

5th ISAF 2025

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Proceedings of Papers



Organized by:
Faculty of Agricultural Sciences and Food - Skopje,
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**Faculty of Agricultural Sciences and Food-Skopje
Ss. Cyril and Methodius University in Skopje,
North Macedonia**



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**V INTERNATIONAL SYMPOSIUM FOR
AGRICULTURE AND FOOD
ISAF 2025**

**8-10 October 2025
Ohrid, North Macedonia**

Skopje, 2026

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PREFACE

The Fifth International Symposium for Agriculture and Food, held on 8-10 October 2025 in Ohrid, brought together researchers, academics, professionals, policymakers, and other relevant stakeholders to exchange knowledge, present research findings, and discuss current challenges and emerging developments in the field of sustainable agriculture and food production.

These Proceedings comprise the scientific papers presented and accepted for publication within the framework of the Symposium. The contributions address a broad range of topics related to below-mentioned topics, reflecting both theoretical developments and practical applications.

The Editors would like to express their appreciation to all authors, reviewers, members of the Scientific and Organising Committees, partner institutions, and participants for their valuable contribution.

The Editors

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The topics of ISAF 2025 are divided into the following scientific sections:

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- Agricultural Economics
- Agricultural Machinery and Sustainable Agriculture
- Apiculture
- Phytomedicine - Plant Protection
- Food Quality and Safety
- Fruit Growing, Viticulture and Wine Production
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ANIMAL BIOTECHNOLOGY

IN SITU AND EX SITU PROTECTION OF THE AUTOCHTHONOUS BOVINE BREEDS

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ABSTRACT

Animal resources were improved throughout the previous century in the direction of greater production and better quality of animal products. In this way, numerous highly productive breeds were created, which could express their productive potential only in much improved conditions of nutrition, accommodation and care. All this caused the disappearance of a large number of old autochthonous, primitive, low-productive, but mostly resistant breeds and strains of autochthonous (domestic) breeds, depopulation of rural and mountainous areas, as well as the abandonment of animal production in marginal areas. Autochthonous breeds play an important place, considering natural potentials, economic and social environment, with historical and cultural heritage, which influenced on rural tradition and agro diversity that developed over the centuries. In this paper are explain the present state of domestic bovine breeds that are locally adapted on our environmental conditions. In the RN Macedonia, there are two representatives of bovine species of autochthonous breeds – Busha cattle and Domestic buffalo. At the end of 2024 there are only 331 registered Busha cattle and 12 domestic buffaloes in the program of book recording. The protection and preservation of autochthonous bovine breeds contributes to the sustainability of agro biodiversity and rural development, through their better use in organic food production and participation in agro tourism by forcing the sustainable concept “from field till table”.

Key words: Animal resources, protection, indigenous (autochthonous or domestic) breeds of cattle

INTRODUCTION

Animal genetic resources (AnGR) include all livestock species, breeds and strains that are of economic, environmental, scientific and cultural interest to agriculture. Preserving the diversity of these resources is essential for ensuring sustainable livestock development, rural livelihoods, culture and tradition as well as environmental stewardship and food security (ERFP, 2022).

According to animal breeds changes in last century, the constant increase in population, which the world is facing today, requires the activation of all natural resources in order to produce sufficient amounts of food. Animal resources were improved throughout the previous century in the direction of greater production and better quality of animal products. In this way, numerous highly productive breeds were created, which could express their productive potential only in much improved conditions of nutrition, accommodation, reproduction, selection and care. The newly created breeds became more and more dependent to a man, less and less resistant and often could not survive in the conditions in which the populations from which they arose had previously lived without problems. All this caused the disappearance of a large number of old autochthonous, primitive, low-productive, but mostly resistant breeds and strains of domestic animals in the world. The depopulation of mountainous areas, as well as the neglect and abandonment of livestock production in marginal areas, unfitting for highly productive breeds, also led to the disappearance of numerous breeds and strains of domestic animals.

In animal husbandry, the problem of preserving low-productive native breeds of domestic animals is still difficult to explain, and this fact makes it difficult to work on improving the use of animal genetic resources in practice. However, agro diversity, including animal genetic resources, in the new concept of sustainable use of genetic resources, occupy a very important place, considering natural potentials, economic and social environment. Macedonia, as a part of the Western Balkans, can be seen as an area where different historical and cultural heritage intertwine, and which greatly influenced the rural tradition and great biological diversity that developed over the centuries. It is reflected in the wealth of species, breeds and strains of domestic animals that are locally adapted to different bioclimatic conditions.

In vivo conservation is the conservation of a breed through the maintenance of live animal populations. It encompasses both *in situ* conservation of breeds within their typical production systems and their *ex situ in vivo* conservation in a controlled environment. *In vivo* conservation

of populations *in situ* is the preferred conservation method. Emphasis is placed on *in situ* programs, because such programs are likely to be the most relevant for long-term *in vivo* conservation objectives (FAO, 2013).

AIM OF THE PAPER

The main objective of this paper is to explain the present state of the genetic resources in cattle representatives in the R.N. Macedonia in 2024, with the explanation of their status, population size, effective size calculation and some recommendations for further activities for their protection. Also, will be presented the In situ – in vivo, as well as Ex situ – in vivo and in vitro conservation of the domestic Busha cattle in 2024 in RN Macedonia.

MATERIAL AND METHODS OF WORK, RESULTS AND DISCUSSION

Cattle production in Macedonia consists of three sectors: small-scale farmers (around 74%) keeping 1-3 cow and mainly producing for home consumption; medium-scale farmers (21%) keeping 10-20 cows with annual production of 4000-6000 kg milk/head and specialized commercial farms (around 5%) with more than 50 heads that produce annually over 7.000 kg milk intended solely for the market.

Table 1. Total no. of cattle in the R. of N. Macedonia, in heads (AFV, 2024)

Year	2020	2021	2022	2023	2024
Total no. of cattle (heads)	152.814	145.299	138.513	128.467	131.200

According to the official statistical data, in 2024 there are totally 131.200 cattle in the R. of N. Macedonia, from which only 2% of domestic Busha cattle, 39% Holstein and more than 50% crossbreeds (Agency of Veterinary and Food-AVF, 2024).

Table 2. Cattle breed distribution in the R.N. Macedonia (AVF, 2024)

Synonym – Breed of cattle	Total No.(heads)	%
Aberdeen Angus – AA	231	0,18
Braunvieh (Brownswiss) - BB	205	0,16
Buffaloes – БИ	25	0,02
Busha – БУ	2445	1,86
Crossbreds – ДР	68065	51,88
Limousine – Л	112	0,09
Brown (Montafon) – MH	2350	1,79
Grey Tyrol (Oberintal) – ОБ	35	0,03
Simmental - CM	6242	4,76
Beef breeds – TP	16	0,01

Holstein - ΦP	50882	38,78
Hereford - XΦ	526	0,40
Charolais - III	66	0,05
Total no. of cattle in 2024	131200	100 %

Organic agricultural production in the Republic of North Macedonia in 2023 is represented from 888 producers. The total area with crop organic production is 4 215 hectares of which 1 475 hectares are areas of organic production in conversion period. With 51% (2 158 hectares), cereals still have the large share of the agricultural area used for organic production (SCS, 2024, European Commission, 2023). The share of organic cattle is 7 528 and in the total number of cattle is 5.1%. The share of organic sheep in the total number of sheep is 20.5% and organic goats are participating with 5.8% in the total number of goats (SCS, 2024).

Table 3.Total organic livestock (no. of heads) in the R. of N. Macedonia (Annual Statistical Review, 2024)

Year	Cattle			Sheep	Goats
	Total organic	organic	in conversion	total organic	Total organic
2021	9 752	7 013	2 739	110 876	5 518
2022	7 821	4 782	3 039	103 940	5 136
2023	7 528	4 204	3 324	120 514	4 966

Table 4. Total cattle heads in organic production in RN Macedonia from 2013 till 2023 (SCS-State Chamber of Statistics, 2024)

Year	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Cattle	2726	2133	4401	3368	4698	6390	7170	8723	9752	7821	7528

From above tables can be considered that in last several years in the R. of N. Macedonia, the total number of cattle in organic production is generally increasing. In 2021, from total 145299 cattle heads, there were 9752 in organic, but in 2023 from total 128467 heads, there are 7528 cattle heads in organic production (Interreg-IPA CBC 2017, AFV, 2024, Rasmus et al, 2024, MAFWE 2024, SCS, 2024).

In the past several decades, as a result of uncontrolled crossing of these cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha.

Busha strains in the R. of Macedonia

There are two main classifications of Busha strains in the R. of Macedonia:

- Classification of strains according to locality: Povardarie strain, Polog strain, Ograzden strain, Prespa (Ohrid) strain, Mariovo strain, etc.

- Classification of strains according to color: Black strain, Brown strain, Red strain, Gray strain, and Tiger strain. This classification is officially accepted from our farmers.

From 2011 till 2024 is active the Programme for Protection of Livestock Biodiversity leaded by the Ministry of Agriculture, Forestry and Water Economy, with the main activities:

- Inventorization,
- Characterization, monitoring of the trends and risks,
- Phenotype analyses,
- Recording of production traits,
- Recording of reproduction traits,
- DNA characterization, and
- Establishment of a gene bank of semen, blood and hair.

In our National Biodiversity Program, in cattle breeding there are two representatives of domestic cattle: 1. Busha cattle and 2. Domestic water buffalo.

Table 5. Total number of controlled Busha cattle in book recording *In situ in vivo* in NRM (2013 - 2024)

Year	Total no. of controlled cattle	Total no. of Busha cattle	Total Busha farms	No. of Busha cattle per farm
2013	1570	730	42	17,38
2014	1962	786	33	23,82
2015	2058	720	42	17,14
2016	2200	898	56	16,04
2017	1260	544	34	15,97
2018	1463	606	39	15,54
2019	2434	654	42	15,57
2020	2129	615	36	17,08
2021	1915	594	32	18,56
2022	2041	438	25	17,52
2023	1688	340	22	15,45
2024	1444	331	24	13,70

Table 6. Total number of domestic buffaloes in RN Macedonia from 1939 till 2024 (ASR, 2024)

Year	Heads, No.	Year	Heads, No.	Year	Heads, No.	Year	Heads, No.
1939	39950	1977	11100	1991	1488	2008	174
1957	20247	1978	9426	1992	1179	2010	92
1960	18919	1979	9343	1993	1125	2012	73
1962	15530	1980	9006	1994	1021	2013	68
1963	17470	1981	9200	1995	1017	2014	70
1964	15663	1982	9020	1996	1013	2015	62
1965	17366	1983	7800	1997	908	2016	55
1966	12645	1984	6750	1998	899	2017	39
1967	13631	1985	4725	1999	725	2018	42
1969	10653	1986	2316	2000	632	2019	50
1973	11255	1987	1780	2001	605	2020	52
1974	9825	1988	1709	2002	599	2021	50
1975	10316	1989	1647	2004	565	2022	52
1976	9768	1990	1602	2006	409	2024	12*

From table 4 and 5 we can see that in *In situ in vivo* program at the end of 2024 in RNM there are totally 331 adult heads of Busha cattle and totally 12 heads of domestic water buffalo.

Table 7. Risk categories according to cattle reproductive capacity (FAO, 2013)

		♀						
Reproductive capacity		≤100	101-300	301-1000	1001-2000	2001-3000	3001-6000	≥6000
Low	≤5							
	6-20							
	21-35							
	≥35							

Note:

	Critical
	Endangered
	Vulnerable
	Not at risk

Effective population size: (abbreviated N_e) the size of a hypothetical idealized population that would generate the values of genetic diversity parameters observed for a given population. The N_e corresponds to the number of breeding animals per generation and is usually smaller than the actual population count. (FAO, 2013, Mijić et al., 2013).

The main calculation of the Effective population size is by this formula: $N_e = \frac{4 \times N_m \times N_f}{N}$,

where: N_e – effective population size, N_m – No. of male reproductive individuals; N_f – No. of female reproductive individuals, and N – total No. of reproductive individuals.

According to the effective size of cattle population, there are 4 levels of risk capacities in cattle: 1. Critical if $N_e \leq 50$; 2. Endangered if N_e is between $N > 50$ and $N_e \leq 200$; 3. Vulnerable if N_e is between $N > 200$ i $N_e \leq 1000$, and 4. Not at risk if $N > 1000$.

Table 8. Effective population size and risk categories of Busha cattle in Macedonia from 2013 till 2024

Year	Number of breeders	Number of males (Nm)	Number of females (Nf)	Effective pop. size (N_e) ($4 \times N_m \times N_f$) / ($N_m + N_f$)	Endangered category (FAO)
2013	42	91	639	318.62	Vulnerable
2014	33	77	709	277.83	Vulnerable
2015	42	66	654	239.80	Vulnerable
2016	56	66	832	244.60	Vulnerable
2017	34	81	463	275.76	Vulnerable
2018	39	49	557	180.15	Vulnerable
2019	42	35	619	132.51	Vulnerable
2020	36	32	583	121.34	Vulnerable
2021	32	47	547	173.12	Vulnerable
2022	25	24	414	90.74	Vulnerable
2023	22	12	327	46.31	Endangered
2024	24	12	317	46.24	Endangered

Table 9. Effective population size and risk categories of domestic buffalo in Macedonia from 2019-2024

Year	No. of breeders	Number of males (Nm)	Number of females (Nf)	Effective population size (N_e) ($4 \times N_m \times N_f$) / ($N_m + N_f$)	Endangered category (FAO)
2019	4	7	28	22.40	Critical
2020	3	6	21	18.67	Critical
2021	3	5	22	16.30	Critical
2022	3	5	20	16.00	Critical
2023	2	4	19	13.22	Critical
2024	2	1	8	3.56	Critical

Table 10. In vitro conservation of Busha cattle and of Domestic buffalo at the end of 2024

Breed	Species	Type of gene material	No. of animals	No. of doses	X - Average per bull
Busha cattle	Cattle	Sperm doses from bulls	8	3143	392.88
Busha cattle	Cattle	Fibers from coat	124	124	1 dose per head
Busha cattle	Cattle	Blood samples	876	876	1 dose per head
Water buffalo	Cattle	Fibers from coat	65	65	1 dose per head
Water buffalo	Cattle	Blood samples	42	42	1 dose per head

In table 10 was presented the *IN SITU - in vitro* conservation was done for both cattle representatives – Busha cattle and domestic buffalo, in the following genetic materials: sperm doses from bulls, fibers from coat and blood samples. In last several years were collected totally 3143 sperm doses from Busha bulls, 124 i.e. 65 fiber samples from Busha i.e. buffaloes and 876 i.e. 42 blood samples from Busha i.e. buffaloes till the end of 2024.

CONCLUSIONS

According to the FAO criteria, the domestic Busha cattle at the end of 2024 is categorized as an ENDANGERED status, but the domestic buffalo as a CRITICAL. By The *IN SITU - in vivo* conservation of domestic cattle in the R.N. Macedonia, at the end of 2024 were registered totally 331 heads of Busha cattle (only adult male and female individuals) and 12 heads of domestic buffaloes. *IN SITU - in vitro* conservation was done for both cattle representatives – Busha cattle and domestic buffalo, in the following genetic materials: sperm doses from bulls, fibers from coat and blood samples. In last several years were collected totally 3143 sperm doses from Busha bulls, 124 i.e. 65 fiber samples from buffaloes and 876 i.e. 42 blood samples from Busha cattle till the end of 2024.

In vivo conservation is the conservation of a breed through the maintenance of live animal populations. *In vivo* conservation of populations *in situ* is the preferred conservation method. *Ex situ in vivo* conservation of cattle takes a crucial place and basic strategy on our farms.

According to the present state of the domestic cattle breed Busha and domestic water buffalo, *in situ* and *ex situ* conservation is necessary for their protection. The protection and preservation of autochthonous bovine breeds contributes to the sustainability of agro biodiversity and rural development, through their better use in organic food production and participation in agro tourism by forcing the sustainable concept “from field till table”.

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SECOND LOCATION RECORD OF *Monoplex parthenopeus* (SALIS MARSCHLINS, 1793) IN THE MEDITERRANEAN COAST OF TÜRKİYE

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ABSTRACT

Konyaaltı Beach is one of Antalya's world-famous beaches. During scuba diving conducted on May 24, 2025, a gastropod species with hair-like structures on its shell was identified under an high density polyethylene (HDPE) pipe that carries seawater from the sea to an aquarium on land. This gastropod was collected and brought to the laboratory of the Faculty of Fisheries at Akdeniz University. In the laboratory, some biometric measurements of the shell were taken, and the species was identified as *Monoplex parthenopeus* (Salis Marschlin, 1793). *Monoplex parthenopeus* had previously been reported for the first time along Türkiye's Mediterranean coast in the Gulf of Antalya, approximately 20 km from this location. With this study, the presence of the species on the Turkish Mediterranean coast is being reported for the second time. Belonging to the family Cymatiidae and known as the giant or hairy triton, *M. parthenopeus* is a gastropod species distributed in the Indo-Pacific and Atlantic Oceans. The fact that this species has now been reported in two nearby locations in the Gulf of Antalya around the same period raises the possibility that global warming may be playing a role.

Keywords: *Monoplex parthenopeus*, record, Gulf of Antalya

INTRODUCTION

Global climate change has recently altered oceanic conditions, influencing species dynamics and ecosystems around the planet. One of the most important effects is rising seawater temperatures. It is reported that global sea water temperatures have risen by 0.159 °C each decade over the last century (Brown et al., 2019). One biological implication of rising temperatures is the change in species distribution known as tropicalization. During these changes, species that can live in higher seawater temperatures expand their suitable habitats, while those that dwell in lower temperatures partially migrate to colder places (McLean et al.,

2021). In recent decades, the occurrence of tropical and subtropical species outside their natural ranges has increased globally (López-Alonso et al. 2023).

The Mediterranean Sea is connected to the Atlantic Ocean via the Strait of Gibraltar and to the Red Sea, an extension of the Indian Ocean, by the Suez Canal. The Mediterranean's biodiversity has been changing rapidly due to increasing water temperature. This alteration is especially noticeable in the eastern Mediterranean region. Numerous Red Sea-originating species have migrated to the Eastern Mediterranean via the Suez Canal and have rapidly spread by adapting to local conditions. Species that have migrated into the Mediterranean are not limited to those originating from the Red Sea. The Mediterranean serves as a habitat for a significant number of species that originated from the Atlantic.

Some species distributed in the Indo-Pacific and Atlantic Oceans are also observed in the Mediterranean. *Monoplex parthenopeus* (Salis Marschlins, 1793), known as the giant or hairy triton, is one of these species. This marine gastropod, belonging to the Cymatiidae family, inhabits in subtropical and temperate seas in both the northern and southern hemispheres. It is distributed over a wide area from the Atlantic Ocean to the Gulf of Mexico, from the Caribbean Sea to Brazil, from the Canary Islands and Angola to the Iberian Peninsula (Beechey, 2014). Moreover, this gastropod species has also been reported in the Indian Ocean and the Red Sea. *M. parthenopeus* lives on rocky, gravelly and muddy seabeds in coastal areas extending from the intertidal zone to a depth of 70 m. The shell is 80-190 mm long, medium-sized, stout, spindle-shaped, and has long and dense hairs on a thick, plate-shaped dark brown cuticle (Gokoglu et al. 2024).

M. parthenopeus is a carnivorous species that mainly feeds on bivalves and echinoderms. This gastropod species causes extensive damage to oyster and mussel beds (Buso et al., 2017). Its saliva contains sulfuric acid chelators, a paralyzing toxin, digesting enzymes, and mucus.

The species exhibits internal fertilization. Following fertilization, the female lays eggs in a cup-shaped capsule over a period of 4 to 6 days. The female cares for the eggs for a month, until they hatch. The larval cycle lasts 175 days, however, the larvae may delay metamorphosis. Therefore, a planktonic life span of up to about 300 days has been observed in the natural environment. This species dispersal capacity in all marine waters. Veliger larvae, carried by ocean currents, can be transported over very long distances (sometimes even over 4000 km) from the original spawning site. Some related species are now known to survive much longer in the larval stage, exceeding what was previously considered a record for larval life span (Couto, 1998).

The first record of this species from the Mediterranean coast of Türkiye was made by Gokoglu et al. (2024). In the reported study, the sample was obtained from an area with a rocky and gravelly bottom structure.

MATERIALS AND METHODS

Antalya City Aquarium is located on Konyaaltı Beach. Along the coastline, an approximately 400 m long HDPE pipe was laid on the sea-bottom (36°52'17.58"N; 30°39'39.09"E coordinates) to draw water from the sea to this aquarium. This pipe acts as an artificial reef in the sea and hosts a rich fauna.

During the scuba dive to this pipe on May 24, 2025, a freshly dead individual of hairy gastropod (Figure 1) was encountered on the pebbled bottom under the pipe at a depth of 12 meters. The gastropod was collected and brought to Laboratory of the Faculty of Fisheries at Akdeniz University, where its length and height were measured with a caliper.

Species identification was conducted according to the works of Wilson (2002), Beu and Raine (2009), Quan Yeo (2014) and Zaminos et al. (2021).

RESULTS AND DISCUSSION

The hairy gastropod species found under a pipe on the pebbled ground at Konyaaltı Beach in Antalya was identified as *Monoplex parthenopeus* (Salis Marschlins, 1793). The shell length of this individual was measured as 83.05 mm, and the shell height was measured as 40.55 mm (Figures 2–3). The thick shell of *M. parthenopeus* has strong canals detailed with spiral ribs containing axial striae and nodules. There are hair-like extensions on the shell (Figures 1 and 2). These findings are consistent with the diagnosis of *M. parthenopeus* by Wilson (2002), Beu and Raine (2009), Quan Yeo (2014), and Zaminos et al. (2021).

M. parthenopeus is a gastropod species that is widely distributed in both the Northern and Southern Hemispheres. It inhabits subtropical and temperate seas and has been reported in marine, brackish, intertidal, reef, rocky or muddy shore, and estuarine habitats (Beu and Raine, 2009)

There are limited studies on the presence of *M. parthenopeus* along the coasts of Türkiye. The first record of the species on the Turkish coast was reported by Okuş et al. (2004) from the

Datça-Bozburun coastline in the Aegean Sea. To date, there is only one report (Gokoglu et al., 2024) documenting the presence of *M. parthenopeus* on the Mediterranean coast of Türkiye.

In a recent study, Zaminos et al. (2021) detected this species in a long-line system within a mussel (*Mytilus galloprovincialis*) cultivation area in the Aegean Sea and suggested that its presence was linked to increasing seawater temperatures.

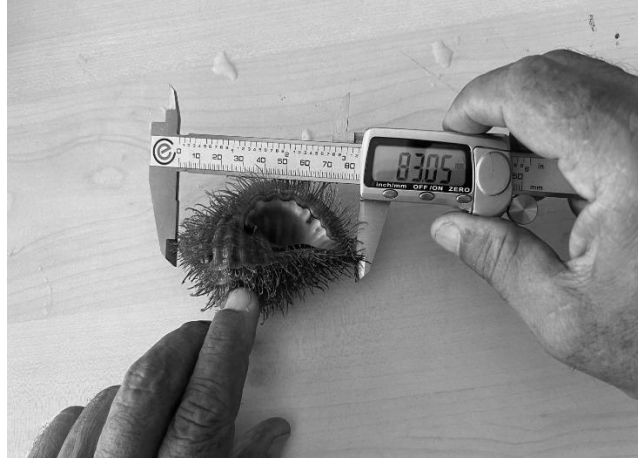


Figure 1: Length measurement of *Monoplex parthenopeus*

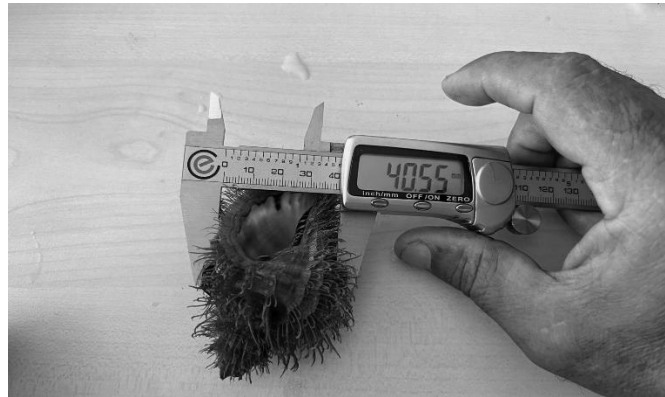


Figure 2: Shell-height measurement of *Monoplex parthenopeus*

CONCLUSIONS

In this study, *M. parthenopeus* was recorded at a depth of 12 meters off Konyaaltı Beach in Antalya Bay. The first Mediterranean record of the species from the Turkish coast was made during a scuba dive approximately 20 km from Antalya Bay. The second record, presented in this study, was made approximately one year after the first, again from Antalya Bay. This recurrence is thought to be related to the effects of global warming. These findings suggest that biodiversity in the eastern Mediterranean is gradually changing.

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IMPORTANCE OF GENOMIC ANALYSIS IN INHERITANCE OF MILK PRODUCTION TRAITS IN HOLSTEIN COWS

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ABSTRACT

This paper presents the theoretical foundations for evaluating breeding values using traditional methods (progeny testing, pedigree, and performance testing) and genomic analysis. A statistical analysis was also conducted comparing two practical methods for assessing the breeding value of Holstein heifers. Cartilage tissue samples from ear tags were collected for genomic analysis from 71 female calves born between early October 2022 and the end of March 2023. The analysis was conducted at the LABOGENA laboratory in France according to USDA-CDCB standards, comparing the chip with DNA samples and evaluating the impact of 60,000 SNP density markers. Breeding value assessment based on ancestry averages was carried out using software for genetic positioning, planning, and mating. The reliability of the genomic analysis method for predicting the ability to transmit milk production traits (PTAM, PTAF, PTAP) in female individuals of the Holstein population ranges between 65–70%. The reliability of the same trait estimates using the pedigree method ranges from 35% to 40%. Average values and coefficients of variation for PTAM, PTAF, and PTAP estimated from ancestry are significantly higher than those estimated from genomic analysis. The correlation between the two methods for assessing breeding value in terms of milk yield, protein yield, and fat yield-expressed through Pearson and Spearman coefficients-is positive and strong.

Key words: breeding value, genomic selection, Holstein-Friesian cattle

INTRODUCTION

The improvement of cattle production from a genetic perspective aims to select animals with desirable phenotypes for important traits and use them as parents for future generations. Since ancient times, humans have attempted to “enhance” certain breeding values of domestic animals, consciously applying selection within livestock populations by deciding which individuals to use for reproduction and to what extent (Ivanković, 2012). Genetic evaluation of an individual candidate for selection refers to the assessment of its breeding value. The breeding value of an individual determines its place within the population and is the main criterion for selection (Jovanovac, 2013).

In the 1970s, breeding work was based on progeny testing, where the breeding value of a bull was evaluated by monitoring the productivity of its offspring (Ivanković, 2012). In cattle breeding, progeny testing is conducted for traits related to milk production and fattening. A bull’s breeding value for milk yield is assessed based on the deviation in milk production of his daughters compared to the average of their peers (Uremović et al., 2002).

During the last two decades of the 20th century, the rapid development of molecular genetics led to intensified research into the structure and function of genomes and genes. Genetic maps of domestic animals were developed, and genetic markers became tools for more effective selection, improvement of key production traits, elimination of hereditary diseases, and greater reliability in breeding programs. By incorporating appropriate genetic markers into breeding programs, it became possible to select genetically favorable individuals at an early age, regardless of gender, body structure, physiological status, or other factors, before economically important traits even manifest.

All of this has led to a better understanding of genetic mechanisms and the development of genomic selection in livestock, especially in cattle breeding (Ivanković, 2005).

Genomic selection involves the use of genetic information obtained through direct genome (DNA) analysis of the animal to describe its breeding value earlier and more accurately. This was made possible by the sequencing of the bovine genome and the development of analytical methods that can quickly and cost-effectively test thousands of gene loci simultaneously and identify a large number of genetic markers.

The aim of this paper is to statistically compare the estimation of breeding values using the traditional method based on ancestry with the method of genomic analysis in Holstein heifers.

MATERIALS AND METHODS

Cartilage tissue samples from ear tags for genomic analysis were collected using specialized Allflex TSUA (Tissue Sampling Unit Applicator) pliers and TSU (Tissue Sampling Unit) kits for sample collection and storage. Biological material was obtained from 71 female calves born between early October 2022 and the end of March 2023. The analysis was conducted at the LABOGENA laboratory in France, following USDA-CDCB (Council on Dairy Cattle Breeding) standards, by comparing the DNA chip with the samples and evaluating the impact of 50,000 SNP (Single-nucleotide polymorphism) density markers.

Breeding value estimation based on ancestor averages was performed using software for genetic positioning, planning, and mating. The software utilizes performance data from male ancestors going back three generations. The estimation of breeding value involves parameters such as previously evaluated breeding values of ancestors (via genomic analysis and/or progeny testing, individual performance), and the inbreeding coefficient calculated from the pedigree as a measure of relatedness and genetic diversity.

Of the 78 breeding values for various traits obtained through genomic analysis and the 33 breeding value estimates obtained based on pedigree (using the genetic positioning software), the correlation between results from both methods was determined for three production traits:

- Predicted Transmitting Ability for Milk Yield (PTAM),
- Predicted Transmitting Ability for Fat Yield (PTAF),
- Predicted Transmitting Ability for Protein Yield (PTAP).

Since the assessment of genetic potential using both methods is based on testing Holsteins in the USA, the unit of measurement for the predicted transmitting abilities is pounds (lbs), not kilograms. Given that $1 \text{ lb} = 0.45359237 \text{ kg}$, all calculations related to determining the correlation between the two data sets in this study were performed using the original unit (pounds-lbs) to avoid potential errors due to conversion and rounding of decimal numbers.

To determine the relationship between breeding values for milk traits (milk yield, milk fat, and protein yield) obtained through the traditional method (pedigree-based) and those

obtained through genomic analysis, correlation methods were used, including Spearman's rank correlation coefficient. Statistical analysis was performed using Microsoft Office Excel.

RESULTS AND DISCUSSION

The true breeding value of an individual for a quantitative trait is an unknown value and is therefore estimated with greater or lesser accuracy. The accuracy of breeding value estimation is influenced by several factors: the type and amount of information, the heritability of the trait, the degree of environmental influence, and the statistical method and model used for estimation. The accuracy of breeding value estimation directly affects animal selection and the overall success of the breeding program (Jovanovac, 2013).

The implementation of genomic analysis into genetic improvement programs for dairy and dual-purpose cattle breeds represents a turning point in selection. Traditional methods previously involved selecting parents based on their own performance, offspring results, half-sibling performance, and pedigree. Back then, the reliability of breeding value estimation for individuals in the population heavily depended on progeny performance data (progeny testing). Genomic selection allows for a significant reduction in generation intervals—more than half, depending on selection goals—which greatly accelerates the rate of genetic change in the population.

To achieve sustainable and competitive development in cattle breeding, and in line with global trends, the Republic of Serbia has initiated the implementation of genomic selection in its cattle industry. The goal of genomic selection is to integrate all available sources of information—phenotype, pedigree, and genetic markers—to maximize the accuracy of estimated breeding values (EBV) and secure genetic progress. Genomic breeding values can be calculated for both sexes at an early age, allowing genomic selection to enhance profitability and accelerate genetic gain in dairy cattle breeding by shortening generation intervals and reducing progeny testing costs. In addition to greater genetic progress, genomic selection also improves pedigree tracking, helps prevent inbreeding, and facilitates faster improvement of traits with low heritability.

Selection based on offspring relies on the production data of the animal's descendants to estimate its breeding value. This form of selection is also referred to as progeny testing (Jovanovac, 2013). A more accurate assessment of offspring phenotypes ensures higher accuracy in estimating the breeding value of their parents, who are candidates for selection. Besides the number of offspring, the accuracy of breeding value estimation depends on the

degree of relatedness between offspring from the same parent and the heritability of the trait (Jovanovac, 2013).

Table 1. Statistical Indicators of Breeding Value Estimation for Traits of Interest

Breeding value assessment	$\bar{x}$ - (lbs)	SD - S_x - (lbs)	Min - (lbs)	Max - (lbs)	IV - (lbs)	CV (%)
PTAM - based on pedigree	999.35	254.30	+68	+1491	1423.00	25.45%
PTAM - based on GA	623.15	384.44	-59	+1568	1627.00	61.69%
PTAF - based on pedigree	43.28	11.09	+13	+64	51.00	25.62%
PTAF - based on GA	30.80	17.85	-15	+64	79.00	57.95%
PTAP - based on pedigree	37.62	6.88	+13	+51	38.00	18.29%
PTAP - based on GA	26.69	9.53	+3	+49	46.00	35.69%

In this study, a statistical analysis was conducted on the correlation between breeding value estimates for milk yield, fat yield, and protein yield traits obtained through pedigree-based methods and results from genomic analysis. Descriptive statistical parameters provide information on average values and variations of individual results relative to the population mean included in the study.

Based on the statistical indicators (Table 1), it can be concluded that the average estimated transmitting ability for all traits based on pedigree is significantly higher than the average estimated transmitting ability for all traits based on genomic analysis.

In the case of breeding value estimation using genomic analysis, high coefficients of variation are observed for the estimated transmitting ability of milk yield (61.69%), fat yield (57.95%), and protein yield (35.69%). The results of breeding value estimates obtained through genomic analysis show significantly greater variability compared to those estimated based on pedigree.

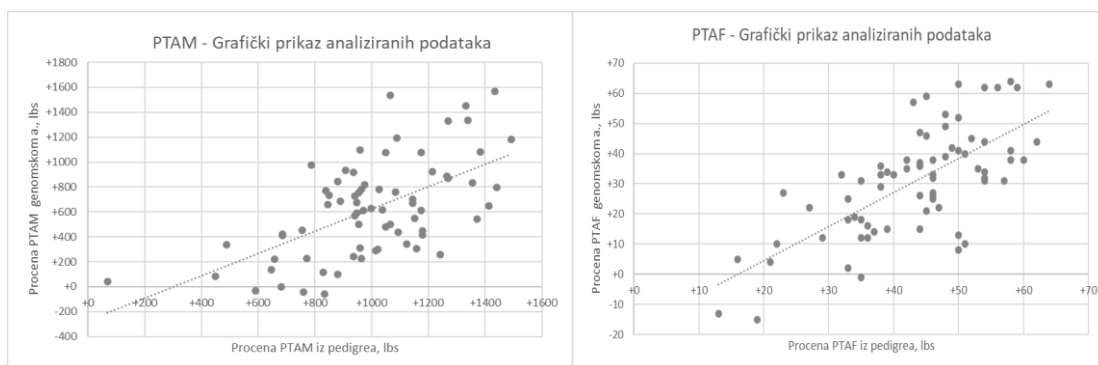


Chart 1. Display of Analyzed Data for
Milk Yield Trait Chart

Chart 2. Display of Analyzed Data for
Milk Fat Yield Trait

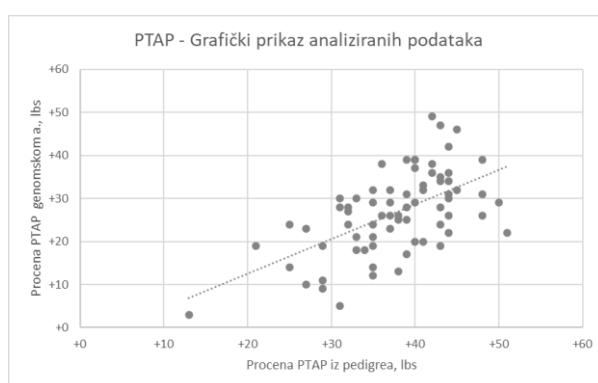


Chart 3. Display of Analyzed Data for Protein Yield Trait

Over the last twenty years, polymerase chain reaction (PCR) and DNA sequencing (determining nucleotide sequences), as fundamental research methods in molecular genetics, have become successful and economically viable for a broader range of scientists (Avisé, 2004). With the decreasing cost of genotyping and the development of microchips (e.g., Illumina SNP50K chip), it has become possible to genotype around 50,000 genetic markers across the entire cattle genome. This opened up the possibility of incorporating an additional source of information into selection work, known as genomic selection (Špehar, 2013).

Pearson's correlation coefficient (r_{xy}) expresses the degree of association between two sets of data, in this case, the results of genomic analysis and the results of breeding value estimation based on pedigree. The independent variable (x) in this case is the breeding value estimation based on pedigree, while the dependent variable (y) is the genomic breeding value, which directly depends on pedigree.

Table 2. Correlation Analysis of Breeding Value Estimation Methods for Traits of Interest

Characteristic x	Characteristic y	r_{xy}	Sr_{xy}	t
PTAM - based on pedigree	PTAM - based on GA	0.5931	0.0969	6.1187***
PTAF - based on pedigree	PTAF - based on GA	0.7008	0.0859	8.1597***
PTAP - based on pedigree	PTAP - based on GA	0.5819	0.0979	5.9429***

In all three examined cases, the correlation coefficient (r_{xy}) is positive and ranges from 0.5819 to 0.7008 (0.5931 for PTAM, 0.7008 for PTAF, 0.5819 for PTAP). Based on the correlation coefficient (Table 2), it can be concluded that the correlation between the two methods for estimating breeding values for milk yield, protein yield, and milk fat yield is positive and very strong. The results of the t-test show that all correlations examined in the study are statistically highly significant (at a significance level of $\alpha = 0.001$).

In all three examined cases, a positive Spearman's correlation coefficient was found, and the association is strong. The results of the t-test show that all correlations examined in the study are statistically highly significant (at a significance level of $\alpha = 0.001$).

Table 3. Spearman's Correlation Coefficient of Breeding Value Estimation Methods for Traits of Interest

Characteristic x	Characteristic y	r_s	t
PTAM - based on pedigree	PTAM - based on GA	0.5451	5.4002***
PTAF - based on pedigree	PTAF - based on GA	0.6538	7.1779***
PTAP - based on pedigree	PTAP - based on GA	0.5654	5.6943***

The accuracy of breeding value estimation depends on the size of the reference population needed for establishing a quality estimate, the heritability of traits, and the relatedness between the selection candidates and the reference population (Scheifers and Weigel, 2012). Meuwissen et al. (2001) state that the use of molecular markers for assessing genetic ability ensures faster genetic gain compared to traditional selection. However, according to Janda's (2011) research, the breeding values of bulls that have been conventionally tested through their offspring are over 75%, which is a higher reliability compared to the reliability of genomic breeding values for young bulls.

CONCLUSION

The genetic potential of production traits in Holstein cows (milk yield, milk fat, and protein yield), due to their economic value and relatively high heritability, has a significant

impact on the profitability of milk production. The reliability of genomic analysis for predicting the ability to transmit milk traits (PTAM, PTAF, PTAP) in female individuals within the Holstein population ranges from 65-70%. On the other hand, the reliability of estimating these traits from pedigree data is 35-40%. Under the conditions of milk production in Serbia, genomic analysis still involves high costs for sample transportation, taxes, and laboratory analysis, which can be financially and time-consuming for farmers. Based on the results obtained in the study, it can be concluded that the average values and the coefficient of variation for PTAM, PTAF, and PTAP estimated from pedigree data are significantly higher than the average values for PTAM, PTAF, and PTAP estimated from genomic analysis. The correlation between the two methods for estimating breeding values for milk yield, protein yield, and milk fat yield, expressed through Pearson's and Spearman's correlation coefficients, is strong and positive.

Using pedigree data for estimating the breeding value of production traits, under the current conditions of milk production in Serbia, can yield positive selection results, but certainly with lower selection effects compared to genomic selection. Therefore, producers should be actively encouraged to introduce genomic analysis into selection practices as a more precise method for determining breeding values.

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AMMONIA EMISSIONS FROM LIVESTOCK PRODUCTION: CHALLENGES, CAUSES AND MITIGATION APPROACHES

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ABSTRACT

Livestock production contributes significantly to the emission of ammonia (NH₃), a gas that negatively affects air quality, soil and water systems and contributes to environmental acidification. According to FAO data and recent research, livestock production is responsible for more than 60% of total ammonia emissions, with cattle production accounting for a significant share. The main source of ammonia is nitrogenous substances from manure, especially urea from urine, which is rapidly converted to ammonia. The level of emissions is influenced by numerous factors: diet composition, digestive efficiency, manure management, microclimatic conditions and animal husbandry. An effective emission reduction strategy includes optimizing nutrition, especially crude protein content, balancing key minerals such as calcium and phosphorus, as well as proper storage and application of fertilizers. Understanding the dynamics of nitrogen within the ruminant organism and the fertilizer system is crucial for reducing losses in the form of ammonia. In addition to conventional measures, precision livestock farming technologies are gaining increasing importance, which enable individual monitoring of production and animal health, thereby contributing to better utilization of nutrients and reducing the emission footprint. A short review of current knowledge indicates the need for integrated approaches that combine feeding, manure management and monitoring technologies to achieve sustainable and environmentally friendly livestock production.

Keywords: ammonia emissions; livestock production; ruminant nutrition; sustainable agriculture; nitrogen

INTRODUCTION

Ammonia (NH_3) is one of the most significant reactive nitrogen compounds in the atmosphere, with profound effects on the environment and human health. It contributes to acidification of soils, eutrophication of aquatic systems, and the formation of fine particulate matter ($\text{PM}_{2.5}$), which is linked to respiratory and cardiovascular diseases (Wyer et al., 2022; Apte et al., 2018; Lelieveld et al., 2015). The agricultural sector is responsible for approximately 93% of total NH_3 emissions in Europe (EEA, 2024), and globally, more than 80% of atmospheric ammonia originates from agricultural sources (Van Damme et al., 2021). Within agriculture, livestock systems are dominant, contributing more than 60% of total emissions, primarily from cattle, swine, and poultry operations (FAO, 2023). Historically, ammonia emissions have not received the same policy attention as greenhouse gases, but recent initiatives such as the EU Farm to Fork Strategy and the UNECE Gothenburg Protocol have established stricter reduction targets (European Commission, 2020). According to Bittman et al. (2014), controlling ammonia losses from manure and fertilizer use is key to closing the agricultural nitrogen cycle and improving nutrient efficiency. Ammonia emissions decreased by only 16% between 2005 and 2022 in Europe (EEA, 2024), indicating slow progress. Exposure to $\text{PM}_{2.5}$ from ammonia-derived aerosols has been linked to diseases such as asthma, chronic obstructive pulmonary disease, and lung cancer (Yu et al., 2000; Lelieveld et al., 2015). Therefore, understanding ammonia dynamics and mitigation opportunities within livestock systems is essential to achieving sustainable agricultural production.

SOURCES OF AMMONIA IN LIVESTOCK PRODUCTION

Ammonia emissions in livestock originate mainly from nitrogen metabolism and urea hydrolysis. Urea excreted in urine is rapidly converted to ammonia by urease-producing microorganisms in manure and bedding (Monteny & Erisman, 1999). The major emission pathways are:

1. Animal housing, due to decomposition of urinary urea and feces;
2. Manure storage, where temperature, aeration, and pH affect volatilization;
3. Field application of manure, when fertilizer remains exposed to the atmosphere before incorporation.

In cattle housing systems, average emissions are around 119 g NH_3 per head per day, with values varying between 24–318 g depending on the housing type, ventilation, and temperature (Lee et al., 2025). For poultry, factors such as litter composition, stocking density,

and air exchange rates play crucial roles (Vitaliano et al., 2024). Research by Sommer et al. (2019) showed that manure handling and application stages account for more than half of total ammonia losses in livestock operations. Volatilization is most intense immediately after manure excretion and during spreading, making those the critical control points for mitigation.

FACTORS AFFECTING EMISSIONS

Ammonia emissions are influenced by a wide range of biological, environmental, and management factors.

- 1. Nutritional and physiological factors:** Feed composition, particularly crude protein (CP) content, has a direct impact on nitrogen excretion. Overfeeding protein leads to excessive urea formation, which is subsequently converted to ammonia. Increased protein intake can result in higher urea excretion and consequently higher emissions (Ndegwa et al., 2008). Studies show that reducing dietary CP by 10–15% while supplementing with synthetic amino acids can lower emissions by 20–40% without compromising productivity (Hristov et al., 2011; Dijkstra et al., 2013). The balance of minerals, especially calcium and phosphorus, also affects nitrogen metabolism and microbial activity in manure (FAO, 2023).
- 2. Housing and microclimate:** Microclimatic conditions such as temperature, humidity, and airflow strongly influence ammonia volatilization. Poor ventilation or warm, humid environments increase emission rates (Wyer et al., 2022). Housing type and flooring design—solid vs. slatted floors—determine how urine and feces mix, influencing urea hydrolysis (Groot Koerkamp et al., 1998). Litter type, manure retention, temperature and humidity have a pronounced effect on volatilization of NH_3 (Preece et al., 2017). Figures for cows show significant differences by facility type (e.g., houses vs. tethering) (20–45 g/day/cow vs 5–27 g/day) in one review (Monteny and Erisman, 1999). Higher temperature and higher air humidity promote volatilization, and reduced ventilation increases the concentration of NH_3 .
- 3. Manure storage and application:** Storage conditions, including duration, cover type, and temperature, are critical for ammonia retention. Period, pH, cover, surface treatment and storage conditions significantly affect the amount of NH_3 released (Vitaliano et al., 2024; Lee et al., 2025). Covering slurry tanks or using acidification techniques (e.g., adding sulfuric acid to lower pH) can reduce emissions by up to 80% (Sommer et al., 2019).

Application methods also play a role: injection or rapid incorporation into soil after spreading minimizes volatilization (Misselbrook et al., 2005).

The complex interactions among these factors indicate that effective mitigation must consider the entire production system, rather than addressing single points in isolation.

MITIGATION STRATEGIES

There are a number of measures that have been shown to be effective in reducing NH_3 emissions from livestock systems. Mitigation approaches are typically categorized into nutritional, manure management, housing, and technological strategies.

1. Nutritional strategies

Optimizing dietary nitrogen supply according to animal requirements is one of the most efficient and economically viable strategies. Reduction of expressed protein in feed, supplements that increase nitrogen utilization and feed optimization can reduce urea excretion and thus NH_3 emissions (Ndegwa et al., 2008). Balancing amino acids, adding urease inhibitors, or improving rumen nitrogen utilization through feed additives (such as probiotics or tannins) can significantly reduce ammonia excretion (Dijkstra et al., 2013; Hristov et al., 2011). For dairy cows, phase feeding—adjusting diet formulation to lactation stages—further enhances nitrogen efficiency (FAO, 2023).

2. Manure management

Manure cooling, frequent removal, and use of covers (e.g., straw, plastic, or geotextile) can substantially decrease volatilization. Covering manure storage, application of acidification, litter selection and regular renewal reduce urine and ammonium accumulation (Vitaliano et al., 2024). Acidification during storage reduces pH and converts ammonium (NH_4^+) into non-volatile forms (Sommer et al., 2019). On-farm solid–liquid separation also minimizes surface area exposure. Field application through injection or trailing shoe systems prevents NH_3 losses compared to broadcast spreading (Misselbrook et al., 2005).

3. Housing design and technology

Modern housing technologies, including air scrubbing systems and slatted floors with urine separation, are effective mitigation options (Vitaliano et al., 2024). Automated monitoring of barn temperature and humidity can optimize ventilation rates and minimize emissions.

4. Precision livestock farming

Precision livestock farming tools, such as sensors for feed intake, respiration rate, and manure accumulation allow continuous monitoring and early detection of inefficiencies (Norton and Berckmans., 2017). Integrating precision livestock farming data with emission models provides real-time feedback for improving feeding and environmental control strategies.

DISCUSSION AND OUTLOOK FOR THE FUTURE

Despite advances in research, challenges remain in standardizing emission factors, measurement techniques, and modeling approaches (Bist et al., 2023). Current inventories often underestimate small-scale farm contributions and regional variability in climate and manure management (Van Damme et al., 2021). Comprehensive mitigation requires a system-based approach, combining nutritional optimization, manure handling improvements, and technological innovations. As noted by Sutton et al. (2013), nitrogen management should be integrated within a “circular nutrient economy,” ensuring efficient recycling while reducing environmental losses. The adoption of precision livestock farming technologies is expected to transform emission management by enabling data-driven decision-making, improving feed conversion ratios, and monitoring animal health indicators (Norton and Berckmans., 2017). Future research should focus on life cycle assessment (LCA) and cost–benefit analyses to evaluate the trade-offs between emission reduction, productivity, and economic viability. Policymakers and farmers alike must align emission reduction goals with practical, farm-level realities.

CONCLUSIONS

Ammonia emissions from livestock production represent a major environmental and public health challenge. The main sources are nitrogen metabolism in animals and volatilization from manure during housing, storage, and field application. Effective mitigation requires integrating feed management, manure storage practices, and technological innovations. Nutritional adjustments, such as reducing dietary protein and optimizing amino acid balance, remain the cornerstone of emission reduction. Advanced manure handling, air treatment systems, and precision farming technologies provide further potential to achieve climate and air quality goals. Sustainable livestock systems of the future must balance productivity and environmental protection, reducing nitrogen losses while maintaining farm profitability.

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EFFECT OF THE LAYING HENS' GENOTYPE AND REARING SYSTEM ON THE SELECTED MEAT QUALITY PARAMETERS

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ABSTRACT

Laying hen meat at the end of the production cycle has low organoleptic and sensory qualities, and for that reason it is not readily consumed and is mostly used as a raw material for the meat industry. As a result, the majority of the research that is now available focuses on the quality of broiler meat, whereas the meat of laying hens has less information. This study aimed to investigate the selected breast and drumsticks meat quality parameters (pH, water holding capacity - WHC, cooking loss - CL and tenderness) of different genotypes (Isa Brown and New Hampshire) and rearing systems (organic and floor) of laying hens at the end of the production cycle. The breast meat of Isa Brown had higher pH, WHC, CL and tenderness compared to New Hampshire hens ($p \leq 0.05$), while the rearing system had no significant effect on these traits ($p \geq 0.05$). It is important to note that a significant interaction of the examined factors was recorded for the pH of breast meat ($p \leq 0.05$). There was no significant effect of any of the factors on the pH and CL of drumstick meat ($p \geq 0.05$). Drumstick meat from Isa Brown had higher WHC compared to New Hampshire hens ($p \leq 0.05$). There was also a significant interaction of the examined factors on drumstick meat tenderness ($p \leq 0.05$).

Keywords: layers, pH, water holding capacity, cooking loss, tenderness.

INTRODUCTION

Poultry meat is one of the most popular meats in the world, with consumption of 132.4 million tonnes in 2021 (Shahbandeh, 2023), and it is expected to represent 41% of all the protein from meat sources in 2030. The global shares of other meat products are lower: beef (20%), pork (34%), and sheep meat (5%) (OECD-FAO, 2021). Consumers are attracted to

poultry due to lower prices, product consistency and adaptability, excellent nutritional value and lack of religious restrictions on its consumption.

It is estimated that around 7% (Kokoszyński et al., 2016) or even 15-20% (Rakonjac et al., 2016) of the world's poultry meat production consists of laying hens at the end of the production cycle and post-laying parent flocks. Meat that comes from laying hens after the production period is not readily consumed due to poor organoleptic and sensory properties (Wegner et al., 2024), and the prices of carcasses and meat of old laying hens on the market are low (Choe and Kim, 2020). This kind of meat represents only a by-product, which is obtained at the end of the completed egg production cycle and represents, above all, an important raw material for the meat industry (Rakonjac et al., 2016) as well as for the production of pet food (Kokoszyński et al., 2016). Processing the meat of old laying hens into diversified value-added products is an important way of obtaining residual value in the egg production industry and represents a wise choice from the perspective of environmental protection (Fan and Wu, 2022).

For this reason, it is important to know some characteristics of this meat, because most of the available data on meat quality refer to broilers.

Water holding capacity (WHC) is defined as the ability of the meat structure to hold water within its fibers, and it can be measured by drip or cook loss (Fanatico et al., 2005). Cooking loss is the amount of moisture released by the meat during cooking when the muscle proteins denature, causing structural changes in the tissue of the meat (Honikel, 2004). Expressible moisture refers to the quantity of water that can be expelled from the meat by the use of force and free drip refers to the amount of water that is lost by the meat without the use of force other than capillary forces. Increase in the water content of muscles, enhancing tenderness, juiciness, firmness, and appearance, and improve the quality and economic value of meat (Mir et al., 2017).

The amount of moisture lost during cooking is determined by the pH of the meat. Meat with a high pH will have a lower cooking loss compared to meat with a low pH (Lawrie and Ledward, 2006) and the resulting meat products will be perceived as being dry (Warriss, 2010). Post-mortem pH decline is one of the most important events in the conversion of muscle to meat, because of its effect on the meat texture, water holding capacity, cooking loss, juiciness, microbial stability and/or shelf-life and colour stability (Honikel, 2004). The rate of pH decline is dependent on the activity of glycolytic enzymes post-mortem and the ultimate pH is determined by the initial glycogen reserves of the muscle at mortem (Lawrie and Ledward,

2006). A low pH is associated with poor meat quality and produces meat with lower WHC and functionality.

Tenderness is considered the most important palatability characteristic of meat and a primary factor of quality by consumers (Destefanis et al., 2007). Tenderness can be evaluated by objective methods, instrumental or sensorial with trained panels, or by subjective methods, with a consumer panel (AMSA, 1995). Toughness or tenderness of meat is affected by various factors of which marbling, connective tissue type and content, enzymatic ageing and muscle shortening are only a few (Miller et al., 1995).

For this reason, this study aimed to investigate the selected meat quality parameters of different genotypes of laying hens from alternative rearing systems at the end of the production cycle.

MATERIAL AND METHODS

The experiment was conducted in a 2x2 factorial design with two laying hen genotypes (commercial hybrid Isa Brown and New Hampshire dual-purpose breed) and two rearing systems (floor and organic). 30 birds were housed per group.

The stocking density in floor rearing system was 2.5 birds/m². The feeding program was designed according to the requirements of laying hens in a conventional production system (Table 1).

Table 1. The chemical composition of the diet for laying hens

	Floor system	Organic system
Chemical composition	%	%
Dry matter	88.38	89.82
Crude proteins	16.79	16.82
Crude fats	5.15	4.31
Cellulose	4.82	4.29
Ash	12.52	12.68
Ca	3.72	3.43
Total P	0.71	0.81
Na	0.17	0.18
Lysine	0.79	0.80
Methionine+cystine	0.68	0.48
Metabolisable energy	11.5 MJ	11.3 MJ

Organic groups had the same stocking density as floor groups in houses, but each hen had and about 5m² pastured outlet. The feeding program was designed according to the regulations for organic production, without additions of synthetic amino acids, vitamins and

minerals, using mostly organic-produced components (Table 1). Feed and drinking water for all four experimental groups was available *ad libitum*.

After the end of the one-year production cycle, six animals per group (a total of 24 layers) were randomly selected. After a fasting period of 12 hours, selected animals were slaughtered. After slaughtering, samples of white (breast muscle) and dark meat (drumstick muscle) were collected. These samples were conducted following examination:

- pH of meat
- Water holding capacity (WHC) (cm²)
- Cooking loss (CL) (%)
- Tenderness (kg)

Samples were used for pH analysis for each treatment using a pH meter equipped with a penetration probe (Hanna HI 83141; Hanna Instruments, USA).

Water holding capacity (WHC) of meat was determined according to Grau and Hamm (1953), where the value of WHC is expressed in cm² of wetted surface.

Cooking loss was calculated as a weight difference in the samples before and after cooking in distilled water in a closed glass container (at 80°C for 10 min). It was expressed as a percentage of the weight of the sample before cooking.

Meat tenderness was determined by cutting force (kg) and was measured after cooking at 100 °C for 10 minutes and cutting the meat into 0.5 x 1 x 2 cm pieces in the direction of the muscle fibres on a consistometer (Volodkevich, 1938). Higher values indicate greater cutting force, and this indicates tougher meat. On the other hand, samples that have lower cutting force values indicate softer meat.

All data were analyzed by ANOVA and LSD test (Stat Soft Inc Statistica For Windows. Version 7.0., 2006).

RESULTS AND DISCUSSION

The breast meat of Isa Brown had higher pH, WHC, CL and tenderness compared to New Hampshire hens ($p \leq 0.05$), while the rearing system had no significant effect on these traits ($p \geq 0.05$) (Table 2)

Table 2. The selected quality traits of breast meat of laying hens

	pH	Water holding capacity (cm ²)	Cooking loss (%)	Tenderness (kg)
Genotype				
Isa Brown	5.68±0.08 ^a	9.47±0.82 ^a	33.11±3.18 ^a	2.76±0.53 ^a
New Hampshire	5.59±0.07 ^b	8.78±0.62 ^b	29.89±1.36 ^b	2.35±0.22 ^b

Rearing system					
Floor		5.64±0.11	9.22±0.94	31.86±3.11	2.45±0.37
Organic		5.63±0.05	9.03±0.64	31.14±2.77	2.66±0.51
Genotype x Rearing system					
Isa Brown	Floor	5.73±0.08 ^a	9.57±0.99 ^a	33.56±3.53 ^a	2.52±0.52 ^b
	Organic	5.63±0.03 ^b	9.37±0.69 ^{ab}	32.66±3.05 ^{ab}	3.01±0.46 ^a
New Hampshire	Floor	5.55±0.04 ^c	8.88±0.81 ^{ab}	30.16±1.37 ^b	2.39±0.12 ^b
	Organic	5.63±0.07 ^b	8.68±0.39 ^b	29.62±1.43 ^b	2.32±0.29 ^b
ANOVA					
Genotype		*	*	*	*
Rearing system		ns	ns	ns	ns
Genotype x Rearing system		*	ns	ns	ns

a-c Values within the column with no common superscript are significantly different ($p \leq 0.05$) * $p \leq 0.05$, ns - not significant.

The significant effect of genotype on the pH of breast meat is in accordance with the results of Ahokpossi et al. (2024) who found significant differences in breast pH ($p \leq 0.05$) on Cobb 500 (5.3) and Goliath (5.95), as well as Liu et al. (2023) between Hy-Line grey (6.00) and Guangyuan grey (5.74). A significant effect of the genotype on this trait is reported by Sampels et al. (2021), Lee and Kim (2021) and Englmaierova et al. (2022). This occurrence may have been caused by the heavier carcass weight, as the hens with heavier carcass weights tended to exhibit higher pre-slaughter stress, leading to a decrease in the glycogen in the meat, especially in older birds (Zaneti et al., 2010). On the other hand, Duvnjak (2023) and Wegner et al. (2024) found no significant effect of genotype on this trait ($p \geq 0.05$). The absence of a significant effect of the rearing system on breast meat pH is consistent with the results reported by Husak et al. (2008) and Semwogerere et al. (2018) on broilers. It is important to note that a significant interaction of the examined factors was also recorded for this trait, so there was no significant difference between the genotypes in the organic system ($p \geq 0.05$), while these differences were significant in the floor ($p \leq 0.05$), which is in accordance with the results of Ozbek et al. (2020).

The breast meat with high pH has a higher water holding capacity than meat with lower pH (Mir et al., 2017), so the breast meat of Isa Brown hens had higher WHC and CL compared to New Hampshire ($p \leq 0.05$). Similar results were reported by Ahokpossi et al. (2024), who found higher WHC and CL in the breast meat of Cobb 500 chickens (WHC 36.12% and CL 34.43%) compared to Goliath genotype chickens (WHC 21.89% and CL 18.41%). The authors explain this by the high free water content of the Cobb 500 breast meat.

A significant effect of genotype on WHC of breast meat is reported by Joubert (2013) and Duvnjak (2023) and on CL by Zaneti et al. (2010) and Pellattiero et al. (2020). The absence

of significant effect of the rearing system on these traits is in accordance with the results published by Joubert (2013) and Sampels et al. (2021).

The breast meat of Isa Brown hens had higher values for tenderness compared to New Hampshire ($p \leq 0.05$), which is related to the size and diameter of muscle fibers. A significant effect of genotype on this trait was found by Aksoy et al. (2021) and Englmaierova et al. (2022).

The examined factors had a significantly much lower impact on drumstick meat, so there was no significant effect of any of the factors on pH and CL ($p \geq 0.05$) (Table 3). Results consistent with these, that the genotype has no effect on pH and CL of leg meat, were also found by Liu et al. (2023). Drumstick meat from Isa Brown had higher WHC compared to New Hampshire hens ($p \leq 0.05$). The drumstick meat of Isa Brown had greater tenderness than New Hampshire hens, however, there was also a significant interaction of the examined factors on this trait, so differences were noted between genotypes only in the organic rearing system ($p \leq 0.05$), while there were no differences in the floor system ($p \geq 0.05$). A significant effect of genotype on tenderness is reported by Ahokposi et al. (2024), as well as Wegner al. (2024) which compared two genotypes of laying hens and found significantly higher tenderness ($p \leq 0.05$) in Lohmann Brown compared to Lohmann White leg muscles.

Table 3. The selected quality traits of drumsticks meat of laying hens

		pH	Water holding capacity (cm ²)	Cooking loss (%)	Tenderness (kg)
Genotype					
Isa Brown		5.85±0.04	12.94±1.23 ^a	38.93±1.21	5.25±1.23 ^a
New Hampshire		5.86±0.05	11.96±0.75 ^b	38.19±1.17	4.40±0.61 ^b
Rearing system					
Floor		5.86±0.04	12.66±1.14	38.81±1.08	4.74±0.88
Organic		5.85±0.06	12.24±1.10	38.30±1.36	4.91±1.23
Genotype x Rearing system					
Isa Brown	Floor	5.86±0.05	13.32±1.25 ^a	39.31±1.25	4.78±1.18 ^{ab}
	Organic	5.84±0.04	12.57±1.21 ^{ab}	38.54±1.15	5.73±1.19 ^a
New Hampshire	Floor	5.86±0.02	12.00±0.53 ^b	38.31±0.63	4.71±0.55 ^{ab}
	Organic	5.87±0.07	11.92±0.98 ^b	38.06±1.61	4.08±0.52 ^b
ANOVA					
Genotype		ns	*	ns	*
Rearing system		ns	ns	ns	ns
Genotype x Rearing system		ns	ns	ns	*

a-b Values within the column with no common superscript are significantly different ($p \leq 0.05$)

* $p \leq 0.05$, ns - not significant.

CONCLUSION

Based on the examination of selected quality traits of hen meat, the following conclusions can be drawn:

- The breast meat of Isa Brown had higher pH, WHC, CL and tenderness compared to New Hampshire hens ($p \leq 0.05$), while the rearing system had no significant effect on these traits ($p \geq 0.05$). It is important to note that a significant interaction of the examined factors was recorded for the pH of breast meat, so there was no significant difference between the genotypes in the organic ($p \geq 0.05$), while these differences were significant in the floor system ($p \leq 0.05$).
- There was no significant effect of any of the factors on the pH and CL of drumstick meat ($p \geq 0.05$). Drumstick meat from Isa Brown had higher WHC compared to New Hampshire hens ($p \leq 0.05$). There was also a significant interaction of the examined factors on drumstick meat tenderness, so differences were noted between genotypes only in the organic rearing system ($p \leq 0.05$), while there were no differences in the floor system ($p \geq 0.05$).

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THE EFFECTS OF ORGANIC SELENIUM ON THE GROWTH OF DAY-OLD CHICKS

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ABSTRACT

The biological function of selenium, which is used in poultry nutrition, comes to the fore only when it is incorporated into selenoproteins. The microelement Selenium, which occupies an active place in glutathione peroxidase (GSH-Px), has the role of defending the cellular system from damage caused by the action of oxygen, improving the immune status of animals, and increasing the production characteristics of chickens. Here, we observe the effects of organic selenium on the presence of selenium in egg homogenate with the addition of 0.30 mg/kg of inorganic selenium, 0.03 mg/kg of organic selenium, and a mixture of organic and inorganic selenium of 0.30 mg/kg (0.15 + 0, 15) to determine the optimal concentration of selenium in eggs and its essential importance on the number of incubated eggs and the quality of hatched chicks. The study found that the presence of selenium in eggs had a positive effect on embryo growth and the length of incubated day-old chicks, which was statistically proven by measuring and comparing experimental (O1) and control group (K), and confirmed that experimental (O1) chicks were longer by 0.69 cm compared to control (K) chicks. This confirmed that the use of organic selenium was rational and had a positive effect on the quality of breeding eggs and the length of incubated day-old chicks.

Keywords: day-old chicks, selenium, egg yolk, length, growth.

INTRODUCTION

The development of a cub in the embryonic phase largely determines its development even after birth. This is especially important in poultry, primarily in broiler production, which is short-lived and does not have enough time to later compensate for some shortcoming that was made in the initial phase of embryonic development of chickens. Therefore, we once again

point out the importance of the quality of one-day-old chickens and a simple method for determining the quality by measuring the length of the chickens. A large number of scientists pointed to the fact that we must follow global trends and innovations in the market of additives, enzymes, trace elements, and probiotics, taking full advantage of their beneficial effects on the growth and health of poultry, to achieve maximum production results and performance of domestic animals (Crump et al., 2002). One of the most commonly used nutrients in poultry nutrition in recent times is selenium (Se). This essential nutrient has been experimentally proven to be used and added to food in small quantities, but has an exceptional and significant effect in achieving production results, both younger and older categories of poultry. It is most often found in two forms, organic and inorganic. Organic selenium is bound to the amino acids of plants, selenomethionine, and selenocysteine, while inorganic selenium is found in the form of various minerals: selenite, selenate, and selenide.

Selenium, together with vitamin E, represents a multicomponent barrier of protection of biological membranes from oxidative degeneration. Selenium is the active ingredient of the enzyme glutathione peroxidase-GSH-px. New research in these areas is aimed at studying the effect of selenium as a food supplement on fertility, egg fertilization, embryonic development, the number of hatched chickens, and the quality of one-day-old chickens. The content of selenium in the tissues depends on the concentration of selenium in the feed, but also the form of selenium. Today, selenium is added to broiler parents in the form of sodium selenite from 0.10 ppm to 0.30 ppm, the same applies to selenomethionines, with recommended doses of 0.30 ppm, the upper limit. Cantor (1997) states that the selenium content in eggs depends on the concentration of selenium in chicken feed, as well as on the form of selenium. He states that the use of organic selenium achieves a 30% higher concentration of selenium in eggs compared to the use of inorganic selenium. The same author and his collaborators confirmed the role and importance of organic selenium in the diet of heavy line parents, stating that the amount of selenium in eggs does not exceed a certain limit when selenium is added in inorganic form, while the concentration of selenium in eggs constantly increases with the addition of organic selenium. et al., 2000). Data on the content of selenium in eggs, their influence on a higher percentage of fertilization and reduced embryonic death, were obtained from several different papers (Hassan, 1987; Gajčević et al., 2009; Surai, 2006; Vukašinović et al., 2006). Pešić et al. (2014) state in their paper that it is very difficult to maintain the level of optimal egg production, their fertilization, and a high percentage of hatching, with the use of adequate poultry food. Jokić (2005) came to similar results and stated that very little is known about the

nutritional needs of embryos during their development, because in the last few days before laying, the chicken's metabolism changes, along with its needs. Hansen (1996) states in his paper that the selenium content from eggs passes into the tissues of chicken embryos during incubation. The highest concentration of selenium was recorded in the liver of day-old chicks and was significantly higher in chickens obtained by laying eggs that were enriched with organic selenium, ie eggs obtained from a parent flock that consumed food enriched with organic selenium. Numerous papers talk about the use, role, and importance of selenium in chicken nutrition, as well as the impact of organic selenium on the production results of chickens (Surai, 2002; Pavlovski et al., 2009; Jokić et al., 2005; Marković et al., 2010a). Therefore, we once again point out the importance of the quality of one-day-old chickens and a simple method for determining the quality by measuring the length of the chickens. A well-known expert in the field of incubation Ron Meijerhof (2006) states that at the end of incubation, the embryo grows an average of 1 cm in 24 hours. Under optimal incubation conditions, the embryo does not have to spend energy reserves to adapt to external conditions, but all the energy is used for growth and development. That is why the length of the chickens is an excellent indicator of the quality of the chickens. Many researchers (Tona et al., 2003; Meierhof, 2006; Molenaar et al., 2007) claim that every millimeter of additional body length of day-old chicks means greater potential and better fattening performance.

The study aimed to determine the optimal concentration of selenium in eggs and its essential importance on the quality of breeding eggs and the length of incubated day-old chicks.

MATERIAL AND METHODS

Animals.

The experiment was performed on the poultry farm ZZ "Crnatovo" in Crnatovo and the incubator station Pile-prom in Novo Selo. The material used in the experiment were fertilized eggs of moulting parental pairs of the heavy line COOB 500 and 120 one-day-old chicks originating from the mentioned parents. The COOB 500 heavy line chickens used in the experiment were subjected to moulting, a method with limited food and water. This method is the most applicable in practice due to its practicality and simplicity. Four different mixtures of concentrated feed were used for poultry nutrition, different for each group. Control group (K) used a mixture in which the mineral vitamin supplement did not contain selenium. The experimental group (O1) used the same mixture in which organically bound Selenium was added in the form of selenomethionine whose commercial name is Sel-Plex™ (Altech Inc, Kentucky, USA) in an amount of 0.30 mg/kg of mixture. The experimental group (O2) in

contrast to the first experimental group received the addition of selenium in the form of inorganic selenium - Sodium selenite 0.30 mg/kg of a mixture, while the third experimental group (O3) received a mixture of mixed organic and inorganic selenium in the amount of, 15 + 0.15 mg/kg) 0.30 mg/kg of mixture.

Determining the length of chickens.

During the experiment, periodic collection of eggs was performed by groups, a certain number of eggs (330 per group, a total of 1,320 pieces) were used for incubation and obtaining one-day-old chicks. After incubation, the length of the body was measured in one-day-old chickens, with a ruler, from the tip of the beak, on the one hand, to the middle toe on the left foot, on the other hand. The rest of the eggs (10 pieces per group, a total of 40 pieces) were used for the analysis of egg content, homogenates, atomic absorption spectrophotometric method via hydride (HG-AAS) on the device Varian Spektra AA-10 with hydride addition VGA-76 by the method Pavlović et al . (2009).

Statistical analysis.

Statistical analyses were performed using IBM SPSS Statistics for Windows v17.0 (SPSS Inc., Chicago, IL, USA). All data were processed by standard statistical methods, the descriptive method for determining the arithmetic means, $\pm$ standard deviation (S.D), standard error of the arithmetic mean (S.E), and analysis of variance (ANOVAs) to examine the statistical difference. Differences in the probability level $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Studies conducted to determine the effect of different levels of selenium and selenium of different origins on the concentration of selenium in egg content proved that there is a statistically significant difference in the groups that participated in the experiment. The analysis of the results showed a higher percentage of selenium in the content of eggs originating from hens that consumed food to which selenium of organic origin was added, as well as selenium obtained by a combination of organic and inorganic selenium. Similar data were obtained by a group of scientists who analyzed body mass and conformational components (Yakubu, A. and M.M. Ari, 2016).

The data obtained from the analysis of egg content show that the concentration of selenium in eggs, primarily in the yolk, is much higher in chickens that consumed selenomethionine and selenium obtained by the synergy of organic and inorganic selenium. It was

also found that the level of selenium in eggs was significantly increased after the addition of organic selenium in the food consumed by the parents. The obtained results are not surprising, given the fact that organic forms of selenium, selenomethionine, are absorbed faster in body tissues and nonspecifically incorporated into proteins, unlike inorganic selenium, sodium selenite, which is passively absorbed in the body with a very low degree of absorption. These claims have been proven in the works of Rutz (2003), Rayman (2004), Payne et al., (2005), Surai et al., (2006), Nollet et al., (2007), Pešić et al., (2015).

Table 1. Selenium content in eggs at the end of treatment, mg/ kg $\pm$ Se

Group	Average	N	Min	Max	SD	SE	Index
Organic selenium (I)	0,31	6	0,31	0,33	0,0303	0,0152	100,00
Inorganic selenium (II)	0,23	6	0,22	0,23	0,0344	0,0172	71,43
Synergies selenium (II)	0,33	6	0,32	0,33	0,0268	0,0134	145,33
Control	0,22	6	0,21	0,22	0,0438	0,0219	65,45

Min – minimum; Max- maximum; N- number of samples; SD – standard deviation; SE- standard error

Also, the analysis of the data showed that the addition of organic selenium in food increases the activity of glutathione peroxidase in eggs, yolk and egg white, which slows down the oxidation process of eggs and prolongs the freshness of eggs. Egg freshness and lack of oxidation of eggs

contribute to a higher percentage of fertilized eggs and a higher percentage of hatched eggs. Similar results were obtained by Surai (2000) in a study conducted on day-old chicks.

Table 2. Statistical significance of differences in tested selenium in egg content

Comparison	Difference	Q	P	Significance	Comparison
Synergies - Control	0,11	4	6,523	0,003	**
Synergies - Inorganic	0,10	4	5,942	0,006	**
Synergies - Organic	0,01	4	0,712	0,957	NS
Organic - Control	0,10	4	5,811	0,007	**
Organic - Inorganic	0,09	4	5,230	0,014	*
Inorganic - Control	0,01	4	0,581	0,976	NS

NS- without statistical significance, *-the significance of differences at the level of 95% ** - the significance of differences at the level of 99%

By presenting the results of the examined groups in Table 3, a difference in the results of measuring the length of day-old chicks was noticed and only recorded in experimental groups that consumed selenium of organic origin compared to groups that consumed food enriched with selenium of inorganic origin or did not consume food with the addition of selenium ($p < 0.05$).

Table 3. The average length of one-day-old chickens – at the end of treatments in cm.

Group	Average	N	Min	Max	SD	SE	Index
Organic selenium (O ₁)	18,57	30	16,80	20,30	0,903	0,165	100,00
Inorganic selenium (O ₂)	18,35	30	16,80	20,10	0,906	0,165	98,79
Synergies selenium (O ₃)	18,34	30	16,40	20,10	0,902	0,165	99,93
Control	17,88	30	16,20	19,80	0,758	0,138	97,50

Min - minimum; Max - maximum; N - number of samples; SD – standard deviation; SE – standard error

When presenting the results of the second parameter, chicken length, differences were found between chickens, which are statistically significant, assuming that every millimeter of additional body length means better production results and broiler performance (Yakubu, A. and M.M. Ari, 2018). Also, a well-developed chicken in optimal incubation conditions consumed the available nutrients from the yolk for the development and growth of organs, while the body left little unused substances and energy in the form of the rest of the yolk sac.

Table 4. Statistical significance of the difference in the length of day-old chicks

Comparison	Difference	Q	P	Significance
Synergies - Control	0,69	4,3330	0,019	*
Synergies - Inorganic	0,24	1,4790	0,834	NS
Synergies - Organic	0,20	1,3960	0,861	NS
Organic - Control	0,47	3,9380	0,025	*
Organic - Inorganic	0,01	0,0833	1,000	NS
Inorganic - Control	0,46	2,8540	0,257	NS

*NS- without statistical significance, *-the significance of differences at the level of 95% ** - the significance of differences at the level of 99%*

This suggests that such chickens are longer than those that contain a larger remnant of the yolk sac and that they are shorter and rounded. This statement is the opinion of many authors

who have established in their work the importance of measuring the length of chickens (Tona et al., 2003; Meierhof, 2006; Molenaar and Rejrink, 2006).

CONCLUSIONS

The obtained results indicate that the use of organic selenium in the diet of broiler parents can affect the quality of their offspring. During the examination, it was determined that organic selenium as pure or in synergy with inorganic selenium affects the percentage of egg content. Examination of the length of the chickens is an important parameter that indicates that every centimeter of the chicken significantly affects the later growth. The results obtained during this study indicate that selenium of organic origin showed a significant effect on the length of day-old chicks. Based on all the presented facts, the use of selenium in the form of selenomethionine in broiler parents is recommended, through which it will indirectly improve performance, obtain quality chicken, which will positively affect



Image 1. Measurement of the length of the sawmill in the incubator station "Pile promet" - Novo Selo
(Source: author data)

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AGRICULTURAL ECONOMICS

THE BUSINESS MODEL IN BULGARIAN AGRICULTURAL COOPERATIVES: REGIONAL DIFFERENCES AND SOCIAL IMPACT

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ABSTRACT

Over the past decade, interest in sustainable and flexible business models has been increasing, including their impact on regional policy and development. A main argument for this is that regional differences in Bulgaria are increasingly deepening and expanding, which leads more and more research to focus on research on business models, often built around values and social impact. Cooperatives, together with other actors in the social economy, are attracting more attention than usual. To respond to this challenge, we draw attention to the activities of agricultural cooperatives and focus on their business model.

After Bulgaria's integration into the EU in 2007 and the accompanying socio-economic challenges transformed the model of territorial development in agricultural cooperatives. This led to a deepening of the processes of depopulation in the rural areas of the country. Unbalanced public support for certain territorial needs influenced the regions in the direction of deepening the development processes based on the market principle, rather than on social needs.

The aim of this paper is to analyze the business model in Bulgarian agricultural cooperatives and to highlight some regional differences. The analysis was developed in accordance with the implementation of the work programme on the project „Cooperative models for doing business in Bulgaria and their potential for implementing innovative management solutions“, financed by the „Scientific Research“ Fund, „Fundamental Scientific Research-2022“ competition. Contract No. KII-06-H65/1 -12.12.2022.

The analysis covers 863 agricultural cooperatives, distributed in 28 statistical planning regions, which are at NUTS-3 level, according to the classification of territorial units and correspond to the districts in Bulgaria. The cooperatives are specialized in growing cereals (excluding rice), legumes and oilseeds. Official data from the Agrostistics Department of the Ministry of Agriculture and Food, and Ciela INFO for the period 2007-2021 were used.

Preliminary results show regional differences in terms of selected economic indicators such as: income, costs, profit, labor productivity and profitability. At the same time, the efficiency ratio of costs and revenues is similar.

Key words:, *cooperative model, economic indicators, regional development, social impact*

INTRODUCTION

Agricultural cooperatives play a significant role in the development of rural areas and the overall competitiveness of the agricultural sector in Bulgaria. They contribute to improving the economic stability of small and medium-sized producers, facilitate access to markets, and ensure more efficient use of production resources. Despite the long tradition of cooperative activity in Bulgarian agriculture, the transformations that occurred after 1990 fundamentally changed their structure, functions, and economic importance.

In recent decades, the cooperative sector has faced numerous challenges related to land fragmentation, limited financial resources, aging membership, and increased competition from private agricultural enterprises. At the same time, the process of integration into the European Union and the implementation of the Common Agricultural Policy (CAP) created new opportunities for modernization and strengthening of cooperatives' market positions.

Analyzing the business model of agricultural cooperatives is of particular importance for understanding their sustainability, efficiency, and adaptability to the changing socio-economic environment. Identifying regional differences provides valuable insights into the influence of local factors such as resource potential, institutional support, and regional development strategies. The problems of competitiveness and the regional economy of agricultural cooperatives in Bulgaria have been studied by a number of researchers (Bachev, et al., 2022; Bashev, 2011; Boevsky, I., & Aliyev, A. B., 2023; Ivanova, 2005; Kopeva, et al., 2012; Koteva, 2011, 2015, 2017; Marinov, 2023; Sarov, 2020, 2023, 2025), etc.

The aim of this paper is to analyze the business model of Bulgarian agricultural cooperatives and to highlight some regional differences in their economic performance and efficiency during the period 2007–2021.

MATERIALS AND METHODS

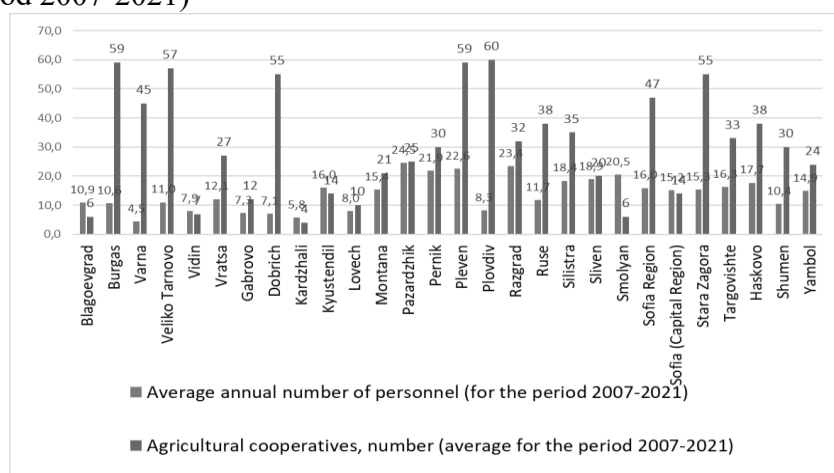
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correspond to the districts in Bulgaria. The cooperatives are specialized in growing cereals (excluding rice), legumes and oilseeds. Official data from the Agrostistics Department of the Ministry of Agriculture and Food, and Ciela INFO for the period 2007-2021 were used. The following statistical and economic indicators were used: number of agricultural cooperatives, number of employees, income, expenses, profit, productivity, profitability, cost-effectiveness ratio, revenue-effectiveness ratio. Correlation between the analyzed indicators was also taken into account.

RESULTS AND DISCUSSION

The general overview of the data is for the period 2007–2021 and covers 28 districts in Bulgaria. The average annual number of personnel is 14.0 people. The total number of agricultural cooperatives is 863 or 30.8 cooperatives on average per district (Figure 1).

Figure 1. Agricultural cooperatives, number and Average annual number of personnel (average for the period 2007-2021)



Source: Own calculations, Ciela INFO

The districts with the highest and lowest average personnel are Pazardzhik – 24.5; Razgrad – 23.4; Pleven – 22.6; Pernik – 21.9; Smolyan – 20.5. Districts with larger or more capital-intensive cooperatives – probably with higher mechanization or specialization. The districts with the lowest personnel are Varna – 4.5; Kardzhali – 5.8; Vidin – 7.9; Dobrich – 7.1 and Gabrovo – 7.3. Smaller cooperative structures are observed here, probably with less land or oriented towards small local markets.

In terms of the distribution of cooperatives, the districts with the largest number of cooperatives are Plovdiv – 60; Pleven – 59; Burgas – 59; Veliko Tarnovo – 57; Dobrich and Stara Zagora – 55. These are traditionally strong agricultural regions, especially Northern and Southern Bulgaria (Thracian Lowland, Dobrudzha). With the fewest cooperatives: are the

districts of Kardzhali – 4; Smolyan – 6; Vidin – 7; Lovech – 10; Gabrovo – 12. Mountainous districts have less agricultural areas and more fragmented land use.

A weak positive correlation is evident between the number of employees and the number of cooperatives. Districts with more cooperatives also tend to have a higher average number of employees, but not always. The correlation coefficient is $\approx +0.25$. For example: Pazardzhik has few cooperatives (25), but a very large number of employees, which indicates the presence of larger structures. In Plovdiv there are many cooperatives (60), but an average small number of employees and smaller cooperatives.

The average size of cooperatives in Bulgaria is small – about 14 employees. Two types of regions are observed: Northern plains (Pleven, Razgrad, Pazardzhik, Dobrich), which are characterized by more intensive agriculture. Mountainous (Smolyan, Kardzhali, Gabrovo), which are characterized by smaller-scale production. Strong deconcentration is also found: there is no strong relationship between the number of cooperatives and their size. At the same time, significant regional unevenness stands out: Northern Bulgaria is more active in cooperative agriculture.

The correlation coefficient in terms of the number of agricultural cooperatives and the average annual number of personnel is 0.02, which means almost no dependence between the two variables. In short, regions with more cooperatives do not necessarily have larger cooperatives in terms of personnel, and vice versa.

In Northern Bulgaria, the centers with developed cooperative agriculture are Pleven, Razgrad, Veliko Tarnovo, Dobrich and Plovdiv, which have the most cooperatives. Grain-producing and mixed cooperatives predominate. The staff is smaller, as the activities are highly mechanized and seasonal. In Southern Bulgaria, there is a more limited number of cooperatives, but a larger average staff, probably with more complex activities (livestock, perennial crops, vegetable production). Districts such as Pazardzhik, Pernik, Smolyan and Haskovo have relatively few cooperatives, but a high number of staff. Mountainous regions compensate with more manpower for smaller areas. On this basis, the following conclusions can be drawn. Southern Bulgaria has fewer, but larger cooperatives (in terms of staff). Northern Bulgaria has more, but smaller cooperatives. Geographical and production differences (grain versus perennial crops and livestock) explain this structure. At the national level: an average of 14 people per cooperative and 31 cooperatives per district - a structure typical of small and medium-sized agribusiness (Table 1).

Table 1. Summary indicators

Region	Average staff	Average number of cooperatives	Characteristics
Northern Bulgaria	13.0 people	39 cooperatives	More, but smaller cooperatives
Southern Bulgaria	15.2 people	23 cooperatives	Fewer cooperatives, but with more staff.

Source: Own calculations, Ciela INFO

For the period 2007–2021, the average cooperative had moderate efficiency, with each worker producing about 75–80 thousand BGN worth of output, and the profit/expenses yielding about 5 times the return.

The districts with the highest profitability coefficient are Ruse – 9.6; Vidin – 9.3; Sofia (capital) – 8.3; Vratsa – 7.8; Sliven – 8.1. (Figure 2). The high profitability here suggests effective management and developed processes of mechanization of the production process, as well as better market orientation of the cooperative structures.

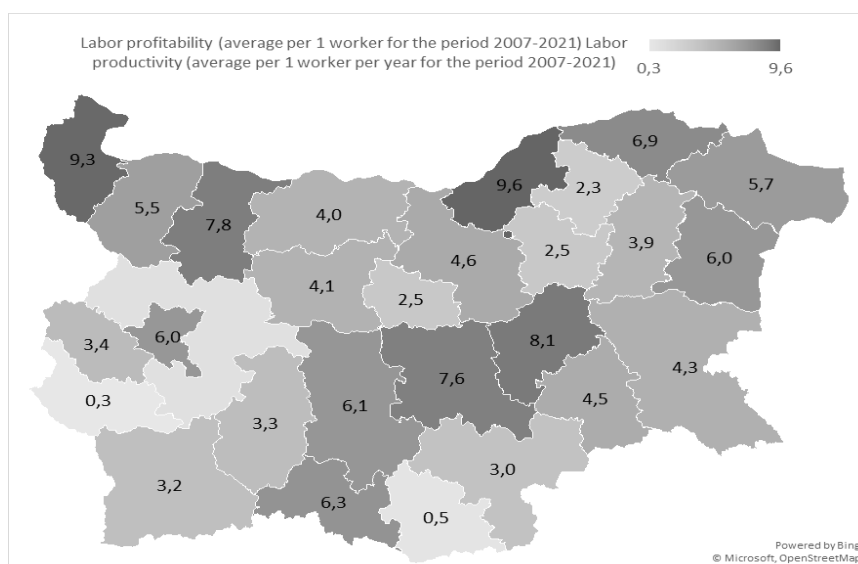


Figure 2. Labor profitability and Labor productivity (average per 1 worker per year for the period 2007-2021).

Source: Own calculations, Ciela INFO

The highest productivity is achieved by cooperatives located in the Dobrich region – 315.2 thousand leva, Veliko Tarnovo – 111.2 thousand leva, Ruse – 88.2 thousand leva, Pleven – 84.4 and Yambol – 81.9. The Dobrudzha region (Dobrich) dominates – this is logical, because large quantities of grain and oilseed crops with high value and low labor costs are produced there. (Table 2). The lowest values in terms of profitability are Kyustendil (0.3) and Kardzhali (0.5). Accordingly, the lowest productivity is Kardzhali (7.6), Pazardzhik (8.8), Sliven (10.6). These are mainly mountainous and semi-mountainous regions, where agriculture is small-scale, labor-intensive and with low returns..

Table 2. Average values (for all areas)

Indicator	Average value
Labor profitability	≈ 5.2
Labor productivity	≈ 75.8

Source: Own calculations, Ciela INFO

Calculations show that the correlation coefficient between profitability and productivity is ≈ 0.47 . This means a moderate positive relationship: Regions with higher productivity tend to have higher profitability, but not always. Examples of this trend are Dobrich - with extremely high productivity, but profitability is moderate (5.7). Next in line is Ruse - high values for both indicators, which is an example of an effective cooperative structure. Sliven region has high profitability, but low productivity. Most likely specialized products with high value (e.g. viticulture),(Table 3).

Table 3. Average values by region

Region	Profitability	Productivity
Northern Bulgaria	5.4	105.0
Southern Bulgaria	4.9	52.1

Source: Own calculations, Ciela INFO

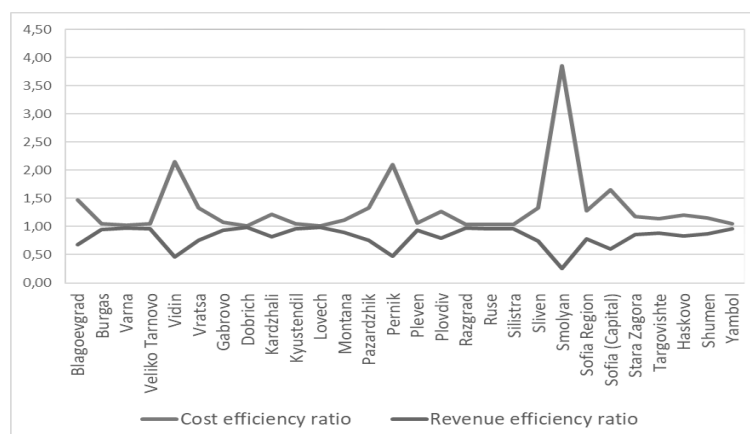
Northern Bulgaria has twice as high productivity and slightly better profitability. This is related to larger agricultural areas, more mechanized farms and the predominance of grain production. Southern Bulgaria is more diversified – more permanent crops, livestock and more labor-intensive activities, which is evidence of lower productivity, but more stable profit per unit of product.

Based on the analysis, the following conclusions can be drawn: Northern Bulgaria is in first place in labor efficiency with 2 times higher productivity. Profitability and productivity are related, but not completely proportional. The regions of Ruse and Dobrich stand out as the most efficient regions. The mountainous regions (Kardzhali, Kyustendil, Smolyan) lag behind due to small farms and weaker mechanization. The general trend is that Bulgarian cooperative agriculture has stable but uneven efficiency by region.

The cost-effectiveness ratio shows how many times the profit covers the costs (Figure 3). The higher it is, the more efficiently the costs are used. The highest values are Smolyan: 3.9 – extremely efficient costs; Vidin: 2.2; Pernik: 2.1 and Sofia (Capital): 1.7. Medium and lower efficiency ($\approx 1.0 - 1.3$): most districts such as Varna, Veliko Tarnovo, Dobrich, Lovech, Razgrad, Ruse, Silistra and Yambol. This means that the costs almost correspond to the revenues and the profit is relatively small.

Revenue efficiency ratio shows how efficiently the revenues lead to profit (profit/revenue). The lowest values (≤ 0.5) are Smolyan: 0.3 – a large part of the revenues are spent; Vidin and Pernik: 0.5 and Sofia (Capital): 0.6 With medium values (0.7–0.8): Blagoevgrad, Vratsa, Pazardzhik, Plovdiv, Haskovo, etc. The highest (≈ 0.9 –1.0) are Varna, Veliko Tarnovo, Dobrich, Kyustendil, Lovech, Razgrad, Ruse, Silistra, Yambol – relatively good profitability of revenues.

Figure 3. Cost efficiency ratio and Revenue efficiency ratio average for regions in Bulgaria (2007-2021)



Source: Own calculations, Ciela INFO

Districts with extremely high profit to cost ratio are Smolyan: although revenues are small (129.8), profit is 96.1 – very high cost efficiency (3.9), followed by Vidin with profit 79.5 at costs 68.7. And Pernik: profit 46.6 at costs 42.3.

Districts with high revenues but low profitability are Dobrich: revenues 1968.1, profit only 32.5 – almost everything is spent. Varna: revenues 1091.2, profit 23.2 – profitability $\approx 2\%$.

Small districts (such as Smolyan, Vidin, Pernik) are distinguished by high cost efficiency, but not always with a large overall impact on the economy. Large districts with high revenues (Dobrich, Varna, Veliko Tarnovo) have low cost efficiency, which indicates that a large part of the revenues is spent. Sofia (Capital) has average revenue and good cost efficiency, but profitability to revenue is moderate.

CONCLUSIONS

The analysis of 863 agricultural cooperatives across the 28 NUTS-3 regions of Bulgaria for the period 2007–2021 reveals a complex and regionally differentiated structure of cooperative farming. Despite the overall stability of the sector, significant disparities are observed in the number, size, and efficiency of cooperatives between Northern and Southern Bulgaria.

The average cooperative employs about 14 workers, indicating predominantly small and medium-sized structures. There is only a weak correlation between the number of cooperatives and the average number of personnel ($r = 0.02$), suggesting that regions with many cooperatives do not necessarily have larger ones. Northern Bulgaria hosts more cooperatives (39 on average per region) but of smaller size, while Southern Bulgaria has fewer but larger cooperatives (15 employees on average). This reflects distinct production specializations: grain and oilseed production in the North versus mixed and labor-intensive farming, including livestock and permanent crops, in the South.

Regional contrasts in performance indicators are pronounced. Northern regions, such as Dobrich, Ruse, and Pleven, demonstrate higher labor productivity (up to 315.2 thousand BGN per worker annually in Dobrich) and slightly higher profitability (average 5.4). In contrast, mountainous regions like Kardzhali, Smolyan, and Kyustendil lag behind due to smaller, fragmented farms, limited mechanization, and higher labor costs. The correlation between productivity and profitability ($r \approx 0.47$) indicates a moderate positive relationship—regions with higher productivity tend to be more profitable, but the connection is not uniform, suggesting that factors such as specialization, market orientation, and cost structure play a crucial role.

The cost efficiency ratio and revenue efficiency ratio further confirm the dual nature of cooperative performance. Smaller regions (Smolyan, Vidin, Pernik) exhibit high cost efficiency—low expenses relative to profit—while large agricultural regions (Dobrich, Varna) show the opposite pattern: high revenues but limited profit margins, indicating higher operational costs or reinvestments. This asymmetry underlines the influence of scale economies and production type on financial efficiency.

Overall, the results demonstrate that Bulgarian agricultural cooperatives have achieved moderate but regionally uneven efficiency. Northern Bulgaria stands out as the more productive and mechanized part of the sector, benefiting from large-scale arable farming and favorable land conditions. Southern Bulgaria, though less productive, maintains more labor-intensive and diversified production structures, offering social stability and local employment.

From a policy perspective, the findings emphasize the need for targeted regional support measures. Investments in mechanization, digitalization, and cooperative management capacity could enhance productivity in Southern and mountainous regions, while Northern cooperatives would benefit from diversification and value-added processing to improve profitability. Strengthening inter-cooperative networks and improving market access would also contribute to more balanced and sustainable cooperative development nationwide.

In conclusion, agricultural cooperatives remain a vital yet heterogeneous part of Bulgaria's rural economy. Their future competitiveness depends on bridging the productivity and efficiency gap between regions through innovation, balanced territorial policies, and continuous adaptation to market and technological challenges.

ACKNOWLEDGMENTS

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THE ROLE AND IMPORTANCE OF TOBACCO IN MACEDONIA – FROM THE PAST TO THE PRESENT

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ABSTRACT

The introductory part of the paper entitled " The place and significance of tobacco in Macedonia – from the past to the present ", is focused on the fact that tobacco occupies a special place in the structure of agriculture with enormous socio-economic significance for our country and in the past decades, the production of tobacco, and especially oriental tobacco in Macedonia, is characterized by enviable quality and world reputation.

Taking into account the importance of tobacco, its production should be directed towards obtaining higher income per unit area and of course higher quality raw material. The agricultural sector in Macedonia is of great importance for the development and advancement of the economy and, together with forestry and fisheries, is the third largest sector (after services and industry) in the country.

The starting point for constructing the theoretical part of the paper will be the reports from the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia, the State Statistical Office, data from the World Bank database, etc.

In the empirical part, the scientific method will be used - statistical analysis conducted on data obtained from survey questionnaires, whereby the data analysis will be carried out with the method of descriptive explanation of the results obtained from the research, with the mandatory application of the comparative method.

In the concluding observations, the socio-economic importance of tobacco for the country, the importance of good organization, planned production and the strategic management approach are highlighted.

Keywords: tobacco production, strategy, sustainable development, world trends, economic indicators

INTRODUCTION

From an economic perspective, tobacco production holds significant importance for our country, which is known as a producer of high-quality oriental-type tobacco. This fact alone highlights how crucial tobacco cultivation is for the economic development of the nation and how deeply tobacco is embedded in the economic growth projections and calculations for the country.

The economic and social relevance of tobacco production is further underscored by the fact that around 30,000 families rely on it as their primary source of livelihood. In this context, it is essential to continue with the established strategy - namely, the ongoing policy of subsidizing tobacco, intensifying negotiations and discussions with foreign investors who could enter this market to bring in knowledge, production technology and incentives to increase the quantities produced, as well as to boost demand for tobacco. This would help improve market conditions and the price agreed upon between producers and buyers.

The entry of major cigarette-producing companies such as Philip Morris and Imperial Tobacco is highly significant for Macedonia. These companies never invest in countries randomly - they base their decisions on detailed analyses that ensure expected returns. Such analysis has revealed to them that Macedonia has strong potential for producing high-quality tobacco, particularly oriental types, and that our country takes care of both tobacco growers and the tobacco industry. This stands in contrast to many European countries where cigarette manufacturing plants are being shut down.

Macedonia must remain focused on developing this sector, which brings over 120 million euros in annual foreign currency inflow. For this reason, a coherent and comprehensive vision for the development of agriculture and rural areas is needed - a well-designed strategy for agricultural development in all its segments, including tobacco production. This means the primary focus should be on institutional development, a new approach to organizational and managerial operations, and technical-technological changes and innovations. Technological advancement and innovation continue to be vital for agricultural and rural development, but they must be supported by appropriate management structures in the sector. In other words, it is necessary to design, implement, and continuously revise the specific measures of economic and agricultural policy that are essential to support investment in agriculture, including:

- Maintaining healthy and stable macroeconomic and trade policies that encourage investment in agriculture;

- Improving the functioning of markets for input production factors (labor, agricultural land, and capital) and outputs at the local, national, and international levels;
- Strengthening and improving the quality of human capital through appropriate education and access to productive resources;
- Establishing a strong institutional environment that enhances market access, ensures the dissemination of information, defines standards and provides an adequate legal and regulatory framework;
- Providing research services that develop productive technologies;
- Improving marketing, transportation and communication infrastructure to support timely access for agricultural producers to seasonal and long-term capital and production inputs, as well as providing strong incentives through agricultural product pricing policies;
- Protecting natural resources and the environment.

The adherence to and implementation of these measures - supplemented and adjusted according to current trends and conditions - would represent a guiding framework for the development of tobacco production in Macedonia.

RESULTS AND DISCUSSION

Macedonia is a country with over 200 sunny days annually, making agricultural production - including tobacco cultivation - highly feasible due to favorable climatic conditions.

On the other hand, the significance of tobacco production as a branch of agriculture is immense and irreplaceable, as it involves 10-12% of the population, and together with the tobacco processing industry, accounts for 16-18% of the total working-age population in Macedonia.

For this reason, it is essential that Macedonia respects and follows its strategy for the development of tobacco production, which is aligned with the directives of the European Union - the integration into which remains our strategic goal.

This strategy includes the following measures: ¹

- Measures for support and state aid (subsidies) to agricultural holdings engaged in tobacco production;
- Ensuring the continuous and sustainable development of tobacco production;
- Increasing competitiveness and maintaining the traditional reputation in the tobacco market.

Chart 1. Three Pillars of the Tobacco Production Development Strategy

Support measures and subsidies for tobacco farms
Support measures and subsidies for tobacco farms
Increasing competitiveness and maintaining the traditional reputation of the tobacco market

According to the potential of Macedonian tobacco, along with the measures undertaken by the state from various aspects, it is expected that in the foreseeable future these will have positive effects on the tobacco sector in Macedonia.

Experts working on tobacco production and its development, based on their projection methods, foresee an increase in tobacco production in the coming period. However, it is important to also emphasize the intentions of the other side. All governmental and non-governmental organizations and associations at the global level that, through their analyses, hold an anti-tobacco stance.

To mitigate these anti-tobacco pressures, the European Union and global progressive policies require that tobacco production in the upcoming period comply with established standards related to public health, the health of agricultural crops, environmental protection and animal welfare. Additionally, it is particularly important for agricultural land to be maintained in good agricultural and environmental condition. This means producing high-quality tobacco that is in market demand, while simultaneously preserving the environment from pollution caused by excessive use of fertilizers, agrochemicals and various waste materials.

In the European Union, if farmers fail to meet these standards, the amounts of direct payments are reduced or completely withdrawn for the year in which the standards are not met.

¹ Tobacco Production Strategy for the Period 2020–2026, Official Gazette of the Republic of North Macedonia No. 267/19, dated 24.12.2019.

Based on the demands of the global market, the scientific and technological advancements in the world, and the available natural, productive and human (labor) potentials, it can confidently be stated that today - and even more so in the future - there will be an increasing need for:

- Raising the quality of tobacco production to a much higher level, taking into account the demands of buyers;
- Efficient and effective production, where emerging and potential problems can be more easily identified and organizational entities can effectively manage them;
- Increasing motivation and job satisfaction among employees (engaged personnel) through better utilization of technical and technological advancements, greater use of tools and mechanization, which largely replace physical labor and reduce the time required for production operations and processes;
- Guaranteeing the quality of tobacco and tobacco product production and ensuring secure market placement;
- Continuously developing research, educational, and advisory activities to raise awareness for the proper implementation of Good Agricultural Practices (GAP);²
- Raising awareness of quality in overall operations among all individuals involved in the company or farm;
- Integrating economic and environmental goals - i.e., increasing income while preserving the environment, maintaining healthy crops and supporting the favorable existence of all living organisms;
- Developing opportunities to create alternative (combined) income through expanding tobacco production with other supplementary products or services;
- Taking care of environmental protection through a publicly declared policy;
- Establishing systems to monitor tobacco production - i.e., developing continuous monitoring mechanisms;
- Developing a program to minimize harmful environmental effects caused by the organization's activities;

² Pashovska Silvana, Miceski Trajko, "Preference management activities based on good agricultural practices in tobacco production", Journal of Agricultural, Food and Environmental Sciences, JAFES, ISSN 2545-4315, pp.132-141 / 2018

- Establishing a system for achieving set goals through data monitoring, internal audits, etc.

Tobacco production in Macedonia, after the transitional period - especially during the last decade of the 20th century - has remained relatively stable in terms of the production of oriental tobacco, which continues to be cultivated without major disruptions. The fluctuations in primary tobacco production that occasionally occurred before and after the transition are the result of a characteristically dry decade, as well as issues related to privatization, economic policies and pricing policies toward this crop.

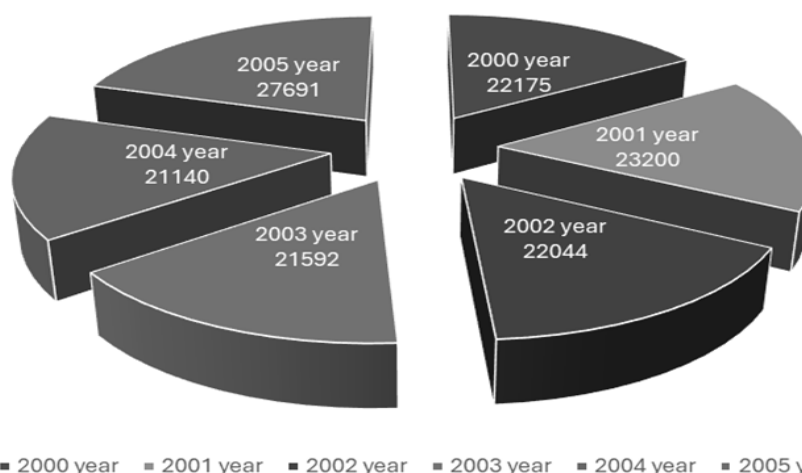
Due to these causal factors influencing the oscillations in tobacco production, concrete measures are being taken - particularly in the form of subsidies - which apply to agricultural production as a whole and to tobacco growing as a part of it.

The tobacco-producing region in Macedonia is characterized by commendable quality and a globally recognized reputation. Efforts are needed to further develop the production and economic policy through the implementation of a development strategy, which includes a pricing policy as a necessary stimulus for tobacco production.

Compared to neighboring countries, Macedonia, as a traditional producer of oriental tobacco, maintains relatively stable production. The country possesses exceptional potential for the cultivation of high-quality, small-leaf, aromatic types of tobacco - that is, oriental tobacco, which enjoys a high degree of global demand and is essentially its own sole alternative.

In the period from 2000 to 2005, there were no major fluctuations in the production of either oriental or large-leaf tobacco types such as Virginia and Burley, which emerged during that time and did not exceed 6% of domestic production.

Chart 2. Structure of Tobacco Production in the Republic of North Macedonia in the period from 2000 to 2005



Source: State Statistical Office – MAKSTAT Database, FAOSTAT, World Bank

The future of tobacco production will always depend on various factors, but the favorable soil and climate conditions, along with the long-standing tradition, form a basis for greater development and year-on-year growth.

In the 2005 harvest, 8,741 hectares were planted and 27,691 tons of tobacco were produced, while the average for this six-year period amounted to 23,600 tons of tobacco.³

In the period from 2006 to 2010, a trend of increasing both the number of tobacco producers and the cultivated tobacco areas is evident in Macedonia. Namely, from 15,808 hectares in 2005, the area planted with tobacco increased to 18,846 hectares in 2010.

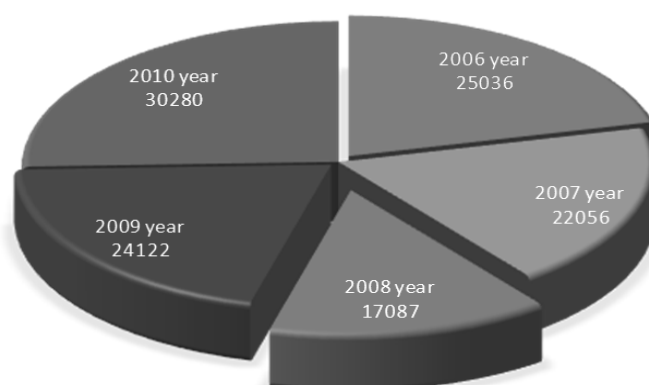
In 2010, 30,280 tons of tobacco were produced in Macedonia, with an average price of 136.6 MKD, while the average for this analyzed period was 19,764 tons of tobacco.

According to research data from the Ministry of Agriculture, Forestry and Water Economy, the State Statistical Office and the MAKSTAT database, the changes occurring in the global strategic development of the tobacco sector in our country can be observed. The increase in land under tobacco cultivation, as well as the growing number of tobacco producers, indicates that year by year, interest in tobacco production is rising, establishing it as a key economic crop that enables employment and generates profit for the Macedonian population.

³ Pashovska Silvana, " The future of Macedonian tobacco is in production according to standards for competitive product with high quality", International Journal of Agriculture, Environment and BioResearch, 07 (02), pp. 92-97, ISSN 2456-8643 / 2022

Furthermore, buyers are seeking continuous tobacco production in order to focus more on purchasing prices and to increase the foreign currency inflow into the country, which protects this so-called "yellow gold."

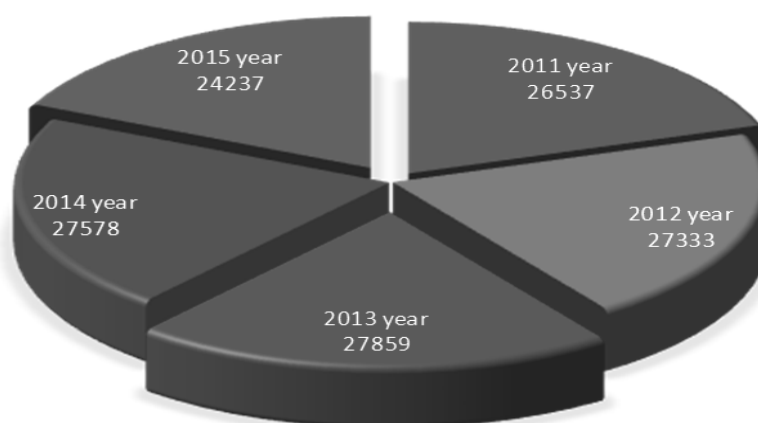
Chart 3. Structure of Tobacco Production in the Republic of North Macedonia in the period from 2006 to 2010



■ 2006 year ■ 2007 year ■ 2008 year ■ 2009 year ■ 2010 year

Source: State Statistical Office – MAKSTAT Database, FAOSTAT, World Bank

In the period from 2011 to 2015, despite fluctuations in global tobacco production, Macedonia stood out among countries in the region by having the highest number of tobacco producers per capita. The tobacco sector engaged around 16% of the working-age population. This highlights, above all, the social dimension of tobacco production, which employs a large labor force and remains a labor-intensive process. It has a greater capacity to absorb workers than any other industrial crop.



■ 2011 year ■ 2012 year ■ 2013 year ■ 2014 year ■ 2015 year

Chart 4. Structure of Tobacco Production in the Republic of North Macedonia from 2011 to 2015

Source: State Statistical Office – MAKSTAT Database, FAOSTAT, World Bank

When it comes to the quantity of Macedonian oriental tobacco in relation to the total global production of oriental tobacco, it can be concluded that our role in this area is quite small. However, our priority must be quality. We must not allow any deviations in quality, nor in the typological and varietal purity of our tobacco. If we want to maintain our exports and retain traditional buyers-especially in this time of misinterpreted varietal liberalism-it is essential to adhere to the legal regulations and acts governing tobacco production and tobacco as an industrial crop.

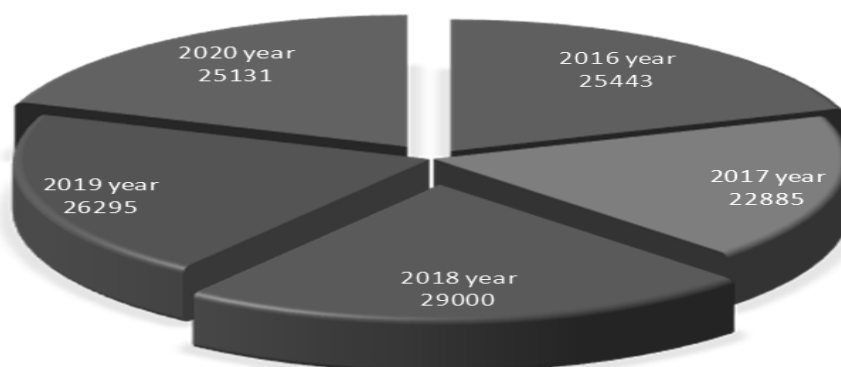
Global policy and the strategic development of the tobacco sector in Europe are closely interconnected. As a candidate for European Union membership and an active participant in the global tobacco market, our country must follow the guidelines and principles of the European integration framework for agricultural development.

In the period from 2016 to 2020, Macedonia was affected by a series of socio-economic, political and economic turbulences which led to uncertainty in the agro-complex. In the 2016 harvest, according to data obtained from the Ministry of Agriculture, Forestry and Water Economy of the Republic of North Macedonia, 16,379 hectares were planted and 25,443 tons of tobacco were produced; in 2017, 22,885 tons of tobacco were produced; in 2018, 25,547 tons of tobacco were produced; in 2019, production amounted to 26,234 tons of tobacco; while in 2020, it amounted to 26,112 tons of tobacco. The average for this period is 25,244 tons of tobacco, which represents an increase compared to the average of the previously analyzed periods and is a good indicator of the progress of the economic policies for tobacco in Macedonia.

If all the measures continuously offered by the European Commissions for the protection and advancement of agriculture, as well as the IPARD funds, are utilized, they will greatly contribute to the developmental course of tobacco production in our country.

In Macedonia, in 2018, 14,802 hectares of tobacco were planted, 34,104 contracts were signed with 9 purchasing companies, and 23,430 registration forms were issued. The total agreed quantity for purchase amounted to over 29,000 tons of tobacco. Compared to the 2017 harvest, 57 more registration forms were issued for tobacco production in 2018, and over 4,700 more contracts were signed. There was also an increase in the planted area of tobacco - by 387 hectares.

In 2019, 14,127 hectares were planted, while in 2020, there was a slight decline to 11,936 hectares of tobacco.

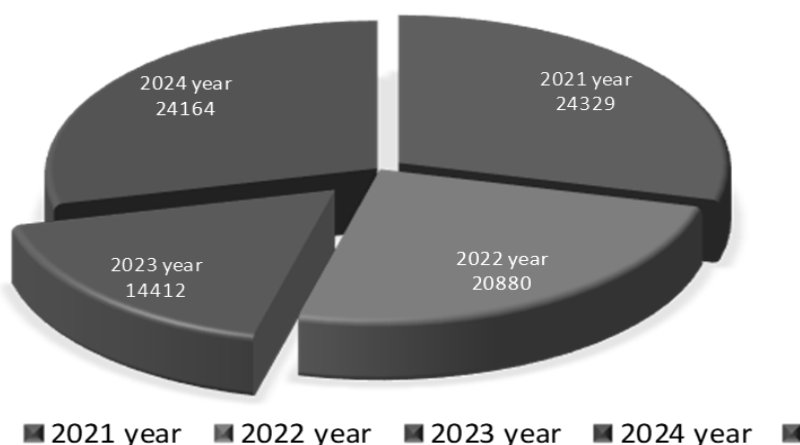


■ 2016 year ■ 2017 year ■ 2018 year ■ 2019 year ■ 2020 year

Chart 5. Structure of Tobacco Production in the Republic of North Macedonia from 2016 to 2020

Source: State Statistical Office – MAKSTAT Database, FAOSTAT, World Bank

The trend of stabilization in tobacco production in Macedonia continues in the following years, specifically from 2021 to 2024, which is clearly visible in Chart 6 below.



■ 2021 year ■ 2022 year ■ 2023 year ■ 2024 year ■

Chart 6. Structure of Tobacco Production in the Republic of North Macedonia from 2021 to 2024

Source: State Statistical Office MAKSTAT – database, FAOSTAT, World Bank

The data in the chart shows a stable trend in tobacco production, with the exception of 2023, when there was a decrease in produced quantities to 14,412 tons. To maintain stability in production, it is necessary to continue the policy of subsidies and incentives by the state. Macedonian tobacco production, supported by government subsidy policies, should increase in the future since production in Greece, Bulgaria, and Turkey will continue to decline - the trend in production of this type of tobacco in Balkan countries shows a further linear decrease. Albania and Serbia have very small and almost negligible quantities of oriental tobacco.

The main strategic priorities of the state regarding the development of tobacco production should be: initial capital, human resources, modern mechanization, consultations with scientific institutions and organizations, and of course, market policy and pricing policy.

Increasing capital investments will contribute to greater socio-economic development of tobacco production in Macedonia. Most of the unemployed population, especially in the Pelagonia region, make their livelihood precisely through tobacco production. Economic and social factors have a particular influence on tobacco production in our country because the ultimate goal of every farming activity is to achieve an economic effect.

In other words, the goal of every state is greater economic and strategic success in the field of active farming of those crops that generate the greatest profit but at the same time create the most employment for the population. Through proper management, huge success is achieved in the work of entrepreneurs and a reduction in production costs, and simultaneously an increase in tobacco productivity as a crop that brings the greatest economic benefit, which flows into the state budget.

CONCLUSIONS

The previous elaborations indicate the fact that efforts are directed towards proving that tobacco production, specifically the production of oriental tobacco, has real developmental potential over a long-term period, viewed from the perspective of global demand for the presence of oriental tobacco types in tobacco blends used in the production of quality cigarettes, as well as for other, broader needs for this type of tobacco on a global level. Additionally, tobacco production in our country provides a condition for improving the social and economic status of a large part of the population, engaging nearly all family members, creating work habits (of course, within the framework of preventing child labor abuse) among young people, thereby possibly steering them away from bad habits and addictions, as well as generally improving the financial benefits for our state.

From a future perspective, tobacco production, with certain fluctuations, shows a stable trend of movements, with slight upward trends in global production, which our country must follow and adapt to accordingly.

For the population oriented towards agricultural production, and especially tobacco production, it is of primary importance to create and build a perception of security and future in this sector, which requires developed management of all activities and appropriate

motivation. Positive stimulation and motivation will ensure greater efficiency and effectiveness in operations, reduce production costs, increase productivity, and improve business results.

The Republic of North Macedonia should not foresee measures to reduce tobacco production because, due to the sensitivity of the socio-economic aspect, this issue should be left for resolution in the future period, after the possible accession of Macedonia to the EU, when tobacco production plans will be aligned with the regulations of the European Union.

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FIELD CROP PRODUCTION

ENERGY POTENTIAL AND PERSPECTIVES OF BIOMASS DERIVED FROM WHEAT, BARLEY AND MAIZE

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ABSTRACT

Plant residues from field crops have the potential to be used as a source of energy, which is the foundation for a different approach on biomass in plant production. In order to achieve energy potential of biomass we used statistical data for 2005-2024 (total arable area, production and yield), literature data (residue product ratio RPR, calorific value CV, dry matter DM), two method for obtaining energy potential (mathematical and BEP) and exponential trend with 15 years forecast. From total arable area (± 517461 ha, 2005-2014), 30.1% were at represented wheat, barley and maize (15.4%, 8.7%, 6.0%) or 155605 ha. The prediction 2025 - 2040 of total arable land show a tendency towards stagnation in maize and barley and a reduction in wheat, total production decrease for wheat and increase for barley and maize, and in terms of yield show a towards growing tendency. Biomass energy potential 2005-2024 shows the highest percentage of total production for wheat (48.5%), compared to barley (28.3%) and maize (23.2%). In terms of yield the highest percentage of biomass achieved in maize (37.6%). Prediction 2025-2040 of total biomass production increases by 7% for barley and 1% for maize, as well as 2% barley and 3% maize in terms of yield biomass in kg/h. The total energy potential of studied crops according to the mathematical model showed 10546 GJ for 2005-2024 and 10546 GJ for forecast period, and was higher than the BEP method (9281 GJ, 9154 GJ) by 1.36%.

Keywords: wheat, barley, maize, biomass, energy potential, forecast

INTRODUCTION

From total energy sources, renewable energy sources in the global economy account for 14%, of which biomass accounts for about 70% (Popp et al., 2021), which in turn is technologically converted into several energy carriers such as electricity, heat, liquid fuels, gaseous fuels, and solid

biomass (T.N.O. Mensah et al., 2025). The EU Renewable Energy Directive (2018/2001/EU) set a new binding renewable energy target for the EU of at least 32% for 2030, revision in 2023, raises the target to 42.5% (<https://energy.ec.europa.eu>). The European Scientific Advisory Board on Climate Change - ESABCC indicates that the demand for bioenergy will increase by over 50% by 2040 in some scenarios, while other scenarios assume a more modest increase, or even no increase above today's levels (<https://www.ecologic.eu/>). As fossil-based carbon is phased out of the EU economy, farmers will have new business opportunities to deliver biomass and bio-based materials in a sustainable manner for different uses in the bioeconomy (<https://eur-lex.europa.eu>). Agricultural biomass from crop residues (stem, straw, husk, cob, husk, husk and pod) refers or is defined as the non-edible plant parts left after harvesting (Ertugrul et al., 2024; R. Lal, 2005). Rather than removing crop residue, plantation biomass - energy crops (sugarcane, switch grass, sorghum, eucalyptus, poplar, and oil palm) refers to grown plants through agricultural practices (R. Lal, 2005, Gravalos et al., 2016). The objective of this research is to assess the agricultural residual biomass and its energy potential of three important field crops in the Macedonia, using two different approaches for evaluating, and growth forecasting in the next 15-year period for prediction of renewable energy sources.

MATERIAL AND METHODS

Evaluation of biomass energy potential is essential for the preliminary assessment of agricultural residues derived from field crops. To determine this two different approaches for potential assessments were adapted from studies by Guzmán-Bello et al., (2023), and Batista et al., (2023). These were incorporated into a mathematical procedure essential and the BEP method. The first formula used is: $E = m \times CV$, where: E - energy potential (J or MJ), m - mass of the biomass (kg or t), and CV - calorific value of the biomass (MJ/kg). The second equation is Biomass Energy Potential Estimation Method (BEP), for determines the theoretical energy potential of biomass: $BEP = \sum_{i=1}^n (M_i \times RRP_i \times HHV_i \times DMC_i)$ where: BEP - the Biomass Energy Potential, expressed in MJ or GJ; n - the total number of agricultural products; M - the annual production; RRP - the residue ratio for the product; HHV - the value of higher calorific value; DMC - the dry matter content (%). Data for total arable and sown areas, total production, and yields from: wheat, barley, and maize, from the period 2005 - 2024 were utilized from the Macedonian Statistical Office ([/makstat.stat.gov.mk](http://makstat.stat.gov.mk)). Data sets from the literature for crop residue ratio (RPR), calorific value (CV) of biomass conversion and dry matter content (DMC) were also used. The results from the two methods was compare and with prediction with growth forecast for 15 year period 2005 -2040.

RESULTS AND DISCUSSION

In assessing the distribution of total arable land nationwide ($\bar{x}$ 517461 ha) from 2005 to 2024, tree field crops constitute 30,1 percent (wheat 15,3%, barley 8,1% and maize 6,0%). The growth forecast for 2025 to 2040 indicates a decrease of 2.8 %, corresponding to 502,766 hectares and 1,6 % of total production, over the ensuing fifteen years. Arable land for wheat records a downward decline from 110000 ha (2005) to above 70000ha (2024) or mean for this period of 79425 ha. Barley maintained a stable area throughout the twenty-year period (45,000 to 50,000ha) with a mean value of 44,994ha. Similarly, maize showed stability yielding an average area of 31,188 ha for the period. Projection to 2040 indicates a modest reduction relative to the mean cultivated areas of barley and rye, but by a pronounced ongoing decline in wheat to below 50,000 hectares. This represents a 54% decrease from the 2005-average area levels and a 37% reduction compared with the average over the past two decades (Fig. 1).

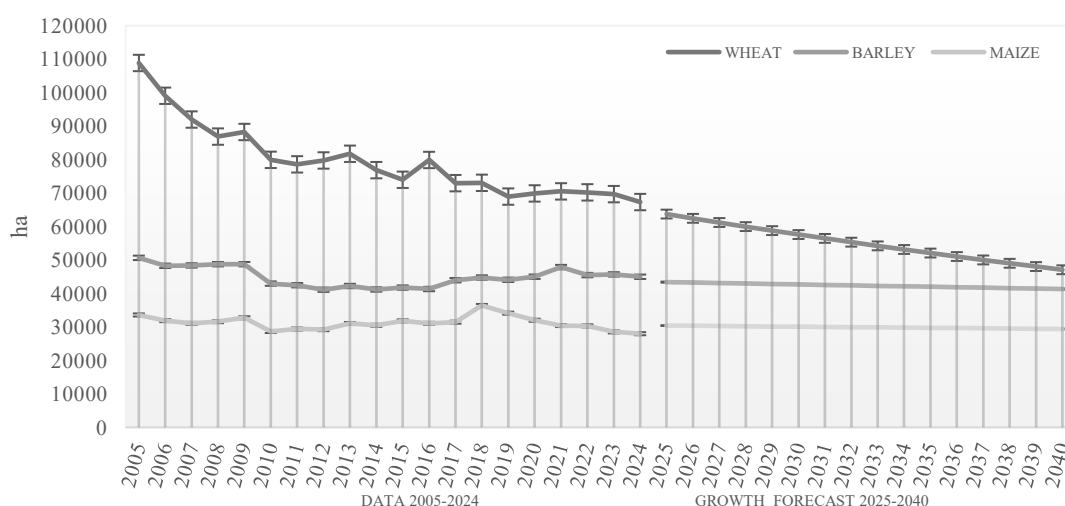


Figure 1. Arable land of wheat, barley and maize from 2005 to 2024, with projections to 2040

Consequently as with arable land and for total production for wheat records a indicate declining from in excess of 330,000 t in 2005 to 220,000 t in 2024, with a mean for the period of 250,842 t. Barley production fluctuated over the twenty-year span, ranging from above 100,000 t in 2012 to a peak of 187,676 t (2018), with an average of 135,353 t. Maize production showed instability, ranging from above 90,000 t (2012, 2017) to 160,000 t in 2009, yielding an average of 137,179 t. Projection for wheat to 2040 indicates a 27% reduction in production to the average of the 2005-2024 period. For barley, total production is anticipated to rise to 47% above the mean by 2040, while maize by 2040 is expected increase by 5.3% (Fig. 2).

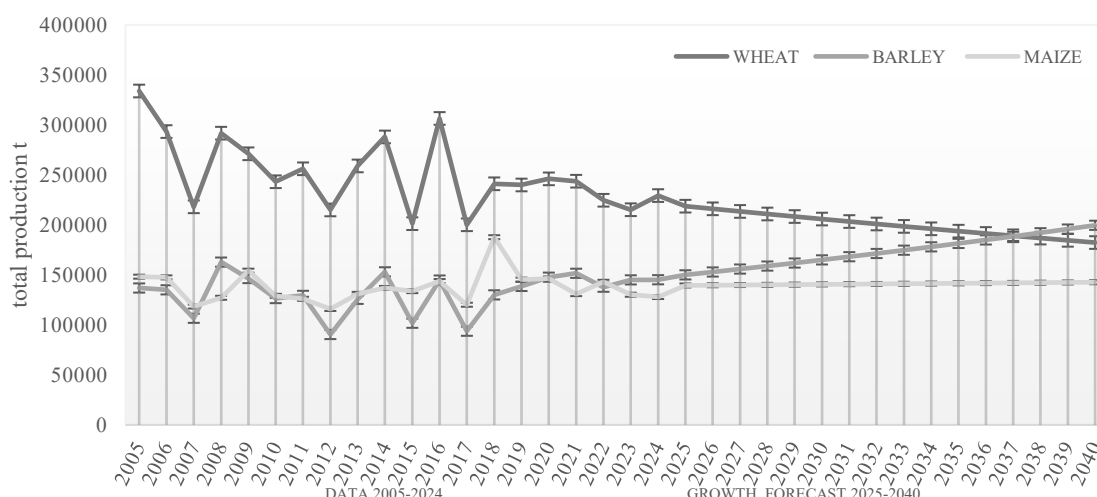


Figure 2. Total production of wheat, barley, and maize from 2005 to 2024, with projections to 2040

Yielding on wheat over twenty-year period showed min yield in 2007 (2402 kg/ha), max in 2016 and mean of 3197 kg/ha. Barley min yield has in 2017 (2138 kg/ha) and max in 2014 (3719 kg/ha) and mean of 2975 kg/ha. Data for maize showed instability from 3836 kg/ha in 2007 to records in 2018 (5164 kg/ha), and mean for this period of 4414 kg/ha.

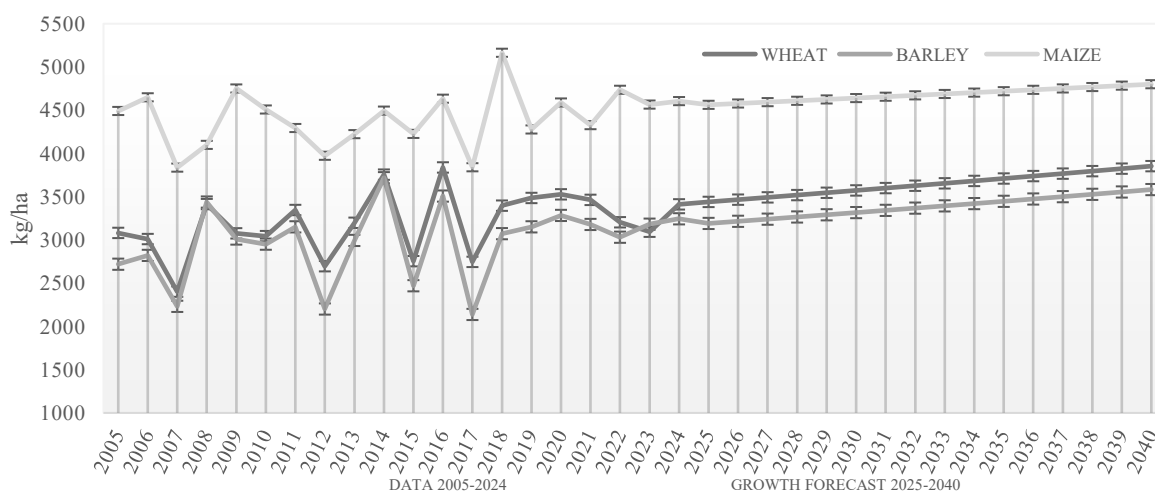


Figure 3. Yield of wheat, barley, and maize from 2005 to 2024, with projections to 2040

Projection for wheat and barley to 2040 indicates a 20% increase in yield relative to the average of the 2005–2024 period, while maize yield is expected to rise by 8.7% (Fig. 3).

In following tables, the literature datasets are presented for factors such: residue-to-crop production ratio (Table 1), biomass calorific value and dry matter content.

Table 1. Literature datasets used for determining residue to crop production ratios, calorific value and dry matter content

	Wheat	Barley	Maize	
RPR	1.30	1.30	1.00	Ericsson and Nilsson (2006)
		1.50		Craham et al. (2007)
	1.20		0.90	Johnson et al. (2006)
	1.34		1.00	Kadam and McMillan (2003)
	1.75	1.75	2.00	Koopmans and Koppejan (1997)
	0.60		1.30	Koukios (1998)
			0.87	Linden et al. (2000)
		1.00	1.00	Nelson (2002)
	1.00	1.24	0.70	Panoutsou and Labalene (2006)
		1.65		Patterson et al. (1995)
		1.25		Petersen et al. (1995)
		1.20		Kaltschmltt and Hartmann (2000)
		1.00	1.00	Walsh et al. (2000)
	1.2	1.19		Cukaliev (2014)
	1.3			Maznevskia (1998)
$\bar{x}$	1.21	1.31	1.06	
Mj/kg	16.54	16.1	17.5	Jóvér et al., (2018), Piskier (2017)
	17.3	16.8	16.8	Piskier (2017), Tyminski (1997)
$\bar{x}$	16.92	16.45	17.15	
DMC %	88%	88%	88%	Wirsenius (2000); Ronzon et al. (2015); Drackley et al. (1985)
DMC num.	0.88	0.88	0.88	

The mean annual total biomass from field crops over the period 2005–2024 was 626,241 t, which is one percent higher than the 15-year forecasts period 2025-2040. Among the crops, wheat exhibited the greatest biomass production (303,519 t), whereas maize produced the smallest quantity (145,410 t). For prediction period 2025 and 2040, there is a marked decline in total biomass production per ton in wheat, decreasing from 303,519 t to 242,013 t. By contrast, barley exhibits a substantial increase, rising from 177,312 t to 227,564 t, while maize shows a minimal increase (Table 2).

Table 2. Total biomass production, yield and % of sum

	Wheat	Barley	Maize	Sum	Wheat	Barley	Maize	Sum
	$\bar{x}$ 2005 - 2024				$\bar{x}$ 2025 - 2040			
Total Arable Land	79425	44994	31188	15560	55017	42356	30013	12738
Total prod. t	25084	13535	13717	52337	20001	17371	14103	51475
kg/ha	3197	2975	4414		3642	3383	4681	
% of total arable	15.3	8.7	6.0	30.1	10.9	8.4	6.0	25.3
Total biomass prod./t	30351	17731	14541	62624	24201	22756	14949	61907
Yield biomass kg/ha	3868	3897	4679	12444	4407	4432	4962	13800
% total prod.	48.5	28.3	23.2	100	39.1	36.8	24.1	12738
% biomass yield	31.1	31.3	37.6	100	31.9	32.1	36.0	51475

*<https://makstat.stat.gov.mk> and literature datasets for RPR residue to crop production ratios (Tab. 1)

The biomass production distribution is greatest in wheat (48.5%), followed by barley (28.3%) and maize (23.2%). This hierarchical pattern persists throughout the 15-year period to 2040, but with a reduction of 9.4% for wheat and corresponding increases of 8.5% for barley and 1% for maize. Regarding biomass yield relative to production, maize achieves the highest percentage at 37.6%, followed by barley and wheat with approximately equal percentages of 31.3% and 31.1%. While for period 2025 to 2040, wheat and barley are projected to exhibit marginal growth of less than one percent, while maize is expected to experience a 1.6% decline in biomass yields per hectare (Tab. 2).

Table 3. Energy potential of biomass (MJ, GJ)

		2005-2024				Growth forecast 2025-2040			
		Wheat	Barley	Maize	Sum	Wheat	Barley	Maize	Sum
Total biom prod.	t	303519	177312	145410	626241	242013	227564	149497	619074
Yield biomass	kg/ha	3868	3897	4679	12444	4407	4432	4962	13800
Approach I EP	MJ/t	5135541	2916782	2493782	10546105	4094860	3743428	2563874	10402161
	MJ/kg/ha	65447	64106	80245	209797	74566	72906	85098	232571
	GJ	5136	2917	2494	10546	4095	3743	2564	10402
	GJ/kg/ha	65.4	64.1	80.2	210	74.6	72.9	85.1	233
Approach II BEP	MJ	4519274	2566775	2194524	9280572	3603481	3294217	2256210	9153909
	MJ/kg/ha/y	57598	56417	70613	184628	65616	64154	74884	204654
	GJ	4519	2567	2195	9281	3603	3294	2256	9154
	GJ/kg/ha/y	57.6	56.4	70.6	184.6	65.6	64.2	74.9	204.7

*Biomass potential in approach I was measurement used means values from literature datasets for RPR and CV (Tab. 1, 2) and for approach II was include and datasets for DM (Tab 1).

The energy potential derived from the two-decade dataset is contingent upon the aggregate arable area occupied by the crops under study. The energy of the biomass from the all examined field crops, shows a total potencial of 10546 GJ in the first approach and where dry matter content is included in the assessment the potential is lower at 9281 GJ, yielding a difference between approaches of 1265 GJ. Wheat cultivated on a total arable area of 79425 hectares yields (Table 2) an energy potential of 5136 GJ under the first approach and 4519 GJ under the second approach. For the other two predominant field crops, barley and maize, the energy potentials are 2,917 GJ and 2,494 GJ in the first approach, and 2567 GJ and 2195 GJ in the second approach, or sum of all three field crops are 10546 GJ and 9281 GJ, respectively. The amount of electrical energy calculated per capita is about 70 GJ per year ($\equiv$ 2.5 t coal, 1.6 t crude oil) (Pabis et al., 2015), which in our case this energy need covers the annual corn harvest which

provides between 70.6 and 80,02 GJ/kg/ha/y (Table 3). The projection for the ensuing fifteen-year period indicates a modest decline in total biomass production, which aligns with a reduction in total arable land (Table 2), but yields of the examined crops are projected to rise (Table 3). Similar outcomes were obtained regarding the energy potential in the first and second approaches, with a theoretical reduction of energy potential by 1.3% or 127 to 144 GJ, projected to occur by the end of the 15-year period in 2040.

CONCLUSION

The potential of the biomass from the primary field crops wheat, barley and maize represented on respectable 30,1% of total arable land, can also be analyzed from perspective as a significant energy potential. Projections for the 2025-2040 period indicate a modest reduction of 2.8 % arable land and 1.6% decrease in total production. The substantial reduction of wheat arable land estimated at 37%, is forecast based on the mean values observed over the preceding two decades. Employing two distinct methodological approaches yields estimated biomass energy potentials of 10,546 GJ (approach I) and 9,281 GJ (approach II). The projected energy potential for biomass exhibits a negligible decline of about 1.3%, corresponding to a reduction of 127-144 GJ by 2040.

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INVESTIGATION OF CHANGES IN GRAIN YIELD AND SOME YIELD COMPONENTS IN HYBRIDS AMONG BREAD WHEAT MUTANTS

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ABSTRACT

This research was carried out to determine the variation in some agricultural traits of the three bread wheat cultivars with different origins (NKÜ Lider, Bezostaja and GK Beke's) and the reciprocal hybrids of their genotypes obtained by applying different gamma ray doses (0, 100, 200, 300 Gy). The experiment was arranged in a randomized complete block design with 3 replicates during the 2017-2018 wheat growing seasons. In the study, plant height, spike length, number of spikelets per spike, number of grains per spike, grain weight per spike varied between 85.93-118.44 cm, 10.69-12.86 cm, 20.4-24.57 no, 37.09-64.8 no, 2.26-3.56 g, respectively. For grain yield, the average of hybrid combinations and mutant populations varied between 445 kg da⁻¹ and 926 kg da⁻¹. The lowest heterosis and heterobeltiosis values for grain yield were observed in the GK Beke's-300×NKÜ Lider-300 (-39.429% and -42.704%, respectively) combination, whereas the highest heterosis and heterobeltiosis values were found in the GK Beke's-100×Bezostaja-100 (42.393% and 40.644%, respectively) hybrid combination. Among the studied genotypes, NKÜ Lider-0×GK Beke's -0, NKU Lider-200×GK Beke's-200, Bezostaja-100×GK Beke's-100, GK Beke's-0× NKÜ Lider-0, GK Beke's-100× NKÜ Lider-100, GK Beke's-100 × Bezostaja-100, GK Beke's-300×Bezostaja-300, Bezostaja-100×NKÜ Lider-100, Bezostaja-200×NKÜ Lider-200, Bezostaja-300×NKÜ Lider-300 hybrid combinations showing high performance in more than one trait were determined as promising combinations.

Keywords: Bread Wheat, Mutation, Combination, Heterosis, Grain yield.

INTRODUCTION

Wheat ranks first among cultivated cereal species worldwide, with a cultivation area of approximately 220 million hectares. However, in terms of production volume, it ranks third with about 799 million tons, following maize (1.24 billion tons) and rice (800 million tons). In Turkey, wheat is a crop of strategic importance, ranking first with nearly 8 million hectares of cultivated area and a production volume of 19 million tons (FAO, 2023).

The world population, which is currently around 8 billion, is projected to reach 8.5 billion by 2030 (Anonymous, 2016). However, in parallel with this increase in global population, arable land is rapidly decreasing due to errors in agricultural practices such as tillage, irrigation, fertilization, and pesticide application, as well as factors including erosion, industrialization, and urbanization. Under these circumstances, increasing grain yield per unit area emerges as a necessity in order to meet the wheat demand of the rapidly growing world population.

Approximately 90% of the areas cultivated with wheat worldwide and 85% of the areas in Turkey are planted with bread wheat, while the remaining areas are used for durum wheat production. In Turkey, bread wheat accounts for the largest share in human nutrition due to its extensive cultivation area and production volume. However, the yield and production per unit area of wheat cultivated in Turkey are not at the desired level. Increasing production is only possible by obtaining higher yields per unit area. Studies have shown that yield improvements can be achieved either by developing high-yielding varieties or by mitigating factors that limit productivity (Bella et al., 1987; Yürür, 1993). Indeed, traditional breeding methods have produced numerous new wheat varieties to date, contributing significantly to increased agricultural production.

The primary goal of plant breeding programs is to generate variation in the desired genotypes, perform selection based on target traits, and propagate genotypes that meet the breeding objectives. Among the breeding methods employed to create variation in different genotypes, hybridization is known to be the most commonly used technique. The purpose of hybridization is to combine the desired traits present in two or more varieties, lines, or species into a single genotype, improve an existing trait, and generate genetic variation (Singh et al., 1990). However, developing new varieties through these traditional breeding methods requires considerable time, labor, and resources. Therefore, attention has shifted toward new approaches that can provide variation more efficiently and rapidly. One such approach is mutation breeding (Olgun et al., 2012). The primary objective of mutation-based plant breeding is to create a rich phenotypic variation using an appropriate mutagen dose and, through positive selection, develop new genotypes with improved traits by altering several key characteristics. The most

notable achievement in plant mutation breeding is the over 3.220 released mutant cultivars worldwide (Bado et al., 2015).

The successful application of mutation can be enhanced through the combination of various methods. It has been observed that irradiating hybrid seeds increases mutation frequency, promotes genetic recombination, and broadens the mutation spectrum. Consequently, this approach provides a greater number of mutants for selection and genetic analysis (Savov, 1991; Weng, 1991; Kajjidoni et al., 2009). Gregory (1956) reported that variation induced by irradiation could be further increased when combined with hybridization. In contrast, Gupta and Virk (1997) indicated that the variation obtained through mutation and hybridization does not always result in additive effects.

In this study, it was aimed to create greater variation in grain yield and some agronomic traits affecting yield by combining mutation technique and classical hybridization technique in bread wheat.

MATERIALS AND METHODS

In this study, three bread wheat cultivars (NKU Lider, Bezostaja and GK Beke's) with different origins, phenological, and agronomic characteristics were used as plant material.

Obtaining mutant and hybrid populations

Seeds of bread wheat cultivars with 13-14% moisture content were irradiated using an "Ob-Servo Sanguis ^{60}Co " gamma irradiation device at a dose rate of 427 Gy/h, with doses of 0 (control), 100, 200, and 300 Gy. Following irradiation, the seeds were stored in a refrigerator at 4 °C until sowing (Akbaý and Ünver, 1986) and were sown in plots in November 2016.

Reciprocal hybridization was performed on the planted plots in May 2017, as shown in Table 1. For each hybrid combination, 12 spikes were emasculated and pollinated. The pollinated spikes were collected during the harvest season in July 2017 and threshed individually by hand to obtain the seeds necessary for growing the F1 and M1F1 generations, which served as the experimental material for the 2017–18 growing season.

Field experiment

The experimental site and growing conditions: This study was carried out in the experimental area of Field Crops Department of Agricultural Faculty of Tekirdağ Namık Kemal University, Tekirdağ, Türkiye during the 2016-2017 and 2017-2018 wheat growing seasons. Tekirdağ district locates at latitude 40° 36'-40° 31' and longitude 26° 43'-28° 08' and

altitude is 10 m. During the 2017-2018 wheat growing season, the total precipitation and the average temperatures were 661.5 mm and 12.1 °C, respectively. In addition, the long-term total precipitation and mean temperature were recorded as 521.2 mm and 11.9 °C, respectively.

In the field experiment, a total of 24 combinations (Table 1), including 6 F₁ and 18 M₁F₁, and 3 bread wheat varieties (NKÜ Lider, Bezostaja and GK Beke's) as their parents were used as material. The seeds of these 24 combinations were sown by hand on November 10, 2017, in 2 m long plots with an intra-row spacing of 5 cm and an inter-row spacing of 20 cm. The experiment was arranged in randomized complete block design with 3 replicates.

Table 1. Reciprocal crosses and parents

NKÜ Lider (L)	Bezostaja (BJ)	GK Beke's (BK)
L (Parent)	BJ (Parent)	BK (Parent)
L-0xBJ-0 (F ₁)	BJ-0xL-0 (F ₁)	BK-0xBJ-0 (F ₁)
L-0xBK-0 (F ₁)	BJ-0xBK-0 (F ₁)	BK-0xL-0 (F ₁)
L-100xBJ-100 (M ₁ F ₁)	BJ-100xL-100 (M ₁ F ₁)	BK-100xBJ-100 (M ₁ F ₁)
L-100xBK-100 (M ₁ F ₁)	BJ-100xBK-100 (M ₁ F ₁)	BK-100xL-100 (M ₁ F ₁)
L-200xBJ-200 (M ₁ F ₁)	BJ-200xL-200 (M ₁ F ₁)	BK-200xBJ-200 (M ₁ F ₁)
L-200xBK-200 (M ₁ F ₁)	BJ-200xBK-200 (M ₁ F ₁)	BK-200xL-200 (M ₁ F ₁)
L-300xBJ-300 (M ₁ F ₁)	BJ-300xL-300 (M ₁ F ₁)	BK-300xBJ-300 (M ₁ F ₁)
L-300xBK-300 (M ₁ F ₁)	BJ-300xBK-300 (M ₁ F ₁)	BK-300xL-300 (M ₁ F ₁)

The experiment was fertilized at different growth stages such as sowing (20.20.0 composed fertilizer), tillering (urea, 46% N), and stem elongation (calcium ammonium nitrate 26% N). The experiment was harvested in July, and data were collected on grain yield and agronomic traits such as plant height, spike length, number of spikelets per spike, number of grains per spike, grain weight per spike.

Statistical analysis

Analysis of variance (ANOVA) for all examined traits were performed according to randomized complete block design using the MSTAT-C statistical software. The means were compared using Duncan's multiple range test (Steel and Torrie, 1980). The heterosis and heterobeltiosis rates of the hybrids were calculated as percentages relative to the mean of the two parents and to the superior parent, respectively (Chiang and Smith, 1967). The statistical significance of the calculated heterosis and heterobeltiosis values was tested by the t-test according to Wynne et al. (1970).

RESULTS AND DISCUSSION

In the study, the mean values and significance groups of grain yield (GY) and agronomic traits such as plant height (PH), spike length (SL), number of spikelets per spike (NSPS), number of grains per spike (NGPS), and grain weight per spike (GWPS), for 24 combinations are presented in Table 2, while the heterosis and heterobeltiosis values are given in Table 3.

Plant height (cm)

The analysis of variance revealed that the differences in plant height among the hybrid combinations were statistically significant at the 1% probability level. The plant height of the hybrid combinations varied between 85.93 cm and 118.44 cm. The lowest value was recorded in the GK Beke's-300×NKÜ Lider-300 hybrid combination, while the highest was observed in the Bezostaja-200×GK Beke's-200 hybrid combination (Table 2). These results are in agreement with the findings of Inamullah et al. (2006), who also reported significant variation in plant height among hybrid combinations.

The heterosis values for plant height among the hybrid combinations ranged from -9.213% to 16.160% (Table 3). One hybrid combination was found to be significantly negative at the 1% level, while 19 hybrid combinations were significantly positive at the 1% level. The lowest heterosis value was observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the highest value was recorded in the GK Beke's-0×NKÜ Lider-0 hybrid combination. The heterobeltiosis values for plant height among the hybrid combinations ranged from -14.250% to 15.599%. Two hybrid combinations were found to be significantly negative at the 1% level, while four combinations were significantly positive at the same level. The lowest heterobeltiosis value was observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the highest was recorded in the GK Beke's-0×NKÜ Lider-0 hybrid combination. With respect to plant height, the hybrid combinations NKÜ Lider-100×Bezostaja-100 and GK Beke's-300×NKÜ Lider-300, which exhibited significantly negative heterosis and heterobeltiosis, can be considered promising for achieving the desired plant heights.

Spike length (cm)

The differences among the hybrid combinations for spike length were found to be statistically significant ($P < 0.01$). In the study, spike length among the hybrid combinations varied between 11.19 cm and 12.86 cm (Table 2). The shortest spikes were observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the longest spikes were obtained

from the NKÜ Lider-0×GK Beke's-0 hybrid combination. Our findings are similar to the spike length values (11.53-14.60 cm) reported in previous studies (Kumar et al., 2017).

As shown in Table 3, heterosis values for spike length in the hybrid combinations varied between -4.782% and 9.164%. Five combinations were significantly positive at the 1% level. The lowest heterosis value for spike length was observed in the GK Beke's-0×NKÜ Lider-0 hybrid combination, whereas the highest value was recorded in the NKÜ Lider-200×GK Beke'-200 hybrid combination. In the study, heterobeltiosis values for spike length ranged from -10.135% to 5.013%. One hybrid combination exhibited significantly negative heterobeltiosis at the 1% level, whereas no combination showed significantly positive heterobeltiosis. The lowest heterobeltiosis value was observed in the GK Beke's-0×NKÜ Lider-0 hybrid combination, while the highest was recorded in the NKÜ Lider-200×GK Beke's-200 hybrid combination. As a result, NKÜ Lider-200×GK Beke's-200 (9.164**), NKÜ Lider-0×GK Beke's-0 (7.886**), and GK Beke's-100×NKÜ Lider-100 (7.732**) hybrid combinations, which showed significantly positive heterosis at the 1% level, were identified as the combinations to focus on for improving spike length.

Number of spikelets per spike

In the analysis of variance, the effects of hybrid combinations on the number of spikelets per spike were statistically significant at the 1% level. In the hybrid combinations, the spikelet number per spike varied between 20.40 and 24.57 (Table 2). The highest number of spikelets per spike was recorded in the Bezostaja-0×GK Beke's-0 hybrid combination, followed by the Bezostaja-200×GK Beke's-200 hybrid combination with 24.52. GK Beke's-300×NKÜ Lider-300 hybrid combination had the lowest number of spikelets per spike. Our findings are also in agreement with those reported by Bibi et al. (2013).

In the study, heterosis values for the number of spikelets per spike in the hybrid combinations ranged from -9.774% to 4.555% (Table 3). The lowest heterosis value was observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the highest value was recorded in the Bezostaja-0×NKÜ Lider-0 hybrid combination. For the number of spikelets per spike, one hybrid combination was found to be significantly negative at the 1% level, while two combinations were significantly positive at the 5% level. The heterobeltiosis values for the number of spikelets per spike in the hybrid combinations ranged from -13.155% to 3.019% (Table 3). The lowest heterobeltiosis value was observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the highest value was recorded in the Bezostaja-0×GK Beke's-0 hybrid combination. In terms of the number of spikelets per spike,

four hybrid combinations were found to be significantly negative at the 1% level and one at the 5% level, while no hybrid combination was found to be significantly positive. As a results, the hybrid combinations Bezostaja-0×NKÜ Lider-0 and Bezostaja-0×GK Beke's-0, which exhibited significantly positive heterosis at the 1% level, were identified as promising combinations for spikelet number per spike.

Table 2. Mean values and significance groups for investigated traits.

Hybrid Combinations	PH (cm)	SL (cm)	NSPS	NGPS	GWPS (g)	GY (kg da ⁻¹)
NKÜ Lider (L)-0 × Gk Beke's (BK)-0	105.69 f-j	12.86 a	23.47 a-h	60.22 a-d	3.56 a	834.33 ab
NKÜ Lider (L)-0 × Bezostaja (BJ)-0	115.44 abc	11.92 a-f	23.31 d-I	62.06 a-d	3.29 a-e	804.33 bc
L-100 × BK-100	103.54 h-k	11.31 e-g	22.57 e-I	48.75 e-I	3.07 a-1	926.00 a
L-100 × BJ-100	109.94 b-h	12.25 a-e	23.11 a-I	64.12 ab	3.30 a-d	680.00 h-k
L-200 × BK-200	99.81 j-l	12.15 a-e	23.45 a-1	52.15 d-h	3.07 a-1	580.00 l-n
L-200 × BJ-200	115.23 abc	11.56 b-g	22.40 f-I	61.24 a-d	3.05 a-1	686.67 g-j
L-300 × BK-300	101.86 i-k	11.53 b-g	22.93 b-I	49.10 e-1	2.99 a-I	604.00 j-m
L-300 × BJ-300	107.22 e-I	11.93 a-f	23.35 a-1	58.82 a-e	3.12 a-1	613.67 I-m
Gk Beke's (BK)-0 × NKÜ Lider (L)-0	107.75 d-1	11.35 e-g	21.87 a-j	48.82 e-1	3.15 a-h	734.33 c-1
Gk Beke's (BK)-0 × Bezostaja (BJ)-0	115.02 abc	11.94 a-f	23.35 a-1	43.85 h-1	2.65 f-j	695.00 e-I
BK-100 × L-100	105.62 f-j	12.54 abc	23.31 a-1	55.23 c-g	3.50 a	714.33 d-1
BK-100 × BJ-100	116.10 ab	11.83 a-f	23.96 a-e	43.32 h-I	2.56 h-j	801.67 bc
BK-200 × L-200	105.11 g-j	11.78 a-g	22.76 c-I	48.17 f-1	2.86 b-j	601.67 j-m
BK-200 × BJ-200	114.76 a-d	11.84 a-f	23.44 a-h	37.09 i	2.26 j	600.33 k-m
BK-300 × L-300	85.93 o	11.19 g	20.40 j	51.67 d-h	2.49 i-j	445.00 o
BK-300 × BJ-300	108.67 c-1	12.20 a-e	23.77 a-g	40.63 ii	2.51 i-j	562.00 mn
Bezostaja (BJ)-0 × NKÜ Lider (L)-0	113.77 a-e	12.48 a-d	24.10 a-d	64.80 a	3.30 a-d	767.67 b-g
Bezostaja (BJ)-0 × Gk Beke's (BK)-0	112.54 a-g	12.24 a-e	24.57 a	45.17 g-I	2.62 f-j	756.00 b-h
BJ-100 × L-100	114.23 a-e	12.48 a-d	23.40 a-h	63.78 abc	3.48 ab	809.33 bc
BJ-100 × BK-100	112.78 a-f	11.43 d-g	23.97 a-e	42.97 h-I	2.59 g-j	783.67 bcd
BJ-200 × L-200	113.53 a-e	12.17 a-e	22.49 e-I	58.83 a-e	3.18 a-h	779.33 b-e
BJ-200 × BK-200	118.44 a	11.91 a-f	24.52 a	39.69 ii	2.38 I-j	695.33 e-I
BJ-300 × L-300	111.81 a-g	12.61 ab	22.93 b-I	64.60 a	3.33 a-d	655.00 i-l
BJ-300 × BK-300	110.35 b-h	11.64 a-f	23.48 a-h	42.16 h-I	2.36 I-j	468.67 o
MSE	12.882	0.305	0.558	27.939	0.101	1822.828
F values	19.308**	2.504**	4.039**	8.504**	3.823**	19.867**

PH: Plant height, SL: Spike length, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, GWPS: Grain weight per spike, GY: Grain yield, MSE: Mean squared error

Number of grains per spike

The results of the variance analysis indicated that the effect of hybrid combinations on the number of grains per spike was statistically significant at the 1% level. In the study, the number of grains per spike of the hybrid combinations ranged from 37.09 to 64.80. The lowest number of grains per spike was determined in the Beke-200×Bezostaja-200 hybrid combination. On the other hand, the Bezostaja-0×NKÜ Lider-0 hybrid combination had the

highest value for the number of grains per spike, followed by the Bezostaja-300×NKÜ Lider-300 hybrid combination (64.60), which was placed in the same statistical group (Table 2). The results obtained in this study are in agreement with the findings of Fellahi et al. (2013).

Table 3. Heterosis (He-%) and heterobeltiosis (Hb-%) values for investigated traits.

Hybrid Combinations	PH		SL		NSPS		NGPS		GWPS		GY	
	He	Hb	He	Hb	He	Hb	He	Hb	He	Hb	He	Hb
L-0×BK-0	13.939**	13.389**	7.886**	1.821	3.347	1.295	-9.088	-10.347	6.907	6.907	9.039*	8,542*
L-0×BJ-0	12.878**	3.692	-2.694	-5.622	1.128	-2.264	0.993	-4.962	0.000	-1.201	26.566**	5,601
L-100×BK-100	9.150**	6.501*	-2.835	-7.899*	-1.656	-4.121	-22.693**	-22.913**	-6.687	-8.358	40.729**	24,129**
L-100×BJ-100	2.336	-6.537**	2.083	-0.244	0.000	-3.143	8.715	1.988	4.762	-1.493	4.455	-8,847*
L-200×BK-200	4.975*	3.409	9.164**	5.013	3.395	1.121	-16.919**	-17.654**	-3.762	-4.361	-13.086**	-18,805**
L-200×BJ-200	7.873**	-1.614	-2.530	-4.778	-3.780	-8.121**	2.016	-1.543	3.741	-4.984	14.445**	10,694*
L-300×BK-300	7.618**	1.647	1.407	0.348	1.415	-2.384	-21.213**	-26.618**	2.397	-1.320	-17.786**	-22,232**
L-300×BJ-300	0.187	-5.807*	0.760	-2.133	1.345	-4.107	3.066	1.906	13.455	11.429	-3.511	-11,405*
BK-0×L-0	16.160**	15.599**	-4.782	-10.135**	-3.699	-5.611*	-26.298**	-27.319**	-5.405	-5.405	-4.030	-4,467
BK-0×BJ-0	12.964**	3.342	3.466	0.590	-0.681	-2.096	-29.705**	-34.718**	-19.453**	-20.181**	8.764*	-9,584*
BK-100×L-100	11.343**	8.640**	7.732**	2.117	1.569	-0.977	-12.417*	-12.666*	6.383	4.478	8.561*	-4,245
BK-100×BJ-100	10.508**	-1.301	4.137	0.939	1.097	0.419	-26.775**	-31.499**	-16.883**	-20.497**	42.393**	40,644**
BK-200×L-200	10.549**	8.900**	5.840	1.815	0.353	-1.854	-23.260**	-23.938**	-10.345	-10.903	-9.839*	-15,771**
BK-200×BJ-200	8.901**	-2.015	3.678	-2.471	-1.471	-3.856	-38.795**	-41.434**	-22.603**	-28.707**	-7.213	-15,959**
BK-300×L-300	-9.213**	-14.25**	-1.583	-2.611	-9.774**	-13.155**	-17.089**	-22.777**	-14.726*	-17.822*	-39.429**	-42,704**
BK-300×BJ-300	7.106**	-4.533*	4.096	0.082	-0.627	-2.382	-34.117**	-39.277**	-12.238	-17.162*	-17.109**	-27,640**
BJ-0×L-0	11.245**	2.192	1.878	-1.188	4.555*	1.048	5.452	-0.766	0.304	-0.901	20.798**	0,788
BJ-0×BK-0	10.528**	1.114	6.066*	3.117	4.509*	3.019	-27.589**	-32.753**	-20.365**	-21.084**	18.310**	-1,648
BJ-100×L-100	6.330**	-2.890	4.000	1.629	1.255	-1.928	8.138	1.447	10.476	3.881	24.321**	8,489*
BJ-100×BK-100	7.348**	-4.123	0.616	-2.474	1.139	0.461	-27.366**	-32.052**	-15.909*	-19.565**	39.195**	37,486**
BJ-200×L-200	6.282**	-3.065	2.614	0.247	-3.393	-7.752**	-1.999	-5.418	8.163	-0.935	29.888**	25,632**
BJ-200×BK-200	12.393**	1.127	4.291	-1.895	3.069	0.574	-34.505**	-37.328**	-18.493**	-24.921*	7.470	-2,660
BJ-300×L-300	4.476*	-1.775	6.503*	3.445	-0.477	-5.832**	13.194*	11.920	21.091**	18.929*	2.987	-5,438
BJ-300×BK-300	8.762**	-3.057	-0.683	-4.512	-1.839	-3.573	-31.636**	-36.990**	-17.483*	-22.112**	-30.875**	-39,656**

L: NKÜ Lider, BK: GK Beke's, BJ: Bezostaja, PH: Plant height, SL: Spike length, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, GWPS: Grain weight per spike, GY: Grain yield, He: Heterosis, Hb: Heterobeltiosis.

Heterosis values of the hybrid combinations ranged from -38.795% to 13.194% (Table 3). The minimum value was recorded in the GK Beke's-200×Bezostaja-200 combination, while the maximum was observed in the Bezostaja-300×NKÜ Lider-300 combination. For the number of grains per spike, fourteen hybrid combinations exhibited significantly negative heterosis at the 1% level, and one combination at the 5% level. In contrast, only one hybrid combination showed significantly positive heterosis at the 5% level. As indicated in Table 3, heterobeltiosis values for grain number per spike among the hybrid combinations varied between -41.434% and 11.920%. The minimum value was recorded in the GK Beke's-200×Bezostaja-200 combination, while the maximum was observed in the Bezostaja-300×NKÜ Lider-300 combination. Regarding grain number per spike, fourteen hybrid combinations were found to be significantly negative at the 1% level, while no combination showed significantly positive heterobeltiosis. The Bezostaja-300×NKÜ Lider-300 hybrid combination, which exhibited significantly positive heterosis at the 5% level, was identified as a promising combination for increasing grain number per spike.

Grain weight per spike (g)

The analysis of variance for grain number per spike indicated that the differences among the hybrid combinations were statistically significant at the 1% level. The grain weight per spike of the hybrid combinations ranged from 2.26 g to 3.56 g (Table 2). The lowest grain weight per spike was recorded in the GK Beke's-200×Bezostaja-200 hybrid combination. The highest grain weight per spike was observed in the NKÜ Lider-0×GK Beke's-0 combination, followed by the GK Beke's-100×NKÜ Lider-100 combination (3.50 g), which belonged to the same statistical group (Table 2).

In the study, heterosis values for grain weight per spike among the hybrid combinations ranged from -22.603% to 21.091% (Table 3). The lowest heterosis value was observed in the GK Beke-200×Bezostaja-200 hybrid combination, whereas the highest value was recorded in the Bezostaja-300×NKÜ Lider-300 combination. Five hybrid combinations exhibited significantly negative heterosis at the 1% level, while one combination showed significantly positive heterosis at the same level. In Table 3, the heterobeltiosis values of the hybrid combinations for grain weight per spike ranged from -28.707% to 18.929%. The lowest heterobeltiosis value was observed in the GK Beke's-200×Bezostaja-200 hybrid combination, whereas the highest value was recorded in the Bezostaja-300×NKÜ Lider-300 hybrid combination. Six hybrid combinations exhibited significantly negative heterobeltiosis at the 1% level, while one combination showed significantly positive heterobeltiosis at the 5% level.

Among the hybrid combinations, Bezostaja-300×NKÜ Lider-300, showing significantly positive heterosis at the 1% probability level and significantly positive heterobeltiosis at the 5% probability level, was considered a promising combination for increasing grain weight per spike.

Grain yield (kg da⁻¹)

The results of the variance analysis indicated that the differences in grain yield among the hybrid combinations were statistically significant at the 1% level. Grain yield of the hybrid combinations ranged from 445 to 926 kg da⁻¹ (Table 2). The lowest yield was recorded in the GK Beke's-300×NKÜ Lider-300 hybrid combination, followed by the Bezostaja-300×GK Beke's-300 combination (468.67 kg da⁻¹), which belonged to the same statistical group. The highest grain yield was obtained from the NKÜ Lider-100×GK Beke's-100 hybrid combination, followed by the NKÜ Lider-0×GK Beke's-0 combination (834.33 kg da⁻¹).

Table 3 indicates that heterosis for the hybrid combinations varied between -39.429% and 42.393%. The minimum value was recorded in the GK Beke's-300×NKÜ Lider-300 combination, while the maximum was observed in the GK Beke's-100×Bezostaja-100 combination. In Table 3, heterobeltiosis values of the hybrid combinations ranged from -42.704% to 40.644%. The lowest heterobeltiosis value was observed in the GK Beke's-300×NKÜ Lider-300 hybrid combination, whereas the highest value was recorded in the GK Beke's-100×Bezostaja-100 hybrid combination. Considering the heterosis and heterobeltiosis values determined in the study, it was seen that GK Beke's-100×Bezostaja-100, NKÜ Lider-100×GK Beke's-100 and Bezostaja-100×GK Beke's-100 hybrid combinations, which have positive significant values, can be considered to increase grain yield (Table 3).

CONCLUSION

In this study, significant variations among the 24 hybrid combinations were observed for plant height, spike length, number of spikelets per spike, grain number per spike, grain weight per spike, and grain yield. Considering the mean values, heterosis, and heterobeltiosis together, the hybrid combinations NKÜ Lider-100×Bezostaja-100 and GK Beke's-300×NKÜ Lider-300 were found to be promising for plant height; NKÜ Lider-0×GK Beke's-0, NKÜ Lider-200×GK Beke's-200, and GK Beke's-100×NKÜ Lider-100 for spike length; Bezostaja-0×GK Beke's-0 and Bezostaja-0×NKÜ Lider-0 for number of spikelets per spike; Bezostaja-300×NKÜ Lider-300 for grain number and grain weight per spike; and NKÜ Lider-100×GK Beke-100, GK Beke-100×Bezostaja-100 and Bezostaja-100×GK Beke-100 for grain yield.

Author Contribution Statement

All authors contributed equally to the preparation of this manuscript.

Conflicting Interest Statement

All authors declare that they have no conflicts of interest regarding this manuscript.

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DETERMINATION OF AGRICULTURAL POTENTIAL OF BREAD WHEAT (*Triticum aestivum* L.) MUTANT DOUBLED-HAPLOID LINES OBTAINED BY COMBINED GAMMA RADIATION AND ANTHER CULTURE APPLICATIONS

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ABSTRACT

The research was carried out to examine the agricultural characteristics of 25 bread wheat genotypes, including 19 mutant double haploid advanced lines developed by anther culture from M₀ populations created by gamma ray, 6 standard varieties, according to the randomized block trial design with 4 replications during the 2015-2016 growing year under Tekirdağ ecological conditions. In the study, quality traits of bread wheat varieties, such as plant height, spike length, number of spikelets per spike, number of grains per spike, number of grains per spike, grain weight per spike, harvest index, thousand grain weight, test weight, grain yield, protein ratio, wet gluten ratio, gluten index and Zeleny sedimentation value, were examined. Based on the data obtained in the experiment conducted, plant height, spike length, number of spikelets per spike, number of grains per spike, number of grains per spike, grain weight per spike, harvest index, thousand grain weight, test weight, grain yield, protein ratio, wet gluten rate, gluten index, Zeleny sedimentation value, averages of bread wheat varieties were 92.20 cm, 10.03 cm, 20.93 units, 41.68 units, 1.74 g, 44.70%, 43.71 g, 77.65 kg hl⁻¹, 753.76 kg da⁻¹ 14.75%, 34.59%, 83.44%, 58.56 ml respectively. As a result, when evaluated together with grain yield and quality characteristics, BSB 150/3, BSB 300/3, BSB 300/12, BSB 350/5, and BSB 300/3 bread wheat varieties have higher yields than other varieties. It was concluded that these high-performance varieties may be the most suitable varieties for our country's bread wheat production regions, especially the Thrace region.

Keywords: Bread wheat, Double haploid, Gamma rays, Grain yield, Quality, Mutant line.

INTRODUCTION

Today, we are approaching the limits of genetic variability that can be used to solve production and quality problems in wheat, a vitally important grain for human nutrition. Furthermore, the world population is rapidly increasing, and it is estimated that by 2050 the population will be approximately 50% larger than today, and that food shortages will increase daily. Among the measures that must be taken to achieve this nutritional increase, the most realistic is to increase yield per unit area. In addition to improving cultural practices, the most crucial step in increasing yield per unit area is the development and introduction of new varieties that are resistant to diseases and pests, of high quality, and have high yield potential. The greater the genetic variability in populations, the more successful the breeder's selection will be. Classical breeding methods, such as crossbreeding between close and distant relatives and between breeds, as well as physical and chemical mutations, are widely used to expand this variability.

As a result of intensive research and development on mutation breeding, over 3,212 new cultivars of 170 plant species have been registered in more than 60 countries. According to FAO (2012) data, Asia (60%) is the continent with the highest production of mutant cultivars, followed by Europe (30%), North America (6%), Africa (2%), and Latin America (2%). Cereals constitute 48% of the plant species produced, ornamentals 20%, food legumes 14%, and vegetables, forage crops, oilseed crops, and trees 3% (Mba, 2013). Approximately 70% of mutant cultivars were directly registered as mutants, while the remaining 30% were developed through combination breeding programs using mutants (Maluszynski et al., 2000). Given that mutation application is a sustainable, flexible, unregulated, non-hazardous, environmentally acceptable, highly effective, and low-cost technology (Kainthura and Srivastava, 2015), more effective use of mutation techniques is necessary in wheat breeding programs. It can normally be identified and selected in the second or third generation after mutagen application (Forster et al., 1996; Jauhar et al., 2009).

Doubled-haploid technology has received the most attention for improving the efficiency of traditional plant breeding methods and shortening breeding time. The advantages of doubled-haploid plant production have long been recognized by plant breeders, and more than 280 cultivars have been developed in this way (Szarejko and Forster, 2007). The use of doubled-haploid techniques will increase the efficiency of selection in mutation breeding and allow for more efficient use of mutations in plant breeding (Snape, 1989). Anther culture has been used in bread wheat to increase aluminum tolerance, improve frost tolerance, disease resistance, and water stress tolerance (Khan et al., 2001; Humphreys et al., 2007; Bakos et al., 2008).

The combination of mutation techniques with in vitro culture, particularly anther culture, is considered an "ideal system" (Xu et al., 2012). Genetic variation can be generated more efficiently in a shorter time, and subsequent selection and propagation of desired genotypes can be achieved, increasing the effectiveness of plant breeding programs (Ahluwalia et al., 2000; Maluszynski et al., 2000). The combined application of these techniques in China and France has resulted in the development of numerous high-yielding, early-season, salinity-tolerant, and drought-tolerant wheat varieties, many of which have been accepted by farmers in wheat production areas (Liu et al., 2009). It has been reported that high gamma ray doses in wheat cause a significant reduction in plant height and spike length (Farak and El-Khawaga, 2013), that when combined with mutation and double haploid technology, it can be used successfully in traits such as plant height, leaf dimensions, flag leaf position, brightness, spike characteristics, heading, ripening time and disease resistance (Giura, 2013), that it can be a potential source for enriching genetic diversity (Cheng et al., 2015), and that new forms with high grain yield and quality potential of wheat have been revealed by Nazarenko et al. (2023).

Studies investigating the agronomic capacity of mutant DH lines in bread wheat are limited. This study will demonstrate the benefits of combining doubled haploid and mutation breeding, and will also explore the potential use of mutant doubled haploids and genetic diversity in agricultural studies.

MATERIALS AND METHODS

Seeds of two bread wheat advanced lines (BSB = Bezostaja x Sarajevo and FA: Flamura80 x Atilla 12), developed by the Department of Field Crops, Faculty of Agriculture, Tekirdağ Namık Kemal University, and included in the seed collection, were irradiated with gamma rays at doses of 100, 150, 200, 250, 300, 350, and 400 Gy (1 gray = 100 rad = 0.1 krad) from a ^{60}Co source at the Ankara Nuclear Research and Training Center of the Turkish Atomic Energy Authority. Nineteen lines selected from single plant rows grown in Tekirdağ Namık Kemal University, Faculty of Agriculture, Field Crops Experiment and Research Area in 2015-2016 growing year and six bread wheat varieties commonly grown in the region were used as material for the production of 120 spontaneous doubled-haploid plants developed from anthers taken for anther culture from M_1 populations generated by different gamma ray doses applied to two bread wheat varieties.

Each line and standard variety were planted separately in the 2015-2016 growing season using a randomized block design, with 2 meter row spacing, 20 cm between rows, and 500 seeds per square meter in three replications. The study was conducted at the Field Crops

Experimental and Research Area of Tekirdağ Namık Kemal University's Faculty of Agriculture. Plant height, spike length, number of spikelets per spike, number of grains per spike, grain weight per spike, harvest index, thousand-seed weight, hectoliter weight, grain yield, protein content, wet gluten content, gluten index, and Zeleny sedimentation value were examined. Variance analysis was performed on the data obtained from the experiment using a randomized block design, and genotype significance groups were then presented separately for the traits studied. The statistical significance of the differences between the means was checked by: The EKÖF (Least Significant Difference) test was performed in the MSTAT version 3.00/EM package program according to the method recommended by Steel and Torrie (1960).

RESULTS AND DISCUSSION

The variance analysis results for the plant height, spike length, number of grains per spike, grain weight per spike, harvest index, grain yield, thousand grain weight, gluten index, Zeleny sedimentation value, and protein content of the genotypes used as material in the study conducted with 25 bread wheat genotypes are presented in Table 1.

Table 1. Variance analysis results of the examined characteristics

<i>Sources of Variation</i>	Replication	Genotype (G)	Error
<i>D.F.</i>	2	24	48
Plant height	3.30	76.08**	3.59
Spike length	0.09	1.38**	0.08
Grain number per spike	11.08	39.90**	3.52
Grain weight per spike	0.06	0.12**	0.03
Harvest index	3.27	7.46**	2.74
Grain yield	211.96	28110.29**	770.64
1000 grain weight	5.34	33.21**	1.72
Glutent index	3.45	386.68**	3.35
Zeleny sedimentation value	3.88	121.65**	4.51
Protein content	0.05	1.69**	0.01

According to the variance analysis results, the differences between the means of the genotypes (mutant doubled haploid lines and varieties) were found to be statistically significant at the 0.01 probability level for the examined traits (Table 1). The results of the least significant difference (LSD) test, which was conducted to reveal these differences between the genotypes, are given in Tables 2 and 3.

In the study carried out in Tekirdağ ecological conditions, bread wheat mutant doubled-haploid lines had average plant heights ranging from 86.9 (BSB 300/22) to 98.8 (BSB 300/4) cm. Ten mutant lines (BSB 150/3, BSB 150/11, BSB 150/34, BSB 300/2, BSB 300/3, BSB 300/4, BSB 300/18, BSB 350/5, BSB 400/2, and FA 150/5) had values above the average, while

nine lines (BSB 150/26, BSB 150/36, BSB 200/5, BSB 200/6, BSB 250/2, BSB 300/12, BSB 300/22, BSB 300/24, and FA 100/2) produced shorter plant heights. A comparison of the standard varieties and the mutant lines reveals that the mutant lines had 3.85% greater plant height than the standard varieties (Table 2).

Table 2. Grain yield, average and significance of some yield components

Genotypes	PH	SL	GNS	GWS	HI	GY
BSB 150/3	95.8 a-e	10.60 b-e	47.5 ab	2.14 a	46.02 a-d	872 abc
BSB 150/11	93.2 ef	9.57 h-k	37.9 h-k	1.72 b-f	45.05 bcd	812 c-g
BSB 150/26	91.9 e-I	9.55 h-k	40.7 e-j	1.62 c-g	43.87 cd	735 hi
BSB 150/34	96.1 ab	10.30 c-g	43.6 b-f	1.95 abc	44.87 bcd	861 a-d
BSB 150/36	90.7 f-j	10.43 c-g	39.7 f-j	1.79 a-f	44.01 cd	790 e-h
BSB 200/5	89.5 g-j	9.25 jkl	44.1 b-e	1.86 a-e	47.11 abc	804 d-g
BSB 200/6	89.2 hij	8.80 l	37.5 ijk	1.39 fg	42.46 d	661 jk
BSB 250/2	92.2 d-I	9.41 i-l	38.9 g-j	1.53 d-g	45.79 a-dj	638 k
BSB 300/2	96.0 a-d	10.40 c-g	38.6 h-k	1.71 b-f	42.78 d	791 e-h
BSB 300/3	93.6 b-g	10.37 bc	39.9 f-j	1.77 a-f	42.99 d	818 c-f
BSB 300/4	98.8 a	10.83 bc	45.4 abc	1.94 a-d	44.13 cd	653 jk
BSB 300/12	91.3 f-I	10.49 c-f	45.9 abc	1.94 a-d	44.02 cd	772 fgh
BSB 300/18	96.2 a-d	11.60 a	43.0 c-g	1.65 c-g	44.56 cd	634 k
BSB 300/22	86.9 j	9.33 jkl	43.8 b-f	1.65 c-g	44.55 cd	703 ij
BSB 300/24	90.4 f-j	9.62 h-k	38.4 h-k	1.66 c-g	44.59 cd	768 fgh
BSB 350/5	97.7 ab	10.70 bcd	44.8 b-e	2.08 ab	44.29 cd	849 b-e
BSB 400/2	97.0 abc	10.51 b-f	41.1 d-i	1.85 a-e	43.59 cd	914 a
FA 100/2	88.6 ij	9.90 f-j	41.9 c-h	1.69 b-f	45.47 a-d	764 f-i
FA 150/5	93.2 c-h	9.55 h-k	49.0 a	1.87 a-e	45.2 bcd	766 fgh
Mean	93.06	10.06	42.19	1.78	44.49	768.68
ESPERIA	74.4 k	9.56 h-k	37.7 ijk	1.23 g	43.33 d	481 l
RUMELI	94.0 b-f	10.08 d-h	45.0 a-d	1.68 b-f	48.30 ab	882 ab
NKÜ LİDER	90.1 f-j	11.14 ab	44.2 b-e	1.88 a-e	48.89 a	740 hi
FLAMURA 85	90.9 f-j	10.00 e-i	41.9 c-h	1.61 c-g	44.58 cd	732 hi
SELİMİYE	88.4 ij	9.07 kl	34.7 k	1.51 efg	43.45 d	652 jk
PEHLİVAN	99.0 a	9.84 g-j	36.9 jk	1.78 a-f	43.75 cd	752 ghi
Mean	89.47	9.94	40.06	1.61	45.38	706.5
LSD ($P \leq 0.05$)	4.158	0.64	4.11	0.41	3.63	60.90

PH: Plant height, SL: spike length, GNS: number of grains per spike, GWS: grain weight per spike, HI: harvest index,

GY: grain yield

Among the mutant doubled haploid lines, three lines (BSB 200/6, BSB 300/22, and FA 100/2) had shorter plant height than the standard variety average. A general evaluation of the mutant doubled haploid lines and the standard variety averages revealed that, in addition to BSB 150/3, BSB 150/34, BSB 300/2, BSB 300/4, BSB 300/18, BSB 350/5, and BSB 400/2, 12 other mutant doubled haploid lines were suitable for plant height traits. Irfaq and Nawab (2001) found that plant height increased in wheat varieties at a dose of 10 kR, but abnormalities occurred at higher gamma irradiation doses (30 and 40 kR). Researchers reported that varieties responded differently to different gamma irradiation doses according to certain criteria.

According to these results, it can be said that the increased plant height in the mutant doubled-haploid lines used may vary depending on the applied gamma ray dose, genotype and ecological factors.

Spike length in bread wheat mutant doubled-haploid lines varied between 8.80 (BSB 200/6) and 11.60 (BSB 300/18) cm. While 10 lines (BSB 150/3, BSB 150/34, BSB 150/36, BSB 300/2, BSB 300/3, BSB 300/4, BSB 300/12, BSB 300/18, BSB 350/5 and BSB 400/2) were above the average, 9 lines (BSB 150/26, BSB 200/5, BSB 200/6, BSB 250/2, BSB 300/22, BSB 300/24, FA 100/2 and FA 100/5) were below the average. When the standard varieties and the mutant doubled-haploid lines were compared in terms of the examined trait, it was seen that the mutant lines had 1.19% more spike length than the standard varieties (Table 2). Since the average of the mutant doubled-haploid line was above the average of the standard variety, it was understood that 10 lines (BSB 150/3, BSB 150/34, BSB 150/36, BSB 300/2, BSB 300/3, BSB 300/4, BSB 300/12, BSB 300/18, BSB 350/5 and BSB 400/2) were promising in terms of spike length trait.

In bread wheat mutant doubled-haploid lines, grain number per spike varied between 37.5 (BSB 200/6) and 49.00 (FA 150/5), while it was above average in nine lines (BSB 150/34, BSB 150/3, BSB 200/5, BSB 300/4, BSB 300/12, BSB 300/18, BSB 300/22, BSB 350/5, FA 150/5), it was above average in 10 lines (BSB 150/11, BSB 150/26, BSB 150/36, BSB 200/6, BSB 250/2, BSB 300/2, BSB 300/3, BSB 300/24, BSB 400/2 and FA 150/2) yielded below the average. When comparing standard commercial varieties with mutant lines, it was observed that the mutant lines had 5.04% more grain per spike than the standard varieties (Table 2). As a result of the overall evaluation, nine lines were determined to be promising for this trait, with grain per spike above the standard line and standards. Furthermore, 12 lines (BSB 150/3, BSB 150/26, BSB 150/34, BSB 200/5, BSB 300/4, BSB 300/12, BSB 300/18, BSB 300/22, BSB 350/5, BSB 400/2, FA 100/2, FA 150/5) yielded above the standard variety average, suggesting that further research on these lines would be beneficial. Our results were similar to the findings of Ahmed et al. (2017), who found that gamma irradiation at 15 kR in wheat caused a significant increase in the number of grains per spike and that 25 kR and lower irradiation doses could be used to improve the yield characteristics of wheat.

Grain weight per spike of bread wheat mutant doubled-haploid lines varied between 1.39 (BSB 200/5) and 2.14 (BSB 150/3) g. While nine lines (BSB 150/3, BSB 150/34, BSB 150/36, BSB 200/5, BSB 300/4, BSB 300/12, BSB 350/5, BSB 400/2, FA 150/5) had above average grain weight per spike, 10 lines (BSB 150/11, BSB 150/26, BSB 200/6, BSB 250/2, BSB 300/2,

BSB 300/3, BSB 300/18, BSB 300/22, BSB 300/24 and FA 100/2) had below average grain weight per spike. Comparing standard commercial varieties with mutant lines, mutant lines show 9.55% greater per-spike grain weight than standard varieties (Table 2). Nine mutants were found to have higher per-spike grain weight than both the line and variety averages, indicating promising results for the trait studied. Furthermore, seven lines (BSB 150/26, BSB 300/2, BSB 300/3, BSB 300/18, BSB 300/22, BSB 300/24, and FA 100/2) were found to be above the standard variety average, suggesting that further research on these lines would be beneficial.

Harvest index in bread wheat mutant doubled-haploid lines varied between 42.46% (BSB 200/6) and 46.02% (BSB 150/3). While ten lines (BSB 150/3, BSB 150/11, BSB 150/34, BSB 200/5, BSB 250/2, BSB 300/18, BSB 300/22, BSB 300/24, FA 100/2 and FA 150/5) were above the average of the mutant lines, 9 lines (BSB 150/26, BSB 150/36, BSB 200/6, BSB 300/2, BSB 300/3, BSB 300/4, BSB 300/112, BSB 350/5 and BSB 400/2) were below the average. When the standard commercial varieties and the mutant lines were compared, it was seen that the mutant lines had 1.96% less harvest index than the standard varieties (Table 2). Among the mutant doubled-haploid lines, 4 lines (FA 100/2, BSB 250/2, BSB 200/5 and BSB 150/3) were determined to be promising in terms of the examined trait because they had a harvest index higher than the standard variety average.

Grain yield per decare of bread wheat mutant doubled-haploid lines varied between 634 (BSB 300/18) and 914 (BSB 400/2) kg da⁻¹. While ten lines (BSB 150/3, BSB 150/11, BSB 150/34, BSB 150/36, BSB 200/5, BSB 300/2, BSB 300/3, BSB 300/12, BSB 350/5 and BSB 400/2) had above average values, 9 lines (BSB 150/26, BSB 200/6, BSB 250/2, BSB 300/4, BSB 300/18, BSB 300/22, BSB 300/24, FA 100/2 and FA 150/5) gave lower grain yield. When the standard commercial varieties and the mutant lines were compared, it was seen that the mutant lines had 8.08% higher grain yield than the standard varieties (Table 2). As a result of the overall evaluation, 10 lines had grain yields above the average for both the line and variety, indicating promising results for the traits studied. Furthermore, four lines (BSB 150/26, BSB 300/24, FA 100/2 and FA 150/5) also had grain yields above the average for the standard variety, suggesting that further research on these lines would be beneficial.

In mutant doubled-haploid lines, thousand-grain weight ranged from 42.46 (BSB 200/6) to 46.02 g (BSB 150/3). Eight lines (BSB 150/3, BSB 150/34, BSB 150/36, BSB 300/2, BSB 300/3, BSB 300/4, BSB 400/2 and BSB 350/5) were above the line average, while 11 lines (BSB 150/11, BSB 150/26, BSB 200/5, BSB 200/6, BSB 250/2, BSB 300/12, BSB 300/22, BSB 300/24, FA 100/2 and FA 150/5) were below the average. A comparison of standard

commercial varieties and mutant lines reveals that mutant lines have a 1000-grain weight per spike by 2.18% more than standard varieties (Table 3). The overall assessment indicated that eight lines were promising for the trait studied, as they had a 1000-grain weight per spike above the average of both the line and variety. Furthermore, four lines (BSB 300/24, BSB 300/18, and BSB 150/11) also exceeded the average of the standard variety, suggesting that further research on these lines would be beneficial.

Table 3. Averages and importance of some quality attributes

Genotypes	TGW	GI	ZSV	GPC
BSB 150/3	46.50 bcd	79 kl	55.3 g-j	16.13 a
BSB 150/11	43.40 ef	82 jk	66.7 abc	15.30 c
BSB 150/26	41.6 f-i	87 ghi	59.0 efg	14.20 fg
BSB 150/34	48.80 ab	76 lm	54.0 hij	14.5 e
BSB 150/36	46.00 b-e	82 jk	53.3 ijk	14.43 ef
BSB 200/5	42.20 fgh	91 d-g	59.3 efg	14.57 de
BSB 200/6	41.70 f-i	84 ij	68.0 abc	16.07 a
BSB 250/2	39.10 ij	88 f-i	61.0 def	15.17 c
BSB 300/2	44.40 c-f	89 e-h	59.3 efg	14.10 gh
BSB 300/3	47.80 ab	74 mn	52.3 i-l	15.63 b
BSB 300/4	44.00 cd	72 mn	49.0 kl	14.17 fg
BSB 300/12	42.20 fgh	85 hij	55.7 g-j	15.13 c
BSB 300/18	43.60 def	95 a-d	65.0 cd	14.10 gh
BSB 300/22	39.3 hij	81 jk	65.3 bcd	14.57 de
BSB 300/24	43.30 ef	82 jk	56.0 f-j	14.53 e
BSB 350/5	46.40 bcd	66 o	52.3 i-l	15.67 b
BSB 400/2	50.00 a	52 p	53.7 ijk	15.77 b
FA 100/2	42.7 fg	96 abc	61.7 de	13.27 i
FA 150/5	42.00 f-i	75 lmn	51.7 jkl	14.00 gh
Mean	43.94	80.84	57.82	14.80
ESPERİA	36.40 j	99 a	70.0 ab	13.83 h
RUMELİ	39.80 ghi	98 a	70.3 a	15.60 b
NKÜ LİDER	46.60 bc	97 ab	61.7 de	14.10 gh
FLAMURA 85	42.90 f	92 c-f	58.7 e-h	14.83 d
SELİMİYE	43.60 def	93 b-e	57.0 e-i	14.50 e
PEHLİVAN	48.60 ab	71 n	47.7 l	14.60 de
Mean	42.98	91.66	60.9	14.57
LSD ($P \leq 0.05$)	2.88	4.01	4.66	0.28

TGW: thousand-seed weight, GI: hectoliter weight, ZSV: Zeleny sedimentation, GPC: grain protein content.

The gluten index in mutant double haploid lines varied between 52.00% (BSB 400/2) and 96.00% (FA 100/2). twelve lines (BSB 150/11, BSB 150/26, BSB 150/36, BSB 200/5, BSB 200/6, BSB 250/2, BSB 300/2, BSB 300/12, BSB 300/18, BSB 300/22, BSB 300/24 and FA 100/2) had values above the average, 7 lines gave lower gluten index. When standard

commercial varieties and mutant lines were compared, it was seen that standard varieties had 11.80% higher gluten index rate than mutant lines (Table 3).

While sedimentation values in mutant doubled-haploid lines were between 51.7 (FA 150/5) and 68.00 (BSB 200/6) ml, 9 lines (BSB 150/11, BSB 150/26, BSB 200/5, BSB 200/6, BSB 250/2, BSB 300/2, BSB 300/18, BSB 300/22, FA 100/2) gave values above the average, 10 lines (BSB 150/3, BSB 150/34, BSB 150/36, BSB 300/3, BSB 300/4, BSB 300/12, BSB 300/24, BSB 350/5, BSB 400/2 and FA 150/5) gave lower values. When comparing standard commercial varieties and mutant lines, it is seen that standard varieties have 5.06% higher values than mutant lines (Table 3). As a result of the general evaluation, 6 lines (BSB 150/11, BSB 200/6, BSB 250/2, BSB 300/18, BSB 300/22 and FA 100/2) had Zeleny sedimentation rates above the average of both lines and varieties, indicating that they are promising in terms of the examined trait. Balkan et al. (2021) found that Zeleny sedimentation values in M₂, M₃ and M₄ mutant populations of three bread wheat genotypes irradiated with different gamma rays were significantly increased in the mutated populations compared to the control.

The protein content in mutant doubled-haploid lines varied between 14.00% (FA 150/5) and 16.13% (BSB 150/3). While 11 lines (BSB 150/26, BSB 150/34, BSB 150/36, BSB 200/5, BSB 300/2, BSB 300/4, BSB 300/18, BSB 300/22, BSB 300/24 FA 100/2 and FA 150/5) had values below the average, 8 lines gave higher protein content. When the standard varieties and the mutant lines were compared, it was seen that the mutant lines had 1.55% more protein content than the standard varieties (Table 3). As a result of the general evaluation, it was determined that 11 lines were promising in terms of the examined trait because they had protein content above the average of both the line and the variety. The results obtained were similar to those of Rahimi and Bahrani (2011), who found that gamma radiation caused an increase in protein content.

CONCLUSION

Based on the data obtained in the experiment conducted, plant height, spike length, number of spikelets per spike, number of grains per spike, number of grains per spike, grain weight per spike, harvest index, thousand grain weight, test weight, grain yield, protein ratio, wet gluten rate, gluten index, Zeleny sedimentation value, averages of bread wheat varieties were 92.20 cm, 10.03 cm, 20.93 units, 41.68 units, 1.74 g, 44.70%, 43.71 g, 77.65 kg hl⁻¹ -1, 753.76 kg da⁻¹ 14.75%, 34.59%, 83.44%, 58.56 ml respectively. As a result, when evaluated together with grain yield and quality characteristics, BSB 150/3, BSB 300/3, BSB 300/12, BSB

350/5, and BSB 300/3 bread wheat varieties have higher yields than other varieties. It was concluded that these high-performance varieties may be the most suitable varieties for our country's bread wheat production regions, especially the Thrace region. In this study, 150/3, BSB 150/11, BSB 150/34, BSB 150/36, BSB 200/5, BSB 300/2, BSB 300/3, BSB 300/12, BSB 350/5 and BSB 400/2 mutant lines were determined as the most promising lines in terms of grain yield. The results obtained show that mutation breeding can be used successfully to improve yield and quality traits in wheat.

Author Contribution Statement

All authors contributed equally to the preparation of this manuscript.

Conflicting Interest Statement

All authors declare that they have no conflicts of interest regarding this manuscript.

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THE EFFECT OF DIFFERENT SEEDING DENSITY ON GRAIN YIELD AND GRAIN QUALITY IN SELECTED POPULATIONS OF KIRIK WHEAT

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ABSTRACT

The study conducted during the 2024-2025 growing season at the Tekirdağ Namık Kemal University Faculty of Agriculture experimental area, involved four different Kırık wheat populations and six seeding densities (200, 300, 400, 500, 600, and 700 m²/seed). The study was conducted using a split-plot design with three replications, with Kırık wheat populations placed in the main plot and the seeding density placed in the subplots. In the study, plots with 17 cm row spacing and 5 meters row length were sowed in six rows using a plot drill. The necessary cultural practices were implemented in accordance with the experimental techniques. The study was examined grain yield per decare, thousand-grain weight, test weight, Zeleny Zeleny sedimentation value, delayed Zeleny sedimentation value, wet gluten, wet gluten index, protein rate, sunn pest damage and embryo black point. According to the obtained data, grain yield varied between 72.00 and 183.00 kg/da. The highest grain yield was achieved at 200-300 seed per square meter, while yield decreases occurred at seeding rates greater than 500 seed per square meter. When quality characteristics were examined, significant changes in quality characteristics were observed depending on the population and seeding densities. In the study, wet gluten rate varied between 26-38%, gluten index varied between 73-97%, test weight values varied between 71.50-80.50 kg/hl, Zeleny Zeleny sedimentation varied between 34-50 ml, delayed Zeleny sedimentation varied between 10-48 ml, sunn pest damage varied between 0.0-3.5%, embryo black point varied between 0.0-1.0% and protein rate varied between 13.60-17.90%.

Keywords: Wheat, Seeding density, Grain yield, Local variety.

INTRODUCTION

Wheat (*Triticum* spp.) is a global staple food and is classified within the genus *Triticum* of the Poaceae (Gramineae) family. The *Triticum* genus encompasses approximately 300

species with a wide diversity (Matsuoka, 2011). Wheat is cultivated in extensive areas worldwide and in Turkey due to its ease of sowing, harvesting, transportation, and storage. Furthermore, its important role in human nutrition increases the demand for wheat cultivation. With its ecological diversity, Türkiye serves as a gene center for many plant species. Türkiye, where wheat was first cultivated and is one of the most important gene centers, possesses a wealth and diversity of local wheats that can be used directly or indirectly in wheat breeding studies. Landraces are groups of individuals with similar appearances, often in a population structure, that survive under natural and artificial selection pressures (Özberk et al., 2016). Inadequate and uneven distribution of rainfall during the wheat growing season causes decreases in yield (Öztürk and Korkut, 2018).

Local wheat is cultivated in limited areas and under varying climatic conditions, and is at risk of extinction as its acreage dwindles. However, it has been used as an important genetic resource in some wheat breeding programs. It has been reported that local wheat varieties collected from Türkiye have been used as parents in the development of many wheat varieties (Skowmand et al., 1984). Altıntaş et al. (2008) emphasized the need to use local bread wheat varieties from Türkiye to create variation in wheat breeding.

Local bread and durum wheats in Türkiye are Karakılçık, Yellow wheat; Polatlı Kösesi; Kobak Wheat, Akçalıbasan, Gülümbür, White Wheat, Sünter, Deli Hüseyin Wheat, Üveyik, Kavılca, Havran Reddish, Erzurum Reddish, Karakılçık; Whitebasak; Einkorn; Kamçı, Beyaz Kelle, Purple Wheat, Akbaş, Red Wheat, Görmez, Black Buckwheat, Grandfather wheat; Çalıbasan, Üveyik; Şahman, Antique Hittite, Zız; Zerun, Şahman, Kunduru Yellow Bursa; Aşure, Menceki; Kırık, Yayla-305, Zerun, Şigon; Köse Wheat; Tir Wheat; They can be specified as Siyez, Gernik, Bağacak, Sorgül, Beyaziye, İskenderi, Mısri, Havrani (Hocaoğlu and Akçura, 2014; Demirel, 2013; Koyuncu, 2009; Sönmez et al., 1999; Çağlar et al., 2006).

It has been reported that thousand grain weight in wheat (Aydoğan and Soylu, 2017) varies depending on genetic structure, ecological factors (Bulut, 2015; Ghaffar et al., 2018), the main stem and tillers in the plant, the number of ears per square meter, the number of grains per ear, and the applied cultural treatments (Abbas, 2017). It has been reported that the protein amount in wheat varies according to the genotype, growing conditions, sowing time, and climatic conditions, and the Zeleny Zeleny sedimentation test is a rapid method that provides an idea about both the protein amount and quality of wheat (Elgün et al., 2012). It has been reported that the Zeleny sedimentation value depends on the genotypic trait as well as the effect of climatic factors (Aydoğan et al., 2010) and sowing time (Çağlar et al., 2011).

Among local genotypes, Kırık wheat populations are particularly preferred for

breadmaking in the Eastern Anatolia Region due to their good bread quality and high protein content. Kırık is a local wheat population cultivated throughout the Eastern Anatolia Region, including in some villages in the provinces of Kayseri, Siirt, Sivas, Elazığ, Malatya, Artvin, and Hakkari (Gökgöl, 1939). The local Kırık wheat variety (*Triticum aestivum* var. delfii), which exhibits alternative characteristics, has an upright spike, a sparse density, and a medium height. The grain is white, with or without awns at the top, and the outer glumes are red. It is highly drought-resistant, medium-yielding, susceptible to lodging, and a medium-early maturaten. Kırık, with its high bread quality, is susceptible to bunt and rust diseases, responds well to fertilizer, and is resistant to grain drop (Ertugay, 1980).

In this study, the changes in yield and quality traits were examined in 6 different seeding density in 4 different Kırık wheat populations and it was aimed to determine the most suitable populations and seeding density in terms of yield and quality.

MATERIALS AND METHODS

The research was carried out in the 2022-2023 production year in Süleymanpaşa District of Tekirdağ province. The experiment was carried out in 4 different kırık populations at 6 different seeding density according to a split plot design with 3 replications. Kırık wheat populations were sown in main plots, with seeding density in sub-plots. Seeds were sown with a drill in plots consisting of 6 rows, with 17 cm row spacing and 5 meters row length, at the Tekirdağ Namık Kemal University experimental area on November 9, 2023. Plots were sown at rates of 200, 300, 400, 500, 600 and 700 seeds per square meter. Pre-emergence herbicides were used for weed control in the experimental area. For fertilization, 20 kg/da 20-20-0 compound fertilizer was applied to provide 5 kg of pure nitrogen and 5 kg of pure phosphorus per decare. Urea fertilizer (46% nitrogen) was applied to provide 7.5 kg of pure nitrogen per decare during the tillering period, and ammonium nitrate fertilizer (33% nitrogen) was applied to provide 4 kg of pure nitrogen per decare before the heading period.

In the study, plots were threshed with a HEGE 160 combine harvester, and the plot yields were converted into grain yield per decare. Variance analysis was performed on the obtained grain yield, gluten content, gluten index, Zeleny sedimentation rate, protein content, test weight, and sunn pest suction rate using the Tarpoggen statistical package program, and the differences between the obtained means were determined using the DUNCAN test.

RESULTS AND DISCUSSION

Variance analysis was performed on the grain yield, gluten rate, gluten index, hectoliter,

Zeleny sedimentation value, sunn pest damage and protein rate values obtained at 6 different seed densities in 4 different populations of Kırık wheat and the results are given in Table 1.

Table 1. Variance analysis results for the examined characteristics

Sources of Variation	DF	Grain yield (kg/da)	Gluten rate (%)	Gluten index (%)	Test weight (kg/hl)	Zeleny Sedim. value (ml)	Sunn pest damage (%)	Protein rate (%)
Block	3	82.95	1.70	80.03	2.17	2.58	0.67	0.44
Populations (P)	3	5353.40**	123.45**	144.79	4.87**	165.36**	2.29**	6.01**
Error1	9	163.32	9.08	39.74	0.55	7.41	0.20	0.19
Seeding density (S)	5	8739.00**	29.83**	161.29**	4.39**	27.83	1.13**	5.89**
P x S	15	301.57**	3.28	46.70	0.77	10.22	0.26	0.48
Error	60	112.98	4.69	29.92	0.75	18.89	0.18	0.98
<i>CV</i>		<i>6.33</i>	<i>6.77</i>	<i>6.35</i>	<i>1.17</i>	<i>10.50</i>	<i>16.07</i>	<i>6.19</i>

According to the results of variance analysis performed on the obtained data, the differences between the populations were statistically significant at the 0.01 level for grain yield, gluten rate, Zeleny sedimentation value, sunn pest damage and protein rate, while they were statistically insignificant for the gluten index. Differences between seeding density were statistically significant at the 0.01 level for grain yield, gluten rate, gluten index, sunn pest damage and protein rate, but were statistically insignificant for the Zeleny sedimentation value. The population x seeding density interaction was statistically significant at the 0.01 level for grain yield, but was statistically insignificant for the other characters. The mean values and the results of the Duncan significance test are given in Table 2 for populations, in Table 3 for seeding density, and in Table 4 for the population x seeding density interaction.

Table 2. Average values and their importance in the examined features

Populations (P)	Grain yield (kg/da)	Gluten rate (%)	Gluten index (%)	Test weight (kg/hl)	Zeleny sedimen. value (ml)	Sunn pest damage (%)	Protein rate (%)
P-1	156.17 c	32.54 a	89.17	75.05a	42.12 ab	2.57 a	16.15 a
P-2	155.29 c	33.33 a	85.38	74.07b	44.13 a	2.93 a	16.46 a
P-3	186.46 a	28.67 b	86.83	74.15b	37.83 c	2.25 b	15.30 b
P-4	173.20 b	33.50 a	83.33	74.57ab	41.42 b	2.84 a	16.13 b

When grain yield and quality traits obtained in different Kırık wheat populations were examined, it was observed that there were significant differences among populations. The highest grain yield was in population P-3 with 186.46 kg/da, followed by population P-4 with 173.20 kg/da. The lowest grain yields were obtained in populations P-1 and P2 with 155.29 and

156.17 kg/da. The gluten rate varied between 28.67% and 33.50%, with the highest gluten rate being 33.50%, 33.33% and 32.54% in populations P-4, P-2 and P-1. The differences among the populations in terms of gluten index values were statistically insignificant. The highest Zeleny sedimentation value was in population P-2 with 44.13 ml, followed by population P-1 with 42.12 ml. The lowest Zeleny sedimentation value, 37.83 ml, was found in the P-3 population, which had the highest grain yield. The lowest value in terms of sunn pest damage, a common pest in the region in bread wheat production, was found in the P-3 population at 2.25%, while the highest value was found in the P-2 population at 2.93%. When the populations were examined in terms of protein rate, the highest protein rates were found in the P-2 and P-1 populations at 16.46% and 16.15%. The lowest protein rate, 15.30%, was obtained in the P-3 population, which had the highest grain yield.

Table 3. Average values and importance of the examined traits at different seed densities

Seeding density (S)	Grain yield (kg/da)	Gluten rate (%)	Gluten index (%)	Test weight (kg/hl)	Zeleny sedimen. value (ml)	Sunn pest damage (%)	Protein rate (%)
200	187.44 ab	30.88 c	90.13 a	75.01a	40.88	2.46 cd	15.49 c
300	194.63 a	31.69 bc	86.69 ab	74.49ab	41.19	2.93 a	15.92 bc
400	180.25 b	33.44 ab	81.81 b	73.86b	42.31	2.84 abc	16.48 ab
500	160.50 c	34.0 a	83.44 b	73.90b	43.50	2.86 ab	16.98 a
600	148.25 d	31.13 c	86.00 ab	74.43ab	40.50	2.53 bcd	15.76 c
700	135.63 e	30.94 c	89.00 a	75.09a	39.88	2.28 d	15.41 c

When the mean values and significance groups of grain yield and quality traits were examined at six different seeding density, the difference between seed densities, except for Zeleny sedimentation, was statistically significant. Grain yield varied between 135.63 and 194.63 kg/da. The highest grain yield was observed at 300 seeds per square meter, and the use of 200 seeds per square meter was in the same statistical group. The lowest grain yield, 135.63 kg/da, was observed at the highest seed rate of 700 seeds per square meter. Grain yield decreased significantly at 500 seeds per square meter or more. The highest gluten rate was 34.0% at 500 seeds per square meter, followed by the use of 400 seeds in the same statistical group. The lowest gluten rate, 30.88%, was in the lowest seed use. Gluten index varied between 81.81-90.13%, the highest index value of 90.13% was obtained by seeding rate 200 seeds per square meter.

Differences between Zeleny sedimentation values were statistically insignificant, with Zeleny sedimentation values ranging from 39.88 to 43.50 ml. The lowest sunn pest damage was

obtained with the highest seed rate of 700 seeds per square meter, with a yield of 2.28%. The highest sunn pest damage was observed with the application of 300 seeds per square meter, with a yield of 2.93%. Protein rate varied between 16.98% and 14.41% at different seeding densities.

Table 4. Average grain yield and importance groups in population (P) x seeding density (Sinteractions)

P x S Int.	Grain yield (kg/da)	P x S Int.	Grain yield (kg/da)	P x S Int.	Grain yield (kg/da)	P x S Int.	Grain yield (kg/da)
3 x 2	214.00 a	4 x 3	184.00 cde	3 x 4	168.00 efg	3 x 6	140.250 ijk
3 x 3	209.75 ab	1 x 2	181.75 c.f	1 x 3	165.25 efg	1 x 6	139.25 ijk
3x 1	208.50 ab	2 x 1	180.25 c.f	2 x 3	162.00 fgh	4 x 6	139.00 ijk
4 x 2	198.25 abc	3 x 5	178.25 def	2 x 4	153.00 ghi	1 x 5	134.75 ijk
4 x 1	190.25 bcd	4 x 4	175.75 def	4 x 5	152.00 ghi	2 x 5	128.00 jk
2 x 2	184.50 cde	1 x 1	170.75 d.g	1 x 4	145.25 hij	2 x 6	124.00 k

The highest protein rate, 16.98%, was obtained with the application of 500 seeds per square meter, followed by the application of 400 seeds per square meter, with a yield of 16.48%. Grain yield values at different seeding density in different populations ranged between 124.00 and 214.00 kg/da. The highest grain yield was found with 300 seeds per square meter in P-3 population, followed by the values obtained with 400 seeds per square meter in P-3 population when using 200 seeds per square meter in P-3 population, 208.50 kg/da and 300 seeds per square meter in P-4 population, which are statistically in the same group. The lowest grain yield was obtained with 124.00 kg/da in P-2 population, 700 seeds per square meter in P-2 population, followed by the values obtained with 128 kg/da when using 600 seeds per square meter in P-2 population, and 134.75 kg/da when using 600 seeds per square meter in P-1 population.

CONCLUSIONS

Based on the evaluation of four Kırık populations at six different seeding density, it was determined that populations P-3 and P-4 were superior in terms of grain yield, with the highest grain yield occurring at 200-300 kg/da. When population x density interaction was considered, it was determined that higher yields were observed with lower seed use per square meter (200, 300 and 1000 seeds), while using 500 or more seeds per unit area reduced yields. It was determined that populations P-1 and P-2 were superior in terms of quality traits, and that quality traits varied with density.

Author Contribution Statement

All authors contributed equally to the preparation of this manuscript.

Conflicting Interest Statement

All authors declare that they have no conflicts of interest regarding this manuscript.

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PRODUCTION CHARACTERISTICS AND NICOTINE CONTENT IN RECOGNIZED ORIENTAL TOBACCO VARIETIES OF THE PRILEP TYPE

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ABSTRACT

In 2023, eight recognized Prilep-type tobacco varieties were planted at the Scientific Tobacco Institute - Prilep: P 10-3/2 (control), NS 72, Prilep 79-94, Prilep 26, Prilep 123/7, P 66-9, P 76/86 and P 90. The experiment was set up in three replications using the Randomized Block System method. The evaluation of the raw tobacco was conducted according to the current Rulebook for the procurement of raw leaf tobacco of oriental aromatic tobacco types (Official Gazette of the Republic of North Macedonia No. 250/2021).

The aim of this study was to examine the production characteristics: g/plant, kg/ha and gross monetary income (MKD/ha), which were also statistically analyzed using the analysis of variance method (LSD test).

According to the obtained data, the yield in kg/ha ranged from 3,145 kg/ha for the P 66-9 variety to 1,550 kg/ha for the control variety P 10-3/2. All varieties were grown under the same soil and climatic conditions. Their gross monetary income ranged from 919 070 MKD/ha for the P 66-9 variety to 227 442 MKD/ha for the control variety. Due to its higher quality, the P 90 variety showed a higher average price per class during evaluation, amounting to 301.6 MKD/kg. The nicotine content in the tested varieties during this harvest was lower, ranging from 0.86% in P 66-9 to 0.33% in the control variety P 10-3/2. This study provides an overview of the genetic potential of the recognized Prilep-type tobacco varieties.

Keywords: yield kg/ha, tobacco, variety, gross income, nicotine.

INTRODUCTION

Tobacco, as an industrial crop, contributes a high percentage to the formation of the national income in the country. From a social perspective, it is significant because it engages a large labor force in the processes of production and processing of raw tobacco, as well as in the refinement of the final product. In our country, around 30,000 households depend on tobacco, and approximately 4,000 workers are employed in tobacco processing factories or tobacco companies.

The raw material from aromatic oriental-type tobacco is primarily intended for large global markets, i.e., for export. The soil and climatic conditions in the country are quite favorable for the production of high-quality aromatic oriental-type tobacco, which is included in the blends of the most premium cigarette brands. According to Karajankov et al. (2007), the cultivated tobacco species *Nicotiana tabacum* L. in our country is divided into three types: fine-leaf oriental (aromatic), additional semi-oriental, and large-leaf (Virginia and Burley).

In recent years, on the cultivated lands of our country, the aromatic oriental tobacco of the Prilep type accounts for about 98%, with only 2% of the Yaka type. Other types such as Djebel, Basmak, semi-oriental, and large-leaf tobacco have almost ceased to be produced in recent years. The Scientific Tobacco Institute regularly maintains in the Gene Bank varieties from different tobacco types that are listed in the National Variety List. Throughout its hundred-year existence, the Scientific Tobacco Institute – Prilep, through selection and breeding, has developed numerous varieties characterized by their own qualitative and quantitative traits. The recognized varieties have taken their place in different regions, and across various harvests, they have been accepted both by producers and tobacco companies.

The aim of this paper is to analyze in detail certain production characteristics of the preserved Prilep-type varieties, presenting data on: grams per stalk, kilograms per hectare, gross monetary income, and nicotine content in the tobacco leaves from the 2023 harvest. The gross monetary income is presented according to the current Regulation (Official Gazette of the Republic of North Macedonia No. 250/2021). The high-quality raw material from these tobaccos, which is almost entirely exported, serves as an incentive to introduce other recognized varieties of this type into production-varieties that are of interest to both producers and processors, i.e., manufacturers, traders, and others.

MATERIAL AND METHODS

In the experimental field of the Scientific Tobacco Institute – Prilep, during 2023, trials were conducted on eight recognized varieties of the Prilep type: P 10-3/2 (used as control), NS 72, Prilep 79-94, Prilep 26, Prilep 123/7, P 66-9, P 76/86, and P 90. The control variety used was P 10-3/2, and the seed material was sourced from the Gene Bank collection of the Scientific Tobacco Institute – Prilep. The conditions for seedling production and all applied agrotechnical measures were the same for all tested varieties.

The soil where the trial was conducted was of colluvial–alluvial type. Autumn plowing was performed at a depth of about 40 cm, and before spring plowing, the soil was fertilized with 250 kg/ha of NPK in a ratio of 8:22:20. The trial was set up in three replications using a Randomized Block System with a planting density of 45×15 cm. During the vegetation period, several supplementary irrigations were carried out as needed.

The harvested tobacco was strung, yellowed, and then dried in sun-curing barns. At the end of the growing season, a hailstorm occurred. The quality assessment of the dried tobacco was conducted according to the Rulebook on the Procurement of Raw Tobacco in Leaf from Oriental Aromatic Types of Tobacco (Official Gazette of the Republic of North Macedonia No. 250/2021).

The adjusted yield per stalk and per hectare was calculated using the Rimker method. The gross monetary income per hectare (MKD/ha) was obtained by multiplying the yield per hectare by the average price per kilogram of raw tobacco.

This paper also presents the nicotine percentage in the raw material of the tested varieties, which was analyzed at the Laboratory for Quality Control of Tobacco and Tobacco Products – L03, according to MKC ISO 2881:2010 Tobacco and tobacco products – Determination of alkaloid content – Spectrometric method.

The data on kg/ha, g/stalk, and gross monetary income (MKD/ha) were statistically processed using Analysis of Variance (ANOVA), following the methodology of Najcheska (2002) and Filiposki (2011).

RESULTS AND DISCUSSION

Yield of dry tobacco per stalk (g/stalk)

The yield of dry tobacco per stalk (g/stalk) is presented in Table 1. The average yield from the three replications ranged from 10.51 g/stalk in the control variety to 21.12 g/stalk in

the variety P 66-9, which achieved an almost 100.95% higher relative difference compared to the control.

A higher yield of 19.54 g/stalk was also recorded in the variety Prilep 79-94, and a similar yield of 19.16 g/stalk was observed in the variety P 90. Variety P 90 also demonstrated good leaf quality during tobacco leaf evaluation, with a relative difference 82.30% higher than the control.

All tested varieties showed a highly significant difference at the 1% level when compared to the control.

According to Mitreski (2012), the lowest average yield per stalk was found in the variety P 12-2/1, with 14.35 g/stalk. Other varieties with lower yield than the control include Prilep 79-94 with 16.94 g/stalk, and NS 72 with 17.00 g/stalk. On the other hand, the variety P 66-9/7 had a higher yield, reaching 21.88 g/stalk.

Gornik (1973), studying the yields of oriental aromatic tobaccos in those years, stated that the average yield for the Prilep type ranged between 9 and 12 grams per stalk.

Table 1. Adjusted yield per stalk of varieties from the Gene bank (g/stalk)

Variety	Average	Difference		
		Absolute	Relative	Rank
P-10-3/2	10.51	/	100.00	8
NS-72	18.74**	+8.23	178.31	5
Prilep 79-94	19.54**	+9.03	185.92	2
Prilep 26	19.10**	+8.59	181.73	4
Prilep 123/7	18.28**	+7.77	173.93	7
P 66 9	21.12**	+10.61**	200.95	1
P 76/85	18.50**	+7.99**	176.02	6
P 90	19.16**	+8.65**	182.30	3

LSD 0.05% = 4.02 g/stalk *
0.01% = 5.53 g/stalk **

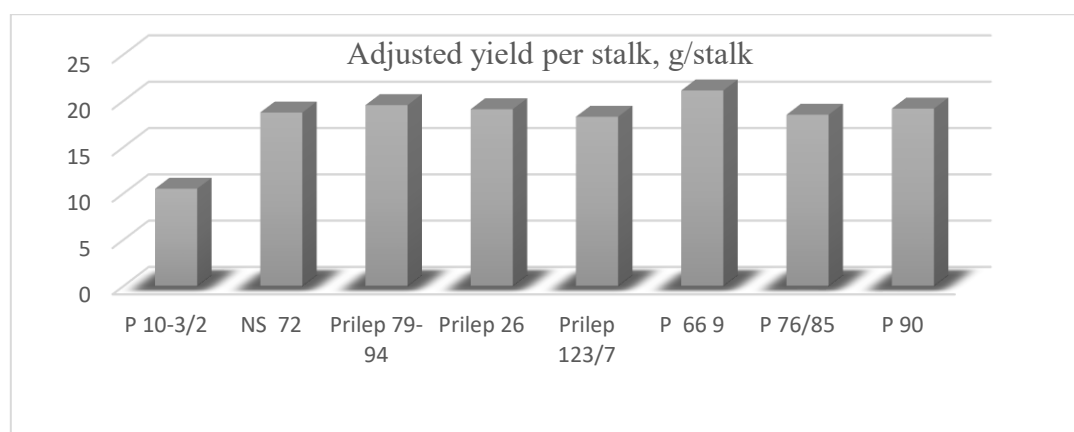


Chart 1.

Adjusted yield per hectare (kg/ha)

According to Table 2, similar to the yield per stalk, the highest yield per hectare was recorded in the variety P 66-9 with 3145 kg/ha, while the lowest was in the control variety P 10-3/2, with 1515 kg/ha.

All other tested lines also showed higher yields per hectare compared to the control. Notably, the variety Prilep 79-94 yielded 2984 kg/ha, and P 90 yielded 2853 kg/ha, both with a relative difference of 82.30% compared to the control.

The fertile variety O. Zlatovrv had a yield of 3094 kg/ha, which is 46.08% higher than the control.

All tested varieties showed a highly significant difference at the 1% level compared to the control.

According to Miroslav D. (2011), depending on environmental conditions, cultivation methods, and applied agrotechnical measures, the dry tobacco yield of the variety P 66-9/7 varies widely, usually ranging from 2000 kg/ha to 3600 kg/ha. Mitreski M. (2012) also reported the highest yield per hectare in his trials for variety P 66-9/7, reaching 3297.45 kg/ha.

Table 2. Adjusted yield per hectare of varieties from the Gene bank, kg/ha

Variety	Average	Difference		
		Absolute	Relative	Rank
P 10-3/2	1515	/	100.00	8
NS-72	2790**	+1275	182.93	5
Prilep 79-94	2984**	+1469	200.92	2
Prilep 26	2844**	+1329	189.14	4
Prilep 123/7	2721**	+1206	172.66	7
P 66 9	3145**	+1670	213.08	1
P-76/85	2754**	+1239	180.51	6
P 90	2853**	+1338	188.31	3

LSD 0.05%= 550.56 kg/ha *

0.01% = 756.70 kg/ha **

Gross monetary income, MKD/ha

Gross monetary income per unit area actually represents a synthesis of the results for the yield and the quality of the tobacco, expressed through the percentage of higher classes and the average price in MKD/ha. From the results presented in Table 3 and Chart 2, it can be seen that

the gross monetary income for the varieties included in the trial is different and ranges from 227,442 MKD/ha in the control variety P 10-3/2 to 919,070 MKD/ha in the variety P 66-9, where the relative difference is 304.09% higher compared to the control.

It should be noted that a key factor in the formation of the gross monetary income in the variety P 66-9 was the high yield per hectare. The variety P 90 (301.6 MKD/kg) was characterized by almost the same average price.

As a result of the higher average price and the good quality of raw tobacco, varieties P 90 and Prilep 123/7 achieved higher gross monetary income compared to the control variety P 10-3/2. The relative difference in the variety P 90 is 278.27% higher than the control, while in Prilep 123/7 it is 261.13%.

It is characteristic that, compared to the control variety P 10-3/2, all varieties in the trial achieved a highly significant difference at the 1% level.

Bogdancheski et al. (1997) state that the standard variety JK 7-4/2 in the Strumica production region had an economic effect ranging from 64,619 MKD/ha to 106,484 MKD/ha in Yaka 323.

In the 2023 harvest, according to the valid Rulebook on the procurement of raw tobacco in leaf from oriental aromatic types of tobacco (Official Gazette of the Republic of North Macedonia No. 250/2021), the purchase prices were as follows: Class I: 300.00 MKD/kg, Class II: 240.00 MKD/kg, Class III: 180.00 MKD/kg, Additional tobacco II/I: 100.00 MKD/kg, Moldy tobacco: 14.00 MKD/kg.

Table 3. Gross Monetary Income (MKD/ha), obtained according to the valid Rulebook for the Evaluation of Raw Oriental Tobacco in Leaf

Variety	Average MKD/ha	Difference		
		Absolute	Relative	Rank
P-10-3/2	227 442		100.00	8
NS 72	787 801**	560 360	346.37	6
Prilep 79-94	793 034**	565 592	348.68	4
Prilep 26	746 588**	519 146	328.25	7
Prilep 123/7	821 361**	593 919	361.13	3
P 66 9	919 070**	691 628	404.09	1
P 76/86	761 847**	534 405	334.96	5
P 90	860 465**	632 891	378.27	2

LSD 0.05%= 296 109 MKD/ ha

0.01% = 409 974 MKD/ha

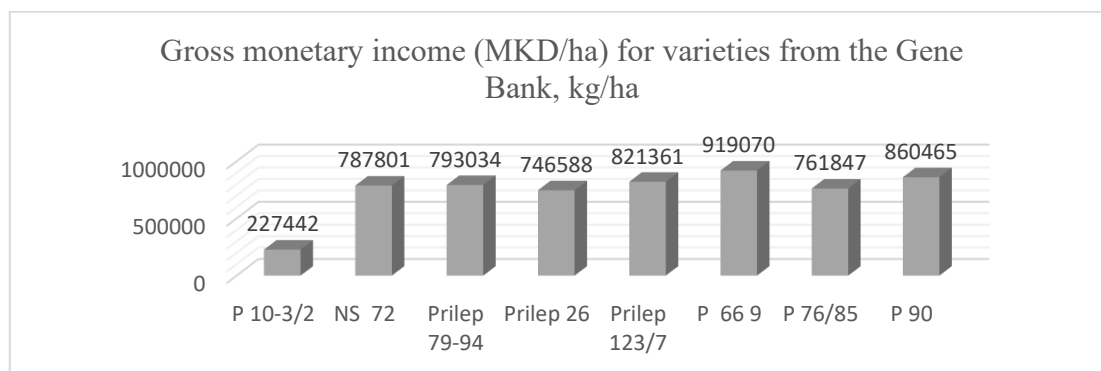


Chart 2.

Nicotine content (%) in varieties from the Gene Bank, 2023 harvest

Chart 3 presents the nicotine content for the 2023 harvest, a year characterized by heavy rainfall and hail. Since nicotine is synthesized in the root system of the tobacco plant (Uzunoski, 1985), and is one of the main alkaloids responsible for tobacco leaf consumption, all tested varieties showed a lower percentage of nicotine in the tobacco leaves.

The lowest nicotine percentage was recorded in the control variety P 10-3/2 (0.53%), while the highest was found in P 66-9 (0.86%) and Prilep 123/7 (0.82%).

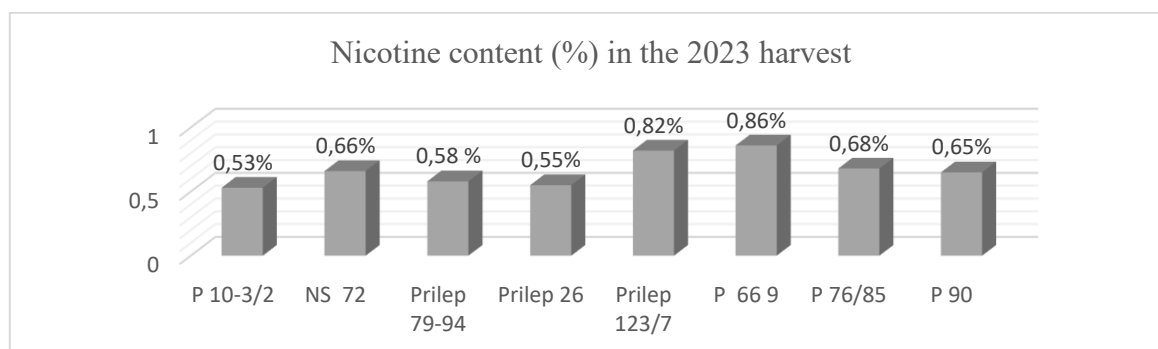


Chart 3.

According to Mitreski (2005), in his research on nicotine content in the variety P 10-3/2, a value of 0.92% was obtained, while in the variety P 23, nicotine reached 1.79%. Mitreski et al. (2005) emphasized that the main chemical components in the Prilep type range as follows: nicotine from 0.8% to 2.3%. Dimitrieski et al. (2011) stated that the variety Prilep P 66-9/7, cultivated in the Prilep region, contains: nicotine 0.96%, proteins 7.18%, and soluble sugars 25.24%. Kochoska (1999) reported a decrease in nicotine percentage from lower to upper insertions; in the variety P 72, nicotine content was 1.77% in the middle stalk positions, and 1.35% in the upper leaves.

CONCLUSIONS

- The average yield per stalk in the trial ranged from 10.51 g/stalk in the control variety to 21.12 g/stalk in the variety P 66-9, which achieved nearly 100.95% higher relative difference.
- The lowest yield per hectare was recorded in the control variety P 10-3/2 (1515 kg/ha), while the highest yield was in the variety P 66-9 (3145 kg/ha).
- Gross monetary income among the varieties included in the trial varied, ranging from 227,442 MKD/ha in the control P 10-3/2 to 919,070 MKD/ha in the variety P 66-9, showing a 304.09% higher relative difference compared to the control.
- The lowest nicotine percentage was found in the control variety P 10-3/2 (0.53%), while the highest nicotine percentages were observed in the tobacco leaves of variety P 66-9 (0.86%) and Prilep 123/7 (0.82%).

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GRAIN YIELD POTENTIALS OF NEWLY IMPROVED OAT VARIETIES IN DIFFERENT SOWING DENSITIES

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ABSTRACT

This study was conducted to investigate the effects of different sowing densities (200, 300, 400, 500, 600, and 700 seeds m⁻²) on grain yield of four oat genotypes, including three commercial varieties (Kahraman, Kırklar and Küçükyayla) and one candidate genotype (YVBD-5) developed by the Trakya Agricultural Research Institute, under the ecological conditions of Edirne. The field experiment was established during the 2019-2020 growing season in a randomized split-plot design with three replications, where the main plots consisted of oat genotypes (Kahraman, Kırklar, Küçükyayla, and YVBD-5) and the subplots consisted of six sowing densities (200, 300, 400, 500, 600, and 700 seeds m⁻²). Analysis of variance revealed that genotype, sowing density, and their interaction had significant effects on grain yield. Results indicated that although not in a strictly linear manner, increasing the sowing density up to 600 seeds m⁻² contributed to grain yield improvement in the tested oat genotypes. Among the genotypes, Küçükyayla produced the highest grain yield (847.78 kg da⁻¹), whereas Kırklar had the lowest (710.72 kg da⁻¹). Furthermore, the effect of sowing density on grain yield was found to vary depending on the genotype. The findings suggest that tillering potential should be considered as a key factor when determining optimal sowing density for oat varieties. Accordingly, for the production of these genotypes in Thrace and other oat-growing regions, sowing densities of 500 seeds m⁻² for Kahraman, 700 seeds m⁻² for Kırklar, 400 seeds m⁻² for Küçükyayla and 600 seeds m⁻² for YVBD-5 would be advisable.

Keywords: Oat, Sowing Density, Grain yield, Variety.

INTRODUCTION

Oat (*Avena sativa* L.), although traditionally used primarily as animal feed, has gained increasing importance in human nutrition in recent years owing to its high nutritional value. Rich in protein, soluble fiber (β -glucans), unsaturated fatty acids, vitamins, minerals, and antioxidants (Mut et al., 2021), oat grains are increasingly utilized in various food products such as bread, biscuits, probiotic drinks, infant foods, and oat flakes (Karaman et al., 2020).

Oat cultivation in Türkiye covers 138,000 hectares, with a total production of 410,000 tons, ranking third among cool-season cereals after wheat and barley (FAO, 2023). In recent years, oat acreage in the country has shown a noticeable increase, particularly due to the development of high-yielding varieties. The Thrace Region accounts for 11.2% of Türkiye's total oat cultivation area and 13.9% of its production. The limited cultivation of oat in Türkiye is primarily attributed to the lack of reliable winter varieties available to farmers and its inability to compete economically with other field crops. Oat is sown as a winter crop mainly in the country's coastal regions, whereas in the major cereal-producing areas, summer oat cultivation results in low yields due to insufficient rainfall (Yürür, 1994).

As with other cereals, oat grain yield is determined by the combined effects of the plant's genetic potential, environmental factors, and cultivation practices. Among the cultivation techniques influencing oat grain yield, sowing density is particularly important. Sowing density is a key crop management factor that affects grain yield by regulating growth, photosynthesis, and yield components, which are critical traits closely related to grain yield performance (Li et al., 2018). It is influenced by genotype, sowing time, seed size, and ecological conditions (Karakuzu and Erbaş Köse, 2025). Previous studies have reported that grain yield in oat is significantly affected by traits such as panicle number per unit area, panicle length, number of grains per panicle, and grain yield per panicle (Mut et al., 2018). Consequently, variations in these traits due to changes in sowing density can have a substantial impact on overall grain yield (Neumann et al., 2007; Altuner and Ülker, 2019; Romitt et al., 2017; Ju et al., 2022; Karakuzu and Erbaş Köse, 2025). Therefore, determining the optimal sowing density under the ecological conditions of the target cultivation area is essential to achieve the desired grain yield in newly developed oat varieties.

The aim of this study was to investigate the effects of different sowing densities (200, 300, 400, 500, 600 and 700 seeds m^{-2}) on grain yield in three commercial oat varieties (Kahraman, Kırklar and Küçükyayla) and one candidate genotype (YVBD-5), and to determine the optimal sowing density for these genotypes.

MATERIALS AND METHODS

Plant materials and field experiments

This study was carried out during the 2019-2020 growing season at the experimental fields of the Thrace Agricultural Research Institute, under the ecological conditions of Edirne. Three commercial oat varieties (Kahraman, Kırklar and Küçükyayla) and one candidate oat genotype (YBVD-5) developed by the Thrace Agricultural Research Institute were used as material in the study. Kahraman, Kırklar and Küçükyayla varieties were registered in 2014, 2014, and 2018, respectively. In the research, six different sowing densities (200, 300, 400, 500, 600 and 700 seeds m⁻²) was applied to these genotypes.

The experiment was arranged as a randomized split-plot design with three replications, with oat genotypes assigned to the main plots and sowing densities to the subplots. Sowing was carried out on October 19, 2019, using a plot-sowing machine. Each plot was 5 meters long, consisting of six rows with a row spacing of 17 cm. Based on soil analysis results, 20 kg da⁻¹ of 20-20-0 compound fertilizer was applied at sowing, 17 kg da⁻¹ of urea (46% N) during the tillering stage, and 20 kg da⁻¹ of ammonium nitrate (26% N) at the stem elongation stage. Weed control was carried out chemically during the tillering stage. All plots were harvested using a plot combine on July 7, 2020.

Climate and soil characteristics of the experimental area

Some climatic data of the experimental location during the oat growth period was recorded as shown in Table 1.

Table 1. Some climatic data of the experimental area in the 2019-2020 growing season.

Months	Temperature (°C)				Precipitation (mm)	
	The lowest	The highest	Mean	Long-term	2019-2020	Long-term
October	10.4	24.1	16.5	14.2	24.2	56.7
November	10.1	18.7	14.0	9.3	40.6	68.8
December	3.7	10.7	6.9	4.5	16.4	75.2
January	-0.9	9.3	3.5	2.0	9.6	62.9
February	2.3	13.7	7.4	5.2	31.8	50.8
March	5.0	17.0	10.5	7.1	38.6	46.2
April	5.6	19.8	12.3	12.7	98.2	49.9
May	12.5	25.6	18.4	17.9	87.2	49.2
June	16.4	30.1	22.7	22.0	46.6	48.9
Average/Total	7.2	18.7	12.4	10.5	393.2	508.6

Source: Meteorological Station Data, Edirne-Türkiye.

When the climatic data of the experimental site for the 2019–2020 oat growing season were examined, it was observed that the monthly temperature values were higher than the long-

term average (Table 1). In addition, the total precipitation during the experimental period was found to be 115.4 mm lower than the long-term average (Table 1).

Based on the soil analysis results, the soil of the experimental area was classified as clay-loam, slightly alkaline, non-saline, and low in organic matter.

Statistical analysis

Analysis of variance (ANOVA) for all examined traits was performed according to the split-plot design using Tarpoggen-Tarist statistical software. Mean comparisons were conducted using the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Grain yield (kg da⁻¹)

In this study, conducted with three oat varieties and one advanced oat line at six different sowing densities, the results of ANOVA for grain yield are presented in Table 2. Besides, mean values and significance group of genotypes, sowing densities and their interaction were given in Table 3.

Table 2. The results of variance for grain yield.

Source of variation	DF	F values
Replication	2	0.4600
Genotype (G)	3	14.4165**
Error-1	6	
Sowing Density (SD)	5	100.4557**
G×SD	15	9.6021**
Error	40	
Total	71	

DF: degree of freedom, **Significant at P<0.01

As shown in the Table 2, the effects of genotype, sowing density, and the genotype × sowing density interaction were found to be significant at the 1% probability level.

Table 3. Mean values and significance groups of genotypes, sowing densities and their interaction for grain yield (kg da⁻¹).

Genotypes	Sowing density (seeds m ⁻²)						Mean
	200	300	400	500	600	700	
Kahraman	649.00 l	725.67 hi	792.33 def	874.67 abc	812.00 d	811.33 d	777.50 b
Kırklar	651.00 kl	627.00 l	734.00 h	716.33 hi	745.33 gh	790.67 def	710.72 c
Küçükyayla	775.67 ef	799.00 de	903.00 a	854.67 c	890.67 ab	863.67 bc	847.78 a
YVBD-5	680.67 jk	701.00 ij	725.67 hi	740.67 gh	802.00 de	766.67 fg	736.11 bc
Mean	689.09 e	713.17 d	788.75 c	796.59 bc	812.50 a	808.09 ab	
LSD (0.01<P)	Genotype (G): 54.56		Sowing density (SD): 15.13		G×SD: 30.25		

The grain yields of the oat genotypes varied from 710.72 kg da⁻¹ to 847.78 kg da⁻¹. The highest grain yield was recorded in the Küçükyayla variety, followed by the Kahraman variety (777.50 kg da⁻¹) and the advanced line YVBD-5 (736.11 kg da⁻¹). The lowest grain yield was observed in the Kırklar variety (710.72 kg da⁻¹) (Table 3). Similar to our findings, Finnan et al. (2018), who evaluated three oat cultivars (Barra, Husky, and Vodka) under five different sowing densities (125, 250, 375, 500, and 625 seeds m⁻²), reported significant differences in grain yield among the cultivars. Similarly, Sarı et al. (2016) indicated that the differences in grain yield among oat cultivars were statistically significant. These differences may have resulted from genotypic variations in grain yield-related traits such as panicle length, number of grains per panicle, grain weight per panicle, and thousand-kernel weight (Naneli and Sakin, 2017; Kahraman et al., 2021).

In this study, grain yield values obtained from different sowing densities ranged between 698.09 kg da⁻¹ and 812.50 kg da⁻¹. The highest grain yield was achieved at a sowing density of 600 seeds m⁻², followed by 700 seeds m⁻² with 808.09 kg da⁻¹. The lowest grain yield was determined at 200 seeds m⁻², followed by 300 seeds m⁻² with 713.17 kg da⁻¹ (Table 3). Our findings demonstrated that grain yield in oat increased linearly up to a sowing density of 600 seeds m⁻², after which it began to decline. Consistent with our results, Karakuzu and Erbaş Köse (2025) reported that grain yield in oat increased with sowing densities up to 300–450 seeds m⁻², but decreased thereafter. Conversely, Finnan et al. (2018) reported that increasing the sowing density from 125 to 250 seeds m⁻² enhanced grain yield, while further increases did not significantly affect yield. Similarly, Ju et al. (2022) found that grain yield increased as sowing density rose from 60 to 180 kg ha⁻¹, but declined at 300 kg ha⁻¹. The reduction in grain yield at higher sowing densities may be attributed to stronger interplant competition and shading, which negatively affect leaf photosynthesis, as previously reported (Ma et al., 2014).

Our findings demonstrated that the interaction between genotype (G) and sowing density (SD) a statistically significant effect on grain yield. The results of the G × SD interaction showed that grain yield ranged from 627.00 to 903.00 kg da⁻¹ (Table 3). The highest grain yield was recorded in the Küçükyayla cultivar at a sowing density of 400 seeds m⁻² (903.00 kg da⁻¹), whereas the lowest grain yield was observed in the Kırklar cultivar at 300 seeds m⁻² and in the Kahraman cultivar at 200 seeds m⁻² (649.00 kg da⁻¹), both belonging to the same statistical group. Our results indicate that grain yields were affected differently by the applied sowing densities of the oat cultivars included in the study. This may be due to the different responses of oat genotypes to sowing densities (Finnan et al., 2018).

CONCLUSION

Sowing density had a highly significant effect on grain yield of oat ($P < 0.01$), and this effect varied depending on genotype. In the present study, increasing sowing density up to 600 seeds m^{-2} generally increased grain yield, whereas further increases beyond this level negatively affected yield. In conclusion, the optimal sowing densities for achieving higher grain yield were determined as 500 seeds m^{-2} for the Kahraman variety, 700 seeds m^{-2} for the Kırklar variety, 400 seeds m^{-2} for the Küçükyayla variety, and 600 seeds m^{-2} for the advanced line YVBD-5.

Author Contribution Statement

All authors contributed equally to the preparation of this manuscript.

Conflicting Interest Statement

All authors declare that they have no conflicts of interest regarding this manuscript.

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FOOD QUALITY AND SAFETY

PRODUCTION OF BIOCALCIUM POWDER FROM FISH BONES AND ITS USE AS A FOOD ADDITIVE

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ABSTRACT

Fish bones are an important waste, especially after the filleting process, which makes up 30% of the total weight. Fish bones are an important mineral source, and 60% of the mineral mass consists of calcium. Calcium has a very important place in human nutrition. Because calcium is the main component of teeth and bones, as well as having physiological functions in the body such as muscle contraction, wound healing, facilitating blood flow, regulating heart rate and transmitting nerve impulses. Since calcium deficiency in the body prevents these functions from being performed, it causes health problems. To prevent this deficiency, calcium is added to food. For this reason, in recent years, researchers have directed their studies on calcium obtained from fish bones, called “biocalcium”. In this presentation, information will be given about biocalcium production stages and its use in the food field. In addition, information will be given about the preliminary results of our ongoing project. In this project, biocalcium production is being studied with different methods. his research was supported by The Scientific Research Projects Administration Unit of Akdeniz University with project number FDK-2024-6699.

Keywords: Fish waste, biocalcium, production methods, food

INTRODUCTION

The processing of seafood produces a significant amount of waste and byproducts. Approximately 45-50% of fish waste consists of internal organs, heads, cut pieces, bones, and skin. This waste not only causes significant environmental problems but also leads to significant losses. The utilization of these wastes, which contain numerous bioactive compounds, can both solve environmental problems and provide economic benefits by being converted into value-added products. New value-added products created through the utilization and conversion of

waste are used in many areas, including food, chemicals, pharmaceuticals, medicine, and cosmetics.

Global fisheries and aquaculture production is reported to be approximately 185 million tons, 88% of which is for human consumption (FAO, 2024). Advances in fish farming and processing technologies have brought about the problem of waste and byproducts. Fish bones, particularly those obtained after filleting, constitute approximately 30% of the total weight of the fish (Lu, 2004). These wastes create significant problems. If waste is left to stand and accumulate, it will degrade very quickly. This causes serious health and environmental problems. Therefore, waste must either be destroyed and rendered harmless or made useful. Traditional waste management includes direct disposal of landfills and the use as animal feed and fertilizer. Today, these have been replaced by the concepts of recycling and recovery. It is expected that the recovery of bioactive compounds will increase profitability. The fish processing industry is faced with the need to develop effective methods for the recovery and utilization of waste. Wastes contain many valuable compounds, including proteins, fats, fatty acids, phospholipids, and fat-soluble vitamins (Rustad et al., 2011; Kim and Mendes, 2006). In recent years, intensive research has been conducted on new methods and technologies to obtain bioactive compounds from various organic raw materials for food and biomedical purposes. There are limited studies on the use of fish bones as an alternative calcium source (Phiraphinyo et al., 2006; Toppe et al., 2006; Tongchan et al., 2009; Boutinguiza et al., 2012). The dry matter of fish bone tissue has a mineral content of 60–70%, primarily calcium phosphate and hydroxyapatite (Gildberg et al., 2002, Barakat et al., 2008), and the calcium content in the mineral mass is at the level of 60% (Kim and Jung, 2007). It is reported that fish bones contain approximately 30–45% protein, 2–32% fat and 15–6% ash, and more than 10% of the fat-free dry matter consists of calcium (Munekata et al., 2020; Toppe et al., 2007).

Calcium is the most abundant mineral of the human body, constituting 1–2% of body weight. An average adult contains 1150–1300 g of calcium. In the human body, 99% of calcium is found in bones and teeth, 1% in extracellular fluids, soft tissues and the circulatory system. Calcium also plays a role in physiological functions such as muscle contraction, wound healing, facilitating blood flow, regulating heart rate, and conducting nerve impulses. Calcium insufficiency in the body can cause a decrease in bone density and a disease called osteoporosis (Trailokya et al., 2017). Calcium deficiency can cause rickets in children and bone fractures in the elderly. These are also associated with vitamin D deficiency. Calcium absorption is affected by factors such as estrogen concentration, age, and vitamin D levels. A decrease in calcium

absorption has been reported with aging (Trailokya et al., 2017). The main sources of calcium are milk and dairy products. However, the high prevalence of lactose intolerance, the emergence of new dietary patterns, concerns about the potential negative effects of dairy on human health, and the decrease in milk consumption in low- and middle-income countries have led to the widespread prevalence of calcium deficiency worldwide (Perez et al., 2023).

Calcium supplementation has been an alternative solution to the problem of calcium deficiency. However, the absorption of pure calcium is low due to calcium precipitation in the form of phosphate salts. Finding new calcium sources to meet the calcium needs of the human body is an important requirement. Chemically, calcium in bones is organized into hydroxyapatite crystals, which form phosphorus. These crystals have very low solubility and must be processed before being added to food. Hydroxyapatite solubilization can be achieved.

With the increasing demand for health-focused products, food products are fortified with a range of health-promoting ingredients. New products with market value have been developed from the often-wasted byproducts of the food processing industry (Wijayanti et al., 2021a). For consumers who have insufficient milk and dairy product consumption due to lactose intolerance, calcium-enriched foods are very important to meet their calcium needs (Kim and Mendis, 2006; Luu and Nguyen, 2009). Various studies have reported that foods such as milk and dairy products, salt, wheat flour, sugar, oil and corn flour are fortified with calcium and calcium salts such as calcium carbonate, tricalcium phosphate and calcium citrate are used for fortification (Sheik et al., 1987; Goldscher and Edelstein, 1996; Tateo et al., 1997; Singh et al., 2007). From the perspective of consumer acceptability and calcium availability, fish bones are effective natural sources of calcium (Phiraphinyo et al., 2006; Hemung, 2013). Various studies have shown that biocalcium powder obtained from fish bones can be used for food fortification purposes in crackers, cookies, sausages, surimi gel, mayonnaise, etc. It has been determined that it does not cause any negative effects on sensory properties (Benjakul and Karnjanapratum, 2018; Hemung and Sriuttha, 2014; Idowu et al., 2019; Karnjanapratum and Benjakul, 2018; Wijayanti et al., 2021b).

In this study, it was aimed to produce biocalcium powder from fish bones by different methods and to determine the characteristics of the obtained biocalcium powder. In this presentation some information will be given about the preliminary results of our ongoing project.

MATERIALS AND METHODS

Sea bass bones were used in the study. The bones were cut lengthwise into small pieces, 8-10 cm long. These bone fragments were then thoroughly washed under running water to remove blood and other contaminants. They were then stored at -20°C until biocalcium powder production began.

Biocalcium Powder Production

To produce biocalcium powder, fish bones stored in deep freezers were placed in the refrigerator to thaw. The raw fish bones were analyzed. Biocalcium powder was produced using various methods.

For biocalcium production, sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂), and surfactant solutions were prepared. Ten different groups were formed consisting of combinations of chemicals used for protein removal, oil removal, and bleaching processes for biocalcium production. These groups were A1X (NaO-Ethanol-H₂O₂), A2X (NaOH-Surfactant-H₂O₂), A1Y (NaOH-Ethanol-NaOCl), A2Y (NaOH-Surfactant-NaOCl), B1X (NaOH + NaOCl-Ethanol-H₂O₂), B2X (NaOH + NaOCl-Surfactant-H₂O₂), B1Y (NaOH + NaOCl-Ethanol-NaOCl), B2Y (NaOH + NaOCl-Surfactant-NaOCl), W (Boiled in water), RB (Dried control sample). Biocalcium production was carried out separately for these groups.

Yield, Moisture, protein, fat, ash, calcium and hydroxyproline contents of biocalcium powders were examined.

The yield of Bio Calcium powder was determined by dividing the weight of bio calcium powder by the weight of raw bone. (Yield (g/100g) = Biocalcium weight / Raw bone weight X100).

The moisture content of the samples was dried in an oven at 105°C until they reached a constant weight, the amount of crude protein was determined by the Kjeldahl method, the amount of crude fat was determined by the extraction method in the Soxhlet system, and the samples weighed in crucibles brought to a constant weight for crude ash were kept in a muffle furnace at 550±5°C until they turned into white ash (AOAC, 1990).

Calcium content was determined using the Thermo Scientific iCAP PRO ICP-OES system. Hydroxyproline content was determined using a UPLC-MS/MS instrument according to the method described by Kivrak et al. (2014).

RESULTS AND DISCUSSION

Yield

Yield is calculated by comparing the weight of the biocalcium powder to the initial weight. It is reported that the higher the yield, the higher the product's economic value and effectiveness (Febriani et al., 2021). Knowing the yield is crucial for determining a product's proper utilization and economic value. In our research, the highest yield was obtained in groups B2Y (44.5%) and B1Y (43.5). Alkaline extraction resulted in lower yields. The lowest yield was observed in groups A2Y (38.9%) and A1Y (38.3), which had the lowest protein content. It shows that the combination of NaOH + NaOCl is better in protein removal. In a study where bone powder was produced from milkfish using water, alkaline, and acidic extraction methods, the highest yield was obtained with the water extraction method (Wulandari and Kusumasari, 2019).

Proximate composition

The moisture content of raw bones was found to be higher than that of biocalcium powders (Fig. 1a). Heating during production removed free water. It has been reported that moisture content plays an important role in maintaining the stability and shelf life of powder products and the moisture content required to extend the shelf life of the powder is a maximum of 10% (Ojo et al. 2017). In the present study, the moisture content of biocalcium powders was found to be quite low. The highest moisture content was obtained by extraction in water. Among the other groups, the highest moisture content was found in the A1Y group. The lowest moisture content was found in the B1X group. Previous studies have reported different moisture contents for bone powders.

While the protein content in fish bone was as high as 27.56%, it decreased significantly after biocalcium production. The boiling method in water did not sufficiently remove protein from biocalcium powder. However, a significant decrease in protein content was observed with the use of the alkaline method. The lowest protein content values were found in B1X (Fig1b). This indicates that the combination of NaOH and NaOCl is more effective in protein removal than using NaOH alone. It has been reported that the use of NaOH in the production of tuna bone powder causes a decrease in protein and fat contents (Nemati et al., 2007).

The fat content of the original bone, which was 1.09%, decreased significantly after being converted into biocalcium powder. The fat in fish bones is lost during the heating process. This loss can extend the shelf life of the powder. The lowest fat content was found in the B2X group (Fig 1c). Surfactants were used in the fat removal process in this group. This indicates that surfactants are more effective than ethanol in oil removal.

High ash contents were found in biocalcium powders obtained by chemical methods. The extraction performed with water had a low ash content (Fig 1d). Compared to raw bone, biocalcium production appears to increase the ash content.

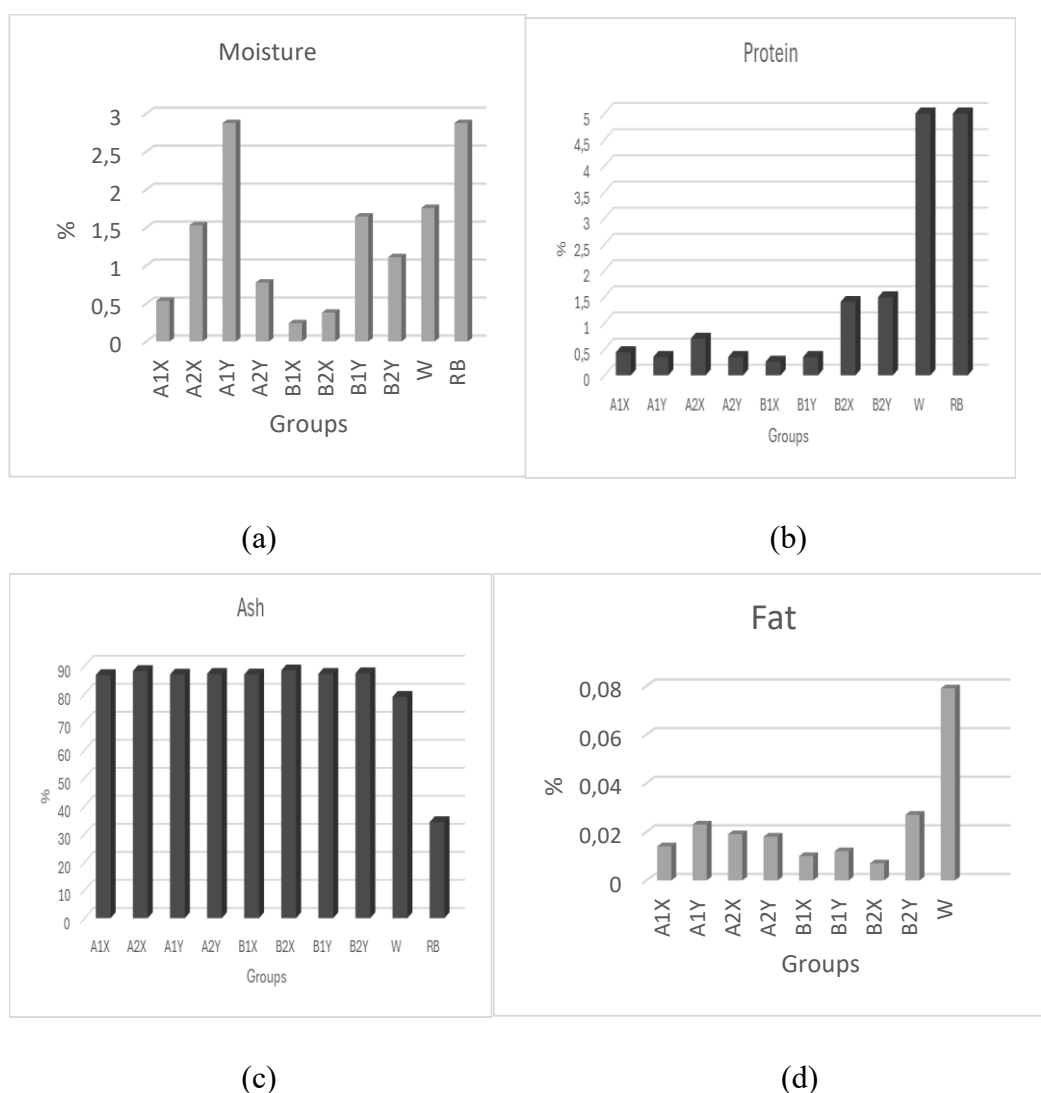


Figure 1. Moisture (a), Protein (b), fat (c) and ash (d) contents of biocalcium powders

Calcium (Ca)

In our study, Ca was found to be 35-38% (Fig. 2). Ca content increased after biocalcium powder production. By removing non-collagenous proteins, Ca became more concentrated in biocalcium powder. The highest Ca content was found in the B1Y (38.30%) group. This group also had the lowest fat and protein content among the groups. This may be due to the lower amount of organic matter, which increased the mineral content. The lowest Ca content was in the A2Y (34.74%) group.

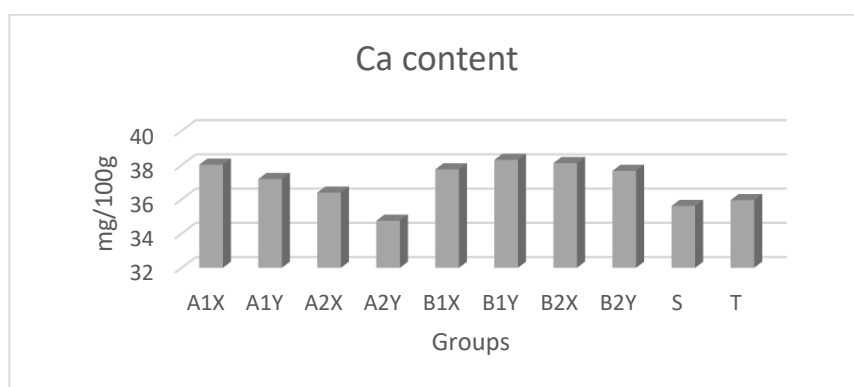


Figure 2. Calcium contents of biocalcium powders

Hydroxyproline

Hydroxyproline is a unique amino acid found in collagen and ensures its biological stability. Because it is found almost exclusively in collagen, the hydroxyproline level is used as a reliable indicator of collagen content in products. The presence of hydroxyproline indicates that collagen protein is formed in biocalcium.

This study examined the hydroxyproline level in bone-derived biocalcium powders and evaluated its relationship with collagen content. Analysis revealed 1.43–1.59% hydroxyproline in biocalcium powders, indicating a collagen content exceeding 10%. These findings suggest that this powder can be considered not only a mineral but also a functional protein (collagen) source. Raw bone had the lowest hydroxyproline content compared to other samples (Fig. 3). The lowest hydroxyproline content in the raw bone sample may be due to the presence of meat, blood, lipids, etc. Increases in hydroxyproline content in biocalcium samples obtained after protein and lipid removal pretreatments indicated that the hydroxyproline content was concentrated.

Analysis results indicate that biocalcium powder is an important food additive not only for its calcium (35–38%) but also for its high collagen content. Collagen is increasingly used in

functional foods and supplements due to its contribution to bone health, connective tissue support, and anti-aging effects. The 1.5% hydroxyproline level indicates that such products can confidently claim "contain collagen."

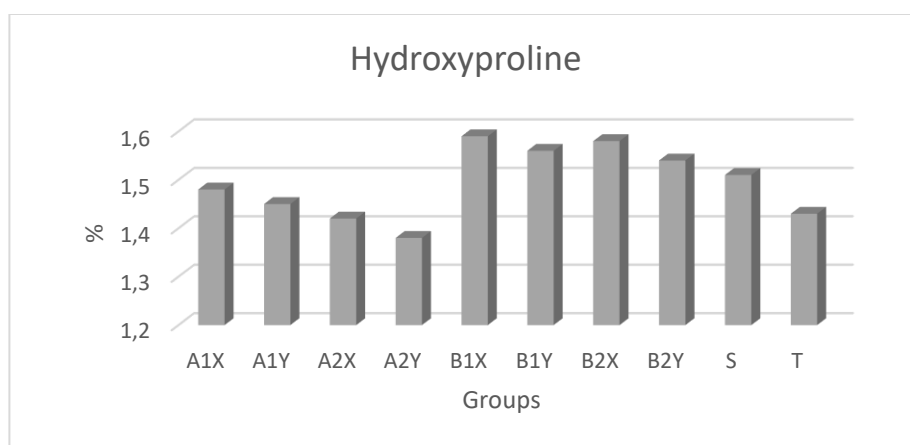


Figure 3. Hydroxyproline contents of biocalcium powders

CONCLUSIONS

In this study, biocalcium powder production methods involving different protein and fat removal processes were compared with the boiling water method. The study showed that used methods in the production of biocalcium powder from sea bass bones is effective due to their high dry matter, ash and calcium and low protein and fat contents of the obtained biocalcium powders. Among the methods, alkaline method was effective in protein removal, while the use of surfactants was effective in fat removal.

Analysis results indicate that biocalcium powder is an important food additive not only for its calcium (35–38%) but also for its high collagen content. The 1.5% hydroxyproline level indicates that such products can confidently claim “contain collagen”.

This study demonstrates that bone-derived biocalcium powder is not only a mineral source but also contains high-quality collagen. The high hydroxyproline level indicates that the product largely preserves its natural collagen structure, reinforcing its potential use in food, nutraceutical, and functional product development.

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HARNESSING PROBIOTICS FROM CABBAGE: A NATURAL DEFENCE AGAINST FOODBORNE PATHOGENS

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ABSTRACT

The use of probiotics as natural inhibitors of foodborne pathogens has gained significant attention in food safety and preservation. This study explores the inhibitory effects of probiotics isolated from spontaneously fermented cabbage against common food pathogens. Spontaneously fermented cabbage, a traditional and widely consumed product in our country, serves as a rich source of beneficial lactic acid bacteria with potential antimicrobial properties. In this research, probiotic strains were identified and characterized for their antimicrobial activity against different pathogenic microorganisms. The filtered sterile supernatant of the bacterial cultures was tested with an agar well diffusion assay; the growth medium had been previously inoculated with different pathogen strains (*Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13393, *Salmonella enterica* ATCC 10708 and *Staphylococcus aureus* ATCC 25922). Laboratory assays demonstrated that these probiotic strains produce bioactive compounds, including organic acids and bacteriocins, which effectively inhibit the growth of foodborne pathogens. Furthermore, the analysis highlighted the stability and potency of these probiotics under various stress conditions, like different concentrations of NaCl, low pH, and emphasizing their applicability in enhancing food safety. The findings suggest that cabbage-derived probiotics represent a promising, natural approach to mitigating risks associated with foodborne illnesses and reducing reliance on synthetic preservatives. This study contributes to the growing body of research on probiotics and their role in sustainable food systems, providing valuable insights into their practical applications in food safety and public health.

Key words: food safety, preservation, spontaneous fermentation, antimicrobial

INTRODUCTION

Foodborne illnesses caused from pathogens such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* continue to remain a global health challenge. The food industry has relied for long on synthetic preservatives to prolong shelf-life of products and to prevent microbial spoilage. Growing scientific evidence supports that these synthetic chemicals including nitrates, benzoates, sulfites, parabens and formaldehyde pose threats such as allergies, hyperactivity, neurological damage, and even cancer [1]. Their extensive use impacts the antimicrobial resistance that raises food safety concerns prompting the search for natural preservatives. Probiotics are living organisms that when ingested act as a supplement that has a positive benefit in human health by improving internal microbial balance [6]. The mechanism based on which these probiotics work is based on their production of useful metabolites such as lactic-acid, acetic acid, propionic, butyric and formic acid. These compounds lower the pH value in the digestive system by producing conditions that don't allow for pathogens to survive. Additionally, they produce hydrogen peroxide and bacteriocins that have an inhibitory influence on a broad spectrum of Gram - positive bacteria [8,9]. Lactic-acid bacteria (LAB) are a diverse group of Gram- positive microorganisms that are known to be aerotolerant, acid-tolerant, usually nonsporulating rod or cocci microorganisms that play an important role in the process of fermentation of food. They exhibit this role by inhibiting spoilage and promoting excellent flavor, aroma and texture of fermented foods [2,3]. LAB can be isolated from many kinds of sources such as milk products, fermented foods, animal intestines and even soil samples [4]. The most common types of LAB include different *Lactobacillus* spp. (*Lb. acidophilus*, *Lb. johnsonii*, *Lb. casei*, *Lb. rhamnosus*, *Lb. gasseri*, and *Lb. reuteri*) and genus *Bifidobacteria* (*Bf. bifidum*, *Bf. animalis subsp. lactis*, *Bf. longum subsp. longum*, and *Bf. longum subsp. infantis*). Spontaneously fermented cabbage, a traditional food in N. Macedonia is a rich source of such probiotics, especially lactic acid bacteria that are able to inhibit the growth of pathogens. This study evaluates lactic-acid bacteria from fermented cabbage for antimicrobial activity against key pathogens and their stress tolerance to stress conditions that includes high concentration of NaCl and low pH, assessing their potential as natural biopreservatives in food safety.

METHODS AND MATERIALS

1. Isolation of LAB

The cabbage used for the preparation of the traditional fermentation process was sampled from Svinishte, Kopacka region, Municipality of Kichevo at an altitude of 690 m. The cabbage was of the *Futoshka* variety and natural sheep manure was used during cultivation. The preparation of the cabbage involved removal of the outer leaves and cutting out the core with a knife so that a tablespoon of coarse non-ionized salt was added. The cabbage was not washed in order to preserve the naturally occurring microorganisms. The cabbages were tightly packed into a 10 L plastic barrel that was previously disinfected with a sodium hypochlorite solution. After 24 hours a saline solution was added (300 g of salt dissolved in 10 L of water) and the barrel was kept closed for 48 hours. Fermentation occurred between November and December of 2022. Weekly, liquid samples of the brine were collected from the middle or bottom of the barrel using a sterile pipette and stored in sterile bottles to monitor the dynamics of the microbial community in the different stages of the fermentation process. Due to high concentrations of microorganisms, samples were diluted using 0.85% NaCl to allow accurate colony analysis [11].

Each diluted sample was plated onto the following media

- MRS agar – selective for lactic acid bacteria (LAB) due to its rich composition, lower pH, and specific salts.
- SDA (Sabouraud Dextrose Agar) – selective for yeasts and molds, due to acidic conditions and high sugar content.
- Nutrient agar (NA) – for isolation of other aerobic bacteria.

Selective media enable targeted isolation of specific microbial groups, which is critical for further analysis. Isolation of pure cultures:

- Plating: The streak plate or dilution method was used to separate individual colonies.
- Repetition: The procedure was repeated up to three times until a pure culture (morphologically homogeneous colony) was obtained.
- Confirmation: Visual or microscopic inspection, as necessary.

2. Antimicrobial activity

The disk diffusion method based [10] with some modifications was used to evaluate the antimicrobial activity of the LAB isolates from the brine. Overnight cultures of pathogenic bacteria that include: *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 13393, *Salmonella enterica* ATCC 10708 and *Staphylococcus aureus* ATCC 25922 were grown in TSB at 30°C. Further, they were diluted to a turbidity of a 3.0 McFarland standard using sterile 0.85% NaCl. A bacterial lawn was prepared by spreading the suspension on TSA plates using a cotton swab. Wells were cut in the agar using a sterile cork borer and filled with 100 µL of sterile supernatant of the LAB isolates grown on MRS agar. All assays were performed three times to ensure reliability of results. The plates were incubated at 37°C for 24 hours and the diameter of inhibition zones around the wells was measured in millimeters. The general guidelines [12] of the zone diameter is as follows:

Table 1: Antimicrobial activity interpretation based on zone of inhibition.

Zone Diameter (mm)	Interpretation
≥ 20	Strong Activity
15–19	Moderate Activity
≤ 14	Weak or No Activity

Note: These thresholds are general guidelines and may vary depending on the specific microorganism and antimicrobial agent used.

3. Salt and acid tolerance

The testing of the viability of the LAB isolates in the presence of 6 % NaCl was tested by inoculating them into a MRS broth containing NaCl to a final concentration of 6% (w/v). Each medium was inoculated with 100 µL of a standardized suspension of the test microorganisms (0.5 McFarland or a defined CFU/mL). The test tubes were incubated at 30 °C for 24–96 hours. For acid tolerance testing, isolates were also inoculated in MRS broth adjusted to pH 2 and pH3 using 1 M HCl solution. Control media were maintained at pH 6.5–7.0. Each medium received 100 µL of a standardized suspension of the LAB isolates. The test tubes were

incubated at 30 °C for 24–96 hours as well. Growth and survival rates for both tolerances were assessed by measuring optical density (OD) at 610 nm using a turbidimeter.

RESULTS AND DISCUSSION

The results (Figure 1) indicate that strains 2.4.1 and 2.4.2 show board-spectrum antimicrobial activity against all tested pathogens with a diameter of inhibition 15 mm, while strains 2.18.1 and 3.5.1 show strong, however selective inhibition of 35 and 27 mm, respectively against *Listeria monocytogenes*. Strain 2.2.1 shows weak and selective activity against *E. coli* with an inhibition zone of 10 mm. This pattern shows differences in the production of the antimicrobial compounds due to their genetic differences or ecological adaptations to the cabbage brine environment. Similar findings are shown in studies [13] where an isolated *Lactobacillus plantarum* KU200656 from kimchi demonstrated a broad-spectrum antimicrobial activity against *Listeria monocytogenes*, *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Similarly, studies [14] of lactic acid bacteria isolated from traditional fermented Korean foods (including fermented cabbage) characterized for probiotic potential show their antimicrobial and stress - resistant properties.

Further, the results (Figure 2) show that the highest halotolerance at NaCl 6% concentration is noticeable for the strain 2.4.2 which shows rapid and continuous growth with its peak measured at the 96 hour. This suggests that this strain has strong adaptation to high-salt conditions. A strong tolerance is noticeable for the strain 2.4.1 which was also detected at the 96 hour measurement. This is consistent with studies [15] on lactic-acid bacteria from fermented vegetables, where halotolerance is a desirable characteristic that ensures survival in these environments that are typical to brine - based fermentation. Moderate tolerance is indicated in LAB strains 2.2.1 and 2.18.1 which growth fluctuates over time but also reaches its peak at the 96 hour measurements. Lastly, strain 3.5.1 shows poor halotolerance with a low and unstable growth indicating that it is unable to thrive under salt conditions. The observed salt tolerance also reflects the metabolic flexibility of these isolates, which could be beneficial in industrial food processing environments where osmotic stress is common.

The results for tolerance in strong acidity, pH 2 (Figure 3) showed that strains 2.4.1 and 2.4.2 have the highest survival, with strain 2.4.2 maintaining stability early and demonstrating a sharp increase measured at the 96 hour. Strain 2.2.1 also recovered strongly after 72 h measurement, while 2.18.1 showed poor tolerance throughout all time intervals. Lastly, strain

3.5.1 a slight increase was measured in the 96 hour interval. From the results it is evident that the most acid-resistant strains are 2.4.1 and 2.4.2. Tolerance on pH 3 results (Figure 4) indicate that in conditions of low acidity i.e. strain 3.5.1 showed exceptionally high survival across all time intervals, peaking at 72 hours, while strains 2.18.1 and 2.4.1 showed strong survival rates also. Their highest survival rate was also measured at the 72 hour interval. These trends indicate that acid adaptation mechanisms enable some of the strains to survive extreme conditions. Studies show [19] similar reports where *Lactobacillus plantarum* isolated from fermented vegetables exhibited excellent tolerance to low pH, maintaining high viability after hours of incubation at pH 2. This tolerance has been attributed to their ability to regulate internal pH, maintain membrane integrity and production of stress response proteins that protect cellular components. For strains such as 2.4.2 that showed intermediate results of acid tolerance, similar to the observations made in studies [19] that found that some *Lactobacillus* strains maintained viability at pH 3 but thrived at pH 2. Overall, strains 2.4.1 and 2.4.1 show strong acid tolerance and are promising probiotic candidates due to their potential to survive gastric passage and provide beneficial effects in the host intestines.

Overall, the antimicrobial activity is likely due to a combination of organic acid production, synthesis of bacteriocin and competition for nutrients. Lactic-acid bacteria are known to produce lactic acids, acetic acid and other metabolites that lower the pH and inhibit pathogen growth. Specifically, bacteriocins which are active antimicrobial peptides that are thermal and pH tolerant and induce pore formation on cells, induced cell cycle arrest which results in destroying internal cell components via enzymatic degradation with material leakage [15, 16, 17]. Studies [18] support that specific bacteriocins such as plantaricin ZJ316, plantaricin Q7 from *Lactobacillus plantarum* yield inhibitions zones of ~25 mm against *Listeria monocytogenes* confirming with the inhibition zone patterns observed in strains 2.18.1 and 3.5.1 (Figure 1).

Table 2: Inhibition Zone Diameters of the Five LAB Strains with the Highest Probiotic Potential Against Foodborne Pathogens

Strains	Inhibition zone diameters (mm)			
	<i>Escherichia coli</i> ATCC 8739	<i>Listeria monocytogenes</i> ATCC 13393	<i>Salmonella enterica</i> ATCC 10708	<i>Staphylococcus aureus</i> ATCC 25922
2.2.1	10	0	0	0
2.4.1	15	15	15	15
2.4.2	15	15	15	15
2.18.1	0	35	0	0
3.5.1	0	27	0	0

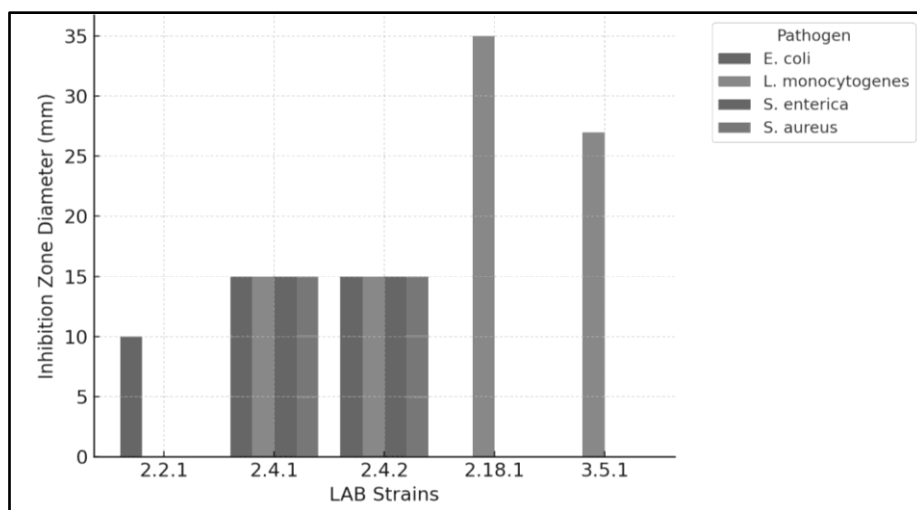


Figure 1: Antimicrobial activity against common food-borne pathogens expressed by diameter of inhibition zones expressed in mm of five LAB isolates

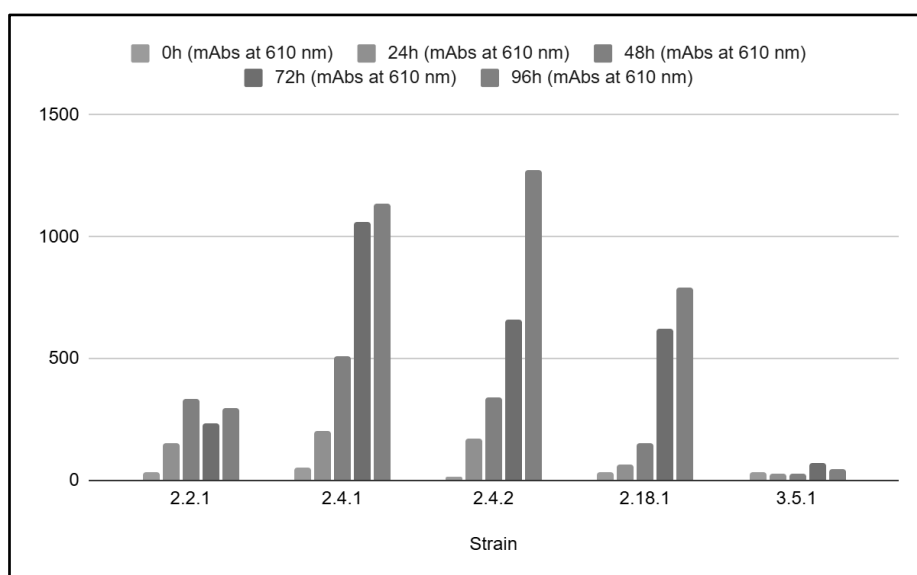


Figure 2: mAbs of 5 LAB strains at 6% NaCL over 96 hours

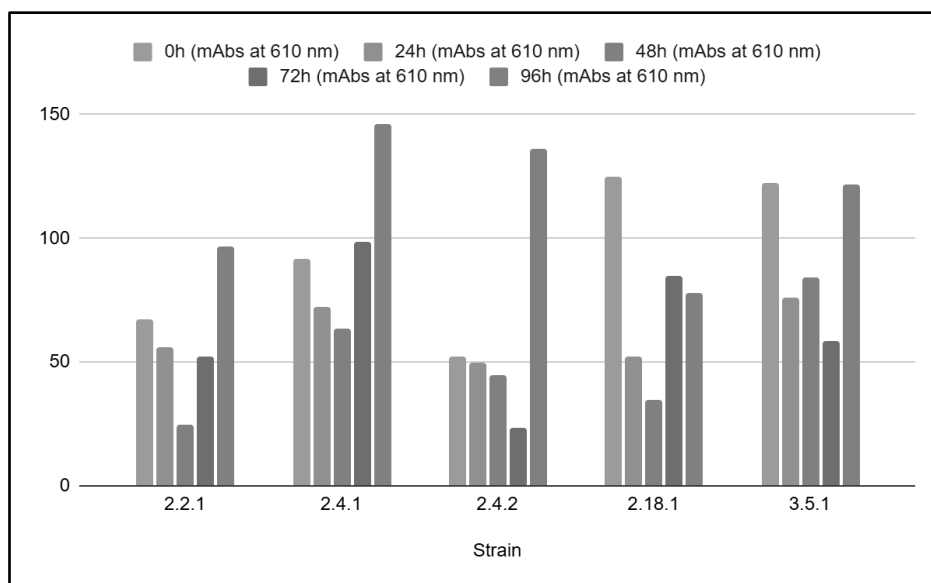


Figure 3: mAbs of 5 LAB strains at pH 2 over 96 hours

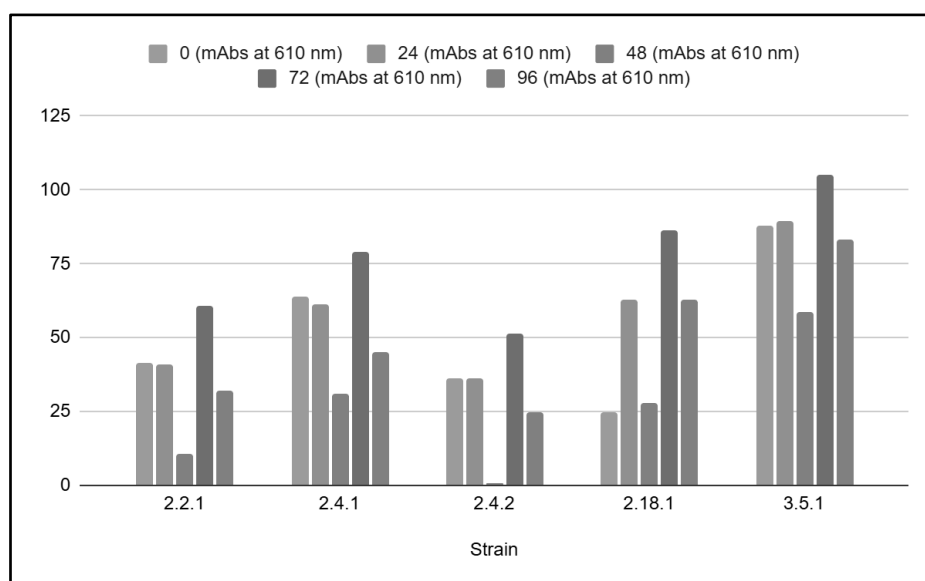


Figure 4: mAbs of 5 LAB strains at pH 3 over 96 hours

CONCLUSIONS

From the results and discussion it can be concluded that strain 2.4.2 is the most promising candidate that showed strong broad-spectrum antimicrobial activity as well as stress tolerance in strong acidic and high salt concentration conditions. Strain 2.4.1 also showed strong broad-spectrum activity with moderate tolerance at strong acidic and high salt concentration conditions. This indicates that strains 2.4.1 and 2.4.2 could be promising probiotic candidates

for industrial applications in food preservation and functional foods. Their antimicrobial activity can reduce the need for synthetic preservatives, thereby aligning with clean-label trends in the food industry. Further, strains 2.18.1 and 3.5.1 showed strong and selective antimicrobial activity specifically to *Listeria monocytogenes* ATCC 13393, however stronger stress tolerance abilities. Lastly, strain 2.2.1 showed limited effectiveness in both antimicrobial activity and stress tolerance environments. Overall, cabbage-derived probiotics are safe, and show high potential of natural alternatives to synthetic preservatives.

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PRODUCTION OF BIOCALCIUM POWDER FROM FISH BONES AND ITS USE AS A FOOD ADDITIVE

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ABSTRACT

Fish bones are an important waste, especially after the filleting process, which makes up 30% of the total weight. Fish bones are an important mineral source, and 60% of the mineral mass consists of calcium. Calcium has a very important place in human nutrition. Because calcium is the main component of teeth and bones, as well as having physiological functions in the body such as muscle contraction, wound healing, facilitating blood flow, regulating heart rate and transmitting nerve impulses. Since calcium deficiency in the body prevents these functions from being performed, it causes health problems. To prevent this deficiency, calcium is added to food. For this reason, in recent years, researchers have directed their studies on calcium obtained from fish bones, called “biocalcium”. In this presentation, information will be given about biocalcium production stages and its use in the food field. In addition, information will be given about the preliminary results of our ongoing project. In this project, biocalcium production is being studied with different methods. his research was supported by The Scientific Research Projects Administration Unit of Akdeniz University with project number FDK-2024-6699.

Keywords: Fish waste, biocalcium, production methods, food

INTRODUCTION

The processing of seafood produces a significant amount of waste and byproducts. Approximately 45-50% of fish waste consists of internal organs, heads, cut pieces, bones, and skin. This waste not only causes significant environmental problems but also leads to significant losses. The utilization of these wastes, which contain numerous bioactive compounds, can both solve environmental problems and provide economic benefits by being converted into value-added products. New value-added products created through the utilization and conversion of

waste are used in many areas, including food, chemicals, pharmaceuticals, medicine, and cosmetics.

Global fisheries and aquaculture production is reported to be approximately 185 million tons, 88% of which is for human consumption (FAO, 2024). Advances in fish farming and processing technologies have brought about the problem of waste and byproducts. Fish bones, particularly those obtained after filleting, constitute approximately 30% of the total weight of the fish (Lu, 2004). These wastes create significant problems. If waste is left to stand and accumulate, it will degrade very quickly. This causes serious health and environmental problems. Therefore, waste must either be destroyed and rendered harmless or made useful. Traditional waste management includes direct disposal of landfills and the use as animal feed and fertilizer. Today, these have been replaced by the concepts of recycling and recovery. It is expected that the recovery of bioactive compounds will increase profitability. The fish processing industry is faced with the need to develop effective methods for the recovery and utilization of waste. Wastes contain many valuable compounds, including proteins, fats, fatty acids, phospholipids, and fat-soluble vitamins (Rustad et al., 2011; Kim and Mendes, 2006). In recent years, intensive research has been conducted on new methods and technologies to obtain bioactive compounds from various organic raw materials for food and biomedical purposes. There are limited studies on the use of fish bones as an alternative calcium source (Phiraphinyo et al., 2006; Toppe et al., 2006; Tongchan et al., 2009; Boutinguiza et al., 2012). The dry matter of fish bone tissue has a mineral content of 60–70%, primarily calcium phosphate and hydroxyapatite (Gildberg et al., 2002, Barakat et al., 2008), and the calcium content in the mineral mass is at the level of 60% (Kim and Jung, 2007). It is reported that fish bones contain approximately 30–45% protein, 2–32% fat and 15–6% ash, and more than 10% of the fat-free dry matter consists of calcium (Munekata et al., 2020; Toppe et al., 2007).

Calcium is the most abundant mineral of the human body, constituting 1–2% of body weight. An average adult contains 1150–1300 g of calcium. In the human body, 99% of calcium is found in bones and teeth, 1% in extracellular fluids, soft tissues and the circulatory system. Calcium also plays a role in physiological functions such as muscle contraction, wound healing, facilitating blood flow, regulating heart rate, and conducting nerve impulses. Calcium insufficiency in the body can cause a decrease in bone density and a disease called osteoporosis (Trailokya et al., 2017). Calcium deficiency can cause rickets in children and bone fractures in the elderly. These are also associated with vitamin D deficiency. Calcium absorption is affected by factors such as estrogen concentration, age, and vitamin D levels. A decrease in calcium

absorption has been reported with aging (Trailokya et al., 2017). The main sources of calcium are milk and dairy products. However, the high prevalence of lactose intolerance, the emergence of new dietary patterns, concerns about the potential negative effects of dairy on human health, and the decrease in milk consumption in low- and middle-income countries have led to the widespread prevalence of calcium deficiency worldwide (Perez et al., 2023).

Calcium supplementation has been an alternative solution to the problem of calcium deficiency. However, the absorption of pure calcium is low due to calcium precipitation in the form of phosphate salts. Finding new calcium sources to meet the calcium needs of the human body is an important requirement. Chemically, calcium in bones is organized into hydroxyapatite crystals, which form phosphorus. These crystals have very low solubility and must be processed before being added to food. Hydroxyapatite solubilization can be achieved.

With the increasing demand for health-focused products, food products are fortified with a range of health-promoting ingredients. New products with market value have been developed from the often-wasted byproducts of the food processing industry (Wijayanti et al., 2021a). For consumers who have insufficient milk and dairy product consumption due to lactose intolerance, calcium-enriched foods are very important to meet their calcium needs (Kim and Mendis, 2006; Luu and Nguyen, 2009). Various studies have reported that foods such as milk and dairy products, salt, wheat flour, sugar, oil and corn flour are fortified with calcium and calcium salts such as calcium carbonate, tricalcium phosphate and calcium citrate are used for fortification (Sheik et al., 1987; Goldscher and Edelstein, 1996; Tateo et al., 1997; Singh et al., 2007). From the perspective of consumer acceptability and calcium availability, fish bones are effective natural sources of calcium (Phiraphinyo et al., 2006; Hemung, 2013). Various studies have shown that biocalcium powder obtained from fish bones can be used for food fortification purposes in crackers, cookies, sausages, surimi gel, mayonnaise, etc. It has been determined that it does not cause any negative effects on sensory properties (Benjakul and Karnjanapratum, 2018; Hemung and Sriuttha, 2014; Idowu et al., 2019; Karnjanapratum and Benjakul, 2018; Wijayanti et al., 2021b).

In this study, it was aimed to produce biocalcium powder from fish bones by different methods and to determine the characteristics of the obtained biocalcium powder. In this presentation some information will be given about the preliminary results of our ongoing project.

MATERIALS AND METHODS

Sea bass bones were used in the study. The bones were cut lengthwise into small pieces, 8-10 cm long. These bone fragments were then thoroughly washed under running water to remove blood and other contaminants. They were then stored at -20°C until biocalcium powder production began.

Biocalcium Powder Production

To produce biocalcium powder, fish bones stored in deep freezers were placed in the refrigerator to thaw. The raw fish bones were analyzed. Biocalcium powder was produced using various methods.

For biocalcium production, sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂), and surfactant solutions were prepared. Ten different groups were formed consisting of combinations of chemicals used for protein removal, oil removal, and bleaching processes for biocalcium production. These groups were A1X (NaO-Ethanol-H₂O₂), A2X (NaOH-Surfactant-H₂O₂), A1Y (NaOH-Ethanol-NaOCl), A2Y (NaOH-Surfactant-NaOCl), B1X (NaOH + NaOCl-Ethanol-H₂O₂), B2X (NaOH + NaOCl-Surfactant-H₂O₂), B1Y (NaOH + NaOCl-Ethanol-NaOCl), B2Y (NaOH + NaOCl-Surfactant-NaOCl), W (Boiled in water), RB (Dried control sample). Biocalcium production was carried out separately for these groups.

Yield, Moisture, protein, fat, ash, calcium and hydroxyproline contents of biocalcium powders were examined.

The yield of Bio Calcium powder was determined by dividing the weight of bio calcium powder by the weight of raw bone. (Yield (g/100g) = Biocalcium weight / Raw bone weight X100).

The moisture content of the samples was dried in an oven at 105°C until they reached a constant weight, the amount of crude protein was determined by the Kjeldahl method, the amount of crude fat was determined by the extraction method in the Soxhlet system, and the samples weighed in crucibles brought to a constant weight for crude ash were kept in a muffle furnace at 550±5°C until they turned into white ash (AOAC, 1990).

Calcium content was determined using the Thermo Scientific iCAP PRO ICP-OES system. Hydroxyproline content was determined using a UPLC-MS/MS instrument according to the method described by Kivrak et al. (2014).

RESULTS AND DISCUSSION

Yield

Yield is calculated by comparing the weight of the biocalcium powder to the initial weight. It is reported that the higher the yield, the higher the product's economic value and effectiveness (Febriani et al., 2021). Knowing the yield is crucial for determining a product's proper utilization and economic value. However, a lower yield for biocalcium powder indicates the product's purity, higher calcium content, and higher bioavailability. In our study, the highest yield was obtained in the B2Y (44.8%) group. Protein and fat levels were also highest in this group. Extraction using NaOH alone resulted in lower yields than the NaOH + NaOCl combination. The lowest yield was observed in the A1Y (38.3%) group. The lower biocalcium yield was due to the lower protein and lipid content of the bone in this group. In other words, protein and lipid removal were more effective in this group. Protein removal in biocalcium production facilitates the conversion of calcium into a soluble and highly bioavailable form. Extraction with water yielded a very high yield (70.9%). This demonstrates that removing protein and fat in water extraction is not sufficient and requires the use of chemicals. In a study where bone powder was produced from milkfish (*Chanos chanos*) using water, alkaline and acidic extraction methods, the highest yield was obtained with the water extraction method (65.57%), similar to our study, followed by acid (50.87%) and alkaline (39.67%) extractions (Wulandari and Kusumasari, 2019).

Proximate composition

The moisture content of raw bones was found to be higher than that of biocalcium powders (Fig. 1a). Heating during production removed free water. It has been reported that moisture content plays an important role in maintaining the stability and shelf life of powder products and the moisture content required to extend the shelf life of the powder is a maximum of 10% (Ojo et al. 2017). In the present study, the moisture content of biocalcium powders was found to be quite low. The highest moisture content was obtained by extraction in water. Among the other groups, the highest moisture content was found in the B2X group. The lowest moisture

content was found in the B1X group. Previous studies have reported different moisture contents for bone powders. These differences may be due to different extraction methods.

When calcium is obtained from natural sources such as bone, the raw material contains significant amounts of collagen and other proteins. Removing these proteins results in higher purity of calcium. Calcium bioavailability increases because proteins can bind to calcium, inhibiting its absorption. Therefore, one step in biocalcium production is protein removal. Alkali is often used for this purpose. Alkali removes proteins from biological materials such as bones by breaking them down, dissolving them, or hydrolyzing them. Removing organic matter such as fat and protein is reported to be an effective way to obtain high-purity TBP (Hemung, 2013). In this study, the protein content of raw bone was found to be 27.56% but decreased significantly after biocalcium production. The highest protein content (11.03%) was determined in the biocalcium powder obtained by boiling in water. This suggests that boiling in water did not sufficiently remove protein from the biocalcium powder. However, a significant decrease in protein content was observed when the alkaline method was used. The lowest protein content values were found in B1X (0.26%) and the highest in B2Y (1.49). The use of NaOH in the production of tuna bone powder has been reported to cause a decrease in protein and fat contents (Nemati et al., 2007).

Raw biological materials contain significant amounts of fat. Purifying calcium without removing the fat in biocalcium production becomes difficult, and the resulting calcium powder may lose its color, odor, and taste. Fats oxidize over time, turning rancid, which reduces product quality. Fat removal is a fundamental step in obtaining pure, odorless, more stable, longer-lasting, and higher-yielding biocalcium. Therefore, we conducted crude fat analyses on the biocalcium powders we produced using various methods. The fat content of the original bone, which was 1.09%, decreased significantly after being converted into biocalcium powder. The fat in fish bones is lost during the heating process. This loss can extend the shelf life of the powder. The lowest fat content was found in the B1Y group (Fig 1c). Surfactants were used in the fat removal process in this group. This indicates that surfactants are more effective than ethanol in oil removal.

In biocalcium powder, ash refers to the total of the product's unburned mineral matter. In other words, ash is the inorganic mineral residue left behind when biocalcium powder is burned at high temperatures (usually 500–600°C). Biocalcium consists primarily of calcium carbonate (CaCO_3) or calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$). These substances remain as ash after the organic portions are burned. The higher the ash content, the higher the mineral (calcium) content.

Higher ash content generally indicates that organic residue is low, combustible materials such as fat and protein have been removed, and the product contains a purer mineral structure. In our study, the ash content in raw fish bones was determined to be 34.26%, while the ash content in water-extracted biocalcium powder was 78.99%, and the ash contents of alkaline-extracted powder ranged from 86.98% to 88.46% (Fig 1d). The lower ash content in water extraction suggests that protein and fat removal was insufficient compared to the other groups. This is confirmed by the higher protein and fat content in this group than in the other groups. No significant difference was found between the ash contents of other alkaline extraction groups.

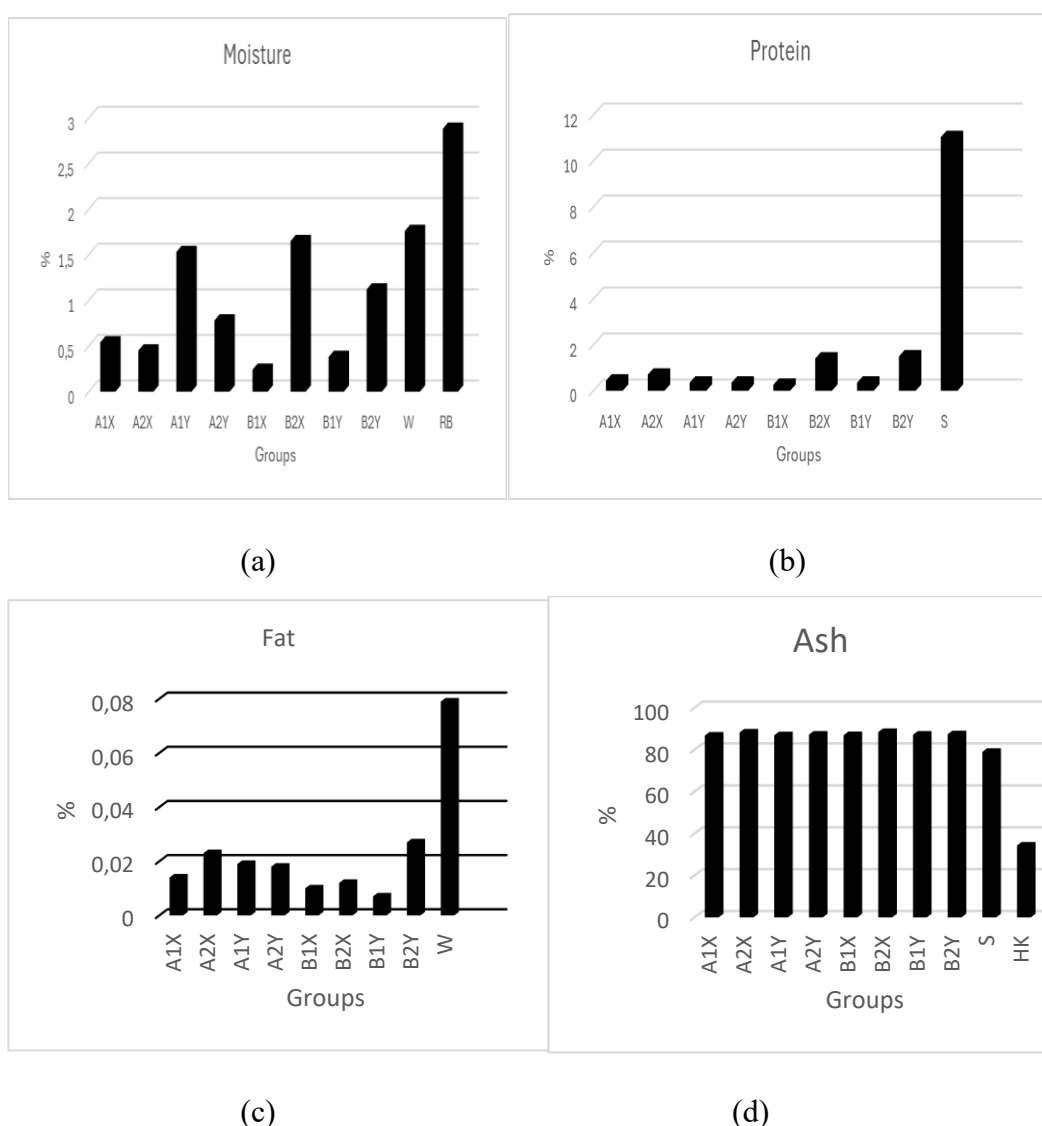


Figure 1. Moisture (a), Protein (b), fat (c) and ash (d) contents of biocalcium powders

Calcium (Ca)

Calcium is one of the most studied minerals related to human health and is also the most abundant mineral in the human body. It is the primary component of hydroxyapatite crystals,

which are associated with bone hardness. The calcium contents of the biocalcium powders obtained in our study ranged from 36.28% to 38.78%. The Ca content in raw bone was determined to be 35.61%. The Ca content of the biocalcium powder extracted in water (35.95%) was found to be lower than that obtained by alkaline extraction. The highest Ca content was found in the B1Y (38.78%) group, and the lowest Ca content was found in the A2Y (36.28%) group (Fig 2). By removing non-collagenous proteins, Ca became more concentrated in biocalcium powder.

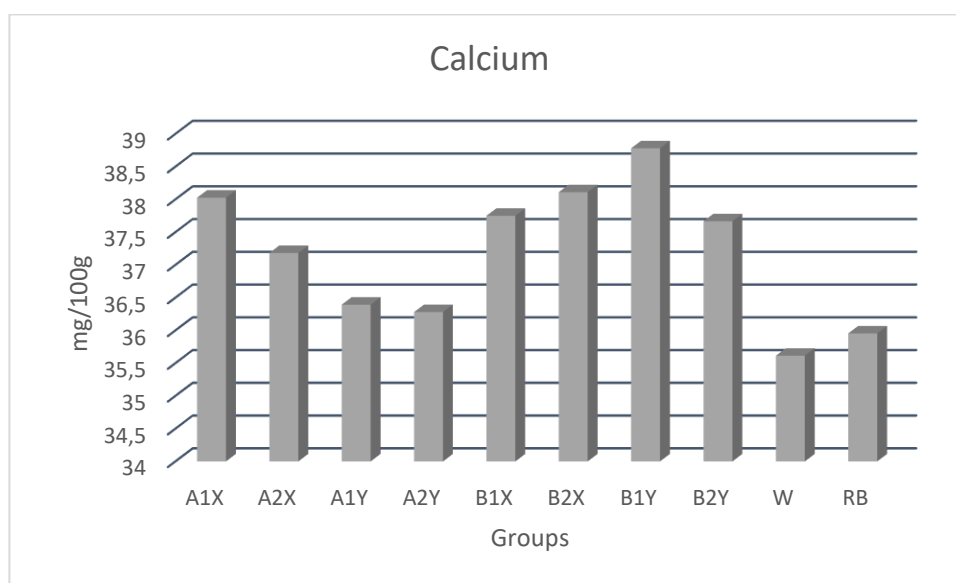


Figure 2. Calcium contents of biocalcium powders

Hydroxyproline

Hydroxyproline is a unique amino acid found in collagen and ensures its biological stability. Because it is found almost exclusively in collagen, the hydroxyproline level is used as a reliable indicator of collagen content in products. The presence of hydroxyproline indicates that collagen protein is formed in biocalcium.

This study examined the hydroxyproline level in bone-derived biocalcium powders and evaluated its relationship with collagen content. Analysis revealed 13.84–15.91 mg/g hydroxyproline in biocalcium powders, indicating a collagen content exceeding 10%. These findings suggest that this powder can be considered not only a mineral but also a functional protein (collagen) source. Raw bone had the lowest hydroxyproline content compared to other samples (Fig. 3). The lowest hydroxyproline content in the raw bone sample may be due to the

presence of meat, blood, lipids, etc. Increases in hydroxyproline content in biocalcium samples obtained after protein and lipid removal pretreatments indicated that the hydroxyproline content was concentrated. The highest hydroxyproline levels were determined in the B1X (15.91 mg/g) and B2X (15.82 mg/g) groups, while the lowest was determined in the A2Y (13.84 mg/g) group.

Analysis results indicate that biocalcium powder is an important food additive not only for its calcium (35–38%) but also for its high collagen content. Collagen is increasingly used in functional foods and supplements due to its contribution to bone health, connective tissue support, and anti-aging effects. The 1.5% hydroxyproline level indicates that such products can confidently claim "contain collagen."

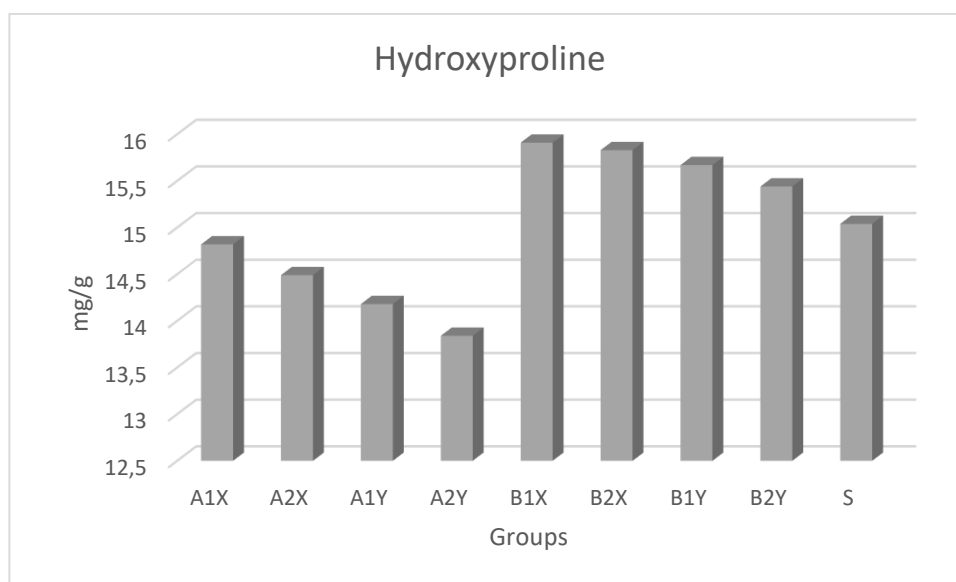


Figure 3. Hydroxyproline contents of biocalcium powders

CONCLUSIONS

In this study, biocalcium powder production methods involving different protein and fat removal processes were compared with the boiling water method. The study showed that used methods in the production of biocalcium powder from sea bass bones is effective due to their high dry matter, ash and calcium and low protein and fat contents of the obtained biocalcium powders. Among the methods, alkaline method was effective in protein removal, while the use of surfactants was effective in fat removal.

Analysis results indicate that biocalcium powder is an important food additive not only for its calcium (35–38%) but also for its high collagen content. The 1.5% hydroxyproline level indicates that such products can confidently claim “contain collagen”.

This study demonstrates that bone-derived biocalcium powder is not only a mineral source but also contains high-quality collagen. The high hydroxyproline level indicates that the product largely preserves its natural collagen structure, reinforcing its potential use in food, nutraceutical, and functional product development.

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TOTAL MONOMERIC ANTHOCYANIN CONTENT AND ANTIOXIDANT ACTIVITY IN FRUITS OF BILBERRY (*VACCINIUM MYRTILLUS* L.)

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ABSTRACT

Bilberry (*Vaccinium myrtillus* L.) is one of the richest natural sources of anthocyanins. Moreover, in bilberries, anthocyanins have been estimated to represent nearly 90% of the total phenolics. These polyphenolic components give bilberry its blue/black color and high antioxidant content. Due to their high levels of phenolics, bilberries have been reported to have multiple biological effects, including anticarcinogenic, anti-inflammatory and antimicrobial activities and lipid-lowering effects. Beneficial effects have also been established in cardiovascular diseases and diabetes, as well as in improved vision and neurodegenerative functions. The aim of this study was to investigate the antioxidant properties, content of total monomeric anthocyanins, indices for pigment degradation, polymeric color and browning index of a bilberry and bilberry extract. The antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and the result was expressed as Trolox equivalents per 100 g of fresh bilberries (mmol TE/100g). Antioxidant activity was 0.34±0.01 mmol TE/100g bilberries. The content of total monomeric anthocyanins was investigated spectrophotometrically by the pH-differential method. Data were quantified as mg of standard cyanidin 3-glucoside equivalents (C3GE) per 100 g of fresh bilberries. The total monomeric anthocyanins content was 403.70±3.97 mg CGE/100 g FW of bilberries and 1821.39±17.89 mg CGE/L of extract. Indices for pigment degradation, polymeric color, and browning were assayed spectrophotometrically. Percent of polymeric color was 23.66%, and browning index

was 0.93. Antioxidant activity of bilberries was significant. The high antioxidant activity matched well with the high anthocyanin content in bilberries.

Key words: Bilberries, Anthocyanins, Antioxidant activity, Total monomeric anthocyanins, Percent of polymeric color, UV/VIS spectrophotometry

INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) is one of the richest natural sources of anthocyanins. These polyphenolic components give bilberry its blue/black color and high antioxidant content, and they are believed to be the key bioactives responsible for the many reported health benefits of bilberry and other berry fruits (Chu et al., 2011). The bilberry plant is a low-growing shrub native to northern Europe, but is now also found in parts of North America and Asia. Bilberry is also known as European blueberry, whortleberry, huckleberry, and blaeberry. Bilberry usually grows in heaths, meadows, and moist coniferous forests, and its growth is favored by moderate shade and moderately humid ground conditions. The bilberry is a small (5-9 mm in diameter) fruit, bluish black in color, with many seeds (Chu et al., 2011; Valentová et al., 2007).

Bilberry contains a variety of phenolic compounds, including flavonols (quercetin, catechins), tannins, ellagitannins, and phenolic acids, but anthocyanins make by far the largest contribution to its phytochemical mix. These naturally occurring phenolic compounds are redox-active antioxidants as well as iron chelators, and are found in red-, blue-, and purple-colored flowers, fruits, and vegetables (Chu et al., 2011). Anthocyanin concentration in the fresh fruit is approximately 0.1-0.5% (Ichiyanagi et al., 2004). Bilberry has higher anthocyanin content compared to other types of berries, such as strawberry, cranberry, elderberry, sour cherry, and raspberry. The total anthocyanin content of bilberry is generally in the range of 300-700 mg/100 g fresh fruit, although this range varies with cultivar, growing conditions, and degree of ripeness of the berry (Chu et al., 2011; Lätti et al., 2008; Može et al., 2011).

Anthocyanins (from the Greek *anthos* for flower and *kyanose* for blue) are water-soluble polyphenolic flavonoid compounds. They are responsible for the pink, red, blue, and purple color of plants. Anthocyanins comprise aglycones - anthocyanidins and their glycosides - anthocyanins. The sugar can be attached at various positions, and can be glucose, galactose, xylose, arabinose, or rhamnose. Anthocyanins also vary in the number and position of hydroxyl and methoxyl groups attached to the core anthocyanidin structure. Therefore, although there are less than 20 naturally occurring anthocyanidins, there are many hundreds of different anthocyanins (Chu et al., 2011; Szajdek & Borowska, 2008). Bilberries are characterized by 15

anthocyanins. These include delphinidin, cyanidin, petunidin, peonidin and malvidin, glycosidically linked to glucose, arabinose or galactose (Lätti et al., 2008; Može et al., 2011).

The antioxidant activity of cyanidin is 4.4 times higher than for ascorbic acid. The content of anthocyanin pigment directly correlates with antioxidant activity of plants. In addition to their antioxidant effects, anthocyanins have been reported to stabilize DNA, modify adipocyte gene expression, improve insulin secretion and sensitivity, and have antiapoptotic, anti-inflammatory, and antibacterial effects. These numerous and potentially highly beneficial effects of anthocyanins make foods rich in these compounds, such as bilberry, potential candidates as “functional foods” and phytotherapeutics (Szajdek & Borowska, 2008).

The official method of anthocyanin pigment analysis is the spectrophotometric pH-differential method (Urbonaviciene et al., 2022). This method is a rapid and easy procedure for the quantitation of monomeric anthocyanins, while other auxiliary spectrophotometric techniques are used to measure the extent of anthocyanin polymerization and browning index (Giusti & Wrolstad, 2001). The pH-differential method permits accurate and rapid measurement of the total anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds (Giusti & Wrolstad, 2001). This method for the quantification of total anthocyanins is widely used for the standardization of natural raw materials (Urbonaviciene et al., 2022). Browning index ($ABS_{520\text{ nm}}/ABS_{420\text{ nm}}$) is a measure of anthocyanin-rich fruit juice pigmentation quality.

Several methods have been developed to determine the antioxidant activity of fruits, vegetables as well as herbs. Two major mechanisms, namely hydrogen atom transfer (HAT) and single electron transfer (SET), are well known in the evaluation of the antioxidant activity against free radicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is one of the methods that utilizes both the HAT and SET mechanism. It is considered to be predominantly based on the electron transfer reaction, whereas hydrogen-atom transfer reaction is only a marginal pathway (Işik et al., 2011).

The antioxidant molecules can quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in the absorbance at 517 nm (Ribeiro et al., 2006; Soares et al., 1997). This method is unsophisticated, cheap and sufficiently accurate and shows the radical scavenging activities of berry extracts, contributed by both anthocyanins and other compounds (Burdulis et al., 2009; Moyer et al., 2002; Sellappan et al., 2002)

The aim of this study was to investigate the content of total monomeric anthocyanins and indices for pigment degradation, polymeric color, browning index and the antioxidant properties of a bilberry and bilberry extract. The content of total monomeric anthocyanins was investigated spectrophotometrically by the pH-differential method. Indices for pigment degradation, polymeric color and browning index were assayed spectrophotometrically. The antiradical properties of these samples were assessed using DPPH method.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from TCI (>97.0%, USA). Potassium chloride (KCl) and potassium metabisulfite ($K_2S_2O_5$) were purchased from Centrohem, (Stara Pazova, Serbia). Sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) was purchased from Alkaloid (Skopje, North Macedonia). Aqueous solutions were prepared using Milli-Q water (Millipore, Bedford, MA).

Bilberry extract preparation

Fully ripe bilberry fruits were collected in Koroška and Škofja Loka woods in Slovenia and then stored for 7 days at the temperature of $-20\text{ }^{\circ}\text{C}$ prior to the beginning of the extraction process. The extraction process was carried out following a modified method previously used by Može et al. (2011). An amount of 600 g of frozen bilberry was first homogenized in 2 L of the ice-cold deoxygenated methanol, and then the homogenate was extracted for 60 min at the ambient temperature with continuous stirring. The obtained extract was filtered using vacuum. The residue was extracted twice for 30 min with 1 L of the ice-cold deoxygenated methanol, and then filtered through using vacuum. Finally, all three extracts were collected, washed in liquid nitrogen and stored at $-20\text{ }^{\circ}\text{C}$.

Total monomeric anthocyanin content

Total monomeric anthocyanins were determined by the pH-differential method (Giusti and Wrolstad 2005). The extract was diluted with 0.025 M potassium chloride buffer, pH 1, and 0.4 M sodium acetate buffer, pH 4.5. After 20 min at room temperature, absorptions at 520 and 700 nm were measured by spectrophotometer (GENESYS™ 10S, Thermo Electron

Corporation, Cambridge, UK). Analysis was performed in triplicate. Total monomeric anthocyanin content was expressed as cyanidin-3-glucoside equivalents (C3GE) using Eq. (1), where MW is the molecular weight of cyanidin-3-glucoside, 449.2 g/mol, DF is a dilution factor, ϵ is the molar extinction coefficient of cyanidin-3-glucoside in aqueous buffer of pH 1.0, 26900 L/mol/cm, and b is the path length of 1 cm:

$$MA = \frac{[(ABS_{520\text{ nm}} - ABS_{700\text{ nm}})_{\text{pH } 1.0} - (ABS_{520\text{ nm}} - ABS_{700\text{ nm}})_{\text{pH } 4.5}] \times MW \times DF \times 1000}{\epsilon \times b} \quad (1)$$

Polymeric color analysis

Percent of polymeric color was determined using the bisulfite bleaching method (Giusti & Wrolstad, 2001). Briefly, 2.8 mL of extract were diluted in water or 0.2 mg/mL potassium metabisulfite. The spectrophotometer (GENESYS™ 10S, Thermo Electron Corporation, Cambridge, UK) was used to collect absorbance readings of triplicate solutions at 420, 520 and 700 nm. Solutions were diluted such that 420 and 520 nm absorbance readings were between 0.2 and 1.0. Equation (2) and absorbance readings of solutions treated with metabisulfite were used to calculate polymeric color. Equation (3) and absorbance readings of solutions left untreated were used to calculate color density. In Eq. (2) and (3), DF was the dilution factor used. The percent of polymeric color was determined using Eq. (4).

$$\text{Polymeric color} = [(ABS_{520\text{ nm}} - ABS_{700\text{ nm}}) + (ABS_{420\text{ nm}} - A_{700\text{ nm}})] \times DF \quad (2)$$

$$\text{Color density} = [(ABS_{420\text{ nm}} - ABS_{700\text{ nm}}) + (ABS_{520\text{ nm}} - A_{700\text{ nm}})] \times DF \quad (3)$$

$$\text{Percent polymeric color} = \frac{\text{Polimeryc color}}{\text{Color density}} \times 100 \quad (4)$$

Determination of browning index

Bilberry extract was diluted with water to bring absorbance values within the instrument range. Browning index was determined as $ABS_{520\text{ nm}}/ABS_{420\text{ nm}}$ (Dorris et al., 2018)

Antioxidant activity assay

The antioxidant activity of the extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method reported by Brand-Williams et al., (1995) and Karori et al., (2007) to determine the free radical-scavenging potential of sample. Briefly, 0.1 mL of the

bilberry extract was mixed with 5 mL DPPH-solution (1×10^{-4} mol/L methanol). Sample blanks were methanol and DPPH-solution. Sample and blank were left in the dark for 30 min at room temperature, after which the absorbance at 520 nm was recorded by spectrophotometer (GENESYS™ 10S, Thermo Electron Corporation, Cambridge, UK). Quantification was performed on the basis of a standard curve and the result was expressed as Trolox equivalents per 100 g of fresh bilberries (mmol TE/100g). Antioxidant activity of bilberry extracts was expressed by percentage of inactivated DPPH reagent using the following equation:

$$DPPH = \frac{A_b - A_a}{A_b} \times 100 \quad (5)$$

where:

A_b = absorption of blank aliquot,

A_a = absorption of solution with analyzed extract.

Statistical data analysis

All measurements were carried out in triplicate, and presented as mean $\pm$ SD.

RESULTS AND DISCUSSION

Total monomeric anthocyanins content

Bilberry is one of the richest sources of anthocyanins, which have multiple biological activities (H. E. Khoo et al., 2017). Bilberries are richer in anthocyanins than other berries such as black and red currants, strawberries, cranberries, lingonberries, and black and red raspberries (Konić-Ristić et al., 2011). For the quantification of total monomeric anthocyanins content in bilberry fruits, the spectrophotometric assay, described by Giusti & Wrolstad (2001) was performed. Spectrophotometric method is widely used for standardization of natural raw material. High amounts of anthocyanin compounds were found in bilberries and bilberry extract. The total monomeric anthocyanins content measured was 403.70 ± 3.97 mg C3GE/100 g FW of bilberries and 1821.39 ± 17.89 mg C3GE/L of extract (Table 1).

Total monomeric anthocyanins content of investigated bilberry samples were in accordance with previously reported findings. For instance, Urbonaviciene and colleagues (2022) reported that the concentration of anthocyanins in bilberry samples ranged from 232.7 to 475.5 mg/100 g FW. Skrede and colleagues (2012) reported that the concentration of anthocyanins in bilberry samples ranged from 429 to 627 mg/100 g FW. The total monomeric anthocyanins content of bilberries from Macedonia was 507 mg/100 g FW (Stanoeva et al.,

2017). Rohloff and colleagues (2015) reported somewhat lower amounts of total anthocyanins in Norwegian bilberries (from 330 to 449 mg/100 g FW), whereas the total monomeric anthocyanins content of Finnish bilberries from 20 different populations were from 350 to 525 mg/100 g FW (Lätti et al., 2008). The differences between our results and the results reported in literature could be due to the environmental conditions, period of harvesting, cultivar variability, or fruit maturity.

Table 1. Total monomeric anthocyanins content in bilberry extract (mg C3GE/L) and bilberries (mg C3GE/100 g FW). Values are presented as means±SD.

No.	pH=1.0		pH=4.5		ABS	Monomeric anthocyanins (mg C3GE /L)	Monomeric anthocyanins (mg C3GE /100g)
	ABS ₅₁₅ nm	ABS ₇₀₀ nm	ABS ₅₁₅ nm	ABS ₇₀₀ nm			
1.	0.780	0.019	0.050	0.019	0.730	1840.72	407.99
2.	0.771	0.020	0.055	0.02	0.716	1805.42	400.16
3.	0.773	0.019	0.053	0.02	0.721	1818.02	402.96
Mean±SD						1821.39±17.89	403.70±3.97

Polymeric color analysis and browning index

Percent of polymeric color of bilberry extract was 23.66±2.40% (Table 2). Oxidative polymerization of tannins by oxygen led to degradation and decrease of red pigments (ABS 520) and increase in yellow pigments (ABS 420); this reaction was named browning index (Zozio et al., 2011). Browning index is a measure of anthocyanin-rich fruit juice pigmentation quality (Dorris et al., 2018). According to the Dorris and colleagues (2018) and Malien-Aubert and colleagues (2001, 2002) expressed the browning index as the absorbance ratio at 520 nm by that at 420 nm (ABS₅₂₀ nm/ABS₄₂₀ nm). The browning index of extract was 0.93 (Table 2). The low browning index values (ABS₅₂₀ nm /ABS₄₂₀ nm) indicate a greater contribution of brown pigmentation to juice appearance and are perceived as lower quality (Ponting et al., 1960). According to Dorris and et al. (2018), the browning index is strongly inversely associated with percent of polymeric color, and positively correlated with content of monomeric anthocyanins.

Table 2. Polymeric color, color density, percent of polymeric color and browning index

No.	Polymeric color	Color density	Percent of polymeric color	Browning index
1.	0.757	2.871	26.37	0.91
2.	0.757	3.475	21.78	0.89
3.	0.758	3.322	22.82	1.00
Mean±SD			23.66±2.40	0.93

Antioxidant activity

The feature that makes bilberry fruit very popular among consumers is their contents of anthocyanins, phenolics and flavonoids, as they exhibit antioxidant activity (G. M. Khoo et al., 2011; Kim et al., 2005) in biological systems.

The EC₅₀ value, defined as the concentration of sample required for 50% scavenging of DPPH radicals under experimental conditions employed, is a parameter widely used to measure the free radical scavenging activity (Cuvelier et al., 1992); a smaller EC₅₀ value corresponds to a higher antioxidant activity (Stajčić et al., 2012).

Anthocyanins are efficient free radical scavengers, due to their ability to inactivate free radicals. Antioxidant activity of bilberries was significant: an EC₅₀ values were 0.3380±0.0111 mmol TE/100g of bilberries and 1.5249±0.0500 mmol TE/L of extract. Because lower EC₅₀ value determines stronger antioxidant effect, this result suggests that the bilberry extract is potent in scavenging free radicals. Anthocyanins represent the majority of all bilberry phenolics, so it is safe to conclude that the antioxidant activity achieved by this extract is accounted mainly by its anthocyanins.

Total antioxidant activity values of investigated bilberry samples were in accordance with previously reported findings. For instance, Stajčić et al., (2012) reported that the DPPH free radical scavenging activity for wild bilberry, (expressed as EC₅₀) was 0.3157±0.0145 mg FW/mL.

CONCLUSIONS

In recent years, a growing demand for healthy food has been noted in the market. Consumers are primarily interested in food which is appealing, helps in preventing various diseases and contains high levels of promoted bioactive compounds. In this work the content of total monomeric anthocyanins, indices for pigment degradation, polymeric color, browning index and antioxidant properties of a bilberry and bilberry extract were determined. Antioxidant activity of bilberries was significant. The high antioxidant activity matched well with the high anthocyanin content in bilberries. The high antioxidant activity indicates that bilberry fruits can be considered as a good source of natural antioxidants that may have potential health effects.

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FRUIT GROWING, VITICULTURE AND WINE PRODUCTION

AMPELOGRAPHIC GRAPE QUALITY PARAMETERS OF TRAMINER CV CLONES GROWN UNDER AGROECOLOGICAL CONDITIONS OF CENTRAL SERBIA WINE REGION

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ABSTRACT

The aim of this study was to determine the ampelographic quality parameters of two Italian Traminer clones (R1 and VCR 6) grown under the agroecological conditions of the Central Serbia wine region and the Grocka subregion. The research was carried out at the Faculty of Agriculture, University of Belgrade, in the experimental field "Radmilovac" (GPS coordinates 44°46'12'' N and 20°44'02'' E). The tested clones were grafted on rootstock *V. berlandieri* x *V. rupestris* – 110 R clone VCR 114. Ampelographic parameters of the bunches and berries (bunch length, width and mass, bunch stem and berry mass, % of berry skin and mesocarp, etc.) were determined on 10 randomly selected bunches. The sugar content of the grape juice-must was determined with the Oechsle hydrometer and the total acidity by the titration method using 0.1 M NaOH. Clone VCR 6 was characterised by a higher average mass of bunches (86.6 g), weight of 100 berries (139 g), weight of berry skins (8.81 g) and sugar content in the grape juice (23.06%), while clone R1 had a higher number of seeds and a higher weight of skins of 100 berries (244 and 7.51 g) and a lower total acidity in the grape juice expressed as tartaric (4.57 g/l). Based on the obtained results, it can be concluded that the tested clones are suitable for growing in the Central Serbia wine region and that, based on the parameters shown, they can be recommended for the production of high quality wine.

Key words: ampelographic parameters, grape, Traminer, R1, VCR 6

INTRODUCTION

According to the latest agricultural census and the census of viticulture areas in the Republic of Serbia, Central Serbia is the largest wine region in Serbia with 13 defined wine subregions. There are 17,118 ha planted with vines and 74,423 individual grape growers. Wine varieties are planted on 13,157 ha, while table varieties occupy 3,961 ha. Viticulture accounts for 0.6% of total agricultural production, with an average yield of $8 \pm 0.23 \text{ t ha}^{-1}$. In the overall

structure of fruit production, grapevine is the most important fruit species in the Republic of Serbia, along with apple, plum, and raspberry (Statistical Yearbook RS, 2023).

Regner et al. (2020) propose that Traminer originated from the hybridisation of two *Vitis vinifera ssp. silvestris* genotypes (type Gmelin), and therefore this variety represents a domesticated wild type. Traminer has played an important role in various breeding programmes and in the development of many European cultivars. There is a very close relationship between Traminer and Pinot. The Gewurztraminer variety was created as a clone of Traminer variety. The isolated Traminer clones had its high potential, which is why it is being propagated in northern Italy, Germany, and other parts of Europe (Nicolini et al., 2019; Ferretti and Febbroni, 2022). Development of Serbian viticulture has been accompanied by introduction of quality clonal material, mainly from Italy, France, and Germany, which is examined at different agro-ecological localities (Pržić and Marković, 2019).

Traminer is a white grapevine variety intended for high-quality wine production. It is a II ripening period variety, maturing 10–15 days after Chasselas. Its name originates from the Tramin area in South Tyrol, Italy. Other synonyms include Savagnin rose, Gewürztraminer, Edeltraube, Roter Traminer, and Traminer rosso (Matijašević, 2021). The R 1 clone was selected in Bolzano in 1969, and VCR 6 in San Michele all'Adige, Italy, in 1996. Both clones have similar yields and smaller clusters compared to the standard variety. The vines are often blended in rose winemaking and can also be used for producing high-quality dessert wines. Clone VCR6 has a superior sensory profile, with floral and apple aromas (VCR, 2025).

The analysis of the mechanical and chemical characteristics of grapes is necessary to ensure the oenological potential of a variety. The proportions of individual structural components of grapes and berries affect the quality of the produced wine. In particular, the proportions of berry skin and seeds, which are rich in phenolic and are an important source of primary aromatic compounds—the main carriers of the wine's varietal characteristics—are significant. Conversely, the proportion of mesocarp, where sugars and acids are dominant accumulated, influences grape juice yield and produced wine, and the wine's balance through the ratio of accumulated sugars to total acids expressed as tartaric (Makuev et al., 2022).

The aim of this paper is to compare the R1 and VCR 6 clones of the Traminer variety by analysing the mechanical composition of bunches and berries and the chemical parameters of grape juice-must, in order to draw relevant conclusions about the production and oenological potential of these clones grown under the agro-ecological conditions of Central Serbia.

MATERIALS AND METHODS

The study was conducted in 2024 at the Faculty of Agriculture University of Belgrade experimental field “Radmilovac” (GPS coordinates 44°46'12" N, 20°44'02" E) using two superior Italian Traminer clones (R 1 and VCR 6). The experimental vineyard, located in the Central Serbia wine region, Belgrade subwine region, Grocka wine area, was planted in 2020 with a row spacing of 2.4 m and 0.8 m spacing between vines within the row. The examined clones (R 1 and VCR 6) were grafted onto the rootstock *V. berlandieri* × *V. rupestris* – 110 R clone VCR 114. The vines were mix pruned according to the single Guyot training system, leaving one spur with two buds and one cane with eight buds.

For laboratory examination, grapes were harvested at full maturity and subjected to mechanical analysis of clusters and berries, as well as analysis of grape juice-must qualitative parameters. Laboratory examinations were conducted at the Department of Viticulture, Faculty of Agriculture University of Belgrade.

The mechanical composition of clusters and berries was determined according to the method described by Marković and Pržić (2020). For this analysis, 10 randomly selected representative clusters were harvested at full maturity. After measuring the mass of the clusters, berries and rachis were separated, and the mass of each was determined. From the selected sample, 100 random berries were chosen for mass measurement. The mesocarp was then removed, and the berry skins and seeds were left at room temperature until dry, after which their mass were measured and the seeds were counted. Other mechanical parameters presented in the paper were obtained by calculation.

The concentration of sugar (%) accumulated in grape juice-must was determined using the Oechsle hydrometer, and the values were calculated using the Dujardin-Salleron formula:

$$\text{Sugar (\%)} = ^\circ\text{Oe} \times 0.266 - 3$$

$^\circ\text{Oe}$ – value of the sugar content in the grape juice measured by the Oechsle scale.

The content of total acids, presented as tartaric acid (g/L) in grape juice-must, was determined by titration using 0.1 M NaOH and phenolphthalein as a colour indicator. The values were determined according to the formula:

$$\text{Total acidity (g/L)} = \text{NaOH used} \times F \times 0.75$$

F – normality factor of the 0.1 M NaOH solution.

The glycoacidometric index - GAI (ripeness index) was calculated from the ratio of sugar to acid content. The pH values were determined using a pH meter.

RESULTS AND DISCUSSION

Agroecological conditions.

The climatic parameters observed in this study refer to the periods 2004–2023 and 2024 (RHSS report and Digital Atlas of Serbia). The mean vegetation temperature (April–October) for 2004–2023 was 19.2°C, and the mean annual temperature was 13.4°C (Table 1). In the year the experiment was established, significantly higher vegetation (21.8°C) and annual (15.9°C) temperatures were recorded. Based on average monthly temperatures, June, July, and August were the warmest months, and in the year of observation, these months also showed significant differences compared to the multi-year average. The coldest months were January and February, which, in the year of observation, had much higher eight-hour temperatures compared to the multi-year average. This can affect the overall development of the so-called small annual vine cycle and phenology as a whole.

Table 1. Mean monthly (Jan–Dec), vegetation (Veg), and annual (Ann) temperatures (in °C)

ts (°C)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Veg	Ann
2004-2023	2.1	4.0	8.2	13.6	18.0	22.1	24.3	23.8	19.0	13.6	8.5	3.8	19.2	13.4
2024	4.1	11.1	12.1	16.3	19.1	25.3	27.7	28.4	20.3	15.2	6.6	4.2	21.8	15.9

The region has a favourable distribution of precipitation throughout the year (Figure 1), with an average annual sum of 648.9 mm and an average sum during the growing period of 402.8 mm for the 2004–2023 period. In 2024, the annual rainfall sum was 660.1 mm, and the vegetative period total was 464.7 mm. The examined climatic values indicate that the region is suitable for grapevine growth and fruiting. In the research year, the distribution of precipitation was uneven, with the highest eight-hour precipitation occurring in May, June, and September. These months, with higher precipitation amounts, deviated significantly from the multi-year average for the same period.

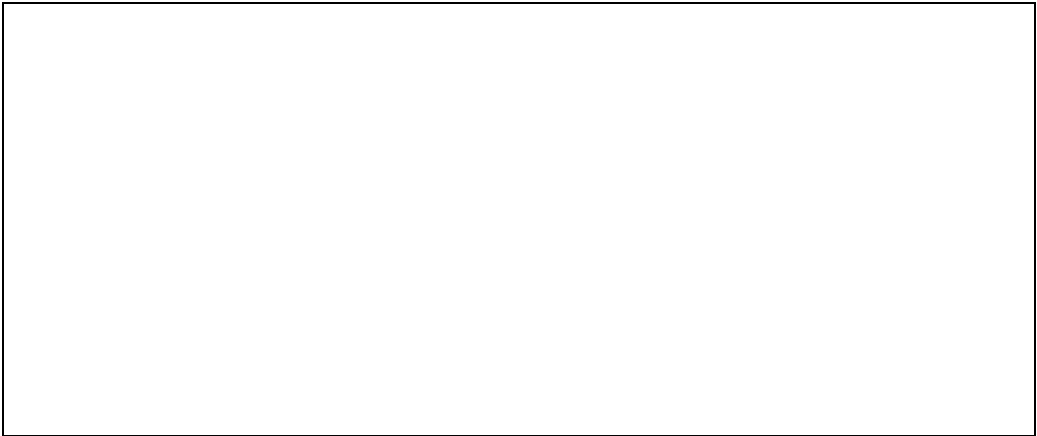


Figure 1. Mean monthly precipitation in the 2004-2023 period and 2024 (mm)

Mechanical composition of berries and cluster.

The mechanical components of grapes had important scientific and practical importance for estimating varietal or clonal oenological potential. The values of mechanical composition of grape and berries mostly depend on the grapevine variety, environmental conditions, and the applied ampelolotechnical measures (van Leeuwen, 2022; Marković and Pržić, 2024). The results of the mechanical analysis of berries and cluster are shown in Table 2 and 3.

Table 2. Mechanical berries composition of Traminer R1 and VCR6 clones

Parameter	R1	VCR6
Berry mass in a cluster (g)	79.60±24.78	79.30±16.73
Stem mass (g)	5.30±1.77	6.30±3.65
Number of berries in a cluster	66.30±19.15	66.30±17.32
Mass of 100 berries (g)	131.00	139.00
Berries skin mass of 100 berries (g)	7.51	8.81
Seed mass from 100 berries (g)	7.00	7.02
Mass of 100 seeds (g)	3.08	2.77
% of mesocarp in a berry	88.92	88.61
% of skin in a berry	5.73	6.34
% of seeds in a berry	5.34	5.05
Berry indicator	78.09	76.56
Berry weight composition indicator	15.51	13.98

There were differences in cluster mass between R 1 and VCR 6 (84.90±26.21 g and 86.60±20.45 g, respectively). Clone R 1 (±26.21) showed significantly greater variation in bunch mass compared to clone VCR 6 (±20.45). The cluster mass for both clones was within the average range for this type of clones, bearing in mind that, according to literary sources, Traminer cluster mass varies between 50 and 120 g (Cataldo et al., 2021; Matijašević, 2021). There were small differences in berry mass per cluster, although clone VCR 6 had a higher stem mass in the cluster (6.30±3.65), with the same number of berries per cluster and seed mass of 100 berries between clones. Clone VCR 6 had a greater average mass of 100 berries (139.00 g) and a greater skin mass of 100 berries (8.81 g), indicating a greater oenological potential of this clone. The same trend was observed with a higher percentage of berry skin (6.34%) and a lower berry weight composition indicator (13.98) for clone VCR 6. A higher content of berry skin contributes to an increased presence of varietal aromas in wines (Galán et al., 2021).

Table 3. Mechanical cluster composition of Traminer R1 and VCR 6 clones (2nd part)

Parameter	R1	VCR6
Cluster mass (g)	84.90±26.21	86.60±20.45
Cluster length (cm)	6.89±0.90	6.29±1.03
Cluster width (cm)	6.21±1.36	7.20±1.69
Berries mass/cluster (g)	79.60±24.78	79.30±16.73
Rachis mass (g)	5.30±1.77	6.30±3.65
Berry length (mm)	12.47±0.58	12.68±0.99
Berry width (mm)	11.91±0.88	10.88±1.35
Average number of seeds in 100 berries	244.00	239.00
Average single berry mass (g)	1.31	1.39
Average mass of 100 seeds (g)	2.87	2.94
Average mass of all berries per cluster (g)	79.60	80.30
Average mass of berries skin per cluster (g)	4.98	5.84
Average mass mesocarp per cluster (g)	69.98	69.80
% of rachis in a cluster	6.24	7.27
% of berries in a cluster	93.76	92.72
% of berries skin in a cluster	5.86	6.74
% of seeds in a cluster	5.47	5.37
% of mesocarp in a cluster	82.43	80.61
Bunch weight composition indicator	15.02	12.75
Bunch structure indicator	4.69	4.16

Chemical composition.

Grapes intended for wine production are harvested at their optimal stage for processing (full maturity). For wine varieties such as Traminer, the content and ratio of accumulated sugar and acids are of great importance. Sugar concentration directly influences the amount of alcohol produced during alcoholic fermentation, which provides structure and balance to the wine and contributes to its preservation (Antalick et al., 2019; Petrović, 2022). Acid content influences taste balance, chemical stability, and pH (Coelho et al., 2018).

Clone VCR6 had a higher content of accumulated sugar (23.06%) and total acids (6.07 g/L) compared to R1 (21.74% and 4.57 g/L, respectively). For comparison, Garić et al. (2025) report a sugar content of 22.51% and total acidity of 7.86 g/L in their Traminer study in the Niš wine-growing subregion (also part of the Central Serbia region). The ability of VCR 6 to accumulate more acids is especially important today, as climate change contributes to lower grape acidity (Plantevin et al., 2024).

Nistor et al. (2021) and Pedri and Petroll (2014) state that the accumulation of sugars and acids is influenced by the climatic conditions prevailing during grape ripening. High temperatures result in high sugar content and low total acidity, producing unbalanced wines

with insufficient freshness. The measured values for both R1 and VCR 6 clones meet the standards for high-quality wine production, with VCR6 showing greater potential for producing high-quality, balanced wines.

Table 4. Chemical composition of the grape must of Traminer R1 and VCR6 clones

Clones	Sugar content		Total acids (g/l)	pH	Glycoacidometric index
	°Oe	%			
R1	93.00	21.74	4.57	3.42	4.76
VCR 6	98.00	23.06	6.07	4.57	3.80

CONCLUSIONS

The analysis of the climatic conditions at the experimental field of the Faculty of Agriculture University of Belgrade "Radmilovac", indicated that in the year of examination (2024), temperature and precipitation were above the multi-year average, especially in June, July, and August which are the months with the highest measured temperatures and the highest level of precipitation.

The analysis of the mechanical composition of clusters and berries revealed greater variation for clone R 1, while clone VCR 6 proved more stable under the conditions at „Radmilovac“. Both clones were within the range of standard values for this type of clones. Clone VCR 6 had a greater cluster and berry skin mass and proportion of berries skin. In terms of the chemical quality of grape juice-must, clone VCR 6 showed higher sugar content (23.06%) and total acids (6.07 g/L).

Both R 1 and VCR 6 clones can be recommended for further planting in this wine region, as they have clearly emphasised oenological potential for the production of high quality wines.

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PROJECTION FOR THE DEVELOPMENT OF VITICULTURE IN RN MACEDONIA FOR THE PERIOD 2021-2040

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ABSTRACT

The forecast for viticulture development in RN Macedonia for the period 2021-2040 was formulated to meet the spatial planning requirements of governmental institutions. The primary indicator for developmental assessment was the vineyard area measured in hectares. This projection integrated data sourced from the State Statistical Office, cross-referenced with annual reports issued by the Ministry of Agriculture, Forestry, and Water Management, relevant viticulture literature, and the finalized 2023 Viticulture and Winemaking Strategy of RN Macedonia. Analytical procedures established in statistical methodologies were employed for data evaluation, complemented by expert insights derived from academic learning and practical field experience.

Findings underscore the extensive growth opportunities in the viticultural sector of RN Macedonia. Spatial planning initiatives anticipate an expanse of vineyards covering up to 38,000 hectares, matching the historical peaks noted in 1981.

The prevailing climate trends, characterized by escalating temperatures, necessitate adaptive measures, notably the establishment of vineyards at higher elevations, mainly within the wine-producing regions of Prespa and Ohrid.

Acknowledging the uncertainties posed by climatic fluctuations on grape cultivation and broader fruit production, a strategic shift from the traditional "French perfection" approach is advocated. Ensuring stability in production entails adopting an "American pragmatic" approach, which prioritizes the cultivation of resilient hybrid grape varieties and native American grapevine species. This strategic pivot aims to safeguard sustainable food sources for the populace amidst evolving environmental conditions.

Key words: RN Macedonia, projection, spatial planning, viticulture.

INTRODUCTION

Viticulture in RN Macedonia presents ongoing opportunities for development. The most advantageous sites for grapevine cultivation are predominantly found in the hilly terrain characteristic of the country's mountainous topography. In RN Macedonia, hilly terrains are plentiful around urban centres and rural areas. These hilly regions typically feature soils of modest fertility, rendering them unsuitable for high-yield agricultural crops and making them ideal for grapevine cultivation without competition from other agricultural activities.

The viticulture and wine industry in the RN Macedonia remains highly attractive to businesses due to abundant opportunities for expanding vineyards in hilly terrain and the integration of modern technologies facilitating automated and mechanized grape production, harvesting, and wine processing. In addition, the prevailing climate changes, characterized by escalating temperatures and necessitate adaptive measures, pushes towards establishment of vineyards at higher elevations, such as the wine-producing regions of Prespa and Ohrid.

The renowned quality of Macedonian wine, combined with the longstanding Macedonian passion for grapevine cultivation, indicates that viticulture holds significant potential for unrestricted growth from 2021 to 2040. Grape varieties cultivated for the production of premium wines typically yield around 10 tons per hectare. This low yield ensures superior wine quality, thereby significantly enhancing the value of grapes during the grape-to-wine processing phase—often increasing their value by at least threefold, and in the case of premium wines, by several tens of times.

In his book "General Viticulture" (Hristov, 2010) compiles historical data revealing the evolution of vineyard areas in the RN Macedonia. In 1912, vineyard coverage was approximately 30,000 hectares. This figure sharply declined to 6,500 hectares during the Grape phylloxera (*Daktulosphaira vitifoliae*) infestation between 1929 and 1934. By 1950, vineyard areas had rebounded to 10,800 hectares and reached a peak of 38,759 hectares in 1981, marking a historical high. According to data sourced from the Ministry of Agriculture, Forestry, and Water Management, there was a consistent decline in vineyard areas until 2010, reaching approximately 20,000 hectares. However, based on our research utilizing data from the State Statistical Office, we observed a subsequent increase in vineyard area from 21,269 hectares in 2011 to 23,776 hectares in 2021.

The Viticulture and Winemaking Strategy (MAFWE 2023) presents a comprehensive vision for advancing RN Macedonia's viticulture sector through technological enhancement. Key initiatives include strengthening the branding of Macedonian wines, establishing geographical indication labels, promoting domestic wines, and fostering wine tourism. The

strategy also prioritizes organizing grapevine growers and winemakers through associations and integrating modern agricultural technologies, such as advanced equipment (tools, machines, implement attachments) for agro-technical operations and harvesting. To support these efforts, state institutions plan substantial subsidies amounting to tens of millions of euros. The Viticulture and Winemaking Strategy addresses technologies for grape and wine production, alongside economic and social considerations. The strategy aligns with European Union standards by enacting a new wine law in 2023 aimed at establishing regulations and ensuring product quality. Mandating good agricultural practices across all production stages is a central focus. Additionally, there is a target to expand organic vineyard areas to cover 5% of total vineyard acreage and to mitigate climate change challenges by establishing new vineyards at higher elevations.

The forecast for viticulture development in RN Macedonia for the period 2021-2040 was formulated to meet the spatial planning requirements of governmental institutions. Spatial allocation considers the strategic placement of different vital crops necessary to meet the nutritional requirements of the population. Each crop is strategically placed in optimal locations to maximize the availability of grapes, fruits, vegetables, spices, grains, fodder crops, and other essentials necessary to sustain the population. This study aims to project vineyard area in the Republic of North Macedonia for the period 2021–2040, based on the anticipated population's demand for grapes and wine, while at the same time considering the needs for other agricultural crops, ensuring that the total agricultural land area is optimally allocated among all crop types.

MATERIAL AND METHODS

Data Sources

Our study relied solely on data obtained from the State Statistical Office (SSO, 2017; SSO, 2022). To ensure the reliability of this data, we cross-referenced it with annual reports issued by the Ministry of Agriculture, Forestry, and Water Management (MAFWE, 2019; MAFWE 2022). Historical trends over the past century were validated using insights from "General Viticulture" authored by Peter Hristov (2010). Our assessment also included alignment with state policies (GOV, 2024), stakeholder objectives, and the developmental trajectory of the viticulture industry outlined in the finalized 2023 Viticulture Strategy (GOV, 2023).

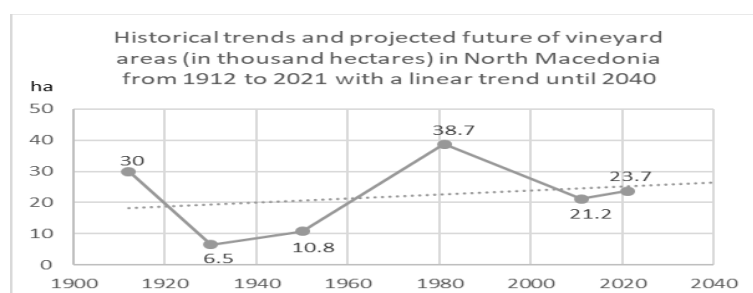
Projection method

The milestones identified in the analysis of the historical data on vineyard areas in RN Macedonia over 110 years (Hristov, 2010) serve as foundational benchmarks for trend analysis, which are further complemented by data from the State Statistical Office for the years 2011 and 2021. In addition, statistical trend analysis was employed to analyse vineyard area data in the RN Macedonia from 2011 to 2021, projecting trends up to 2040. Statistical trend analysis was employed to analyse vineyard area data in the RN Macedonia from 2011 to 2021, projecting trends up to 2040. Six trendlines were utilized: linear, exponential, logarithmic, power, second-degree polynomial, and exponential, evaluated under three scenarios—optimistic, realistic, and pessimistic (Ristevski & Tevdovski, 2008). Each of them was cross-checked with the expert opinion based on spatial planning principles, evident and expected climate change impact, policy settings (policy objectives and respective financial support), etc. to come up with the final projections.

Spatial planning was geared by identifying regions capable of effective supplying essential raw materials, particularly wine and table grapes, to support the viticulture sector. Expert viticulturists select specific sites for grapevine cultivation based on a variety of criteria. The principal metric employed to project viticulture development is the size of vineyard areas quantified in hectares, representing the most prevalent dataset within the existing literature.

RESULTS AND DISCUSSION

A linear trend analysis covering a span of 130 years forecasts a gradual expansion of vineyard areas in the RN Macedonia, reaching an estimated 26,000 hectares by the year 2040 (Figure 1).



(Source: Hristov, 2010; processed by the authors)

Figure 1. Historical trends (1912-2021) and projected vineyards areas in RN Macedonia until 2040 (000 ha)

The projections from the trend analysis forecasting vineyard areas from 2021 to 2040 indicate that vineyard areas are expected to range from a minimum of approximately 25.5

thousand hectares, based on logarithmic and power trendlines, to a maximum of around 30 thousand hectares, according to linear and exponential trendlines.

Under the realistic scenario aligned with the exponential trendline, projections indicate that the total vineyard area in the RN Macedonia will reach 29.2 thousand hectares by 2040. Significant increases are anticipated in the Southeast region by approximately 4.2 thousand hectares and in the Vardar region by 2.3 thousand hectares, while decreases are expected in all other wine-growing regions.

Table 1. Projection of vineyard areas (1000 ha) in RN Macedonia until 2040

Trend	Formula	R ²	Year				
			2021	2025	2030	2035	2040
Linear	$y = 272,15x + 21720$	0.6067	23.3	25.5	26.9	28.3	29.6
Exponential	$y = 21717e^{0,012x}$	0.6061	23.3	25.7	27.3	29.0	30.8
Logarithmic	$y = 1288\ln(x) + 21272$	0.7965	23.3	24.7	25.1	25.4	25.6
Power	$y = 21287x^{0,0568}$	0.8003	23.3	24.7	25.1	25.4	25.7
2nd degree polynomial	$y = 78,337x^2 + 1133,9x + 19997$	0.9284	23.3	20.5	13.2	2.1	0.0

(Source: SSO, 2017; SSO 2022; processed by the authors)

Table 2. Projection of vineyard areas in 2040 by regions (using exponential alignment with real projection (1000 ha))

Regions	Vineyards	Real Projection	Difference 2040-2021	Optimistic Projection	Secure Areas Proposal with Spatial Planning
	2021	2040	2040	2040	2040
Vardar	10.7	13.1	2.3	15.8	16
Southeast	6.1	10.3	4.2	12.8	13
Skopje	1.9	1.7	-0.2	2.2	2.3
East	1.6	1.4	-0.07	2.1	2.1
Northeast	1.3	1.2	-0.05	1.3	1.4
Pelagonia	0.8	0.4	-0.3	0.8	1.5
Southwest	0.6	0.5	-0.04	0.6	1.5
Polog	0.06	0.1	0.03	0.1	0.2
TOTAL	23.3	29.2	5.9	35.9	38

(Source: SSO, 2017; SSO 2022; processed by the authors)

In the optimistic scenario aligned with the exponential trendline, by 2040, all wine-growing regions in the RN Macedonia are expected to see increases in vineyard areas. The Southeast region is projected to expand by 100%, and the Vardar region by 50%. The total vineyard area is forecasted to reach 35.9 thousand hectares, which is approximately 3 thousand hectares less than the historical maximum of around 38 thousand hectares recorded in 1981.

The spatial planning projection for viticulture in RN Macedonia over the next two decades (2021-2040) aligns closely with the strategic goals outlined in the Viticulture and Winemaking Strategy (GOV 2023).

CONCLUSION

The spatial planning proposal for viticulture in RN Macedonia is based on several critical factors: strong commitment, historical background, and statistical projections.

A strong commitment from all stakeholders within the viticulture sector for further advancement and development, aligned with the objectives outlined in the 2023 Viticulture and Winemaking Strategy.

Historical data covering 110 years of vineyard areas in RN Macedonia have shown fluctuations from approximately 6 thousand hectares to a peak of 38 thousand hectares. These historical trends indicate a projected increase towards approximately 26 thousand hectares by the year 2040.

Based on the assessments, we anticipate an increase in vineyard area in RN Macedonia by 2040, ranging from a minimum of 25 thousand to a maximum of 30 thousand hectares, with an optimistic scenario projecting up to 35 thousand hectares. Effective spatial planning is crucial for managing these areas, particularly given the extensive hilly landscapes and aiming to align with the historical maximum of 38 thousand hectares achieved in 1981. The largest vineyard expanses are expected in the Vardar and Southeast regions.

Spatial planning initiatives should prioritize the development of vineyards in the Ohrid and Prespa regions, identified as highly promising for producing premium white wines, and in conditions of global warming. We recommend allocating a minimum of 2 thousand hectares for these regions, not only as a strategic measure to enhance wine quality, but also as a cost-effective measure in mitigating climate change impacts by establishing vineyards at higher altitudes.

Moreover, spatial planning plays a crucial role in wine branding, forming a core strategy in viticulture. Precise vineyard positioning tailored to specific names, climatic conditions, soil

characteristics, and enriched by regional historical context encapsulated in the concept of terroir, is fundamental to effective wine branding strategies.

Effective spatial planning should lead to the establishment of vineyard regionalization in RN Macedonia, essential for facilitating wine branding. The last regionalization effort occurred more than 55 years ago, necessitating a new one due to noticeable climate changes impacting various parameters related to climate and soil conditions.

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COULD THE SIDE SUBSTITUTE THE KING? THE IMPORTANCE OF FLOWER POSITION IN AN APPLE CLUSTER

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ABSTRACT

With the increasingly pronounced effect of climate change, frequent spring frosts are inevitable in recent seasons. In apples, the king flowers in clusters, which are considered the most valuable, are the first to be damaged, while the lateral ones are more likely to survive and ensure yield. This study aimed to reveal whether the position of the flower in the cluster matters concerning yield efficiency. The experiment was conducted in 2023, in an apple orchard of cultivar 'Fuji' grafted on M9 rootstock. Hand thinning was performed, retaining only the king or side (5th or 6th position in the cluster) flower at full bloom (KingF and SideF) and during fruit development when king fruit was 7mm in diameter (King7 and Side7). On control trees, clusters were left unthinned. At harvest, all treatments resulted in an increase in fruit size compared to the control, which had the highest proportion (80 %) of unmarketable fruits (<70 mm). Treatments KingF and King7 slightly reduced the proportion of fruits <70 mm compared to the SideF and Side7, but the yield and number of larger fruit classes did not differ among applied treatments. The moment of thinning was also not impactful. This indicates the equal potential of the side and king fruits to provide marketable yield in specific circumstances.

Keywords: *Malus domestica*, 'Fuji', fruit thinning, yield efficiency, fruit size

INTRODUCTION

The competition between the central fruit in an apple cluster, also known as the 'king', and the lateral ones is well established. The king fruit dominates, as it is normally pollinated and set first, resulting in more than 60% of cases remaining in the cluster after the June drop (Jakopič et al., 2016). Conversely, the side fruits at the 5th and 6th positions in the cluster have a significantly lower chance of survival during the process known as the 'June drop'. When laterals survive, due to the king's dominance, tend to be smaller in size and weight (Goffinet et

al., 1996; Starkus et al., 2024) and exhibit inferior fruit quality (Jakopic et al., 2015). Consequently, thinning methods are applied to favour the retention of the king fruit in each cluster, ensuring the desired fruit size and yield (Radivojevic et al., 2023).

Understanding positional differences within an apple cluster is also essential in terms of frost resistance. As winter dormancy ends, the apple tree becomes more sensitive to low temperatures, with generative buds being most vulnerable to frost injury (Salazar-Gutiérrez et al., 2016). Furthermore, as the blooming period begins, frost tolerance decreases as each phenological phase progresses (Szalay et al., 2019). The flower in the central position of the cluster opens first, while the lateral flowers develop subsequently, resulting in a threefold higher frequency of frost injuries in the king flower compared to the lateral ones (Choi et al., 2025). In the context of climate change, the risk of spring frost has increased in recent years, not only due to more frequent frosts but also because of warmer winters that lead to earlier blooming (Pfleiderer et al., 2019). Since only 5-10% of flowers need to be fertilised to ensure a regular yield in apple trees (Starkus et al., 2024), there is generally no loss of yield after a frost, on account of remaining mostly lateral flowers. While some studies underestimate lateral fruit potential, others have shown their greater development capability when competition in the cluster is reduced by removing the king or the subsequent lateral fruits (Mert et al., 2007; Miranda et al., 2005). Therefore, this study aimed to determine whether side flowers/fruits in clusters can substitute the king in terms of yield and fruit quality.

MATERIALS AND METHODS

The research was conducted in an apple orchard of cultivar 'Fuji' (Figure 1), located in Mala Ivanča, Sopot, Serbia (44.60° N, 20.61° E; 204 m a.s.l.), in 2023. The orchard was established in 2013 with a spacing distance of 3.2 m × 0.8 m, resulting in 3,906 trees per hectare. Cultivar 'Fuji' was grafted on M9 rootstock, and trees were trained as slender spindles. The orchard was covered with a black anti-hail net, supplied by a fertirigation system, and maintained through standard practices. The experiment was set up as a randomised block design with three repetitions, containing one tree within each. Uniform trees regarding vigour and the number of generative buds were selected.



Figure 1. The experimental orchard of the apple cultivar 'Fuji' during the full bloom in 2023 (a); the cluster with a single king flower – KingF (b); the cluster with a single lateral flower – SideF (c).

Hand thinning was performed at two moments: full bloom (21 April 2023) and when the central fruit in a cluster was 7 mm in diameter (2 May 2023). On both occasions, each cluster on the tree was thinned in order to retain only the king flower that opened first or the lateral flower that opened last (Figure 1b,c). Unthinned trees were left as a control (Table 1). The fruits were harvested on October 13, and classified according to their diameter into the following categories: <60 mm (pygmy), 60-70 mm, 70-80 mm, and >80 mm. The total number of fruits and the total yield per tree were determined, as well as per fruit size category. The average fruit weight was calculated based on the total fruit number and yield per tree for each treatment. Return bloom was assessed by counting the number of generative buds in the following spring.

The obtained data were analysed using SPSS 17.0 Software and one-way ANOVA. The significance of the differences between means was determined by the LSD post hoc test at $p \leq 0.05$.

Table 1. Description of the parameters in the performed treatments.

Treatment	Moment of thinning	Target position
Control	Unthinned	Whole cluster
King F	Full bloom	Central flower in a cluster
Side F	Full bloom	Side flower in a cluster
King 7	Average fruit size of 7 mm	Central fruit in a cluster
Side 7	Average fruit size of 7 mm	Side fruit in a cluster

RESULTS AND DISCUSSION

The thinning treatments, as expected, resulted in a significant reduction in total fruit number per tree compared to the unthinned control (Table 2). Consequently, a decrease in fruit number led to a redistribution in size categories and an increase in fruit weight. As a result, the control treatment recorded 46.3 pygmy fruits, while in all other treatments, the presence was negligibly low. The number of fruits in a category of 60-70 mm in diameter, which are considered unmarketable, was also the greatest in the control. Treatments SideF and Side7 recorded twice greater number of this fruit category in comparison to KingF and King7, significantly differing from each other. However, they were comparable regarding larger fruit size classes (70-80 and >80 mm) and average fruit weight.

The clusters thinned to a single fruit, either the king or the side, increased the average fruit weight by 30 to 40 % in relation to fruits that had competition in the cluster. The potential of fruit to attain weight differs based on its position within the cluster (Jakopic et al., 2015), and also depends on competition, i.e. number of fruits per cluster (Mert et al., 2007; Miranda et al., 2005). It was established that the response can vary depending on a cultivar as well, as Mert et al. (2007) suggested a cultivar-related intensity of the king's dominance effect on the lateral fruits. Another study also reported the existence of „king dominant“ and „non king dominant“ cultivars, with the latter showing the same potential of the king and side flower for reaching size in a single flower cluster, while in the former, lateral fruits stay inferior (Goffinet & Lakso, 2017). For cultivar 'Fuji', weight at harvest declined with the concurrence, whereas for the single king or lateral fruit, an equal size was confirmed (Ferree et al., 2001). This is in line with our results, whereby although the king fruits in both KingF and King7 treatments gained more weight, they did not statistically differ from the corresponding side treatments. Additionally, our results oppose the statement that the most distal lateral fruits can not reach the king size (Goffinet & Lakso, 2017), given that exactly the last flower/fruit in the cluster was retained in both SideF and Side7 treatments. Based on all, 'Fuji' could potentially be classified as a „non king dominant“ cultivar.

Table 2. The number of fruits per tree of cultivar 'Fuji', thinned to different treatments, according to fruit size categories.

Treatment	Number of fruits tree ⁻¹					Fruit weight (g)
	<60mm (pygmy)	60-70 mm	70-80 mm	>80 mm	Total	
Control	46.3±7.54a	174.3±1.45a	52.3±17.85	0.0±0.00b	273.0±15.50a	144.6±1.39b
KingF	0.0±0.00b	15.3±3.18c	41.0±11.59	82.7±7.45a	139.0±12.42bc	237.9±10.24a
SideF	4.0±2.00b	29.3±5.46b	56.7±7.26	84.3±20.85a	174.3±17.03b	210.5±16.29a
King7	0.3±0.33b	10.0±3.79c	35.0±15.72	74.0±2.08a	119.3±17.94c	246.4±21.46a
Side7	1.0±1.00b	21.0±5.03bc	62.7±12.00	57.0±4.04a	141.7±17.49bc	209.5±11.1a
Significance	***	***	ns	***	***	**

Means ± standard error in the column followed by different letters indicate significant differences at $p \leq 0.05$. ns not significant; *, **, *** significant at 0.05, 0.01, and 0.001, respectively.

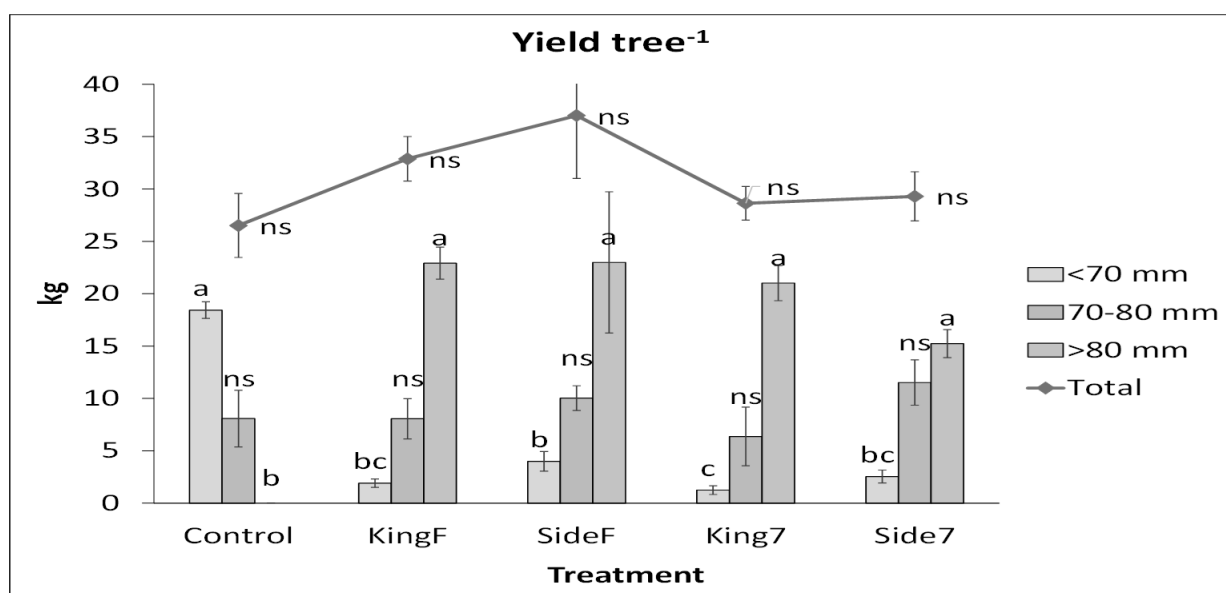


Figure 1. Distribution of fruit size classes of cultivar 'Fuji' represented as the yield per tree

Figure 1 shows the distribution of yield (kg) per tree according to fruit size category. Consistent with the distribution of fruit number, a significant reduction in unmarketable fruits in the total yield was recorded for hand thinned trees. The control treatment produced 70% of the smallest fruit size category in the total yield, whereas in thinned trees, its proportion ranged from 4% (King7) to 11% (SideF). However, this was the only category where notable differences were observed between the side and king fruit treatments; for the larger fruit size classes, treatments were comparable. The total yield per tree was also similar, but the distribution of fruit size distinguished treatments with single fruit in the cluster from the unthinned control. Mert et al. (2007) observed an increase in yield following the removal of the king fruit, but fruit weight decreased due to a still high number of fruits per cluster. With increasing cluster thinning intensity, yield also decreases to a certain level, but the average fruit

weight increases (Miranda et al., 2005). Furthermore, by leaving a single fruit in the cluster, dominance is no longer evident (Ferree et al., 2001). Among single-clustered treatments, SideF achieved the highest total yield (32.9 kg tree⁻¹), with 89% of fruits over 70 mm in diameter. The other three treatments were similar, each displaying over 90% marketable fruits in the total yield. Considering all together, the equal potential of both king and lateral fruits within the cluster, when competition is eliminated, is observed. The return bloom, representing the number of generative buds in the following year, was negligibly low, with no differences between the unthinned control and thinned treatments (Table 3). This indicates that reducing to a single fruit per cluster was not sufficient to prevent alternate bearing, due to the final fruit number and the preferences of the Fuji variety to alternate bearing. For instance, a target fruit number per tree of 90 was desirable for the ‘Golden Delicious’ cultivar to provide a regular yield (Radivojevic et al., 2023).

Table 3. The return bloom of cultivar ‘Fuji’ in spring 2024, following hand thinning treatments.

Treatment	SideF	KingF	Side7	King7	Control	Significance
Return bloom	0.33±0.33	2.00±2.00	0.67±0.67	0.33±0.33	0.00±0.00	Ns

Data are means ± standard error. ns not significant.

CONCLUSIONS

Based on the obtained results, the following conclusions can be drawn:

- There were no differences between the treatments in total fruit number and yield, but the distribution of fruit size classes improved, with a reduction to a single fruit per cluster.
- The side and king fruits showed equal potential to provide marketable yield when competition in the cluster was eliminated.
- King and lateral fruits in the apple cluster can attain comparable fruit weight at harvest.
- The moment of thinning was not impactful in our study.
- Reducing to a single fruit per cluster was not sufficient to prevent alternate bearing in the Fuji cultivar.

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GENETICS, BREEDING AND GENETIC RESOURCES

BASIC MORPHOLOGICAL CHARACTERISTICS OF ORIENTAL VARIETIES FROM THE TYPES YAKA, DJEBEL AND BASMAK

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ABSTRACT

Seven oriental varieties, owned by the Scientific Tobacco Institute– Prilep (three varieties of Yaka, two of Djebel and two of the Basmak type), were examined for morphological traits: plant height with inflorescence, number of leaves per plant and dimensions of the leaves from the middle belt. The experiment was set up at the Experimental Field at STI-Prilep, in 2022, in a randomized block design with four replications, using traditional agricultural practices. Measurements were taken during the budding and flowering stages of plant development. The results were statistically processed.

The aim of the research is to study the morphological traits of oriental genotypes, to determine the stability of the population through their variability and to highlight the best among the selected assortment.

The examined varieties showed a high degree of homogeneity, as a result of their homozygosity. The coefficient of variation of the traits was less than 10%, which is a sign of their stability. In terms of morphological characteristics, all varieties belong to certain types of tobacco. The varieties JK 141 1 and JK 10-7/ JK 10-7/1 dominated in terms of the number of leaves per plant. JK 261 is distinguished by the largest leaves in the middle zone of the plant, while JK 10-7/1, JK 141 and Dzebel N01 are distinguished by the smallest dimensions. The paper offers valuable data for producers and buyers of oriental tobacco.

Keywords: oriental tobacco, quantitative traits, variability.

INTRODUCTION

Plant breeding is a long-term successive process based on phenotypic and genotypic studies of available plant genotype material. The most common purpose of the studies are

morphological traits that are related to yield. These are varietal characteristics that are greatly influenced by changes in the external environment. In order to draw more reliable conclusions, multi-year research is necessary.

A large number of papers have been published by breeders whose subject of research is tobacco. For example: Ramachandra et al. (2015), performed genetic analysis of 62 genotypes in Nipaniat Belgaum, Karnataka – India, for several morphological traits. The best among the parents were Vairam, MS NPN-190, MS A-119 and Kunkumatri. The hybrids MS PL-5 x Vairam and MS GT-4 x Thangam were superior to the control variety. Mitreski and Korubin-Aleksoska (2015), examined some morphological traits in oriental tobacco varieties, and announce that Prilep Basma 82 had the highest average leaf number, and the lowest length and width of the middle belt leaves. Dimitrieski and Miceska (2015), offer information about quantitative traits of varieties from the oriental type Yaka, and as the best variety point out Yaka b 65 - 82/1. Kinay and Yilmaz (2016), conducted experimental trials with hybrids obtained by semi-diallel crosses between the varieties: Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa, in the Tokat province of Turkey, and found an average heterosis of 28.4%. The heterotic effect for stalk height and dry matter yield was 4%. Korubin – Aleksoska and Ayaz (2016 - 16), investigated height of the stalk with inflorescence, leaf number, length of the middle belt leaves and dry mass yield per stalk in five autochthonous tobacco varieties of types: Prilep, Djebel and Yaka tobaccos, and five commercial oriental varieties of Prilep tobacco (P-23, P-84, NS-72, P-66-9/7 and P-79-94). Differences between the genotypes in the investigation period were highly significant, which is a sign of their mutual genotypic and phenotypic diversity. Korubin – Aleksoska and Ayaz (2016 - 17), studied ten oriental tobaccos of the types: Basmak (MK-1, MK-2, MB-2, MB-3, MS-8/1, MS-9/3 and YZ-7), Prilep (Prilep Basma-82), Djebel (Xanthi Djebel-1) and Yaka (YV 125/3), for some more important quantitative traits, and gave precise knowledge of the new type Basmak in Macedonia and the Balkans from genetic, morphological and agronomic aspects. All Basmak varieties are characterized by stability and uniformity as a result of their homozygosity. Korubin – Aleksoska and Arsov (2016) studied five old domestic oriental varieties of the types: Prilep, Djebel and Yaka, and five commercial oriental varieties of the Prilep type for the number of leaves over a two-year period. The authors determined highly significant differences between the variants for the studied trait, as well as their high stability and homozygosity. Korubin – Aleksoska et al. (2018), examined ten genotypes of oriental tobacco, including three varieties and seven newly created lines, for the height of the stalk without inflorescence and the number of leaves per stalk, in order to evaluate the variability and stability of the variants and to designate the most superior

ones in the selected assortment. They found that the top three lines had a high degree of homogeneity. Vitanova and Dyulgersk (2018), studied eight genotypes of oriental tobacco Nevrokop for height of plants and number of leaves, during the three year period. All new lines outperformed the standard variety Nevrokop 1146 in the studied traits. Krumovgrad 90 variety had the highest number of leaves, and the lowest Line 1. The newly-selected lines with the highest value are Line 3 and Line 2, which can successfully be included in future breeding programs. Kinay et al. (2016), studied the effect of heterosis on yield in oriental tobaccos, with special emphasis on those grown in the Black Sea region of Turkey. The two-year research included a semidiallel of seven genotypes and 21 F1 hybrids, in an experiment set up in two locations (Erbaa and Bafra). The best yield and quality were shown by Xanthi-2A x Katerini and Nail x Katerini, grown in Erbaa, and Nail × Katerini and Katerini × Erbaa, grown in Bafra. Kurt et al. (2021), examined the characteristics of 21 lines and four standard varieties, in an experiment set up under different environmental conditions, in order to select the most stable lines – candidates for promising varieties. The ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 were the most stable lines from Basma tobacco type. The ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 were the most stable lines from Basma tobacco type. Malinova, S. (2021), studied two new lines of oriental tobacco and three varieties of high-quality tobacco ecotype Dupnica (Rila 82, Dupnica 160 and Rila 544 - standard), in the field of the Tobacco Experimental Station - Rila, at the Institute of Tobacco and Tobacco Products - Markovo, for the most important morphological traits and for the quality of dry tobacco. The data show that lines 505 and 506 have significantly higher yield and quality compared to the control variety. Aleksoski et al. (2023), performed three-year studies for number of leaves per stalk and the length, width and area of the leaves from the middle part of the stalk – traits directly related to the yield in the offspring of the first generation, in four mother parent tobacco varieties of different types (three of the Prilep type and one of the Basmak type), one pollen donor parent Burley B-1-91, and their four F1 hybrids. The hybrids MS 8/1 x B-1/91 and P 18-50/4 x B-1/91 represent very interesting material for future tobacco breeding activities. Spasova-Apostolova et al. (2023-15), in a three-year experiment set up in the village of Markovo, investigated the most important quantitative traits in four tobacco genotypes of the Krumovgrad ecotype - the most preferred by commercial companies in Bulgaria. They found that in terms of height, the lines belong to the group of oriental tobaccos. All had a greater number of leaves than the control variety Krumovgrad 90, and Krumovgrad 72 is distinguished by the biggest number. Line 71 is distinguished by a greater length of the

14th and 21st leaves. Lines 71 and 73 had the highest average yield (kg/da). Lines 72 and 73 have the highest percentage of 1st class.

The aim of this paper is the analysis of the most important morphological traits of seven oriental tobacco varieties from the types Yaka, Djebel and Basmak, placed in a comparative experiment in 2022, at the Scientific Tobacco Institute – Prilep. Our intention is, by popularizing the data obtained, to offer tobacco buyers, as well as tobacco producers, varieties known for their quality and the pleasant aroma.

MATERIALS AND METHODS

In 2022, thirty varieties of tobacco were sown at the Experimental Field at the Scientific Tobacco Institute - Prilep. The seeds were sown in cold-treated beds, on plots of 1 m², using 0.3 g for each variety separately. The agrotechnical and protective measures applied during seedling production were the same for all varieties. The area for planting the experiment was prepared with one autumn and three spring plowings. Soil fertilization was applied with 250 kg/ha NPK (8:22:20), with the second spring plowing. The experiment was set up on 3.06.2022 and appropriate agrotechnical and protective measures were applied. The aim was to renew and maintain the collection of seed material, located in the Gene Bank of STI-Prilep. The tobacco was planted manually, at a distance of 45 cm from row to row and 12 cm from stalk to stalk in the row, in 4 rows or 104 stalks of each variety separately. The varieties were cultivated manually twice and irrigated 4 times with a drip system during the growing season. In order to obtain healthy seed material, appropriate protective measures were taken.

The oriental varieties of the following types were used as material for this work: Yaka (JK 10-7/1, JK 261 and JK 141). Djebel (Djebel N01 and Djebel 38) and Basmak (MK-1 and MB-3). Ten plants per replicate were measured, which means 40 plants per variety. Measurements of quantitative traits (height of the plant with inflorescence, number of leaves per plant, length, width and area of the leaves from the middle belt of the plant) were made in the budding and flowering stages, and the results were statistically processed. The area of the leaves was calculated by multiplying the length, width and the coefficient $k=0.6354$. At the end of the vegetation period, seed material was also collected.

RESULTS AND DISCUSSION

Today, in the Republic of North Macedonia, the oriental types Yaka, Djebel and Basmak are produced on very small areas. The Department of Genetics, Selection and Seed Control at

the Scientific Tobacco Institute - Prilep is responsible for their maintenance. The varieties belonging to the mentioned types are distinguished by a particularly pleasant aroma. The wishes and needs of tobacco buyers are variable, so breeders should be ready at any time to offer seed material in accordance with the current trends.

In our work, we popularize original data on the morphological traits from the varieties , which we believe will be useful for producers and buyers of oriental tobacco.

Height of plants with inflorescence

The highest among the studied genotypes is YK 261 (148 cm) and the lowest is Djebel N01 (114 cm). In terms of this characteristic, all varieties belong to the listed tobacco types. The standard deviation ranges from 2.6 cm (YK 261) to 7.8 cm (Djebel N01), and the coefficient of variability - CV from 1.7% (YK 261) to 6.8% (Djebel N01). The coefficient of variation in all varieties is lower than 10%, which is a sign of uniformity of their populations.

The values for height of the plants with inflorescence are shown in Table 1.

Number of leaves per plant

The number of leaves per plant is the most studied morphological trait, included in all selection programs, because it is directly related to the yield.

The highest number of leaves is distinguished by YK 141 (57). Second ranked is YK 10-7/1 (52) and the variety with the lowest number of leafes is YK 261 (26). The standard deviation ranges from 0.5 cm (YK 261 and Basmak MK 1) to 1.5 (YK 141). The coefficient of variation ranges from 1.3% (Basmak MK 1) to 5% (Basmak MB 3). In all varieties, CV is lower than 10%, which indicates the fact that the trait is uniform.

The values for the number of leaves per plant are shown in Table 1.

Length of the leaves from the middle belt of the plant

The longest leafes from the middle belt of the plant has YK 261 (28.9 cm) and the variety with shortest leafes is YK 10-7/1 (19.5 cm). The statistical processing of the data showed values for the standard deviation ranging from 0.3 cm (YK 141) to 1.1 cm (MB-3). The coefficient of variation has percentage values from 1.5% (YK 141) to 4.5% (MB-3). Low variability (CV < 10) is an indicator for the stability of the trait in all studied varieties.

Width of leaves from the middle belt of the plant

The widest leaves has YK 261 (14.4 cm) and the narrowest leaves has YK 10-7/1 (12 cm). The standard deviation ranges from 0.35 cm (YK 141) to 0.64 cm (MK-1), and the CV ranges from 2.68% (YK 141) to 4.85% (MK-1). The CV in all varieties is lower than 10%, which is a sign that this is a stable trait.

Leaf area of the middle belt of the plant

The biggest leaf area was observed in YK 261 (264 cm²), and the smallest in YK 10-7/1 (149 cm²). The standard deviation ranges from 7.6 cm² (YK 10-7/1 and YK 141) to 11.8 cm² (Djebel 38), and the coefficient of variability from 3.1% (YK 261) to 5.6% (MK-1). For this trait, the CV in all varieties is lower than 10%, which is a sign of its stability and uniformity.

Table 1 shows the dimensions (length, width and area) of the leaves from the middle belt of the plant

Table 1. Morphological traits of varieties from the types Yaka, Djebel and Basmak (2022)

Varieties	Height of plants with inflorescence (cm)			Number of leaves per plant			Dimensions of the leaves from the middle belt of the plant				
	$\bar{x}$	σ ($\pm$)	CV (%)	$\bar{x}$	σ ($\pm$)	CV (%)	Length (cm)	Width (cm)	Area (cm ²)		
	$\bar{x}$	σ ($\pm$)	CV (%)	$\bar{x}$	σ ($\pm$)	CV (%)	$\bar{x}$	$\bar{x}$	$\bar{x}$	σ ($\pm$)	CV (%)
1. YK 10-7/1	125	6.3	5.1	52	1.3	2.6	19.5	12.0	149	7.6	5.1
2. YK 261	148	2.6	1.7	26	0.5	2.1	28.9	14.4	264	8.2	3.1
3. YK 141	135	5.3	4.0	57	1.5	2.7	23.9	12.8	194	7.6	3.9
4. Djebel N ⁰ 1	114	7.8	6.8	32	0.6	2.0	24.0	13.0	198	10.1	4.9
5. Djebel 38	137	4.6	3.4	41	0.7	1.8	26.5	13.0	219	11.8	5.4
6. MK-1	136	5.2	3.8	38	0.5	1.3	24.3	13.2	204	11.5	5.6
7. MB-3	119	4.4	3.7	29	1.4	5.0	24.9	12.7	201	9.1	4.5

CONCLUSIONS

From the obtained data, we drew the following conclusions:

- The variety YK 261 with inflorescence was the highest, and had the largest dimensions of the leaves from the middle belt. This variety has the smallest number of leaves. The variety YK 141 has the largest number of leaves. The smallest dimensions of the leaves from the middle belt were examined in YK 10-7/1.

- All examined genotypes showed a coefficient of variability (CV) lower than 10%, which means that the variability of the properties is insignificant, i.e. these are highly heritable properties in stable uniform varieties.

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PHYTOMEDICINE-PLANT PROTECTION

RACE DIVERSITY IN THE POPULATIONS OF *PUCCINIA TRITICINA* IN THE AREAS OF THE BULGARIAN BLACK SEA COAST

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ABSTRACT

Leaf rust caused by *Puccinia triticina* is a major fungal pathogen on wheat in Bulgaria and in years with suitable climatic conditions and presence of susceptible varieties it can considerably reduce yields. Sometimes, in the warmer, more humid and coastal regions, more aggressive pathotypes may occur, and if the cultivars grown in these regions are susceptible to the pathogen, the wheat production may be compromised. This study presents data on the *Puccinia triticina* pathotypes most widespread along the Bulgarian Black Sea coast during vegetative growth periods 2022/23 and 2024/25. Thirteen phenotypically different pathotypes were identified. With the highest frequency were pathotypes PKTTR (20.8%) and TKTTR (12.5%). This study also presents data on the efficiency of the *Lr* genes in these regions. As absolutely efficient were identified genes *Lr* 2b, *Lr* 9, *Lr* 19, *Lr* 22b, *Lr* 41, *Lr* 43 and *Lr* 47. High efficiency was found in genes *Lr* 20, *Lr* 40 and *Lr* 48. With good efficiency were genes *Lr* 25, *Lr* 29, *Lr* 39 and *Lr* 42, and satisfactory was the efficiency of genes *Lr* 2c, *Lr* 24 and *Lr* 51. All other investigated genes were either with low efficiency or absolutely inefficient. The monitoring studies were crucial for the early detection of new virulent races with the potential to cause devastating epidemics. The presented data can contribute to the better understanding of the population structure of *Puccinia triticina* and the pathogen's migration in the Black Sea region, as well as to the development of efficient strategies for control, including choice of cultivars carrying a combination of several resistant genes, which can protect the crop from the early stages to its harvesting.

Key words: Black Sea Coast, effectiveness, *Lr* genes, leaf rust, pathotypes.

INTRODUCTION

Wheat is a key crop in Bulgarian agriculture and economy. It is among the most widespread and traditional crops in the country. An average of 1-2 million ha are annually sown with wheat. Bulgaria produces considerably higher amounts of wheat than it consumes and the export to primarily Near East countries and the EU is a stable source of income for the national economy. The development of sustainable and competitive wheat production is significant for the future of agriculture in Bulgaria.

Wheat is however frequently attacked by leaf rust (*Puccinia triticina*), which is one of the most widespread and economically important diseases on this crop in Bulgaria. The disease affects photosynthesis and causes accumulation of sugars and proteins in grain. The attacked plants often form smaller spikes and the reduced test weight is a key parameter of quality.

The area of the Bulgarian Black Sea coast is characterized by higher humidity and long-lasting dew, especially in spring and the beginning of summer. The alternation of humid spring periods with fogs, dew and wind are the perfect conditions for the development of leaf rust. The higher crop density, the later date of sowing and the use of susceptible cultivars increase the risk of disease occurrence. This is the reason for the lower yields from these coastal areas. The annual observations on the efficiency of the isogenic lines, the breeding of resistant varieties and studies on the pathogen's virulence are important measures for prevention and control of leaf rust (Volkova et al., 2022).

The aim of this investigation was to determine the models of virulence of the *Puccinia triticina* isolates, collected from the coastal areas of the Black Sea on the territory of Bulgaria, and to study the genetic variability in the population of the pathogen during vegetative growth seasons 2022/23 and 2024/25, as well as the efficiency of the individual *Lr* genes in this region.

MATERIAL AND METHODS

The samples of plant material were collected from the Bulgarian coastal zones of Black Sea along the line from location Shabla to location Burgas during vegetative growth seasons 2022/23 and 2024/25 (Table 1). The samples contained flag leaves with uredospore infection from different cultivars at each location. The leaves with uredospores were dried at room temperature and placed in an exsiccator, then refrigerated at 4° C until their development and analyzing for pathotype differentiation. The analysis was carried out at the Plant Protection Laboratory of Dobrudzha Agricultural Institute – General Toshevo (DAI). From each sample,

collected from the respective location, 3 monospore isolates of *P. triticina* were obtained, following the procedure for work with rusts (Ivanova, 2012).

Table 1. Samples of uredospore material collected from different locations along the Black sea coast of Bulgaria.

2022/23		2024/25	
Location	Number of samples	Location	Number of samples
Staro Oryahovo	1	Tulenovo	1
Goritza (Varna)	1	Shaba	1
Burgas	1	Kichevo (Varna)	1
		Goritza (Varna)	1
		Burgas	1

Single uredospore isolates were tested for virulence to 20 isogenic lines of Thatcher, each containing 1 gene for resistance to the pathogen. The Thatcher lines were included in 5 sets, each of 4 differentiators: set 1- *Lr* 1, *Lr* 2a, *Lr* 2c, *Lr* 3; set 2 – *Lr* 9, *Lr* 16, *Lr* 24, *Lr* 26; set 3 - *Lr* 3ka, *Lr* 11, *Lr* 17, *Lr* 30; set 4 – *Lr* B, *Lr* 10, *Lr* 14a, *Lr* 18; set 5 – *Lr* 3bg, *Lr* 14b, *Lr* 20, *Lr* 28.

All 52 isogenic lines, including the differentiation sets, were sown in tin boxes, and cultivar *Michigan amber* was involved as a check variant. Eight to nine days old seedlings were inoculated with the uredospores of each isolate for each set of differentiators.

The response of the isogenic lines and differentiators was read 9-12 days after inoculation. The isolates, which produced infection type 0, 0;, 1, 2, 0-1, 0-2 with small to medium uredospores with necrosis and/or chlorosis, gave immune response and were classified as avirulent, while the isolates which produced infection type 3 and 4 with medium to large uredospores, without chlorosis and necrosis, were classified as virulent. Each isolate was given a 5-letter code based on virulence/avirulence to each of the 5 sets with 4 differentiators in accordance with the North American nomenclature for virulence to *P. triticina* (Long & Kolmer, 1987) and modified by Kolmer et al. (2013).

A criterion for breeding value of the *Lr* genes was considered their efficiency. The efficiency of each isogenic line carrying a single gene for resistance was calculated as percent of avirulent isolates according to their total number, using the formula

$$\text{Gene efficiency (\%)} = \frac{\text{Number of avirulent isolates}}{\text{Total number of isolates}}$$

Total number of isolates

RESULTS AND DISCUSSION

The Black Sea Coast is a specific zone in Bulgaria characterized by mild climate with warmer winters and cooler summers in comparison to the continental regions of the country. The proximity of the Black Sea and the sea breeze influence the climate of the coastal areas. The amount and regime of rainfalls depend on the atmospheric circulation and the availability of land and sea. The average annual precipitation is about 450 mm. The absolute air humidity is higher than the rest of the country. The winds are strong, from the north and north-west, frequent and occasionally continuous. All these characteristics – the higher humidity and the strong winds in these coastal areas – are favorable for the development and cross-contamination with *P.triticina* uredospores. A total of 24 monospore isolates were developed from the collected samples (9 during vegetative growth season 2022/23 and 15 - during 2024/25). The analysis carried out revealed that the virulent diversity of *P. triticina* along the Black Sea coast included 13 phenotypically different pathotypes presented in Table 2.

Table 2. Virulent phenotypes of *P. triticina* and frequency of occurrence determined in the coastal areas of Black Sea

Pheno type	Virulence genes	2022/23		2024/25		Total	
		No	%	No	%	No	%
PKTTR	1,2c,3,3ka,3bg,10,11,14a,14b,16,17,18,24,26,28,30,B	4	16.6	1	4.2	5	20.8
TKTTR	1,2a,2c,3,3ka,3bg,10,11,14a,14b,16,17,18,24,26,28,30,B	2	8.3	1	4.2	3	12.5
RKTTR	1,2a,3,3ka,3bg,10,11,14a,14b,16,17,18,24,26,28,30,B	1	4.2	1	4.2	2	8.3
THTTT	1,2a,2c,3,3ka,3bg,10,11,14a,14b,16,17,18,26,28,30,B	0	0	2	8.3	2	8.3
PHTTR	1,2c,3,3ka,3bg,10,11,14a,14b,16,17,18,26,28,30,B	0	0	2	8.3	2	8.3
RKTTT	1,2a,3,3ka,3bg,10,11,14a,14b,16,17,18,20,24,26,28,30,B	0	0	2	8.3	2	8.3
THTTTR	1,2a,2c,3,3ka,3bg,10,11,14a,14b,16,17,18,26,28,30,B	1	4.2	1	4.2	2	8.3
MHTTR	1,3,3ka,3bg,10,11,14a,14b,16,17,18,26,28,30,B	1	4.2	0	0	1	4.2
THTTM	1,2a,2c,3,3ka,3bg,10,11,14a,16,17,18,26,28,30,B	0	0	1	4.2	1	4.2
RKTTQ	1,2a,3,3ka,3bg,10,11,14a,14b,16,17,18,24,26,30,B	0	0	1	4.2	1	4.2
RKTRK	1,2a,3,3ka,10,11,14b,16,17,18,20,24,26,28,30,B	0	0	1	4.2	1	4.2
RHTTR	1,2a,3,3ka,3bg,10,11,14a,14b,16,17,18,26,28,30,B	0	0	1	4.2	1	4.2
RHTTM	1,2a,3,3ka,3bg,10,11,14a,16,17,18,26,28,30,B	0	0	1	4.2	1	4.2

Pathotype PKTTR was with the highest percent of distribution (20.8%), followed by pathotypes TKTTR (12.5%) and pathotypes RKTTR (8.3%), THTTT (8.3%), PHTTR (8.3%), RKTTT (8.3%) and THTTTR (8.3%). Four of the pathotypes (PKTTR, TKTTR, RKTTR and THTTTR) were identified during both vegetative growth periods (2022/23 and 2024/25). Eight

pathotypes (PHTTR, RKTTT, THTTT, THTTM, RKTTQ, RKTRK, RHTTR and RHTTM) were found only during the second period and were missing during the previous one, while pathotype MHTTR was identified only during season 2022/23 and was absent during 2024/25. According to unpublished data, the pathotypes predominant on the territory of Bulgaria are TKTTR (15.9%), THTTR (14.3%) and PKTTR (9.5%). Gulyaeva (2020) reported that pathotype THTTR was present in the Russian population of *P. triticina* during 2001-2018, and pathotype THTTM, which was registered in samples from India, Nepal and Bhutan, according to Bhardwaj et al. (2021) has been the predominant pathotype in India for the last 20 years. Sohail (2015) also reported that pathotype THTTM was among the five predominant pathotypes in the *P. triticina* population in Pakistan. According to data by Kudinova et al. (2024), pathotypes THTTR, PHTTR and TKTTR were the most frequent pathotypes of *P. triticina* in the southern parts of Russia. Ukraine belongs to the Black Sea basin and is a major producer and exporter of grain. The distribution of virulent races of *P. triticina* in Ukraine is highly important due to its proximity to Bulgaria and the easy migration of the pathogen. Babayants et al. (2015) pointed out that during 2013-2014, 12 phenotypes were identified on the territory of Ukraine, the most predominant ones being TGTT (24%) and TJTT (8%). Their investigations showed that virulence for gene *Lr* 19 was not found, while the virulence for gene *Lr* 9 was about 13%. They also reported low frequency of virulence for genes *Lr* 29 and *Lr* 47. Often the transfer of uredospores by the air currents may happen also from the south.

A study carried out during 2009 – 2010 in the regions of West Marmara, Black Sea and the Eastern Mediterranean of Turkey identified 44 virulent phenotypes, the most frequent pathotype in 2009 being FHPTQ (15.4%), and in 2010 the most frequent was FCMSS (16.7%), (Kolmer et al., 2013). The authors pointed out that virulence for genes *Lr* 9 and *Lr* 24 was not found during this period.

Increased occurrence of this disease in the recent years was also observed in several regions of Romania. About 20 single isolates were identified with comparatively low but valid genetic polymorphism in East Romania in the regions of Fundulea, Livada and Pitesti (Dinu et al., 2016). The study was based on RAPD and SSR analyses and it was found out that the wheat genotypes available in these regions were with race-specific resistant profile.

The results from our investigation confirmed that the coastal areas of the Black Sea are an active zone of pathogen evolution of leaf rust. The frequent occurrence of new races requires adaptability approach to the breeding of new wheat varieties.

This study presents the efficiency of 52 isogenic lines based on cultivar Thatcher, which contain a single gene for resistance each. According to unpublished data, absolute efficiency of

genes *Lr* 9, *Lr* 19, *Lr* 41, *Lr* 43 and *Lr* 47 was determined for the entire territory of Bulgaria. The data on the Black Sea region revealed that absolute efficiency in juvenile stage was also found for genes *Lr* 2b and *Lr* 22 b. Therefore, these genes can be considered a priority for incorporating in new varieties. Genes *Lr* 20, *Lr*40 and *Lr* 48 were determined as highly efficient for this region (70-95%). With good efficiency (50-70%) were genes *Lr* 25, *Lr* 29, *Lr* 39, *Lr* 42. Satisfactory (30-50%) was the efficiency of genes *Lr* 2c, *Lr* 24 and *Lr* 51. All other genes were with low efficiency or were absolutely inefficient. Table 3 presents the efficiency of the individual *Lr* genes in the Black Sea region during vegetative growth seasons 2022/23 and 2024/25

Table 3. Efficiency of *Lr* genes to *P. titicina* in the Bulgarian Black Sea Coast during 2022/23 and 2024/25 growing seasons

<i>Lr</i> genes	2022/23		2024/25				<i>Lr</i> genes	2022/23		2024/25			
	Avirulence isolates	%	Avirulence isolates	%	Total number	Average %		Avirulence isolates	%	Avirulence isolat es	%	Total number	Average %
1	0	0	0	0	0	0	27+31	0	0	1	6.6	1	4.2
2a	5	55.5	2	13.3	7	29.2	28	1	11.1	1	6.6	2	8.3
2b	9	100	15	100	24	100	29	4	44.4	11	73.3	15	62.5
2c	2	22.2	7	46.6	9	37.5	30	0	0	0	0	0	0
3	0	0	0	0	0	0	32	0	0	1	6.6	1	4.2
3ka	0	0	0	0	0	0	33	0	0	0	0	0	0
3bg	0	0	1	6.6	1	4.2	34	0	0	1	6.6	1	4.2
9	9	100	15	100	24	100	35	0	0	0	0	0	0
10	0	0	0	0	0	0	36	0	0	0	0	0	0
11	0	0	0	0	0	0	37	0	0	0	0	0	0
14a	0	0	1	6.6	1	4.2	38	0	0	0	0	0	0
14b	0	0	2	13.3	2	8.3	39	6	66.6	8	53.3	14	58.3
15	0	0	0	0	0	0	40	9	100	11	73.3	20	83.3
16	0	0	0	0	0	0	41	9	100	15	100	24	100
17	0	0	0	0	0	0	42	7	77.7	7	46.6	14	58.3
18	0	0	0	0	0	0	43	9	100	15	100	24	100
19	9	100	15	100	24	100	44	1	11.1	0	0	1	4.2
20	9	100	9	60.0	18	75.0	45	0	0	0	0	0	0
21	0	0	0	0	0	0	46	2	22.2	2	13.3	4	16.6
22 A	0	0	0	0	0	0	47	9	100	15	100	24	100
22 B	9	100	15	100	24	100	48	7	77.7	13	86.6	20	83.3
23	0	0	1	6.6	1	4.2	51	6	66.6	5	33.3	11	45.8
24	1	11.1	8	53.3	9	37.5	52	2	22.2	5	33.3	7	29.2
25	4	44.4	8	53.3	12	50.0	60	0	0	2	13.3	2	8.3
26	0	0	0	0	0	0	63	0	0	1	6.6	1	4.2
B	0	0	0	0	0	0	64	0	0	2	13.3	2	8.3

Genes *Lr* 9 and *Lr* 19 have maintained their absolute efficiency on the territory of Bulgaria, including the Black Sea region, for a long period of time. According to data by Ivanova (2012), the first pathotypes overcoming the resistance of these two genes, were registered during 2005 – 2007. Since then, their efficiency remains absolute.

Volkova et al. (2022) presented data on the efficiency of the *Lr* genes at adult and juvenile stages on the territory of Russia during 2011-2020. Their data indicated that lines with genes *Lr* 9, *Lr* 42 and *Lr* 43+*Lr* 24 remained immune for a period of 10 years at all stages of the plant development, while lines with genes *Lr* 19, *Lr* 24, *Lr* 29, *Lr* 36, *Lr* 37, *Lr* 38, *Lr* 43, *Lr* 45, *Lr* 47 and *Lr* 50 demonstrated efficiency in field trials, but at juvenile stage immune lines were not identified during 2011-2020. Volkova et al. (2020) also reported increased frequency of virulent

isolates on the territory of Russia for genes *Lr* 14a, *Lr* 16, *Lr* 19, *Lr* 21, *Lr* 28, *Lr* 30, *Lr* 33, *Lr* 40, *Lr* 45, *Lr* W and *Lr* 50 during 2018-2020. The studies of Babayants et al. (2015) showed that genes *Lr* 9, *Lr* 19, *Lr* 29 and *Lr* 47 were highly efficient for the territory of Ukraine both at juvenile stage and at adult plant stage, while genes *Lr* 24, *Lr* 42, *Lr* 50, *Lr* 51 and *Lr* 56 were efficient only at adult stage.

CONCLUSIONS

The areas along the Bulgarian Black Sea coast have unique climate – they are frequently exposed to strong winds, higher air humidity and temperatures, which facilitate the distribution of *P. triticina* uredospore material. This, combined with a varied set of wheat varieties carrying different genes for resistance, explains the high rate of race variability in the *P. triticina* populations in this region.

As a result from the investigation carried out, 13 phenotypically different pathotypes of *P. triticina* were identified, which demonstrated adaptability to widely distributed varieties during season 2022/23 and 2024/25. With highest percent of distribution were pathotypes PKTTR (20.8%) and TKTTR (12.5%). Absolute efficiency was established for genes *Lr* 2b, *Lr* 9, *Lr* 19, *Lr* 22b, *Lr* 41, *Lr* 43 and *Lr* 47. Genes *Lr* 20, *Lr* 40 and *Lr* 48 were highly efficient in this region. With good efficiency (50-70%) were genes *Lr* 25, *Lr* 29, *Lr* 39 and *Lr* 42. Satisfactory (30-50%) was the efficiency of genes *Lr* 2c, *Lr* 24 and *Lr* 51.

The monitoring studies on *P. triticina* are decisive for the early detection of new virulent races with the potential of devastating epidemics. The data from this study showed that the interregional migration of the *P. triticina* pathotypes was high. Some races were probably transferred by the air currents or through sowing material. Therefore, regional coordination is required in the monitoring and the varietal policy. These are the more specific aspects of such a necessity:

- 1) Regular observations on the crops during the months from April to June;
- 2) Developing a regional database with the *P. triticina* pathotypes common for the Black Sea basin (including Bulgaria, Romania, Ukraine and Turkey).
- 3) Systematic varietal resistance in accordance with the landraces – use of local adapted varieties which demonstrate higher resistance to the pathogene along the Black Sea coast under actual conditions.

The presented results can contribute to the understanding of the structure of the population and the migration of the pathogene in the Black Sea basin and can be useful for the development

of efficient strategies for control, including choice of resistant varieties of wheat and the application of suitable practices for crop management.

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VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

RESEARCH OF LETTUCE GROWING IN FLOWING WATER CULTURE ON THE DİCLE RIVER

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ABSTRACT

The study was conducted to investigate the feasibility of growing lettuce on the surface of the Dicle River, which is in constant flow and where no agricultural activities are carried out, for the purpose of evaluating the river. The study was conducted in the 2023 growing season on the Dicle River and along the riverbank under field conditions. To observe different results based on variety, early lettuce varieties (*Lactuca sativa*) ‘Iceberg, Curly, and Yedikule’ were used in the research. In the study, seedlings placed on styrofoam were cultivated by fixing them on the river, while seedlings were planted simultaneously in plots prepared along the river as a control group to set up the experiment. The parameters examined in the study were total plant weight, marketable head weight, marketable yield, total number of leaves, plant and root lengths, water-soluble dry matter content, titratable acidity, pH, 0-4 damage index, relative water content of leaves, membrane damage in leaves, leaf area index, chlorophyll and carotenoid contents, and color contents in leaves (L*, a*, b*) were investigated. When the data obtained at the end of the study were examined, it was seen that lettuce varieties grown in different environments were more advantageous in terms of many parameters studied compared to soil cultivation. It can be said that lettuce grown on rivers is superior to soil cultivation in terms of quality and market value.

Keywords: Aquaponic, Cultivation, *Lactuca sativa*, River water, Yield, Quality

INTRODUCTION

Securing basic food supplies for humans and animals is becoming increasingly difficult. The main reasons for this situation include the growing world population, the gradual depletion of agricultural land or its encroachment by non-agricultural areas (factors), and the depletion of scarce resources in the universe as a result of the convergence of multiple problems such as global warming. In response to this problem, the scientific community has embarked on new

quests to find alternative solutions. One solution is to emphasize soil disinfection processes and the quantity and variety of fertilizers used. However, it has been observed that the fertilizers and pesticides used in these processes do not improve soil quality; rather, they cause a decline in soil fertility and environmental pollution. Meanwhile, the amount of fertilizer applied per unit area has increased, making it necessary to apply more fertilizer to the soil. This has led to the accumulation of harmful residues in plants and humans, as well an increase in diseases. As a solution to the problem of both the depletion of soil on Earth and the inability to obtain the desired quantity and quality of produce from a given area, research has been begun on soil-less cultivation methods. Originally developed as a solution for infertile soils under cover, soilless cultivation has evolved over time and is now practiced in open field conditions as well. Soilless cultivation is a more efficient production method than soil-based agriculture, with production costs half as high (Özkan, 2014). Many greenhouses around the world are now being designed with soilless cultivation systems to increase profits. Due to insufficient agricultural land and unfavorable climate conditions for feeding the rapidly growing world population, soilless cultivation has increased worldwide (Pardossi et al., 2011). Soilless agriculture is developing rapidly in countries such as the Netherlands, Belgium, Italy, and Japan. Although this form of agriculture dates back approximately 60 years in our country, numerous studies have been conducted on this growing practice from many different perspectives in recent years, and research continues (Gül, 2008; Batem, 2013). Soilless cultivation is primarily used to grow garden plants, especially vegetables and ornamentals. It is a method of growing plants using nutrient solutions in a medium that supports their roots (Varış, 1991). The International Society for Soilless Cultivation (ISOSC) defines it as "the cultivation of non-aquatic plants in completely inorganic media supported by nutrient solutions." Soilless agriculture is also referred to as soilless culture, nutrient culture, or chemical culture (Sevgican, 2003). Soilless culture, which has been developed with technology, enables sustainable agriculture by significantly reducing the use of chemical pesticides and intensive fertilizers. This has become a production system advocated for environmental reasons (Hernanz et al., 2007; Hernanz et al., 2008; Cecatto et al., 2013). Soilless agriculture is an agricultural model that effectively and efficiently meets plants' nutritional and water requirements at optimal levels using nutrient solutions without causing stress to the plants. A mixture of two or more materials is typically required when preparing the growing medium in soilless agriculture to prevent adverse conditions and increase plant resistance to stress (Çıtak et al., 2007; Özer & Uzun, 2013). In this cultivation method, the main goal is to increase productivity and product quality. In line with all these objectives, soilless farming is a technique used in areas where there is no soil or

where the soil is unsuitable for plant cultivation (e.g., rocky, stony areas, shallow soil depth, salinity, high lime content, high and low pH, disease and pest infestation, etc.), and it can be defined as a system that provides a perfect environment (physically and chemically) for the plant root zone and guarantees increased productivity and quality by minimizing risks in production (Bozköylü et al., 2010). Soilless cultivation involves growing plants in stagnant or flowing nutrient solutions or solid media fed by nutrient solutions (Güneş et al., 2012). These systems provide the necessary water and nutrients to the root environment in the required amounts or apply nutrients to the leaves (Gül, 2008). Examining areas in Europe where soilless agriculture is practiced, rockwool and coconut fiber have been reported as the most used substrates for tomato cultivation, which has the highest production (Peet and Heuvelink, 2005). In Turkey, substrates such as coconut fiber, rockwool, and perlite are used as growth media (Toprak & Gül, 2013). In our survey studies conducted to investigate greenhouses practicing soil-less agriculture in Diyarbakır, it was determined that the most used substrate is peat moss + perlite. Even greenhouses initially established with soil have been converted to soil-less systems, as have newly established greenhouses. However, due to natural events and the depletion of scarce resources worldwide, the sources used to heat greenhouses have become costly, making production in greenhouses expensive. Soilless cultivation has sometimes been limited due to costs and implementation issues. In this regard, scientists and producers have resorted to different methods and alternative applications. From this perspective, there has been a shift toward growing plants in rivers to reduce increasing costs and to apply soil-less cultivation in open fields. Considering consumer demands and ease of implementation, lettuce cultivation has emerged as a leading application. Therefore, transforming the idea of using river water as a cultivation medium is expected to accelerate the creation of new agricultural areas and employment opportunities. The Dicle River's proximity to the city and its parallel course with agricultural lands, the absence of agricultural activity on the river thus far, the novelty of the application, and the lack of additional costs for producers when using river water are all factors that make the application mandatory. The high consumption of lettuce in the region, its availability throughout the year, and the fact that the Dicle River is calm and does not have a high flow rate have also led to the idea that growing lettuce on the river surface will contribute significantly to agriculture and employment and will be a new practice in the region, which is why this study was conducted.

MATERIALS AND METHODS

The study was conducted during the spring growing season of 2023 on the Dicle River and in the open fields adjacent to it in Diyarbakır province. To observe variety-specific differences in lettuce (*Lactuca sativa*), the research used the early-maturing varieties 'Iceberg,' 'Curly,' and 'Yedikule.' Seedlings of these varieties were obtained from a specialized seedling company in Antalya.

The trial's establishment:

The study was conducted in two environments: the Dicle River (aquaculture) and open fields (soil cultivation). Divided plots were used according to the trial design, with four replicates and 72 seedlings used for each application. For the experiment on the river surface, 18 holes (5–7 cm in diameter) were drilled at 20-cm intervals in the styrofoam to accommodate the seedling baskets. The holes were then filled with a pumice-perlite mixture, and the baskets containing the seedlings were placed in them. This ensured that the seedlings remained stable on the water's surface. Thus, 18 seedlings on each polystyrene block constituted one repetition, and four polystyrene blocks constituted four repetitions in the experiment. Within the scope of the experiment, the seedlings were transferred to the water surface on March 10. On the same date, the control group seedlings were planted in the ground along the riverbank, and the same fertilization and spraying methods were applied.

The Hoagland nutrient solution was applied as fertilizer once every two weeks using a backpack sprayer. During fertilization, the polystyrene blocks were pulled to the edge of the water, held in place by ropes, fertilized, and returned to their original positions. To combat diseases and pests, seedlings were pulled to the edge of the water and visually inspected during fertilization. Since no diseases or pests were observed, no pesticides were applied. To determine the composition of the river water, analyses were performed at the Diyarbakır Food Control Laboratories on the river water before the experiment and three weeks after it was set up. At the end of the study, the harvested plants were brought to the pomology laboratory at our faculty, where measurements and observations were taken. The UVVIS SP-3000 Plus Spectrophotometer was used for the analysis.

The measurements and observations taken are listed below;

Total Plant Weight (g): Plants were harvested with their roots in the experiment. They were washed in running water to clean them. Then they were weighed.

Marketable Head Weight (g): After removing the outer leaves and roots of the harvested plants, the average marketable head weight was measured.

Total Number of Leaves (number per plant): The total number of leaves on the harvested plants in the experiment was counted and recorded.

Marketable Leaf Count (count/plant): All edible leaves on the plants were counted and recorded.

Marketable yield (ton/da): The marketable yield obtained from a strawberry field or plot was first calculated based on the field or plot, and then the calculation was applied to one decare.

Vitamin C (mg/100 g): According to Cemeroğlu (1992), 100 g of the sample were weighed and an equal amount of 6% metaphosphoric acid solution was added and blended with the sample. Then, 25 g of the homogenized sample was transferred to a 100 mL flask. The flask was filled to 100 mL with a 3% metaphosphoric acid solution. The sample was thoroughly shaken and filtered. Then, 10 mL of the filtered sample was titrated with 2.6 dichlorophenolindophenol solution until a pink color appeared. The acid in the sample was calculated using the following equation:

$$\text{Ascorbic acid (mg/100 g)} = (V \times F \times 100)/W$$

V: Amount of 2,6-dichlorophenolindophenol solution consumed in titration (ml)

F: The factor of the 2,6-dichlorophenolindophenol solution, or the amount of ascorbic acid equivalent to one milliliter of the solution (milligrams).

W = Amount of sample contained in the filtrate used in the titration (g).

Water-soluble dry matter (%): After the water is removed from the plant samples in the laboratory, one to two drops are placed on the prism of the digital refractometer, and the water-soluble dry matter is expressed as a percentage.

Titrateable acidity (%): According to Cemeroğlu (1992), 10 mL of the plant samples, from which the water had been removed, were titrated with a 0.1 N NaOH solution until the pH reached 8.1. The titration results were calculated as a percentage of citric acid using the following formula:

Titrateable acidity (%): Titrateable acidity was calculated as follows:

$$\text{Titrateable acidity (\%)} = (V.F.E.100)/M$$

V: Amount of 0.1 N NaOH solution consumed (mL)

F: Normality of the base used in the titration. If the normality of the solution is 0.1, then $F = 1$.

E: Equivalent acid amount of 1 mL of 0.1 N NaOH (g).

M: Actual amount of the titrated sample (mL or g).

pH: The plant samples, which were taken separately for each parcel, were ground in a blender to extract the water. The pH of the resulting plant juice was then measured using a pH meter.

Green Evening 0-4 Scale Assessment: This scale was used to describe visible symptoms of potential stress in plants. In stress and tolerance screening studies, scores ranging from 0 to 4 were assigned based on the following symptoms (Demir et al., 2012):

No effect (control plants): 0

Slowing of plant growth, local yellowing, and leaf curling (1).

Leaves showing 25%-50% necrotic spots and falling off (2).

Leaves showing 50-75% necrosis and death (3); and

Leaves showing 75-100% necrosis and death (4).

Severe necrosis in leaves at a rate of 75-100%, or complete plant death (4).

Leaf water content (%): At the end of the experiment, the fresh weight of the plant leaves was recorded. After soaking the leaves in pure water for four hours, they were removed from the water, dried, and their turgor weights were determined. The leaves were then dried in a 65°C oven for 48 hours, and their dry weight was recorded in grams. Leaf water content was calculated based on our data by substituting values into the following formula (Sanchez et al., 2004; Türkan et al., 2005).

Membrane Damage in Leaf Cells: Four leaves from the growing tip of each plant were used at the end of the experiment, with two plants from each replicate. Membrane damage was calculated by measuring the amount of electrolyte released from the cell (Fan and Blake, 1994; Długołęcka and Kacperska-Palacz, 1978).

For this purpose, leaf discs with a diameter of 1 cm were soaked in ionized water. They were soaked for 4 hours. After that, their EC was measured. Then, the same discs were heated at 100°C for 10 minutes, after which the EC value of the solution was measured again. The percentage change relative to the control was calculated using the following formula:

$$\text{Membrane Damage Index} = (L_t - L_c / 1 - L_c) \times 100$$

L_t : EC of the stressed leaf before autoclaving/EC after autoclaving

L_c : EC of the control leaf before autoclaving/EC after autoclaving.

Leaf Area Index: The leaf area index (LAI), expressed as leaf area per unit area (leaf area/m²), was measured at the end of the experiment using a Licor LAI-220 Plant Canopy Analyzer on at least six plants in each replicate.

Fresh and dry weights of green plant parts (g): At the end of the experiment and at the end of the vegetation period, the entire green part of the plant was weighed on four plants in each replicate. Then, it was dried at 70°C for 48 hours until a constant weight was reached, after which the dry weight was measured.

Chlorophyll a content: Samples of 0.25 g were taken from lettuce leaves, homogenized in 80% acetone in a dimly lit place away from direct light, filtered, and the extract was made up to 25 mL with acetone. The prepared samples were read at a wavelength of 663 nm (Lichtenthaler and Wellburn, 1983; Zengin, 2007; Amira and Qados, 2011; Kabay, 2014; Alp and Kabay, 2017).

$$\text{Chlorophyll a (mg/g)} = (12.7 * 663 \text{ nm}) - (2.69 * 645 \text{ nm}) * V / W * 10,000.$$

Chlorophyll b content: Samples of 0.25 g were taken from lettuce leaves, homogenized in 80% acetone in a dimly lit area away from direct light, filtered, and the extract was made up to 25 mL with acetone. The samples were read at a wavelength of 645 nm (Lichtenthaler & Wellburn, 1983; Zengin, 2007; Amira & Qados, 2011; Kabay, 2014; Alp & Kabay, 2017).

$$\text{Chlorophyll b (mg/g)} = (22.91 * 645 \text{ nm}) - (4.68 * 663 \text{ nm}) * V/W * 10,000.$$

Total chlorophyll content: 0.25 g samples of plant leaves were homogenized in 80% acetone in a dimly lit area away from direct light. The samples were filtered and the extract was made up to 25 mL with acetone. The prepared samples were read at a wavelength of 652 nm (Arnon, 1949; Lichtenthaler & Wellburn, 1983; Zengin, 2007; Amira & Qados, 2011; Kabay, 2014; Alp & Kabay, 2017).

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}.$$

Carotenoid Amount: 0.25 g of samples were taken from plant leaves, homogenized in 80% acetone in a dimly lit place away from direct light, filtered, and the extract was made up to 25 mL with acetone. The prepared samples were read at a wavelength of 470 nm.

$$\text{Carotenoid (mg/g)} = (1000 * 470 \text{ nm}) - (3.27 * \text{chlorophyll a}) - (104 * \text{chlorophyll b}).$$

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Determination of Proline Content: Proline extraction was performed using 0.5 g of fresh leaf material. Absorbance values of standards containing 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mmol of proline were read at a wavelength of 520 nm. A proline standard curve was then created based on these values. The proline amount was calculated in mmol according to this standard curve.

Color Changes in Plant Leaves: L*, a*, and b* values were determined using a PCE-TCR 200 portable color measurement device to detect color changes resulting from different planting dates and soil-based versus soil-free cultivation.

Nitrate analysis: This was performed as a service at the Science and Technology Application and Research Center affiliated with our university.

Statistical analyses: The data obtained were subjected to variance analysis using the JUMP 7.0 software package. Means with significant differences were grouped using the LSD multiple comparison test (JUMP, 2007).

RESULTS AND DISCUSSION

The data obtained from this study investigating lettuce cultivation using different varieties in river surface water culture are presented below. The data for all examined parameters are given in Tables 1, 2, and 3.

Table 1 shows that lettuce grown in the river is taller than lettuce grown in soil, regardless of variety. Yedikule lettuce was found to have a plant height of 18.01 cm in the river and 15.50 cm in soil. For the Iceberg variety, the plant height was 14.27 cm on the river and 12.44 cm in soil. For the Curly variety, the plant height was 15.09 cm on the river and 10.63 cm in soil. Cultivation on the river was found to be statistically significant ($p < 0.01$) compared to cultivation in soil for all three varieties. Similar results were observed for plant root length, stem diameter, L*a*b* color values, titratable acidity, carotenoid content, plant and root wet and dry weight, plant pH, and leaf water content. In other words, higher results were found in river-based cultivation than in soil-based cultivation for each of the above parameters.

Examining the vitamin C content of the plant leaves collected at the end of the trial (Table 1) revealed no statistically significant difference between river and soil cultivation for the Yedikule and Iceberg varieties. However, a statistically significant difference ($p < 0.01$) was found in the curly lettuce variety. The vitamin C content of curly lettuce grown in the river was found to be higher than that of lettuce grown in soil and other lettuce varieties (15.97).

Examining the plant samples taken at the end of the experiment revealed that the amount of proline secreted by the plants as a defense mechanism under stress conditions was higher in all three varieties grown in soil (Table 1). The proline content of plants grown in the river

averaged around 1.00 for all three varieties, while the average for plants grown in soil was around 1.23–1.24.

At the end of the trial, the yield values of the varieties in different growing environments were examined and found to be statistically significant ($p < 0.05$). Generally, the yields of plants grown in the river were higher than those grown in soil. The highest yield for the Yedikule lettuce variety was 786 tons/da for plants grown in the river and 679 tons/da for those grown in soil.

Examining the data in terms of plant weight revealed that the highest weight was obtained from plants grown in the river for all three varieties. All three lettuce varieties were found to be statistically significant in terms of plant weight ($p < 0.01$). For the Yedikule variety, the average weight of lettuce grown in the river was 158.37 g, while the average weight of lettuce grown in soil was 139 g. The average weight of iceberg lettuce grown in the river was 161 g, while the average weight of lettuce grown in soil was 134 g. The average weight of curly lettuce grown in the river (175 g) was also higher than that grown in soil (146 g).

Table 1. Statistical results of some data obtained from the study

Sort	Application	Plant height (cm)	Root length (cm)	Body diameter (mm)	Vitamin C (mg/100g)	l *	a *	b *	Titrateable Acidity (%)	Leaf area
Yedikule	Up the river	18.01± 0.13a	17.96± 0.25a	14.48± 0.11a	11.69± 0.06a	51.17±0.28 b	-7.42± 0.32a	32.41± 1.22 a	2.71±0.01 b	105.34± 2.37 a
	Soil	15.50±0.13 b	10.70±0.25 b	8.51±0.11 b	11.74± 0.06a	70.11± 0.28a	-6.10± 0.32a	32.02± 1.22 a	3.07± 0.01a	107.28± 2.37 a
	p value	<.0001**	<.0001**	<.0001**	0.7051	<.0001**	0.0578	0.8766	<.0001**	0.7032
	CV	6.73	9.46	5.09	1.66	2.56	-25.91	20.75	0.89	7.05
	LSD	14.87	19.96	16.05	0.67	50.73	7.28	24.34	0.98	25.87
Iceberg	Up the river	14.27± 0.11a	10.37± 0.12a	11.60± 0.23a	5.53± 0.16a	48.86±0.47 b	-7.50±0.18 b	27.86± 0.42a	2.70±0.01 b	93.21± 2.73 a
	Soil	12.44±0.11 b	8.21±0.12 b	8.71±0.23 b	5.53± 0.16a	61.61± 0.47a	-5.46± 0.18a	28.36± 0.42a	3.04± 0.01a	98.65± 2.73 a
	p value	<.0001**	<.0001**	<.0001**	0.4601	<.0001**	<0.0001	0.5593	0.0001**	0.3752
	CV	4.53	7.03	12.60	2.89	4.62	-15.54	8.21	1.21	9.01
	LSD	5.34	6.21	9.01	1.29	35.19	6.56	8.5	0.9	32.65
Curly	Up the river	15.09± 0.11a	15.97± 0.33a	10.84± 0.10a	15.97± 0.33a	54.41±0.35 b	-7.66±0.20 b	31.89± 0.31a	2.70±0.01 b	107.60± 9.23 a
	Soil	10.63±0.11 b	10.50±0.33 b	8.67±0.10 b	10.50±0.33 b	60.31± 0.35a	-6.44± 0.20a	33.06± 0.31a	3.07± 0.01a	87.33± 9.23 a
	p value	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	0.0076	0.0826	<.0001**	0.3337
	CV	4.73	13.84	5.55	13.84	3.31	-15.27	5.25	0.89	29.94
	LSD	1.02	1.57	0.86	1.57	2.06	1.17	0.06	1.00	112.64

Continued from Table 1

Sort	Application	Chlorophyll a	Chlorophyll b	T. Chlorophyll	Carotenoid	Marketable Head Weight (g)	Proline	Marketable Yield (tons/da)	Dry root (g)	Plant dry (g)
Yedikule	Up the river	0.44± 0.00 a	0.61± 0.00 a	1.22± 0.00 a	1.66± 0.00 a	128.29± 1.28 a	0.99±0.02 b	786.59± 11.88 a	4.20± 0.02a	14.40± 0.03a
	Soil	0.07±0.00 b	0.06±0.00 b	0.07±0.00 b	0.38±0.00 c	109.66±1.28 b	1.23± 0.02a	679.92±11.88 b	2.68±0.02 b	12.59±0.03 b
	p value	<.0001*	<.0001*	<.0001*	<.0001*	0.0019	0.0063	0.0109	<.0001*	<0.0001
	CV	1.58	0.029	0.015	0.027	3.39	6.73	5.12	2.55	1.29
	LSD	0.04	1.89	0.95	0.50	51.65	0.71	312.29	4.08	4.86
Iceberg	Up the river	0.16± 0.01a	0.20± 0.00 a	0.29± 0.00 a	1.01± 0.00 a	137.05± 2.81 a	1.03±0.01 b	849.72± 17.42 a	3.97± 0.01a	13.34± 0.04a
	Soil	0.06±0.01 b	0.08±0.00 b	0.14±0.00 b	0.76±0.00 b	111.24±2.81 b	1.24± 0.01a	689.66±17.42 b	3.05±0.01 b	11.10±0.04 b
	p value	0.0073	0.0008	0.0008	<.0001*	0.0101	0.0012	0.0101	<.0001*	<0.0001
	CV	12.16	3.81	3.45	0.12	7.16	3.62	7.16	1.99	1.97
	LSD	0.033	0.024	0.018	0.034	8.54	0.58	466.86	2.45	6.02
Curly	Up the river	0.97± 0.01a	1.01± 0.00 a	1.20± 0.00 a	2.26± 0.00 a	145.57± 1.05 a	1.00±0.01 b	902.55± 6.50 a	1.72± 0.01a	17.06± 0.13a
	Soil	0.43±0.00 b	0.54±0.01 b	0.93±0.00 b	1.34±0.00 b	130.00±1.05 b	1.27± 0.01a	805.99±6.50 b	1.17±0.01 b	11.67±0.13 b
	p value	0.0002	0.0002	<0.0001	<.0001*	0.0018	0.0008	0.0018	<.0001*	<0.0001
	CV	0.037	1.72	0.25	0.12	2.41	4.14	2.41	4.77	4.77
	LSD	2.10	0.035	0.027	0.031	43.18	0.76	267.42	1.47	14.44

Continued from Table 1

Sort	Application	Damage Scale	Total Plant Weight (g)	Root weight (g)	Number of Marketable Leaves (Pieces)	Total number of leaves (pieces)	TSS (%)	pH	Leaf Relative Water Content (%):	Membrane Damage
Yedikule	Up the river	1.07± 0.14a	158.37± 0.35a	42.02± 0.16a	8.87± 0.24a	12.73± 0.25a	4.07± 0.04a	5.92±0.02 b	67.49± 0.33a	7.47±0.05 b
	Soil	1.53± 0.14a	138.45±0.35 b	26.76±0.16 b	6.07±0.24 b	9.53±0.25 b	3.69±0.04 b	6.82± 0.02a	51.50±0.33 b	8.31± 0.05a
	P value	0.1103	<,0001*	<,0001*	<0.0001	<0.0001	0.0006	<0.0001	<,0001*	<0.0001
	CV	57.66	1.29	2.55	17.24	12.29	6.02	1.99	3.07	3.25
	LSD	3.14	28.55	40.76	8.81	9.87	1.32	2.44	43.09	2.44
Iceberg	Up the river	0.93± 0.16a	160.77± 0.53a	39.17± 0.13a	8.53± 0.16a	12.73± 0.21a	5.60± 0.09a	6.10±0.02 b	55.97± 1.54 a	7.72±0.21 b
	Soil	0.87± 0.16a	133.81±0.53 b	30.12±0.13 b	7.60±0.16 b	10.73±0.21 b	4.82±0.09 b	6.79± 0.02a	39.59±1.54 b	9.67± 0.21a
	P value	0.8358	<,0001*	<,0001*	0.0104	0.0003	0.0009	<0.0001	0.0001*	0.0004
	CV	96.07	1.97	1.99	10.72	9.93	9.67	2.10	17.69	13.35
	LSD	3.15	31.66	24.22	4,001	6.81	2.76	1.9	53.99	6.69
Curly	Up the river	1.27± 0.21a	175.02± 0.90 a	17.06± 0.13a	8.47± 0.28a	8.80± 0.25a	3.54± 0.03a	6.12±0.01 b	48.92± 0.48a	7.50± 26.12 a
	Soil	1.67± 0.21a	146.15±0.90 b	11.67±0.13 b	6.47±0.28 b	7.00±0.25 b	3.14±0.03 b	7.00± 0.01a	41.51±0.48 b	60.95± 26.12 a
	P value	0.3605	<,0001*	<,0001*	0.0028	0.0033	<0.0001	<0.0001	<,0001*	0.3235
	CV	79.01	3.07	4.77	20.25	17.64	5.59	0.97	5.83	417.90
	LSD	0.71	0.04	1.28	1.59	1.98	0.29	0.06	3.3	130.46

Table 2. shows the elemental content of the following elements in river water: B, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Ni, As, Se, Sb, Zn, Cd, Hg, and Pb. Elements were analyzed for B, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Ni, As, Se, Sb, Zn, Cd, Hg, and Pb in water samples taken at the beginning and end of the experiment. Statistical analysis revealed that the concentrations of some elements in the water samples taken when the lettuce was planted increased or decreased compared to the concentrations in the water samples taken at the end of the season. No change was observed in elements such as boron (B), zinc (Zn), cadmium (Cd), mercury (Hg), and lead (Pb). Research indicates that the greater the increase in parameter values, the greater the extent of damage. Therefore, it can be concluded that the presence of these elements in the water, in terms of their Na, Mg, Al, K, Ca, Fe, Ni, and Sb content, has reached a harmful level. However, it should be noted that river water is in constant flow and cannot have stable content. Additionally, the ecosystem can be affected by environmental factors. Additionally, the presence of various processing facilities near the river will make its content even more unstable.

Table 2. Content of water samples taken from the river

Sample sample	11B (ppm)	23 Na (ppm)	24 mg (ppm)	27 Al (ppb)	39 Ka (ppm)	44 Ca (ppm)	52 Cr (ppb)	55 Mn (ppb)	56 Fe (ppb)	57 Fe (ppb)	60 Ni (ppb)	75 As (ppb)	78 Se (ppb)	121 Sb (ppb)
Head of work	0.03± 0.00 a	8.06±0.00 b	12.00± 0.00 a	29.37±0.00 b	1.41±0.00 b	50.38±0.00 b	0.24± 0.00 a	0.35± 0.01a	16.82±0.00 b	33.38±0.00 b	0.85±0.00 b	0.76± 0.00 a	0.28± 0.00 a	0.22±0.00 b
Working Middle	0.03± 0.00 a	9.21± 0.00 a	13.50±0.00 b	32.79± 0.00 a	1.80± 0.00 a	51.76± 0.00 a	0.04±0.00 b	0.25±0.01 b	19.57± 0.00 a	39.20± 0.00 a	0.97± 0.00 a	0.64±0.00 b	0.19±0.00 b	11.33± 0.00 a
P Value	0.0705	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0098	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV	1.277228	0.015749	0.012767	0.009518	0.080217	0.004736859	1.252083	10.74901	0.003886	0.006666189	0.034729	0.314571	0.810008	0.020488
LSD	0.01	3.07	4.01	9.15	1.04	3.7	0.54	0.28	7.37	15.57	0.34	0.35	0.25	28.11

Continuation of Table 2.

	66 Zn (ppb)	111 Cd (ppb)	201 Hg (ppb)	208 Pb (ppb)
Head of work	<0.000	<0.000	<0.000	<0.000
Working Middle	<0.000	<0.000	<0.000	<0.000
P Value				
CV				
LSD				

Table 3 shows the heavy metal content of lettuce leaves. At the end of the experiment, we attempted to determine the amounts of certain heavy metals in the leaves. When lettuce grown on the river was compared with lettuce grown in soil, it was found that the heavy metal content was generally higher in all three types of lettuce grown on the river. The higher heavy metal content in river-grown vegetables is thought to be due to transported soil and other waste. Despite using the same water as in soil cultivation, the difference is thought to be due to soil's ability to eliminate heavy metals. Examining the data on lettuce content reveals that metals above 1.00 ppm are generally harmful. Therefore, it can be concluded that all three types of lettuce have harmful levels of arsenic (As) and lead (Pb).

Table 3. Some heavy metal contents of lettuce leaves

Sort	Application	As (ppm)	Cd (ppm)	Hg (ppm)	Pb (ppm)
Yedikule	Up the river	1.81± 0.01a	0.24± 0.00 a	0.01± 0.00 a	3.99± 0.00 a
	Soil	1.73±0.01 b	0.21±0.00 b	0.01±0.00 b	3.93±0.00 b
	P value	0.0062	0.0131	0.0204	0.0001
	CV	1.22	4.84	34.61	0.14
	LSD	0.21	0.09	0.03	0.14
Iceberg	Up the river	1.63± 0.01a	0.24± 0.01a	0.02± 0.00 a	4.50± 0.03a
	Soil	1.60± 0.01a	0.22± 0.01a	0.01± 0.00 a	4.31±0.03 b
	P value	0.1116	0.1475	0.1636	0.0286
	CV	1.60	8.03	65.39	2.02
	LSD	0.13	0.08	0.05	0.59
Curly	Up the river	1.96± 0.00 a	0.34± 0.00 a	0.02± 0.00 a	4.24± 0.02a
	Soil	1.93±0.00 b	0.31±0.00 b	0.01± 0.00 a	4.19± 0.02a
	P value	0.0042	0.0238	0.1183	0.2368
	CV	0.40	0.01	37.42	1.33
	LSD	0.08	0.09	0.03	0.23

CONCLUSIONS AND RECOMMENDATIONS

Upon examining the data obtained at the end of the study, it was found that different lettuce varieties grown in river environments generally contribute to more parameters than those grown in soil. Therefore, it can be concluded that lettuce grown in rivers is superior to soil cultivation in terms of quality and market value.

Therefore, it was concluded that river cultivation, one of the study's objectives, would contribute positively compared to soil cultivation. In this context, the study demonstrated that hydroponic and aquaponic cultivation will prevent disruption to lettuce cultivation due to the gradual loss of agricultural land.

The Dicle River is the heart of Diyarbakır and is used for both drinking water and irrigating agricultural land. The famous Hevsel Gardens, located within the World Cultural Heritage Site (UNESCO) where the province's summer and winter vegetables are produced, are also supplied by this river. Unfortunately, the decline in fertility observed in all agricultural soils, as well as the intensive use of fertilizers and pesticides, is also increasing in our province. In this regard, we anticipate that cultivating lettuce in the water flowing in the Tigris River will free us from our dependence on soil and reduce the amount of fertilizers and pesticides used. It is believed that this will encourage producers to adopt cleaner, more environmentally friendly production models.

One supporting study could involve growing lettuce on agricultural land adjacent to the area where river farming will take place. This would allow for a comparison of yield, quality, and production costs. If cultivation on the river is carried out at a lower cost as predicted, subsequent studies will apply the research on winter vegetables (lettuce) to summer vegetables. It is planned to serve as a model for local farmers due to its advantages. The aim is to contribute to agriculture and animal husbandry, the main sources of livelihood in our province experiencing migration. The successful results obtained from cultivating lettuce and cucumbers in two different vegetation periods using soil-less, still, and flowing water cultures in the greenhouses of our faculty will be evaluated as a flowing water culture using river water. It is intended to serve as an exemplary model applicable to other parts of our country in the near future. This will eliminate the increasing costs of greenhouse heating or cooling and reduce dependence on greenhouses while enabling lettuce cultivation in Diyarbakır on the river, independent of soil, as is done in open field conditions. Products are anticipated to reach the market quickly, leveraging the advantage of a shorter vegetation period in soilless agriculture, as previously achieved and supported by scientific data. Lettuce cultivation is particularly widespread in the Hevsel Gardens, and lettuce consumption holds special cultural significance in the region. Historically, the Hevsel Gardens met most of Diyarbakır's lettuce needs, and the lettuce grown there is called “Hevsel lettuce” (Pirinç et al., 2016). Therefore, it can be said that lettuce cultivation and consumption in the region have great potential.

Many studies support the idea that hydroponic cultivation offers higher quality and appeal than soil cultivation and reduces the vegetation period. Thus, it is important to recognize that growing lettuce in water will bring it to market faster and help feed more people.

Due to global seasonal changes, countries' rainfall patterns have shifted and water resources have begun to decline. Cultivation in rivers is also believed to prevent water evaporation. Since more water is used in soil cultivation than in hydroponic cultivation, water savings are also expected. This study can be considered an open-air application of salad and lettuce cultivation in "aquaculture," which is currently trending upward and is mostly practiced in closed environments. It is believed that providing suitable conditions for growing lettuce in open aquaculture will offer more natural and less costly production opportunities.

These systems are expected to contribute to our country's economy and should be implemented in more regions as model applications. As these applications become more widespread, the "Protected River," containing cleaner, less polluting waste, will enable plant production and underwater life in the Dicle River and many other similar locations. Controlled water flow and regulated facilities that discharge waste into the river will produce "clean rivers and environmentally friendly agricultural products."

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EFFECT OF LED LIGHTING ON THE GROWTH AND DEVELOPMENT OF LEMON BALM (*MELISSA OFFICINALIS* L.) EXPLANTS UNDER *IN VITRO* CONDITIONS

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ABSTRACT

Lemon balm (*Melissa officinalis* L.) is a plant from the Lamiaceae family, commonly known as lemon balm or lemon herb due to its lemon-scented leaves. It is widely cultivated for its culinary and medicinal properties and has broad applications in various processing industries.

Light is a key factor influencing plant physiology, growth, development, and chemical composition. Two main types of lighting are used in plant cultivation: SL-type (sunlight) and FAL-type (artificial light). FAL-type lighting allows precise control of growth conditions and supports stable, year-round production regardless of seasonal or climatic changes.

This study aimed to examine the effect of LED lighting on the growth and development of lemon balm (*Melissa officinalis* L.) explants under *in vitro* conditions. Fluorescent light was used as the control, while red, blue, and a combination of red and blue LED lights were tested as treatments. Morphological traits were evaluated, including number of leaves, root development, number of lateral shoots, plant height, and fresh and dry biomass.

The results indicated that red LED light promoted the greatest plant height and the highest percentage of root development. The combined red and blue LED treatment resulted in the highest number of leaves and lateral branches, as well as the greatest average fresh and dry mass. Statistically significant differences in plant growth and development were observed across treatments, suggesting that LED lighting can serve as an effective alternative to conventional fluorescent lighting under *in vitro* conditions.

Keywords: *in vitro* culture, LED lighting, *Melissa officinalis*, morphological traits, plant growth.

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is a plant belonging to the family Lamiaceae. Its common names are “lemon balm” or “lemon herb,” referring to the lemon scent released when the leaves are rubbed. It is cultivated worldwide due to its culinary and medicinal properties and has broad applications in various processing industries. It plays an important role in phytotherapy for reducing stress and anxiety, possesses antioxidant properties valuable in pharmaceutical applications, and is widely used in the production of perfumes, cosmetics, food products, and herbal teas. Its leaves are also consumed fresh as salad vegetables in different parts of the world.

Plant tissue culture, or *in vitro* culture, involves the cultivation of plant parts (explants) separated from the mother plant on an artificial nutrient medium under sterile, controlled laboratory conditions. Due to the small size of explants, plant propagation through *in vitro* culture is often referred to as micropropagation (Pierik, 1987; Hartmann et al., 1990). This technique allows rapid multiplication of plants under uniform conditions and provides a suitable system for studying the effects of environmental factors, such as light quality, on plant growth and development.

Light is one of the key factors influencing plant physiology, growth, development, and chemical composition. The most significant effect of light in plants is on photosynthesis, which utilizes photosynthetically active radiation (PAR), encompassing wavelengths of 400–700 nm.

Red (600–700 nm) and blue wavelengths (420–460 nm) are the most effective in driving photosynthesis due to the absorption capacity of chlorophyll a and b, which capture light (Rihan et al., 2022)

In general, there are two types of plant illumination: SL-type (sunlight) and FAL-type (fully artificial light). FAL-type allows precise control of plant growth conditions, thereby enabling stable production regardless of season or climate. Recently, light-emitting diodes (LEDs) have been increasingly used as a light source in FAL-type systems because they consume less electricity and are more durable. LEDs can emit specific wavelengths, with red and blue light being particularly effective for maximizing chlorophyll absorption. Red and blue LEDs can therefore be successfully applied to support photosynthesis and light-dependent plant responses (Teklić, 2012.).

Plants absorb red and blue light, which exert the greatest impact on their growth and development. Red light generally stimulates the formation of adventitious shoots in most plants and is important for the development of the photosynthetic apparatus, whereas blue light contributes to chlorophyll formation, chloroplast development, stomatal opening, enzyme synthesis, and photomorphogenesis (Goh, 2009; Kang et al., 2008).

The influence of LEDs on growth parameters, morphology, yield, and the edible quality of plants has been the subject of increasing scientific research. Moreover, numerous studies have confirmed the effects of LEDs on chemical composition, such as vitamin C content, soluble sugars, chlorophyll concentration, protein levels, as well as antioxidant activity in certain plant species. Despite the broad application of LEDs in plant research, information regarding their effects on *Melissa officinalis* L. under *in vitro* conditions remains limited. Previous studies on lemon balm have primarily focused on its phytochemical composition and medicinal value, whereas the influence of different light spectra on its *in vitro* growth and development has not been sufficiently addressed.

Therefore, the aim of this study was to investigate the effects of different LED light spectra on the growth and development of lemon balm (*Melissa officinalis* L.) explants under *in vitro* conditions.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory for certification of seed and planting material (*in vitro* division) of the Institute of Horticulture, Faculty of Agriculture, University of Banja Luka. As plant material, young *Melissa officinalis* L. plants already established in *in vitro* culture were used. Explants were grown in 100 ml Erlenmeyer flasks containing 40 ml of Murashige and Skoog (MS) medium. Explants were cultured under a 16/8-hour light/dark photoperiod at $21 \pm 1^\circ\text{C}$ in growth chambers, with light provided by fluorescent tubes as a control and three types of LED lights. Each treatment group under different lighting conditions consisted of 28 explants distributed into 4 replications (7 plants per replication), resulting in a total of 112 plants across all four lighting treatments.

The prepared nutrient medium was sterilized in an autoclave for 20 minutes at 121°C . On the following day *Melissa officinalis* L. explants were inoculated into the culture medium under sterile conditions in a laminar flow cabinet using sterile laboratory instruments. Each individual explant was gently placed into the medium with its basal part using forceps, thereby establishing the *in vitro* culture. To prevent contamination, each Erlenmeyer flask was properly

sealed. The flasks were subsequently placed under different lighting conditions to monitor the growth and development of the young lemon balm plants.

Plant growth and development were observed over a period of nine weeks, with regular inspections to prevent any potential contamination. Once the explants reached a defined stage of growth and fully occupied the Erlenmeyer flasks, morphological parameters were measured and recorded. These included plant height, number of leaves, root development, and number of lateral branches. Following the assessment of morphological parameters, the fresh and dry biomass of the young plants was determined using an analytical balance, with values recorded to two decimal places. Drying of plant material was performed in an oven at 70°C until a constant weight was achieved.

RESULTS AND DISCUSSION

For data processing, the statistical software package VV-Stat (Vukadinović, 2017) was used. Differences between treatments were examined using one-way analysis of variance (ANOVA), with differences between individual treatments determined at the significance levels of 95% ($p \leq 0.05$) and 99% ($p \leq 0.01$).

To determine the effect of different light conditions on the growth and development of lemon balm (*Melissa officinalis* L.) explants, statistical analysis of the recorded morphological growth and development parameters was performed, and the following results were obtained.

During the experiment, morphological parameters of growth and development of lemon balm (*Melissa officinalis* L.) explants were measured under *in vitro* conditions. The obtained mean values and the results of the analysis of variance (ANOVA) for all analyzed morphological parameters are presented in Table 1. At the end of the experiment, the fresh and dry mass of the explants were recorded, and their mean values together with the corresponding analysis of variance are shown in Table 2.

The presented data indicate that different light conditions exerted distinct effects on the analyzed morphological parameters, suggesting that light quality plays a significant role in modulating the growth and development of lemon balm explants *in vitro*.

Table 1. Effect of different light on the morphological growth parameters of lemon balm (*Melissa officinalis* L.) explants under *in vitro* conditions (K – fluorescent light; T1 – blue LED; T2 – red LED; T3 – combined blue and red LED). Means followed by different letters (a–d) indicate significant differences according to the LSD test ($P \leq 0.05$).

Morphological growth parameters of lemon balm		Plant height (cm)	No of leaves	No of lateral shoots	Root development (%)
Treatment variant	Control (K)	5.80 ^a	13.79 ^s	1.47 ^a	0.61 ^a
	Treatment	6.54 ^{a.b}	16.07 ^b	1.89 ^{a.b}	0.64 ^{a.b}
	Treatment	7.23 ^b	18.64 ^c	2.36 ^{b.c}	0.79 ^b
	Treatment	5.53 ^a	22.28 ^d	2.96 ^c	0.64 ^{a.b}
Average		6,28	17.70	2.17	0.67
<i>Analysis of variance - F</i>		3.666383 [*]	28.6001 ^{**}	11.05301 ^{**}	1.522696
LSD	0.05	1.2315	2.1008	0.5930	ns
	0.01	ns	2.9453	0.8314	ns

ns= not significant

Different light conditions had a statistically significant effect ($p \leq 0.05$) on the height of *Melissa officinalis* explants. The greatest average shoot height was recorded in plants grown under treatment T2, which was 30.74% higher compared to the lowest average height observed in treatment T3. In pairwise comparison of the control plants with the three different light treatments, a statistically significant difference ($p \leq 0.05$) was confirmed only between the control (K) and the treatment with red LED light (T2). In pairwise comparisons among different light treatments, a highly significant difference ($p \leq 0.01$) was found only between treatments T2 (red LED light) and T3 (a combination of blue and red LED light).

The number of leaves and the number of lateral branches were both significantly affected ($p \leq 0.01$) by the different light conditions. The highest average number of developed leaves per explant was recorded in plants grown under treatment T3, under the combination of blue and red LED light, being 61.57% higher compared to the lowest average number of leaves per explant recorded in the control plants (fluorescent light). In pairwise comparison between the control and treated plants, a statistically significant difference ($p \leq 0.05$) was confirmed between the control and treatment T1, while a highly significant difference ($p \leq 0.01$) was found between the control and treatments T2 and T3. In pairwise comparisons among different light treatments, a statistically significant difference ($p \leq 0.05$) was confirmed between treatments T1 and T2, as well as between T2 and T3, whereas a highly significant difference ($p \leq 0.01$) was observed between treatments T1 and T3.

The difference between the highest (T3 – 2.96) and the lowest (K – 1.47) average number of developed lateral branches amounted to 101.4% in favor of treatment T3. In pairwise

comparison between the control and treated plants, a highly significant difference ($p \leq 0.01$) was confirmed between the control and treatments T2 and T3, while no statistically significant difference was observed between the control and treatment T1, although the number of lateral branches per explant was higher in T1 (1.89) compared to the control (1.47). In pairwise comparisons among different light treatments, a statistically significant difference ($p \leq 0.05$) was confirmed only between treatments T1 and T3.

The effect of different light conditions under *in vitro* culture did not have a statistically significant impact on root development of *Melissa officinalis* explants. In pairwise comparison between the control and treated plants, as well as among individual treatments, a statistically significant difference ($p \leq 0.05$) was confirmed only between the control and treatment T2.

Hernandez and Kubota (2012) found that lamps with different ratios of blue and red diodes do not significantly affect the morphological parameters of tomato growth and development up to the second leaf stage. These results are similar to those of our study, where different LED lighting conditions had varying effects on the examined parameters. However, it is important to note that this research was conducted under *in vitro* conditions and on a nutrient medium, which represent entirely different growing conditions compared to previous studies conducted on conventional substrates and in protected environments.

Table 2. Effect of different light on the fresh and dry mass of lemon balm explants (*Melissa officinalis* L.) under *in vitro* conditions (K – fluorescent light; T1 – blue LED; T2 – red LED; T3 – combined blue and red LED). Means followed by different letters (a–d) indicate significant differences according to the LSD test ($P \leq 0.05$).

Treatment variant		Fresh mass (g)	Dry mass (g)
Control (K)		4.07 ^a	0.40 ^s
Treatment (T1)		5.41 ^{b,c}	0.65 ^{b,c}
Treatment (T2)		5.27 ^b	0.54 ^c
Treatment (T3)		7.21 ^d	0.72 ^{b,d}
Average		5.49	0.57
Analysis of variance - F		12.54594**	19.33041**
LSD	0.05	1.1235	0.0979
	0.01	1.5752	0.1372

ns= not significant

Table 2. presents the analysis of variance for the fresh and dry mass of lemon balm explants developed under *in vitro* conditions, under the influence of different lighting conditions.

Different light conditions had a highly significant effect ($p \leq 0.01$) on both the fresh and dry mass of *Melissa officinalis* explants. The highest average fresh mass of explants was recorded in plants grown under treatment T3, which was 77.15% higher compared to the lowest

fresh mass observed in the control plants. In pairwise comparisons between the control group and the three different light treatments, a statistically significant difference ($p \leq 0.05$) was confirmed between the control (K) and both treatment T1 and treatment T2, while a highly significant difference ($p \leq 0.01$) was found in comparison with treatment T3. Among the different light treatments, a statistically significant difference ($p \leq 0.05$) was observed between treatment T1 and treatment T3, as well as between treatment T2 and treatment T3. No statistically significant difference was found between treatments T1 and T2.

The highest average dry mass of explants was recorded in plants from treatment T3, plants grown under a combination of blue and red LED light. This value was 80% higher compared to the lowest recorded dry mass, which was observed in the control plants grown under fluorescent lighting. In pairwise comparisons between the control group and the three different light treatments, a highly significant difference ($p \leq 0.01$) was confirmed between the control (K) and both treatment T1 and treatment T3, while a statistically significant difference ($p \leq 0.05$) was found in comparison with treatment T2. Among the different light treatments, a highly significant difference ($p \leq 0.01$) was observed only between treatments T2 and T3. No statistically significant differences were found between treatment T1 and either treatment T2 or treatment T3.

Heo et al. (2002), in a study investigating the effects of a mixed light-emitting diode (LED) spectrum on the growth and development of *Ageratum houstonianum*, *Calendula officinalis*, and *Salvia pratensis* seedlings, applied blue, red, and far-red light under controlled conditions over a 28-day period. Both the combination of blue and red light and the fluorescent light treatment (control) resulted in an increase in seedling dry mass. The number of flower buds and the flowering of *ageratum* and meadow sage were also enhanced under the combined blue and red light. Similarly, in our study, the highest average fresh and dry mass of *Melissa officinalis* explants, as well as the highest number of leaves and lateral branches, were recorded in plants grown under the combined spectrum of blue and red light (treatment T3).

Mints grown under red and blue LED light showed higher fresh and dry mass compared to those grown under white LED light (Sabzalian et al., 2014). Lin et al. (2013) reported that previous studies have demonstrated the effectiveness of red and blue LED light combinations for plant growth and development. These authors investigated the effects of red, blue, and white LED light on the growth, development, and quality of lettuce. Their study found that root and shoot mass, as well as the freshness and sweetness of lettuce, were greater in plants exposed to a combination of red, blue, and white light compared to those grown under fluorescent lamps.

CONCLUSIONS

Based on the obtained results we can conclude that red LED light promoted the greatest plant height and the highest percentage of root development. The combined red and blue LED treatment resulted in the highest number of leaves and lateral branches, as well as the greatest average fresh and dry mass.

In this study, light was identified as the most important factor influencing the growth and development of *Melissa officinalis* explants under *in vitro* conditions. LED lights offer numerous benefits, including a long lifespan, low heat and UV radiation emission, adaptability to low temperatures, and the absence of mercury, making them environmentally friendly and suitable for use in laboratory conditions. Under *in vitro* conditions, the application of different LED light treatments resulted in statistically significant differences in the growth and development of lemon balm plants. Based on these findings, this type of lighting can be recommended as a viable alternative to conventional fluorescent lighting.

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