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CONTENT

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ENVIRONMENTAL EFFECTS ON PHYSIOLOGICAL INDEX OF BLACK ALDER (*Alnus glutinosa* [L.] Gaertn.*)* DOMINANT TREES IN CENTRAL BOSNIA

SUMMARY

Alnus glutinosa (L.) Gaertn. appears naturally as stands, fragments, patches or river lines mainly around rivers and streams in central Bosnia. Considering spatial frame (mountainous on the headwaters and hilly valley afterward) arises the question of how *A. glutinosa* responds to exposed climate, hydrology and site conditions. We identified twenty tree temporary sample plots on five sites and conducted measurements of tree dominant trees per plot related to tree structural characteristics (diameter et breast height, height, crown projection) and registered the chlorophyll content index (CCI) in leaves of dominant trees monthly in season 2022. To examine structural, climate, hydrology and site effects on CCI quantity and dynamic we used descriptive statistics, analysis of variance and Kruskal – Wallis test. Obtained results indicated that dominant trees of *A. glutinosa* reach similar tree dimensions but physiologically responded differently depending on climate factors (mean annual temperature and maximal precipitation) and water (mean annual water flow and water level) fluctuations pointing out site-dependent responses. We notified increase in CCI on the sites exposed to reduced maximal precipitation and shortage of water at the second half of season. Our results confirmed *A. glutinosa* ability to resist climate challenges and contribute to ecological services on different sites in central Bosnia.

Keywords: black alder, dominant trees, chlorophyll content index, annual variation.

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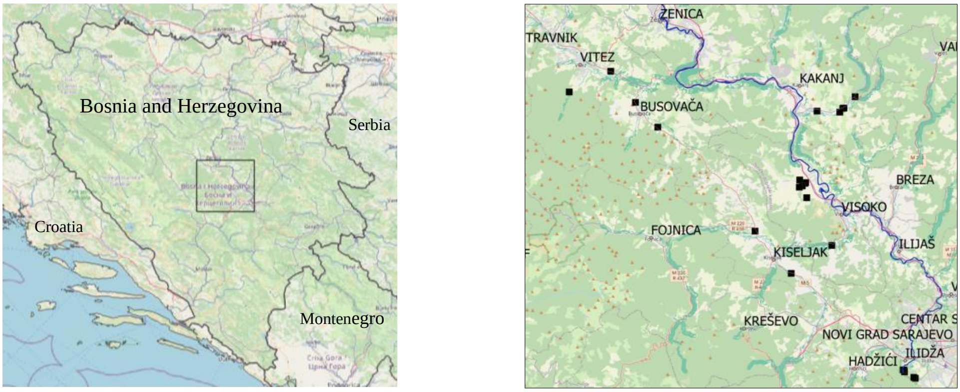
INTRODUCTION

Black alder (*Alnus glutinosa* (L.) Gaertn.) is a tree species situated in different conditions and on different site types across Europe, from central Scandinavia on the north, to the North Africa on the south. Development types of black alder communities range from highly productive closed canopy forest stands (Poland, Croatia) to environmentally important small stands and scattered groups of trees on riversides and planes (Mediterranean region). Similar site conditions for black alder are found in internal Dinarides in the Western Balkans (Vukelić *et al*., 2006; Barudanović, 2007; Laganis, 2007; Rakonjac *et al*., 2009; Spalević *et al*., 2013; Simovski *et al*., 2019). Dominant black alder habitat is situated on plane, riparian areas with available ground water mainly but the species appears also on hilly and lower mountain ranges with changeable environmental impacts. Mandák *et al.* (2016) reported about black alder genetic pools in Europe identifying occurrences of black alder on hilly and lower mountain ranges in internal Dinarides in central Bosnia, at the north of Montenegro and at the east and central Serbia. Although, many studies have been done about black alder interaction with environment in riparian zones on large planes, little literature is available on its interaction on hilly and lower mountain range zones. Recently, black alder situated in hilly and lowland valleys is exposed to rapid urbanization (land-use change and human pressure) and climate changes (temperature increase and water shortage). Like all native plants, black alder is exposed to different environmental conditions influencing its functional responses. Main environmental influences are related to orography, edaphic and climatic characteristics as well as tree and stand structural characteristics. Recently, chlorophyll content index (CCI) non-destructively registered in tree leaves has been promoted as a physiological index (a functional treat) reflecting the interaction between trees and the environment (Hendry *et al*., 1987; Terzi *et al*., 2010; Zhang *et al*., 2011; Talebzadah and Valeo 2022). Several studies confirmed that environmental pressures reflect on CCI index differently between different tree species within ecotypes of single species (Tenkanen *et al*. 2019). Changes of CCI were observed for several tree species during the vegetation season indicating environment x tree species interaction (Demarez *et al*., 1999; Gond *et al*. 1999; Uvalle Sauceda *et al*., 2007; Mõttus *et al*., 2014; Şevik *et al*., 2015; Croft *et al*., 2017; Tenkanen *et al*., 2019; Atar *et al*., 2020). Previous study about black alder reported about high CCI with increasing trend achieving the highest value before leaves falling at the end of vegetation season (Laganis, 2007).

The main aim of this study was to examine CCI annual variation related to main climate factors (temperature and water regime) and different site conditions in central Bosnia. We formulated the following research questions: (i) is average CCI affected by tree dimensions (tree height, DBH, crown area), orography (altitude, slope), climate factors (mean temperature, mean precipitation) and water regime (water level, water flow rate), (ii) is CCI intra-annual variation affected by climate and water regime variables and (iii) is intra-annual CCI variation site dependent. This study may lead to a better understanding of black alder resilience on hilly and lower mountain range areas what is relevant for sustainable forest and environmental management.

MATERIAL AND METHODS

Study area. The selected sites are all located at the subsection of the Bosna River basin examined in this study stretches from Sarajevo and flows north toward the city of Zenica where the Lašva River joins it in central Bosnia and Herzegovina within a smaller area of 1,512 km^2 (41.2 km by 36.7 km) from 43°81’ to 44°14’ N and 17°77’ to 18°29’ E (Fig. 1a). (Fig. 1b).



a) b)

Figure 1. Bosnia and Herzegovina with study area (a), Central Bosnia – sampling plan (black square - sample plots) (b)

Here are noticed changeable site conditions: (a) mountainous vs. plane and riverside positions, (b) waterlogged vs. low water level and low water flow rate sites, (c) protected landscape vs. sites under human pressures with sporadic industrial air and water pollution incidents and (d) natural site condition vs. sites surrounded with agricultural land near settlements.

The dominant forest types in the study area with complex petrographic composition (especially tertiary flyschoid complexes) are beech forests, oak forests and oak and hornbeam forests with mosaic spatial distribution (Stefanović *et al*., 1983). The soil types in the study area, stretching from the spring of the Bosna River downstream, are combinations of fluvisols, dystric and eutric cambisols, calcomelanosols and calcocambisols (Ćesir, 2022).

The climate for this region is humid continental tempered by the Adriatic Sea, with an average annual temperature of 10 °C, while minimal average temperatures occur in January (-0.5 to -4.3 °C) in Sarajevo and Zenica, respectively, and maximum temperatures in July/August (20 to 26 °C). Precipitation differs significantly between Sarajevo and Zenica, where in Sarajevo it ranges from around 64 mm in February to 91 mm in September, while in Zenica it ranges from 34 mm in September to 81 mm in May (Federal Hydro-meteorological Institute). The mean, maximal and minimal temperatures and precipitation were recorded monthly at two metrological stations: at the headwaters on the south (central Bosnia) and at the station near the Bosna River 100 km far away on the north.

Data acquisition. Temporary 23 sample plots varying in size from 60 to 300 m^2 with an average area of 190 m^2 were selected within black alder dominant sites. The plots are situated along a longitudinal gradient and are exposed to various environmental and anthropogenic conditions. The elevation of the sample plots ranged from 385 to 500 meters above sea level (m.a.s.l), while the slope ranged from 0 to 5.6°.

The plant communities examined in this study were all black alder dominant, appearing in two general forms: at the Bosna River headwaters as the forest stand (Fig. 2a), and the other sites as isolated stands or groups of trees along waterways (Fig. 2b).

(a)

(b)

Figure 2. Black alder sites: Closed forest stand (a), River line tree formation (b)

Sample plots are clustered on five sites following a longitudinal gradient. Site 1 is located at the Bosna headwaters, below Igman mountain with a shaded position and colder climate (Ilidza). Sites 2 and 3 occupy sunny exposed planes near fertile agricultural fields around Bosna River (Visoko) and near the industrial area (Kakanj). Sites 4 and 5 are situated in uphill positions, but Site 4 is closer to the river (Kiseljak) while Site 5 occupies drier positions (Busovaca). The dominant and neighboring trees were selected at the beginning of spring (March and April 2022) during flowering. The diameter at breast height (DBH), height and crown area of the three largest trees were measured in April 2022. Descriptive statistics of tree, orography and climatic variables are presented in Table 1.

In-situ measurements related to the chlorophyll content in leaves were determined using a CCM-200 hand-held chlorophyll meter (Opti-Sciences, Massachusetts, USA) and registered as CCI between April and October 2022.

Methods. The data analysis began by conducting descriptive statistics to examine the mean, standard deviation, maximum and minimum values for various variables, including tree structural variables, orography variables, climate variables and a physiological variable: Chlorophyll Content Index (CCI). Descriptive statistics were also calculated for chlorophyll content per month. Next, correlation analyses were conducted using both Pearson’s and Spearman’s correlation coefficients. The strength of correlations was examined between CCI and the variables as well as between CCI, climate variables and water variables during the season. To assess the normality of distribution, the Shapiro – Wilk test was performed. Furthermore, the differences in structural, functional, and environmental variables, as well as CCI values between sites, were examined using both parametric ANOVA and non – parametric Kruskal – Wallis test. The intra–annual CCI variations across different sites were analyzed using repeated – measurement ANOVA, considering Mauchly’s sphericity test with Huynh – Feldt correction applied. All statistical analyses were performed using the “dplyr” package (R Statistical Software version 4.2.2; R Core Team 2022).

RESULTS

Correlations between trees' structural and environmental variables with chlorophyll content index in leaves of dominant trees

Table 1. Descriptive statistics (n = 23).

Variable	Mean	Standard deviation	Minimum	Maximum
Tree structural variables				
Tree height (m)	18.43	4.42	9.90	25.50
Diameter at breast height (cm)	38.93	11.84	20.00	72.20
Crown projection area (m^2)	53.21	33.69	5.11	142.08
Orography variables				
Altitude (m.a.s.l)	449.96	35.85	384.40	500.00
Slope (^{o})	1.95	1.70	0.50	5.64
Climate variables				
Mean annual temperature (^{o}C)	14.04	0.70	12.68	15.02
Mean maximal precipitation (mm)	57.73	0.93	56.63	60.28
Mean annual water level (cm)	99.77	60.19	9.30	230.90
Mean annual water flow rate (m^3/sec)	22.03	11.88	3.60	36.10
Chlorophyll content index	27.53	7.31	14.4	40.6

Table 1 provides important findings regarding the descriptive statistics of the variables examined. These descriptive statistics provide an overview of the central tendency, variability, and range of values for each variable. High standard deviation can be seen for crown projection area (33.69), altitude (35.85) and mean annual water level (60.19), whereas for mean annual temperature and mean maximal precipitation standard deviation is quite low (respectively 0.70, 0.93).

Table 2 presents the correlations between the chlorophyll content index (CCI) and the variables examined. What is worth noting, there is a significant moderately positive correlation between mean annual temperature and CCI, with Pearson's correlation coefficient of 0.42 and Spearman's correlation coefficient of 0.45. There is also a significant correlation between mean annual water flow rate and CCI that is moderately positive, with Pearson's correlation coefficient of 0.41. Spearman's correlation coefficient was 0.38.

The findings show that tree height, altitude, mean annual temperature, and mean annual water flow rate may have more notable associations with chlorophyll content, while the other variables show weaker correlations.

Table 2. Correlations between chlorophyll content index and variables

Variable	Pearson's	Spearman's
Tree height (m)	-0.20	-0.19
Diameter at breast height (cm)	0.12	0.13
Crown projection area (m^2)	0.01	-0.03
Altitude (m.a.s.l)	-0.26	-0.23
Slope (^{o})	0.18	0.10
Mean annual temperature (^oC)	0.42*	0.45*
Mean maximal precipitation (mm)	-0.12	-0.17
Mean annual water level (cm)	0.03	-0.02
Mean annual water flow rate (m^3/sec)	0.41*	0.38

Chlorophyll content index intra-annual variation. Table 3 provides the dates of data acquisitions and summary statistics for chlorophyll content per month. The chlorophyll content tends to increase from May to August, reaching its peak in August (maximum = 59.9, minimum = 16.2), and then gradually decreases in September and October. The range of chlorophyll content values also tends to widen from May to August, indicating greater variability in chlorophyll levels during this period. On average, the highest chlorophyll content is observed in September (32.88).

Table 4 presents the correlation matrix, which explores the relationships between the chlorophyll content index (CCI) and various climate and water variables during the season. Notably, significant correlations are observed between CCI and specific variables. First, there is a moderate positive correlation between CCI and mean maximal precipitation (0.47), indicating that higher levels of precipitation are associated with higher chlorophyll content. Secondly, a very strong negative correlation is observed between CCI and mean annual water level (-0.83). This finding suggests that higher water levels are linked to lower chlorophyll content. Furthermore, a very strong negative correlation is observed between CCI and mean annual water flow rate (-0.82). This implies that higher rates of water flow are associated with lower chlorophyll content.

Table 3. List of dates of data acquisitions and summary statistics for chlorophyll content per month

Month	Day of the Year	Mean	St. dev.	Min.	Max.
May	139	22.89	7.90	9.7	32.7
Jun	154	24.65	7.05	10.7	36.3
July	200	27.24	8.99	12.7	43.3
August	229	31.20	11.00	16.2	59.9
September	249	32.88	10.35	11.9	47.9
October	289	27.44	7.55	15.6	41.1

Table 4. Correlation matrix for chlorophyll content index, climate and water variables during the season

Variable	Temp.	Precip.	Water level	Water flow rate
Mean annual temperature (^{o}C)	1			
Mean maximal precipitation (mm)	-0.12	1		
Mean annual water level (cm)	-0.03	0.04	1	
Mean annual water flow rate (m^3/sec)	0.06	-0.03	0.99	1
Chlorophyll content index	-0.09	0.47*	-0.83*	-0.82*

Abbreviation: Precip. - precipitation.

Figure 3a shows that there are no significant differences in mean air temperature in the southern and northern regions of the study area.

In Figure 3b differences in mean precipitation can be noticed. In May, June, July and September there was more precipitation in the north. Much dissimilarity is seen especially in May. On the other hand, in January, April and August there was more precipitation in the south. In Figure 3c substantial disparity can be observed. The water level was much higher in northern regions. Moreover, in Figure 3d higher water flow rate in the north can also be seen. From February to June this rate is the highest.

Spatial and annual variability of CCI. Table 5 presents the descriptive statistics for various variables measured at different study sites. The table provides a summary of the measurements taken at each site, allowing for comparisons and identification of any differences or patterns among the variables across the study sites.

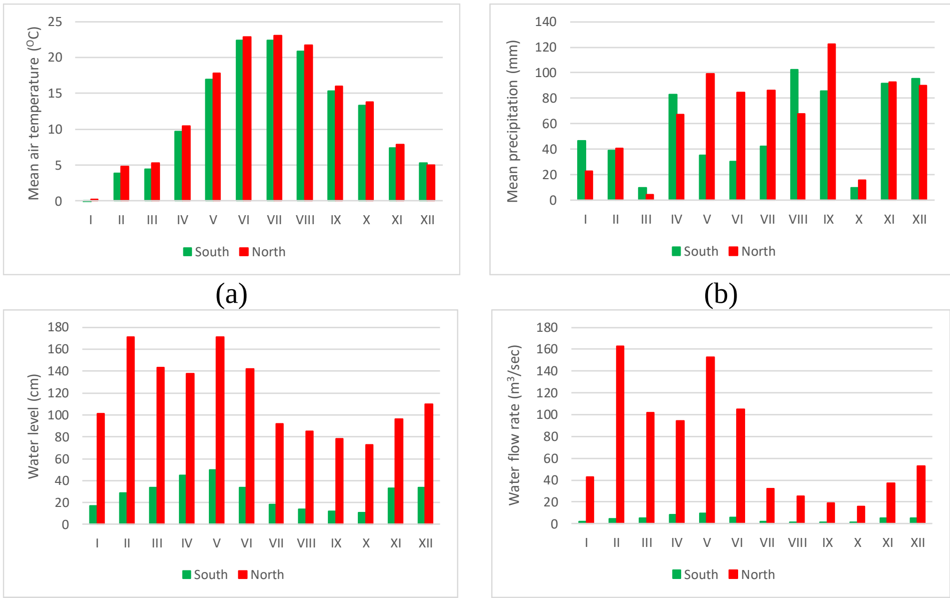


Figure 3. Climate and water intra-annual dynamics on the south and the north of the study area (a) mean air temperature, (b) mean precipitation, (c) water level, (d) water flow rate.

A significant variation in tree height, diameter at breast height, and crown projection area among the different sites can be seen. Site 2 has the tallest trees with an average height of 20.8±2.4 meters, while Site 3 has the shortest trees with an average height of 13.2±4.5 meters. Site 1 has the largest crown projection area with an average of 63.5±24.2 square meters.

The altitude and slope exhibit notable differences across the sites. Site 1 has the highest altitude at 496±2.6 meters above sea level, Site 5 has the lowest altitude at 419±37.2 meters above sea level. Site 4 has the steepest slope with an average of 3.4±1.9 degrees.

Mean annual temperature and mean maximal precipitation show slight variations among the sites. Site 4 has the highest mean annual temperature of 15.0±0.1 degrees Celsius, while Site 1 has the lowest mean annual temperature of 13.4±0.1 degrees Celsius. The mean maximal precipitation ranges from 57.0±0.3 to 58.5±1.3 millimeters, with no significant differences observed. There are significant differences in mean annual water level and mean annual water flow rate among the sites. Site 5 has the highest mean annual water level at 185.7±31.6 centimeters, while Site 1 has the lowest mean annual water level at 9.9±0.4 centimeters. Site 2 has the highest mean annual water flow rate at 32.4±2.1 cubic meters per second, while Site 1 has the lowest mean annual water flow rate at 3.9±0.3 cubic meters per second.

The chlorophyll content index shows higher values at Site 1, whereas the lowest values are at Site 4.

Overall, the findings from Table 5 highlight the significant differences in tree structural variables, orography, water variables and chlorophyll content index among the study sites, while climate variables and show relatively consistent values across the sites.

Table 5. Descriptive statistics of tree structural variables, orography, and annual climate and CCI mean values per site

Variable	Site 1	Site 2	Site 3	Site 4	Site 5
Number of plots/trees	5	6	3	5	4
Tree structural variables					
Tree height (m)	18.9±6.2	20.8±2.4	13.2±4.5	16.8±1.4	20.3±4.5
Diameter at breast height(cm)	36.5±5.3	42.1±11.5	25.9±7.9	41.8±18.5	43.4±5.9
Crown projection area (m^2)	63.5±24.2	62.3±31.9	21.7±15.3	58.3±48.7	51.0±32.7
Orography variables					
Altitude (m.a.s.l)	496±2.6	422±10.6	468±24.7	449±37.2	419±37.2
Slope (^{o})	1.0±0.8	2.1±2.2	0.9±0.4	3.4±1.9	1.9±1.2
Climate variables					
Mean annual temperature (^{o}C)	13.4±0.1	14.4±0.1	13.6±0.5	15.0±0.1	13.5±0.5
Mean maximal precipitation (mm)	57.2±0.1	57.0±0.3	57.7±0.9	58.5±0.7	58.5±1.3
Mean annual water level (cm)	9.9±0.4	97.0±6.5	110.8±44.3	117.7±12.0	185.7±31.6
Mean annual water flow rate (m^3/sec)	3.9±0.3	32.4±2.1	13.9±2.2	32.0±3.1	22.7±4.0
Physiological variable					
Chlorophyll content index	496±2.6	422±10.6	468±24.7	419±37.2	449±37.2

Table 6 provides a summary of the significant values based on the chosen tests for different variables. If the data was found to follow a normal distribution based on the Shapiro – Wilk test, the analysis of variance (ANOVA) test was performed in addition to the Kruskal – Wallis test. However, if the data did not follow a normal distribution, only the Kruskal – Wallis test was conducted.

Table 6. Summary of significant values based on chosen test

Variable	Shapiro-Wilk test	Normal Distribution	Test	*p*-value
Tree structural variables				
Tree height (m)	*p* = .586	Yes	ANOVA	.110
			Kruskal – Wallis	.091
Diameter at breast height (cm)	*p* = .088	Yes	ANOVA	.282
			Kruskal – Wallis	.158
Crown projection area (m^2)	*p* = .089	Yes	ANOVA	.483
			Kruskal – Wallis	.225
Orography variables				
Altitude (m.a.s.l)	*p* = .055	Yes	ANOVA	<.001*
			Kruskal – Wallis	.003*
Slope (°)	*p* < .001	No	Kruskal – Wallis	.473
Climate variables				
Mean annual temperature (°C)	*p* = .077	Yes	ANOVA	<.001*
			Kruskal – Wallis	<.001*
Mean maximal precipitation (mm)	*p* = .006	No	Kruskal – Wallis	.009*
Mean annual water level (cm)	*p* = .089	Yes	ANOVA	<.001*
			Kruskal – Wallis	<.001*
Mean annual water flow rate (m^3/sec)	*p* = .004	No	Kruskal – Wallis	<.001*
Physiological variable				
Chlorophyll content index	*p* = .774	Yes	ANOVA	.063
			Kruskal – Wallis	.050·

The ANOVA and Kruskal – Wallis tests done on tree structural variables, both yield non-significant *p*–values, suggesting no significant differences among the groups. Considering orography variables, for altitude the ANOVA and Kruskal – Wallis tests show highly significant *p*–values (respectively, $p < .001$, $p = .003$), showing significant differences in altitude among the groups. For slope, Kruskal – Wallis test yields a non–significant *p*–value ($p = .473$). On the other hand, all climate variables have highly significant *p*–values indicating significant differences among the groups. For chlorophyll content index, ANOVA test yields a non–significant *p*–value ($p = .063$), while Kruskal – Wallis test shows a *p*–value of .050, indicating a borderline significant difference in chlorophyll content index among the groups.

These findings indicate that there are significant differences among the groups in terms of altitude, mean annual temperature, mean maximal precipitation, mean annual water level, and mean annual water flow rate. However, there are no significant differences observed in tree height, diameter at breast height, crown projection area, slope, and chlorophyll content index among the groups.

Figure 4 illustrates the temporal variation of the chlorophyll content index (CCI) across different sites. It visually represents the fluctuations in CCI values over time at each site.

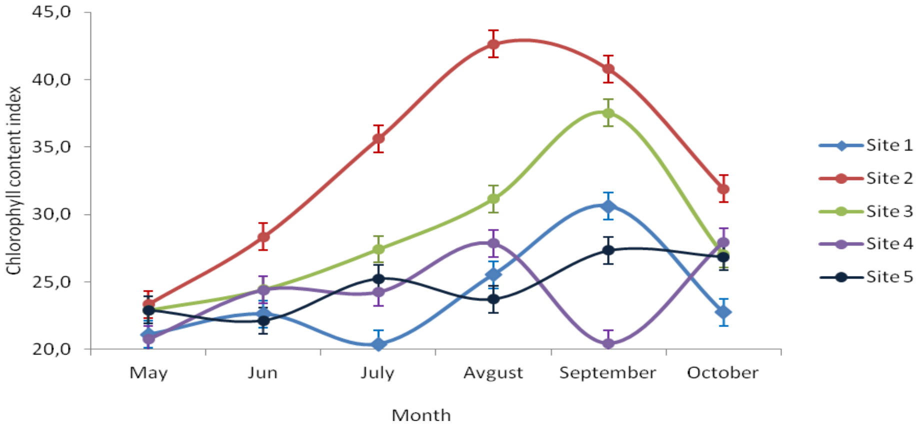


Figure 4. Profile plot month x site with LS confidence intervals

In Table 7, the results of the repeated measures analysis of variance (RM ANOVA) considering Mauchly's sphericity test with applied Huynh–Feldt correction are presented.

The variation between sites was assessed, and it yielded a non–significant F–value ($p = .063$; $F = 2.71$), indicating that there were no significant differences in the dependent variable among the different sites.

The within–subjects error variation (within the tree factor) was calculated. The sum of squares was 4395.57, with 18 degrees of freedom, and the mean square was 244.199. The obtained F–value was statistically significant ($p < .001$; $F = 10.30$), indicating that there were significant differences in the dependent variable within the tree factor.

Table 7. RM ANOVA considering Mauchly's sphericity test (W=0.16, p=0.01) with applied Huynh–Feldt (0.96) correction

Source of variation	Sum of squares	d.f.	Mean square	F	*p*-value
Site	2650.18	4	662.545	2.71	.063
ERROR(Tree)	4395.57	18	244.199	10.30	<.001*
Month	1924.20	4.8	401.24	16.23	<.001*
Month*Site	921.645	19.2	48.05	1.94	.020*
ERROR(Month)	2133.39	90	23.70		

The variation between months was examined, and it showed a highly significant F–value ($p < .001$; F = 16.23), which means that there were significant differences in the dependent variable across different months.

The interaction effect between month and site was evaluated. The F–value was statistically significant ($p = .020$; F = 1.94), showing that there was a significant interaction effect between the month and site factors on the dependent variable.

Notably, Site 2 exhibits the highest CCI values throughout the observed period. In contrast, Sites 1, 4, and 5 demonstrate relatively lower CCI values. Specifically, Site 1 exhibits the lowest CCI values in July, Site 4 in September, and Site 5 in June (Figure 4).

Trend analysis showed significant linear and quadratic trends for CCI changes (Table 8). Trends were significant for sites 1, 2, 3 and 5.

Table 8. Trends of chlorophyll content index on different sites

Site	Trend	t	*p*-value
Site 1	linear	2.46	.016*
	quadratic	-1.37	.175
Site 2	linear	3.52	<.001*
	quadratic	-4.04	<.001*
Site 3	linear	3.35	.001*
	quadratic	-2.29	.024*
Site 4	linear	1.59	.116
	quadratic	-0.64	.523
Site 5	linear	2.40	.018*
	quadratic	-0.51	.612

Sites 1 and 5 exhibited a linear increasing trend present on non-disturbed sites (Atar, 2020) while trends were non-linear with extreme values in the second period of the growing season on sites 2 and 3 (Fig. 4).

DISCUSSION

Chlorophyll content in forest tree leaves. Changeable environmental and climatic conditions influence chlorophyll content and its seasonal dynamics in forest trees (Croft *et al*. 2017; Atar *et al*. 2020). Mainly, chlorophyll content was investigated for deciduous tree species (Bassow, 1997; Demarez *et al*., 1999, Richardson *et al*., 2002) using different instruments. Brown *et al*. (2022) found that CCM-200, among other instruments, represents a suitable choice for CCI determination of some deciduous tree species (ash, beech, silver beech, hawthorn, red maple and sycamore). In the present study, CCI achieved mean of 28.4 units ranging from 10 to 60 in fully developed leaves of black alder during the growing season. In the greenhouse experiment, Sever (2018) reported CCI means in two-year-old oak leaves in control and drought treatment with 13.1 and 21.3 values respectively. Brown *et al*. (2022) reported CCM-200 recordings ranged from 5 to 65 differing between ash, beech, silver beech, hawthorn, red maple and sycamore. Also, similar CCI ranges are presented for hawthorn, hazel and beech in Brown *et al*. (2022) study. Related to the reported results measured by CCM-200 instrument, black alder CCI mean of dominant trees is higher than in silver birch (Tenkanen *et al*. 2019). Assuming similarity between chlorophyll content registered with CCM-200 and other instruments (SPAD-502) mean CCI value of black alder is lower than in oaks (*Quercus petrea* Liebl. and *Q. robur* L.), beech (*Fagus sylvatica* L.) and hornbeam *(Carpinus betulus* L.) leaves (Demarez et al. 1999) and twelve deciduous tree species (*Quercus hartwissiana* Steven, *Ginkgo biloba* L., *Fagus orientalis* Lipsky, *Quercus castaneifolia* C. A. Mey., *Ulmus minor* Mill., *Cinnamomum camphora* (L.) Sieb., *Liquidambar orientalis Mill.*, *Acer negundo* L., *Quercus pubescens* Willd., *Quercus rubra* L. *and Aesculus hippocastanum* L.) in Turkey (Atar *et al*., 2020).

Intra-annual variation of chlorophyll content index of black alder trees. Related to chlorophyll content annual profile, it is known that deciduous trees begin leaf development with strongly increasing chlorophyll content from April to May. Then, between June and August chlorophyll content reaches a stable level and starts to decline from September to October/November (Demarez *et al*., 1999). Annual profiles of chlorophyll content differ between tree species. Demarez *et al*. (1999) studied an annual variation of leaf chlorophyll content of a temperate forest (oak, beech and hornbeam). Croft *et al*. (2017) investigated trembling aspen (*Populus tremuloides* Michx.), red maple (*Acer rubrum* L.), bigtooth aspen (*Populus grandidentata* Michx.) and white ash (*Fraxinus americana* L.) describing slow increase at the start of the season until values stabilize in the middle of the growing season and declining during leaf senescence. The highest CCI values in the middle of the growing season achieved bigtooth and trembling aspen while ash and maple reached lower CCI values. Taulavuori (2006) reported that *Alnus glutinosa* maintains higher chlorophyll concentration and higher ratios of chlorophyll to other pigments which result in prolonged photosynthetic activity in the growing season.

We observed almost full leaf development in the first half of May starting from the beginning of May which was not so early, but we noticed intensive leaf development till the end of August as well as active leaves on the crown base in September too. High CCI values were stable during the long period and a sharp decrease appeared at the end of overall vegetation activities (the end of September to the middle of October). Our CCI results are consistent with other authors' findings related to long and stabile black alder leaf functionality.

Also, we determined a significant annual change of CCI values from the beginning (15 May) and the end (29 October) of the growing season with higher CCI (difference of 17 CCI units). Atar *et al*. (2020) compared chlorophyll contents between the beginning and the end of the growing season for twelve tree species in Turkey and reported significant changes and higher CCI at the end of the growing season for all tree species except *Quercus hartwissiana* Steven, *Cinnamomum camphora* (L.) Sieb., and *Ginkgo biloba* L.

Intra-annual variation of chlorophyll content index of black alder trees on different sites. Considering chlorophyll content on different sites, the black alder appears on various site types such as waterlogged, plateau and riverside sites (Claessens *et al*., 2010) affecting physiological processes and tree responses (Miller, 2012). Talebzadeh and Valeo (2022) stated that chlorophyll content is a species-specific feature that could be upgraded with the assumption that any change in chlorophyll content in a plant is a reflection of weather or environmental impacts on specific sites. Zielewich *et al*. (2020) found that evaluation and monitoring of chlorophyll content in tree leaves reflected site-dependent environmental stressors, weather, climatic and anthropogenic conditions.

In our study, sites differ greatly although CCI mean differences were not significant ($p = 0.06$).

Sites 1 and 2 differ in environmental conditions. Site 2 having the highest CCI values is characterized by plain, sunny positions surrounded by fertile agricultural land while Site 1 is situated at headwaters with a colder climate and hilly positions. Also, sites differ in hydrological conditions. The lowest mean values for water level and water flow rate are registered on Site 1 while higher water levels and water flow rates were present on other sites. Many studies related differences in physiological processes and tree responses to hydrological conditions of sites in interaction with the period of growing season (time).

Related to annual changes in chlorophyll content, environmental variation of different conditions occurs as a complex interaction and species characteristics vary accordingly (Miller, 2012). Annual changes of tree physiological activity depend on the species identity, climate, and site conditions (Hooper *et al*., 2005; Jucker *et al*., 2016). Tenkanen *et al*. (2019) obtained a significant effect of the interaction of dates and sites on CCI means for silver birch in northern Europe.

In the present study, significant differences are obtained for CCI means registered at different sites at different dates. Those differences appeared in July, August and September. In each month, Site 2 reached the highest CCI mean while Site 1 achieved the lowest value or belonged to the group with statistically lower CCI means. In addition, analyzing climate factors, we noticed that Site 1 had lower mean air temperature and maximal precipitation in the first part of the growing season which could contribute to lower CCI. Analyzing water supply conditions, we observed lower water levels and flow rates at Site 1. Hydrological conditions on other sites had two characteristic periods: in the first part of the growing season water level and water flow rate were very high and then in July declined sharply by more than 50%. Recent studies connected CCI increase in forest trees with environmental stress, more specifically with low water level (drought effect) (Arend *et al*., 2016; Hagedorn *et al*., 2016; Sever *et al*., 2018). According to Hagedorn *et al*. (2016), additional photosynthetic activity appears to overcome drought stress after a drought period. In our study, on Site 2 sharp CCI maximal increase appeared shortly after the water decline achieving maximal value and then decreasing progressively.

CONCLUSIONS

Black alder is addressed as an important tree species adaptable to different site conditions in riparian, hilly and lower mountain areas. This study examined CCI annual variation related to main climate (temperature, precipitation) and ground water (water level and flow rate) condition variations on different sites in central Bosnia. Annual variation of climate and water conditions affected annual CCI in interaction month vs. site resulting in CCI significant increase on sites with reduced water quantities. Although climate and water conditions differ on sites, dominant trees reach similar tree dimensions (DBH, height and CPA) as well as mean CCI. It seems that black alder dominant trees resist changeable annual environmental effects what confirm their functionality in similar conditions. Forest management should support sustainable functioning of black alder providing regular water supply and stabile water regime reducing water stress and maintaining conditions for regular black alder growth. Primary strategic and planning objectives should be complemented with the protection of the valuable black alder native habitats and those at highest risk.

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ENTRY BY TESTER BIPLOT MODEL FOR EVALUATION OF SOME KABULI CHICKPEA GENOTYPES BASED ON SEVERAL MULTIPLE TRAITS

SUMMARY

The aim of investigation was to evaluate the pattern of genetic variation in Kabuli chickpea genotypes through various traits under semi-arid rainfed circumstances. Trial was performed to evaluate the response of 50 Kabuli chickpea genotypes via a randomized complete block layout with three replicates. The entry by tester (genotype by trait) biplot which explained 66% of the variability indicated that the important traits for a favorable genotype in semi-arid environments were seeds' number of pod and pods' number of single plant. The biplot model introduced some desirable chickpea genotypes as good for a trait or a category of traits; genotype 27 for chlorophyl content, genotype 26 for seed yield (SY), SP and PP, and genotype 36 for plant height (PH), days to maturity (DM), pod's weight (PW), hundred seed weight (HSW), plant dry weight (PDW), and plant fresh weight (PFW). Based on an ideal assumptive genotype (entry) position, genotype 1 followed to 2, 10, 16, 17, 23, 26, 33 and 34 were ideal regarding the distinction ability and typical potential. According to an ideal assumptive trait (tester) position, PH, PDW, and PFW were more discriminative and typical traits. The responses of chickpea genotypes regarding SY indicated that genotype 26 following to 3, 17 and 27, were the most desirable and can be advised for commercial cultivar release process.

Keywords: distinction potential, discriminative ability, selection, seed yield.

INTRODUCTION

After cereals, legumes are the second source of human food and are considered as a valuable food supplement for cereals. They play an important role in providing human food requirements and regarding their nutritional

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importance, chickpea is cultivated in 49 countries of the world with an area of about 15 million hectares and a production of more than 18 million tons (FAOSTAT, 2022). Chickpea as a diploid (2n=2x=16) species belongs to the Fabaceae family, which ranks second in the world among legumes with mean performance of about 1500 kg ha-1. Chickpea is a very excellent supply of zinc, iron, and fiber and have about 27% protein, 6% fat and 57% carbohydrates and is important in terms of the calcium content (Gupta et al. 2019). This crop stabilizes agricultural systems, especially in rotation with cereals, by improving soil fertility. There are two main types of chickpeas, Desi and Kabuli, the Desi type has small, dark-colored seeds with a hard coating, but the Kabuli type has larger, light-colored seeds and a soft coating. According to FAOSTAT (2022), the harvested area of chickpea, Iran (430 thousand hectares) ranks fifth in the world and ranks ninth in terms of production (176 thousand tons), but in terms of performance per unit area (400 kg ha-1), Iran ranks the last locations in the world (45th), which is very low compared to the global average yield and the highest yield performances (China and Jordan with more than 5000 kg ha-1). About 90% of the chickpea cultivation area in Iran is rainfed, and due to the occurrence of drought during flowering period, which is the sensitive stage of the crop, this stress has a remarkable impact on reducing the yield.

Despite of the large cultivation area of chickpea in world, the production quantity in most of countries is very low and there is a remarkable gap between production potential and actual production. The important cause for this minimum performance of chickpea is the cultivation of local cultivars, as well as using the old agricultural practices (Sellami et al., 2021). Environment, genetic and their interaction are the main components that determine crop’s performance quantity and quality, so the main goal in selecting breeding criteria should be focused on the genetic factors. Therefore, most of the efforts of breeders are directed to discovering ways to increase the genetic potential to select the best genotypes, so the population should have a favorable diversity, and knowledge of this diversity requires germplasm evaluation. In order to estimate genetic diversity, different types of markers including morphological, biochemical and molecular ones are used by breeders. The most important components of chickpea yield include the pods’ number, the seeds’ number, and the hundred seed weight; so, by improving these characteristics, yield can be improved (Joshi et al., 2018). One of the effective ways to obtain efficient information about genetic diversity is the use of the various statistical multivariate models, which makes it possible to make favorable and suitable identifications suitable genotypes for crossing. Principal components analysis is a powerful method in reducing many numbers of attributes to a restricted number of independent and uncorrelated components.

A restricting factor in the chickpea improvement program is its limited genetic base, which makes it difficult for the breeders to generate new cultivars. Therefore, to improve the effectivity of the chickpea improvement project, it is necessary to expand the genetic base of chickpea. Also, determining the characteristics of the germplasm in terms of desired traits facilitates the creation of populations that are designed to achieve specific goal. Thus, the knowledge about genetic structure of the target traits, such as heritability and correlations of traits, is important for designing a successful breeding program. High yield performance, earliness, taller plant height and large seed size are key and economically important traits in the chickpea breeding (Roorkiwal et al., 2018). The taller plant height cultivars are suitable for mechanical harvesting and play an effective role in minimizing production costs (Singh et al., 2022). Also, seed size is an important attribute for international trade, so that large types have a higher price in the market. Related traits to yield performance and stress tolerance are controlled by several genes, so the phenotypic expression of the desired characteristics is largely affected by their genotype as well as environmental effects and their interactions. A positively significant correlation was observed for seed yield with the pods' number of plant, the hundred seed weight, the seeds' number of plant and the harvest index, so they had a positive impact on increase of yield performance (Gediya et al., 2019). Also, there is a positively significant association between the seeds' number, the pod's number, the primary branches, the secondary branches and the weight of one hundred seeds with yield performance (Zali et al., 2011).

According to Meena et al. (2010), chickpea yield indicated a positively association with the pods' number, biomass, harvest index and the branches' number, and indicated a negatively relation with the flowering initiation. In another investigation to explore the interrelationships of chickpea yield performance and its main related traits using different statistical methods, plant height, biomass and the pods' number were the major traits affecting chickpea yield; so, by choosing these traits, high performance can be achieved (Kayan and Adak, 2012). Yucel et al. (2006) showed that yield performance, number of primary shoots and harvest index had the most genetic changes among chickpea genotypes. In investigating the genetic variation among 15 chickpea genotypes, the seeds' number and the hundred seed weight showed a positively significant influence on yield performance and justified 96% of the variation in the fitted regression mode. Mardi et al. (2003) in exploring the genetic diversity of chickpea by the morphological traits, showed that the pod weight and the seeds' number have good genetic diversity and their selection leads to the high performance. In another study that was conducted on 104 cultivars of Kabuli chickpea, high diversity in terms of morphological characteristics were found (Fazeli and Cheghamirza, 2011). The aim of present research is to investigate the genetic variation of advanced genotypes of chickpea, to analyze the association between agronomic-morphological characteristics and to select the most superior genotypes for commercial cultivar release as well as use in future genetic improvement projects.

MATERIAL AND METHODS

In this research, 50 Kabuli chickpea genotypes from the breeding program of Iran's Dryland Agricultural Research Institute were examined in terms of different phenological, morphological, yield-related traits based on the chickpea standard descriptor Biodiversity International. They were selected based on their high performance and pervious years. Field operations included manure application and plowing in autumn, as well as adding decomposed manure plowing, disc harrow, and leveling in spring were performed in field located in Gavshaleh, Saqqez, Iran (36°19′N 46°19′E; altitude 1476 m). Planting was carried out on May 3 in the form of a randomized complete block layout in three replicates. Genotypes were cultivated in plots consisting of 4 lines, one meter long, with a distance between rows of 20 25 cm and the distance of seeds on the row approximately 8 cm. The weeding was applied manually in several stages and other operations according to the usual routine of the region. The land was irrigated three times because rainfall was sufficient during growing season. The days to maturity (DM) was recorded. Ten randomly selected were used to measure plant height (PH), days to maturity (DM), chlorophyl content (CHL), number of pods per plant (PP), pod's weight (PW), number of seeds per pod (SP), hundred seed weight (HSW), plant dry weight (PDW) and plant fresh weight (PFW). At physiological maturity, seed yield (SY) was measured from two central rows.

The dataset was analyzed through an entry by tester biplot model via the GGEbiplot application which shows graphic grasp from interaction structure of measured traits (testers) across genotypes (entries) as:

$$\frac{Y_{ij} - \overline{Y_j}}{SD_j} = \sum_{n=1}^{2} \Phi_n \Psi_{in} \Omega_{jn} + R_{ij}$$

Y_{ij} is the mean of entry (genotype) i for tester (trait) j, $\overline{Y_j}$ is the mean of entries for tester j, SD_j is the standard deviation of tester j for entries, Φ_n is the eigenvalue for PC (principal component) n, Ψ_{in} and Ω_{jn} are values for entry i and tester j on PC n, R_{ij} is the error term of the fitted equation related to entry i for tester j. Also, for obtaining symmetrical scales of testers and entries, the eigenvalue is corrected through vectors' absorption, so a normal presentation of testers and entries ($\Psi_{in}^* = \sqrt{\Phi_n \Psi_{in}}$ and $\Omega_{jn}^* = \sqrt{\Phi_n \Omega_{jn}}$) is happened. The entry by tester interaction biplots are generated by these symmetric scales, and each genotype (entry) or trait (tester) is shown by a special sign. Thus, a graphic interpretation of the relations among genotypes and traits as well as their interaction can be provided.

RESULTS

The first and second derived PCs, accounted for 66% of the dataset variability (Fig. 1), whereas the first PC contributed 41%, and the second PC contributed 25% to the fitted variance. This fitted variability of the entry by tester interaction indicated the role of non-crossover and crossover types of interactions, so ranks of chickpea genotypes across traits are changing, which is in accordance with the report of Sellami et al. (2021) in chickpea and investigation of Kizilgeci et al. (2019) in lentil, emphasizing the problem of getting an indirect reaction to selecting process across genotypes without reading the effect of the entry by tester interaction.

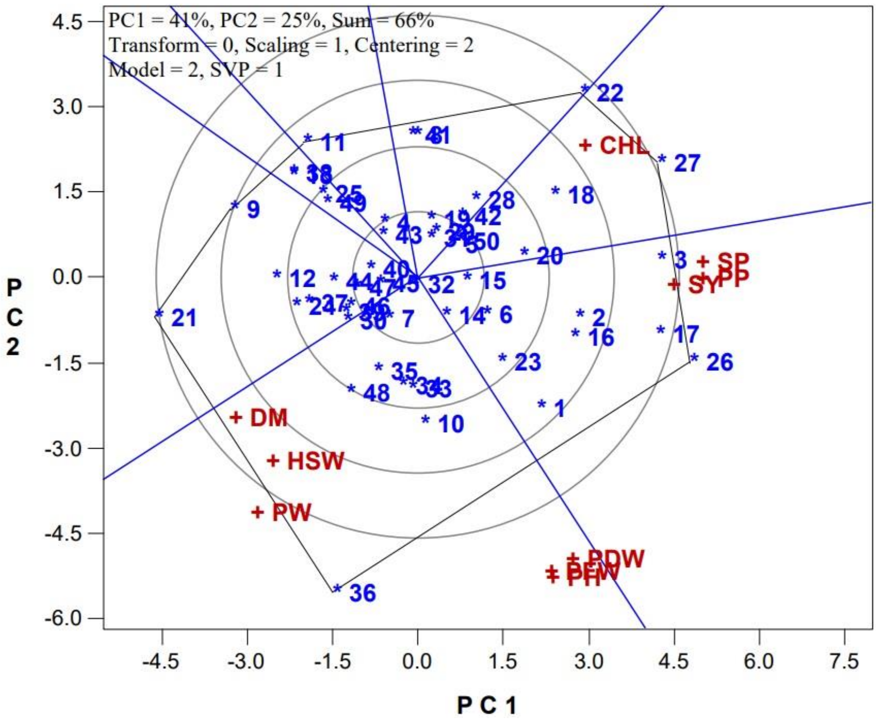

Fig. 1. Which entry (genotype) wins which tester (trait).

Regarding the role of the entry by tester interaction in our chickpea genotypes, this research benefits biplot representation facility, as advised by Yan et al. (2019), as a suitable procedure, because it provide the effective exploration of genotypes and traits. Fig. 1 indicates visual behavior of chickpea genotypes and shows which genotypes were better than others in target traits. Most traits, consist on plant height (PH), days to maturity (DM), pod's weight (PW), hundred seed weight (HSW), plant dry weight (PDW), and plant fresh weight (PFW), were grouped in the section of genotype 36 while seed yield (SY), number of seeds per pod (SP), and number of pods per plant (PP) were located in a distinct section, with the genotype 26 followed by 17 as the winning genotype. Also, genotype 27 was the best genotype for chlorophyl content (CHL), but the other four vertex genotypes (9, 11, 21 and 22) were not the excellent for the chickpea traits (Fig. 1). It seems that yield performance in chickpea is more related to the number of seeds and pods per plant instated the other traits like hundred seed weight. Although, Gediya et al. (2019) reported the positive role of the number of seeds and pods per plant on seed yield, but they found similar positive role for the hundred seed weight while we could not find such relation. However, the entry by tester biplot method prepared clear insights into the response of genotypes, revealing their potential across measured traits and facilitated the detection of the best genotypes. Thus, for commercial cultivar release, genotype 26 followed by 17 can be considered after their testing under multi-environmental trials for insurance their adaptability and yield stability abilities.

The distinction ability of a genotype for the measured traits and the typical potential for showing the symbolic properties of a genotype based on the traits for chickpea genotypes can be identified by an assumptive genotype as ideal position (Fig. 2), the best genotypes are close to this position; but genotypes of the other side are the worst.

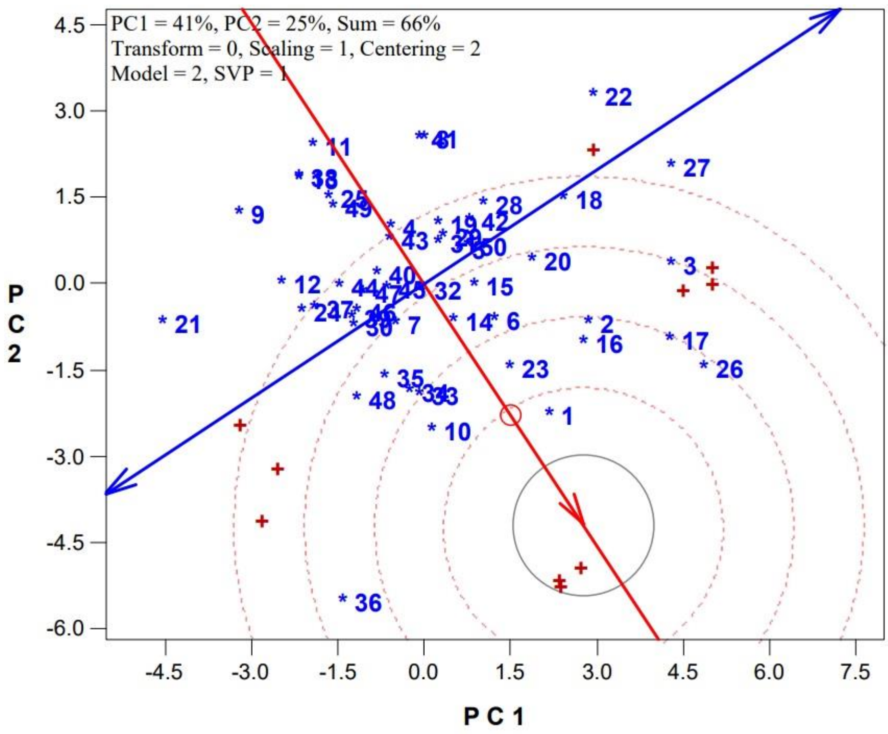

Fig. 2. Ranking entries (genotypes) based on testers (traits).

Based on Fig. 2, genotype 1 followed to 2, 10, 16, 17, 23, 26, 33 and 34 are ideal because they are the closest entries to the ideal position while genotypes 9, 11, 21 and 22 are on the other side and far from this position, so they are the most undesirable genotype regarding the distinction ability and typical potential. The identified ideal genotypes can be assumed as ideotypes which indicated high magnitude of most traits, so the problem appears once the associations of traits are not always significant. Thus, these issues are serious in a chickpea genetic improvement project, in which seed yield and quality characteristics like protein content or other yield components are important for breeding and usually the association between the seed yield and quality characteristics are low or even negative. finally, for genetic improvement projects, strategies that embrace application of multivariate statistical tools specially with graphic presentations have more importance toward the ideotype determination in chickpea.

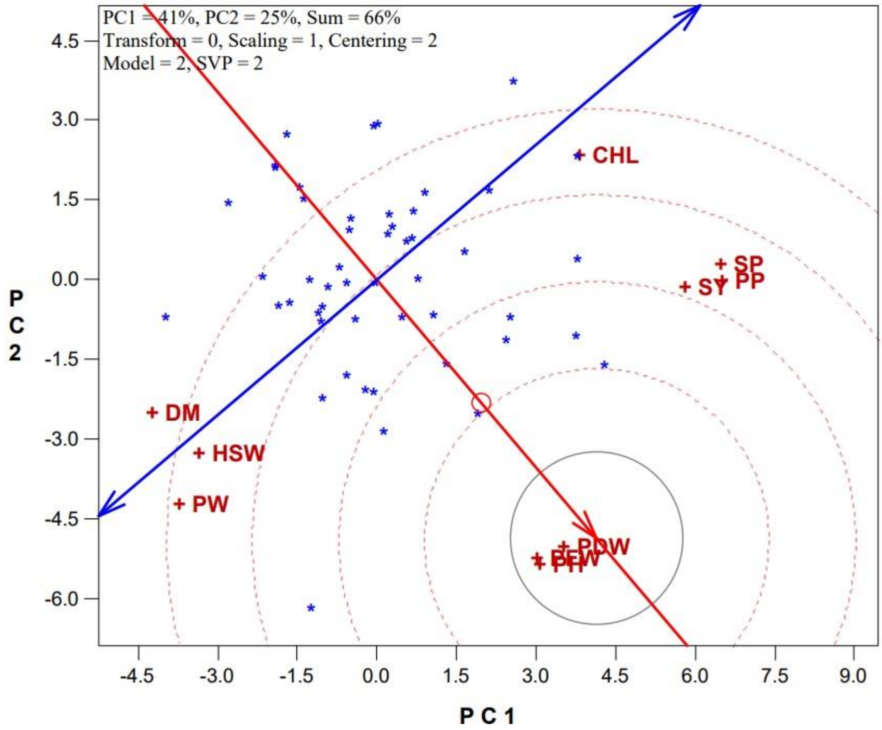

Fig. 3. Ranking testers (traits) based on discriminative and representativeness potentials.

A trait discriminative potential is the magnitude of standard deviation and the larger amount, the higher potential of the trait which is shown an assumptive trait as ideal position of measured traits (Fig. 3), so the best traits are close to this position; but traits of the other side are the worst. Thus, it can be proposed that plant height (PH), plant dry weight (PDW), and plant fresh weight (PFW) are more discriminative traits. However, the discriminative potential of the other remained traits of chickpea except days to maturity (DM) were higher than average of the discriminative potential, so they can discriminate the differences among chickpea genotypes (Fig. 3). Also, the typical potential for indicating the symbolic properties of a trait is measured by the its angle with the axis of the average trait, and the small angle demonstrate the more typical potential of the target trait. Thus, the more desirable traits (PH, PDW and PFW) had a very small angles with this axis and showed more typical potential. In contrast the other traits (DM, CHL, PP, PW, SP, HSW and SY) had large angles with the axis of the average trait and indicated relatively lower typical potential (Fig. 3)

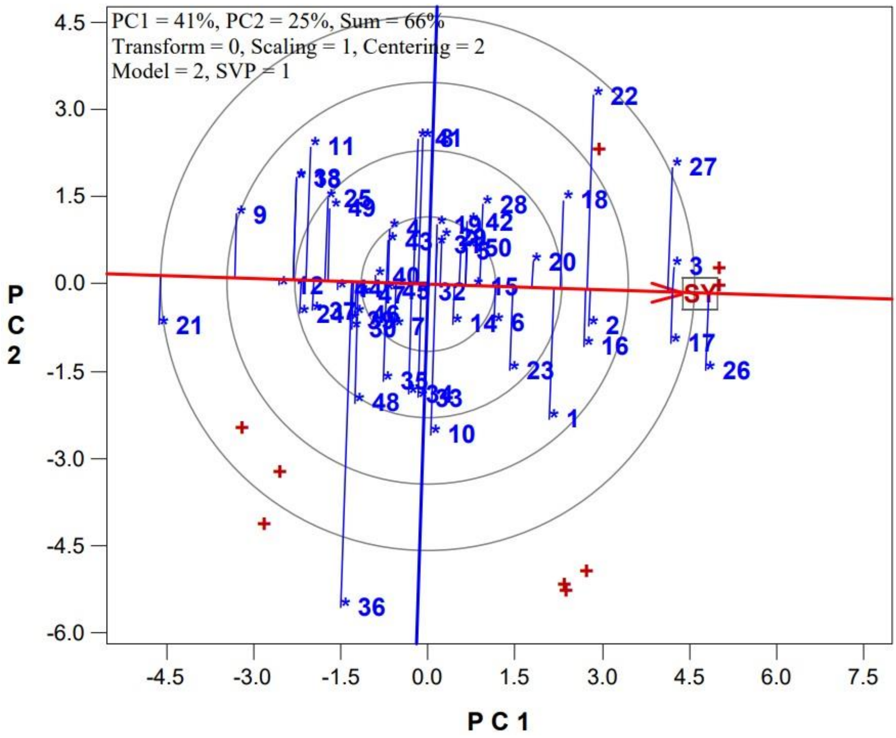

Fig. 4. Examining the performance of/at seed yield (SY).

The responses of chickpea genotypes in the term of seed yield performance as final goal trait are examined in Fig. 4, where a horizontal axis is defined as the line of seed yield and the arrow indicates the axis direction. Therefore, genotype 26 following to 3, 17 and 27, were the most desirable chickpea genotypes considering of the seed yield performance while genotypes 21 and 9 were the most undesirable in this characteristic (Fig. 4). The distance of genotypes from the horizontal axis is the index of standard deviation, so smaller distances are more desirable for selection, thus genotypes 3 and 17 can be advised with low variability. For example, genotype 36 had the low seed yield even lower than average performance (vertical axis) and had more distance from the horizontal axis, so indicated more variability, thus it is one of the most undesirable genotypes.

DISCUSSION

The genetically background of released chickpea cultivars is relatively narrow, which shows a restriction factor for genetic improvement projects. Thus, the assessment of chickpea genotypes is important is essential to barricade erosion in plant materials and manage genetic improvement tasks. We observed relatively high variation in the chickpea genotypes, in accordance with the other reports that have confirmed good diversity in chickpea (Joshi *et al.,* 2018; Aswathi *et al.,* 2019). The employed entry by tester interaction biplot model explained relatively sufficient amount of observed variation (about two third) and verified the exitance of both simple (increasable) and complicated (intersecting) types of interaction among chickpea genotypes and measured traits. Such interaction types have been reported for other legumes like dry bean (González *et al.,* 2006) and several *Vicia* L. species (Kökten *et al.,* 2010). The observed simple and complicated interaction types demonstrated different rankings of chickpea genotypes regarding traits, so selection of the best genotypes is relatively difficult for the semi-arid environments. Thus, it would not be easy to obtain an indirect reaction, ignoring the interaction effects because the genotype by trait interaction causes it hard to choose the most superior genotypes. However, various interaction types have major consideration in genetic improvement projects because they decrease the genetic (Rowntree *et al.,* 2013).

Although, genotype 36 was acted better than other genotypes in most traits but genotype 26 was the high yielding genotype with high amounts of seeds' number of pod and pods' number of plant. However, the main yield components of chickpea yield are hundred seed weight, seeds number of pod and pods' number of plant while we could not show the role of hundred seed weight. This may be due to the fact that our chickpeas genotypes were advanced lines and their seed size was almost large for marketable properties, so this trait did not show any remarkable variation in these genotypes. Also, the nine genotypes of section genotype 26 were similar to this genotype but the similarity of genotypes 2, 3, 16 and 17 was more because they indicated low distance from genotype 26, so they can be used for commercial cultivar release propose. Therefore, they must be entered in multi-environmental trials for assessment of adaptability and yield stability potential and then analyzed by statistical methods.

We found that genotype 1 following to genotypes 2, 10, 16, 17, 23, 26, 33 and 34 had high distinction ability of traits as well as showed typical potential for symbolic ability of a chickpea genotype. These genotypes can be used in future investigations for detecting the relationships among traits of chickpea. If the first principal component of the entry by tester biplot model has significant association with effects of genotypes, the ideal position of genotypes shows its higher values to obtain high performance while the low absolute values of the second principal component indicates the low variability in reaction of the ideal position. The efficiency of the ideal position tool for identification of the most favorable genotypes based on multiple traits has been demonstrated by Sabaghnia *et al.* (2016) on spinach (*Spinacia oleracea* L.) and Rahimi *et al.* (2019) on quince (*Cydonia Oblonga* Miller). However, such properties for behavior of the first and the second principal components has been proved in multi-environmental trials while it may be change in other two-way datasets, thus Yan and Rajcan (2002), for overcoming this problem, another model, which is used in this investigation, was suggested, in which the first principal component is substituted by predication of the linear regression model of the tester-centered data on the genotype effects so that the original scores are the genotype effects and make the model more explicable.

The plant height as well as dry and fresh weight of single plant were identified as the most discriminative traits which can discriminate the differences among chickpea genotypes. Also, their typical potentials for illustrating of the symbolic characteristics of traits were high, so evaluation of chickpea genotypes based on these traits result in more reliable findings and can detect variations among chickpea genotypes. Similar to the ideal position of genotypes, the ideal position of traits indicates the discriminate ability as well as representative potential of traits, so its high values the good discriminate ability of a tester or trait while the low absolute values of the second principal component indicates the better representative potential. The proficiency of the ideal position tool for identification of the most favorable traits has been demonstrated by Baljani *et al.* (2015) on safflower and Porkabiri *et al.* (2019) on tobacco. This option of biplot model is used for our entry by tester dataset based on regression adjustment of the primary biplot model (Yan and Rajcan, 2002). Due to importance of seed yield, the performance of studied genotypes was examined in regards of this final target trait, so genotype 26 following to genotypes 3, 17 and 27, were identified as the most favorable chickpea genotypes. Also, considering the variability, genotypes 3 and 17 were low variable genotypes and so they can be advised for further efforts.

CONCLUSIONS

Among the ten traits of chickpea used in present investigation, yield is related with seeds' number of pod and pods' number of plant and they could be used in selecting the most favorable genotypes. Regarding the ranks of traits based on discrimination and representative properties, plant height as well as dry and fresh weight of plant are identified as the best traits. Evaluations of several traits aid in detection the best genotypes which will be helpful to breeders to develop new cultivars with the higher yield performance epically in rainfed circumstances of semi-arid environments. Also, genotypes 3 and 17 were the most favorable genotypes so they can be advised for cultivar release.

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CONVENTIONAL AND CONTEMPORARY PRACTICES IN MONTENEGRIN OLIVICULTURE: RELYING ON TRADITION HEADING TOWARDS AN INNOVATIVE FUTURE

SUMMARY

Olive growing and olive oil production has been an ingrained tradition in the coastal areas of Montenegro for more than three millenniums. However, various problems such as complex terrain configuration, scattered land, elderly households, urbanization and poor infrastructure led to olive orchards abandonment during the last century. On the other side, climate changes enabled olive growing to shift in more northern areas of Montenegro and that restimulated farmers to revitalize their centennial olive trees, to connect oliviculture with tourism and to implement innovations in this sector. Therefore, our team analyzed the degree of conventional and contemporary practices in Montenegrin olive growing and olive oil production. Local farmers practice olive growing as a secondary activity or as a hobby, very rarely as a primary business (8.33%). Moreover, 88.33% out of interviewed farmers implement rainfed agriculture, relying solely on annual precipitation level. They made more significant progress in olive oil production than in olive growing during the last two decades. One half of the interviewed farmers start harvesting olives in October, when there are less than 25% of olives turning black and 79% of olive producers harvest by hand or use manual and/or electric shakers. Contemporary practices are implemented in post-harvesting techniques and all farmers process their olives 48 hours (the latest) after the harvest, while more than 73% use mechanical process of cold extraction on 2-phase decanters. Finally, 23% of the farmers concluded that olive oil waste management is highly important issue that has to be regulated by law.

Keywords: olive, olive oil, tradition, innovation, Montenegro

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INTRODUCTION

Traditional olive orchards account for a large share of the area under olives in the EU, particularly in marginal areas and therefore, olive growing can be described as a low-intensity production system, associated with old trees, giving small yields and receiving low inputs of labor and materials (Duarte *et al*., 2008). Olive oil is one of the most globally recognized high-value agricultural product. Europe is the top olive oil producer, with 67% of total production and 4 million hectares cultivated, mostly in Spain, Italy and Greece, at the top three leading producers of olive oil (Rapa and Ciano, 2022). With Portugal, those four countries produce more than 95% of total EU olive oil production of 12.5 million tons annually (Patsios *et al*., 2021). It is estimated that the following countries will be the leading forces in future production of olive oil, with 29.93%, 9.08%, 9.06%, 9.00%, and 8.90% of the world's production provided by Spain, Greece, Turkey, Morocco, and Italy, respectively. These five countries will account for 65.96% of the world's production level (Kurtoğlu *et al.*, 2024*)*.

Gagour *et al.* (2024) reports that olive oil is widely recognized as a main component in the Mediterranean diet owing to its unique chemical composition and associated health-promoting properties. The health advantages of extra-virgin olive oil are ascribed mainly to the antioxidant ability of the phenolic compounds (Dini *et al.*, 2020*)*. Olive oil storage is a critical post-processing operation that must be optimized to avoid oxidation. Owing to its great commercial value on markets, olive oil is a target to adulteration with other vegetable oils (Gagour *et al.*, 2024).

Olive growing and olive oil production has always been an ingrained tradition in Montenegro. Historical manuscripts on olive production, preservation and protection on the coast of Montenegro, dating back to 11^{th} and 13^{th} century, testify the importance of olive growing in this region (Perović *et al.*, 2007). The witnesses of the long lasting olive growing tradition in Montenegro are millennial olive trees, such as Old Olive Tree at Mirovica in Bar and Big olive in Ivanovići, near Budva (Lazović *et al.*, 2007). Olive orchards in Montenegro are mainly located on the slopes of mountain massifs of Orjen, Lovćen and Rumija, on hilly terrains (85%), up to the 500 meters above sea level. Suitable ecological conditions prevailing in the coastal zone of Montenegro enabled olive to become the leader among fruit species, covering about 3200 ha (Lazović *et al.*, 2018), with about 436.000 productive trees (Statistical Year Book of Montenegro, 2012). Based on the olive diversity, known for numerous autochthonous varieties (domestic and domesticated) area of Montenegrin coast may be divided in two sub-areas: Bar sub-area (municipalities of Ulcinj, Bar and Budva) and Boka Kotorska sub-area (municipalities of Tivat, Kotor and Herceg Novi) (Miranović K., 2007).

One of the crucial tasks in olive growing development is comprehensive characterization of the local olive assortment on morphological and molecular level, as well as recognition and segregation of the clones from the main olive varieties (Lazović *et al.*, 2014). According to Lazović *et al*. (2000), olive growing in Montenegro is mostly on extensive (70%) or marginal bases (28%), while the intensive agro-technics apply in only 2% of olive orchards. Average age of olive trees is from 150 to 200 years. Trees are from 7 to 10 meters high, going up even to 15 meters, which indicates non-suitability for intensive agricultural practices, such as efficient pruning, pest control and harvesting. Lazović *et al. (2014)* reported that plan protection highly influences olive growing, olive production and olive oil quality; however, is conditioned by the terrain structure, traditional olive groves and small properties. Knežević *et al. (2017)* reported that the areas suitable for olive cultivation in Montenegro are expected to shift northwards, and to the higher altitudes, due to global warming that would anticipate the flowering period of olives.

To improve the consumption of domestic olive oil, there is a need for more affordable prices and better marketing, as well as additional education on the importance of consumption and its health benefits and nutritional value (Jovanović and Joksimović, 2020). Ali *et al.* (2024) recommend to address the challenges and needs within the olive sector, which include awareness and training among farmers, infrastructure improvement, adequate storage and packaging facilities, standardized quality testing, and competitive pricing, through training programs, conducting surveys for data collection, increasing awareness of the health benefits of olive oil and establishing loan schemes and private-sector investments. Furthermore, encouraging the local production of olive oil extraction machinery and upgrading technology can help reduce reliance on imports (Ali *et al.,* 2024).

Considering the fact that Montenegro is pre-accession candidate to European Union, significant funds are available for projects implementation in domain of agriculture. Unfortunately, available grants are still not sufficiently utilized, which entails the necessity for connecting the possibilities with the stakeholders in terms of capacity building on local and national level (Markoč M., 2020).

MATERIAL AND METHODS

In this study, different segments of oliviculture have been compared among sixty farmers located in the southern Montenegro in order to assess the degree of conventional and contemporary practices in olive growing and olive oil production.

Onsite interview method was used in order to collect the data. The comprehensive survey sheet considered eight pillars of oliviculture: General information on farmers (I); General information on agricultural holdings (II); General information on olive orchards (III); Cultivation and maintenance of olive orchards (IV); Olive processing and olive oil production (V); Institutional and professional support (VI); Future investments in olive orchards (VII); Needs of olive growers' for further growth and development (VIII). This well-structured survey sheet was more than useful for the detailed analysis of both conventional and contemporary practices in Montenegrin olive growing.

The interviews with farmers have been performed onsite from January to December 2023, at territory of Bar municipality. Statistical tables and area charts were used in order to present and discuss the obtained results. Finally, desk research method and comparison method were used in order to display the data in this paper.

RESULTS AND DISCUSSION

Farmers on the territory of Montenegrin coast engage in olive growing as a secondary activity or as a hobby, very rarely as a primary business. Therefore, only 5 out of 60 respondents (or 8.33%) stated that olive production is their main occupation. For as many as 63% of respondents, income from olive growing contributes less than 10% of the total household budget, and which indicates the worrying fact that residents seldom perform this activity in professional manner (Figure 1). Furthermore, this research has shown that the lack of young labor is one of the most expressed problems of Montenegrin agriculture (35% of interviewed farmers are retired and above 60 years old).

In order to ensure strategic development of Montenegrin oliviculture, it is important to implement innovative technologies with the support of relevant public and private institutions. However, in order to receive governmental support, it is necessary for farmer to register their agricultural household with the relevant ministry. Anyway, this survey showed that 30% of the farmers has not been registered yet, while there are only 6.67% of registered legal entities in domain of olive production.

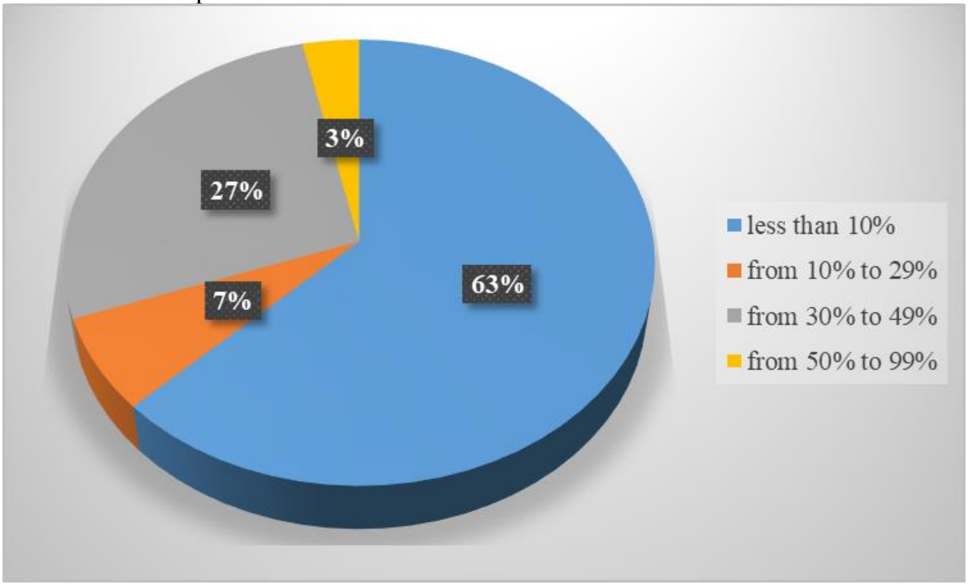

Figure 1: Contribution of olive growing and olive oil production at the total income of agricultural household

Lazović *et al.* (2017) stated that variety *Žutica* predominates in Montenegrin olive growing area with 65% (about 98% in Bar sub-area), followed by Crnica (14.8%), Sitnica (5.5%), Lumbardeška (6.6%) and Šarulja (4.5%), while the other varieties are present in about 2% within Montenegrin olive growing area. This research confirmed previously obtained results since 86.67% farmers grow Žutica, while the most common introduced olive varieties in Bar municipality are Arbequina, Leccino, Frantoio, Picholine, Maurino, Coratina, Arbosana, Ascolana Tenera. Montenegrin farmers grow olives predominantly in monoculture (71.67 %).

However, it is a devastating fact that 88.33% out of interviewed farmers implement rainfed agriculture, relying solely on precipitation level during the year. This is particularly worrying since in Montenegro there is an expressed climatic phenomenon of resource paradox – the least amount of precipitation occurs in the period when the plants have the highest water needs that leads to drought and has negative impact on the quantity and quality of the yield (Markoč M., 2020). Furthermore, merely 20% of farmers apply cultivation methods regularly, such as tillage, fertilization, pruning and pest and disease protection. This lead to the conclusion that olive growing in Montenegro is still closer to extensive than intensive, which is caused by presence of autochthonous assortment prone to alternate bearing; complex relief and terrain configuration, as well as insufficiently developed infrastructure (27%).

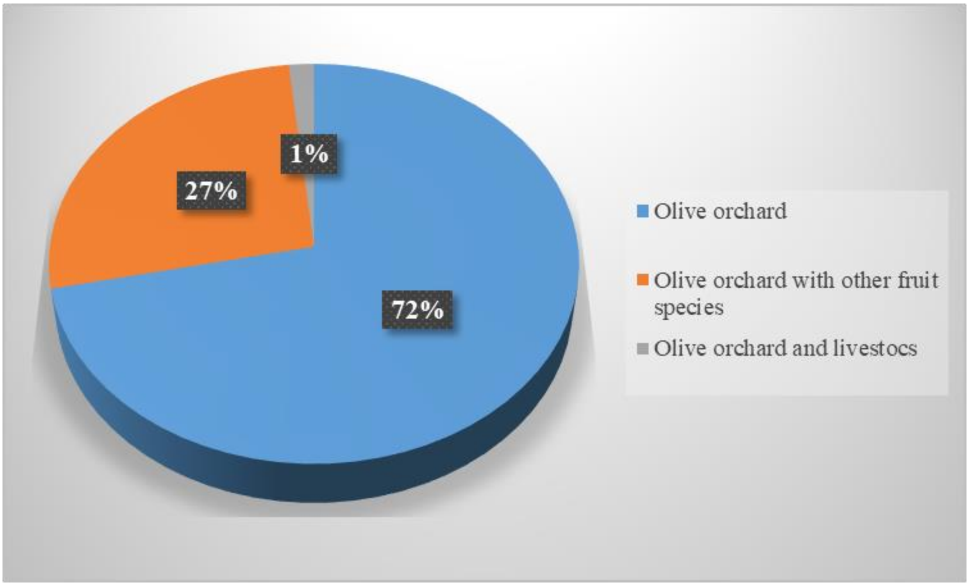

Figure 2: Monoculture system of olive growing in analyzed area

Regarding the results of soil and leaf analysis in olive orchards in southern Montenegro, it is generally recommended to decrease fertilization with potassium, but in some orchards to increase the nitrogen. Since the content of iron, and in the most cases of magnesium, was below optimal, the foliar fertilizers should be applied. In saline and calcareous soils, the application of organic fertilizers could improve nutrient uptake, transport and availability to the plant (Topalović *et al.,* 2020).

Montenegrin producers made more significant progress in secondary production (olive oil production) than in primary production (olive growing) during the last two decades. Therefore, 50% of interviewed farmers start harvesting olives in October (when there are less than 25% of olives turning black) and end harvesting in November (when there are around 50% of olive turning black), while 79% of olive producers harvest by hand or use manual and/or electric shakers (Figure 3 and Figure 4). Finally, contemporary practices are implemented in post-harvesting techniques as well, and all farmers process their olives in 48 hours (the latest) after the harvest.

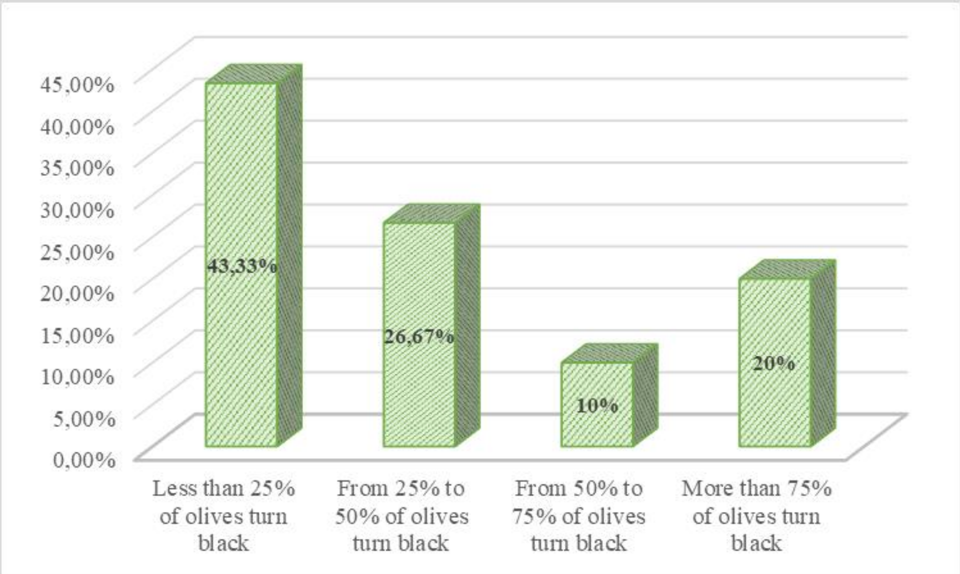

Figure 3: Determination of the beginning of the harvest according to the fruit ripening stage in analyzed olive growing area

The most popular method of olive oil production is mechanical process of cold extraction on 2-phase decanters and is practiced by 73.33% surveyed farmers (Figure 5).

Furthermore, 48.33% producers store their olive oil in tanks made of stainless steel; however, 43.33% farmers still use plastic canisters for these purposes, suggesting improper storage and preservation techniques (Table 1). Except extra-virgin and virgin olive oil, Montenegrin farmers produce table olives, olive paste, olive oil with aromatic plants (such as addition of St. John’s Wort) and souvenirs made of olive wood. One of the concerns that was raised during this research regards olive oil sale, since 38.33% out of interviewed farmers practice doorstep selling, within their agricultural holding (Table 2).

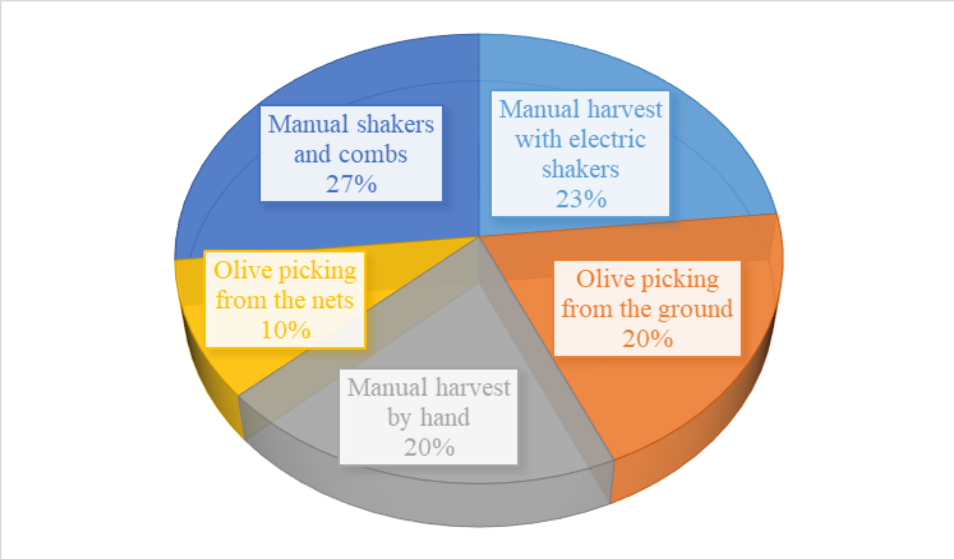

Figure 4: Harvesting techniques in analyzed olive growing area

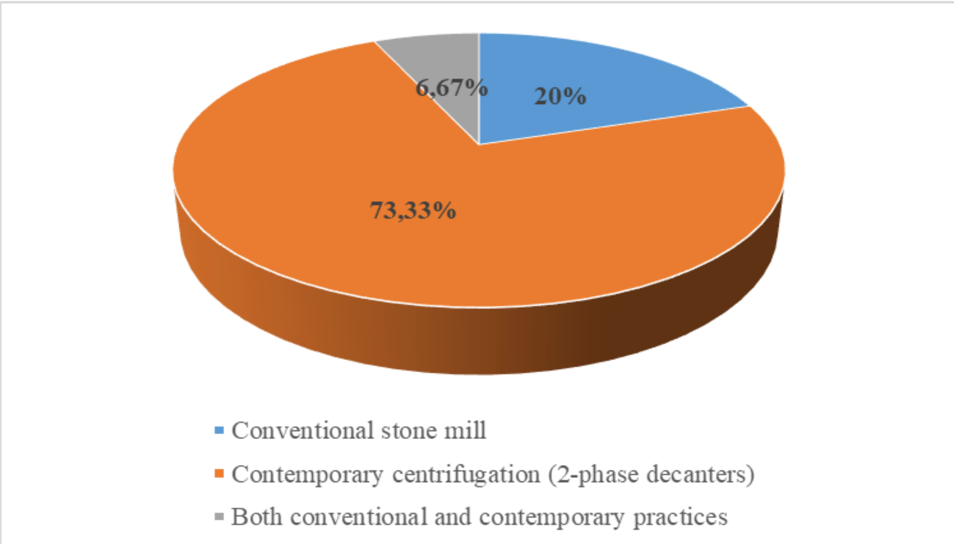

Figure 5: Conventional and contemporary technologies of olive oil production in analyzed olive growing area

Table 1: Olive oil storage and bottling practices

Olive oil storage tanks		**Olive oil bottling**	
Plastic containers	43.34 %	Plastic bottles	18.33 %
Glass containers	8.33 %	Glass bottles with etiquette	28.33 %
Stainless steel container	48.34 %	Glass bottles without etiquette	43.34 %

Table 2: Olive oil sale channels

Olive oil sale	
Directly on agricultural holding / In olive orchard	38.33 %
Marketplaces and fairs	36.67 %
Supermarkets and HORECA sector	6.67 %
Domestic usage (no sale)	16.33 %

Regarding farmers' satisfaction with advisory services, municipal and ministry support and activities of local growers' associations, this research provided us with interesting results (Table 3). Firstly, 28 out of 60 respondents (47%) is satisfied with advisory services that are on disposal; however, 24 out of 60 respondents (40%) considered that support of ministry and municipality is under average or not satisfactory at all. Non-governmental local growers' associations are bearers of olive growing improvement in Montenegro and 73.3% of farmers are satisfied or very satisfied with their level of activity and contribution to oliviculture development in coastal Montenegro. Also, 38 out of 60 interviewed farmers are members of local growers' association. 30% of interviewed farmers plan to expand their land areas under olive trees, mostly cultivating foreign olive varieties.

Table 3: Level of farmers' satisfaction with institutional support

Local and national advisory services	
unsatisfactory	16.67 %
average	36.67 %
satisfactory	46.66 %
NGOs – Local growers' associations	
unsatisfactory	1.67 %
average	25.00 %
satisfactory	13.34 %
very satisfactory	60.00 %
Municipality of Bar and Ministry of Agriculture, Forestry and Water management	
unsatisfactory	11.67 %
average	28.33 %
satisfactory	1.67 %
very satisfactory	35.00 %

This research examined farmers' needs for further work and progress in olive production as well (Table 4). Therefore, in domain of olive orchard raising, 31 out of 60 respondents or 51.67% need to acquire higher knowledge regarding proper selection of olive assortment and pollinators. In domain of olive orchard cultivation, 25 out of 60 respondents (41.67%) do not have innovative skills in proper pest and disease protection. Also, 25% of farmer needs more training in regenerative pruning of centennial and millennial olive trees. Nevertheless, in domain of olive oil production, 22 producers (36.67%) asked for more intensive education in order to understand better the technological process of olive processing and olive oil production. Also, 28.33% of farmers emphasized that are willing to attend specialized courses on organoleptic assessment of virgin olive oils. Regarding post-production techniques, farmers expressed the importance of proper olive oil storage throughout the year. One third of the farmers invest in marketing of their olive oil and other olive products, while 23.33% of the farmers stated that olive oil waste management is highly important issue that has to be regulated by law, since Montenegro is pre-candidate country for accession to the European Union.

Table 4: Needs of olive growers' for further growth and development

Raising olive orchard	
Analysis of soil characteristics in olive orchard	21.67 %
Business plan development for olive cultivation	21.66 %
Proper selection of olive assortment and pollinators	51.67 %
Proper sampling for soil and leaf agrochemical analysis	5 %
Maintaining olive orchard	
Application of organic fertilizers and manure	6.67 %
Irrigation of olive orchard	6.67 %
Soil tillage and management practices in olive orchard	1.67 %
Organic olive growing	18.33 %
Regenerative pruning of ancient olive trees	25.00 %
Integral pest and disease protection of olive trees	41.67 %
Olive oil production	
Olive oil packaging	6.67 %
Conventional and contemporary processing techniques	16.67 %
Study visits of olive oil factories abroad	11.67 %
Specialized courses for olive oil quality assessment	28.33 %
Olive oil production technology	36.67 %
Mechanization in olive growing	
Atomizers and sprinklers in olive growing	18.33 %
Pruning residues crushers	25.00 %
Electric shakers	28.33 %
Motor saws and cutters	28.33 %
Equipment and supplies	
Olive nets for harvesting	28.33 %
Olive crates from harvesting to milling	40.00 %
Olive oil storage tanks	31.67 %
Post-production techniques	
Marketing of olive and olive oil	33.33 %
Olive oil branding and registering trademark	23.33 %
Olive waste valorization	43.34 %

CONCLUSIONS

This research gave an overview of conventional and contemporary agricultural practices in olive growing and olive production of Montenegro. Therefore, olive growing in Montenegro is still closer to extensive than intensive, which is caused by presence of autochthonous assortment prone to alternate bearing; complex relief and terrain configuration, as well as insufficiently developed infrastructure. On the other hand, local farmers significantly improved their skills and knowledge regarding high quality olive oil production.

Also, this research examined farmers' needs for further work and progress in olive production as well. There is an expressed need to acquire higher knowledge regarding proper selection of olive assortment and pollinators and more training in regenerative pruning of centennial and millennial olive trees. Furthermore, farmers asked for more intensive education in order to understand better the technological process of olive processing and olive oil production and emphasized that are willing to attend specialized courses on organoleptic assessment of virgin olive oils.

Given the convenient Mediterranean climate conditions, suitable soils for olive growing, availability of land for further olive expansion, touristic attractiveness that enables placement of olive products, Montenegrin olive growing has yet to be used at its full capacity. Some of the improvements that should be carried out as soon as possible are the establishment of olive growers cadaster, increment of the production of olive seedlings, successful estimation of seasonal pests and diseases attack, expansion of olive growing area, as well as further regeneration of ancient olive trees.

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IMPROVEMENT IN SUSTAINABLE PRODUCTION OF MEDICINAL HERBS - MINT, LEMON BALM AND MARSHMALLOW

SUMMARY

The paper presents the results of the gross margin of three lines of production of medicinal herbs: mint, lemon balm and marshmallow. The importance of improving the production of these herbs has been presented in the form of gross margins. All three herb species achieved the highest gross margins in 2023, which was the criterion for choosing them for the research. The structure and participation of individual variable costs for all three production lines has been presented in relation to total variable costs. A sensitivity analysis was performed, in order to study the impact of price and yield changes (10% and 20% increase and decrease in all three production lines, and the impact of changes in the two largest costs for each production line, 10% and 20% increase and decrease in costs). Based on all the analyses, it can be concluded that the production of mint, lemon balm and marshmallow is very profitable, where around EUR 4,000–EUR 7,500 per ha of profits can be achieved.

Key words: Medicinal herbs, gross margins, production.

INTRODUCTION

The Balkan Peninsula is one of the most important centers of biodiversity in Europe, but the use of plant species in the traditional medicine of some Balkan regions has remained insufficiently researched. Medicinal herbs in previous periods had a much more versatile use (Živković *et al., 2020*).

In terms of biological diversity, Serbia is one of the 158 best centres in the world, and medicinal herbs are an integral part of Serbia's rich plant potential. Due to its favourable climate and soil, Serbia is suitable for cultivation and more

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intensive production of medicinal herbs on larger areas. The production of medicinal herbs is a great opportunity for development of small farms in rural areas of Serbia. There are around 700 types of medicinal and aromatic herbs in the Republic of Serbia, 420 of which have been officially registered, and about 270 in circulation. Regardless of great opportunities that the Serbian plant sector has in the country's economy, as well as many resources in the form of export, processing, packaging, and cultivation of medicinal herbs, it has not still sufficiently represented (Kostadinović *et al.,* 2013).

In the Republic of Serbia, the following medicinal, aromatic and spice herbs are mostly grown: chamomile and mint, on 500 ha, parsley on 100-500 ha, calendula on 100 ha, lemon balm on 50-100 ha, and basil on 10-50 ha. There are also marjoram, thyme, marshmallow, fennel, sage, oleander, celery, oregano, coriander, white scallion, valerian, which is grown in Banat on 50-100 ha, yellow gentian, selenium, and comfrey, commencing to grown due to prospects of its export to Switzerland

In recent years, there has been a high demand for medicinal herbs, in order to trade them as industrial raw materials to be further processed into medicines, cosmetic and hygienic products, spices and various extracts (Popović *et al.*, 2021; 2022a). The advantages of growing these herbs lie in having value-added products on farms, in diversification of production, obtaining quality raw materials, and in more rational use and revitalization of agricultural soil resources, since these herbs are also grown on soil of poorer quality and in hilly and mountainous areas that are completely acceptable for this production from the ecological aspect. Furthermore, growing medicinal herbs preserves rare and endangered plant species, especially those whose collection is prohibited or limited. Due to its available natural resources, Serbia is suitable for intensive cultivation of medicinal herbs, but farmers are advised to contract the sale beforehand. Moreover, the production and processing of medicinal herbs produces significant amounts of waste material that can be used as natural fertilizer in organic agriculture, significantly contributing to the implementation of the principles of circular bioeconomy and good agricultural practice. The production of medicinal herbs is in accordance with the principles of organic agriculture and sustainable rural development (Ugrenović *et al., 2015*).

The traditional use of medicinal herbs while respecting the principles of modern science has proven the presence of significant compounds in medicinal herbs with high biological activity. The goal of improving this production, apart from increasing farmers' profit, is the development of food products that have a positive effect on human health, prevent and reduce the risk of disease. The use of medicinal herbs and their extracts in the food industry has created a wide technological potential for the development of new technological processes and products for different purposes. The market for medicinal herbs has been growing at a rapid pace across the world. Farmers in Serbia should organize the production of those medicinal herbs that traditionally thrive well in our agro-ecological conditions and achieve good quality. Moreover, it would be good if the process of production of medicinal herbs (Popović *et al*., 2018; 2021; 2022a; 2022b; Petrović *et al*., 2021; 2022; Burić *et al.*, 2023; Stevanović *et al.*, 2023; Vasileva *et al.*, 2023; Filipović *et al*., 2021; 2023), inclined as much as possible to the direction of organic agriculture (Radojković *et al., 2017*). The aim of this study is to show the results of the gross margin of three lines of production of medicinal herbs: mint, lemon balm and marshmallow, as well as the importance of improving the production of herbs in question.

MATERIAL AND METHOD

In order to assess the economic effect of certain lines of production of medicinal herbs, the paper presents the gross margins for three lines of production: mint, lemon balm and marshmallow. The methods comprised an economic analysis of the production of medicinal herbs, primarily calculations based on variable costs (gross margin), according to which the researchers determined the basic economic parameters of production (value of production, variable costs and gross margin) per unit area. When determining the optimal volume and structure of production, calculations are often based on variable costs, and the obtained indicators can be used as a good financial instrument for making business decisions (Gogić, *2014;* Petrović *et al.*, *2021*).

The data on which the gross margins for mint, lemon balm and marshmallow production were calculated were obtained from data on the production of the medicinal herbs collected and analysed by the Institute for the Study of Medicinal Plants "Dr Josif Pančić" (ISMP), in Belgrade, Serbia. The reason for choosing these three species of medicinal herbs is that they achieved the highest gross margin in 2023. Furthermore, the method of sensitive analysis was used to determine which of the parameters is sensitive to change, and to determine the impact of those changes on the gross margins.

RESULTS AND DISCUSSION

Table 1 shows the results for the gross margins of the investigated medicinal herbs in the production year of 2023.

As shown in Table 1, it can be concluded that the highest value of production and the highest gross margin is recorded in the production of marshmallow. The value of marshmallow production in 2023 was EUR 24,000, while the gross margin per unit area amounted to EUR 7,545. The value of mint and lemon balm production was at a similar level amounting to EUR 7,200 and EUR 7,500, respectively, while the gross margins of the productions in question were also at a similar level (EUR 3,742 and EUR 3,938 per hectare). Although the production value and gross margin of marshmallow was highest compared to the other two productions, the share of gross margin in the value of marshmallow production was 31.44%, while the share of gross margin in the value of mint and lemon balm production was around 52%. The reason for this ratio lies in significantly higher variable costs in marshmallow production than in the other two productions.

Table 1. Gross margins of production of the medicinal herbs in 2023 (per ha)

Herbs	Production value (EUR)	Variable costs (EUR)	Gross margin (EUR)	Share of gross margin in production value (%)
Mint	7,200.00	3,458.00	3,742.00	51.97
Lemon balm	7,500.00	3,562.00	3,938.00	52.51
Marshmallow	24,000.00	16,455.00	7,545.00	31.44

Source: Authors' calculation based on the data from ISMP

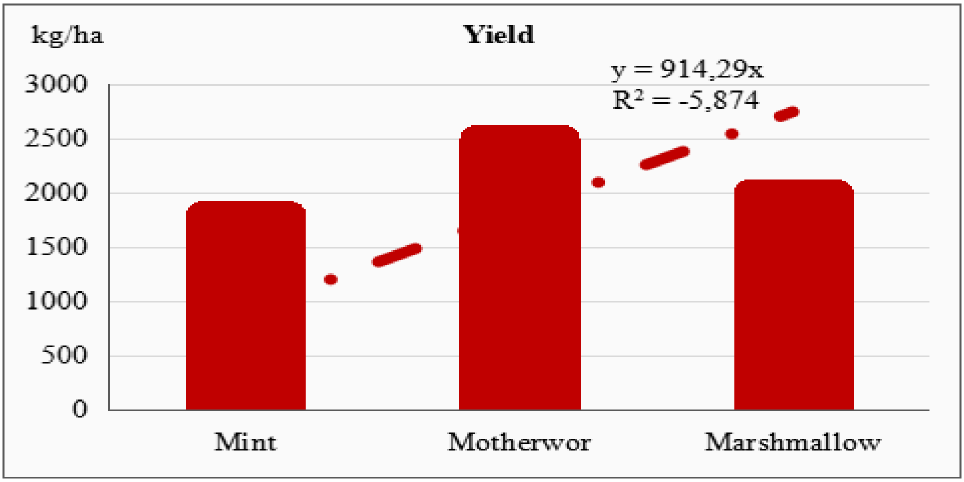

Figure 1. Yield of Mint, Motherwort and Marshmallow

Table 2 shows the structure and participation of individual variable costs in the production of the medicinal herbs in 2023.

Table 2 shows data on the amount and structure of variable costs in the production of mint, lemon balm and marshmallow. It can be concluded that in production of all three herbs, the cost of labour comprised the largest share of costs. In the production of mint, the labour costs made 37.59% of the total variable costs, in the production of lemon balm 40.71%, and in the production of marshmallow as much as 82.04%.

The second largest cost was the cost of planting material in the production of mint and lemon balm, amounting to 23.13% and 19.09% of the total variable costs of the production of these herbs. With marshmallow, the situation was slightly different, and after labour costs, the second largest variable cost was the cost of fuel (6.78%). Therefore, it can be concluded that all three productions of medicinal herbs are highly intensive, which is confirmed by the fact that the cost of labour was the highest in the total variable costs.

Table 2. Structure and participation of individual variable costs in the production of selected medicinal herbs in 2023 (per ha)

Herbs	Mint	Lemon balm	Marshmallow
Mineral fertilizers	376.00	350.00	460.00
	10.87%	9.83%	2.80%
Means of protection	76.00	76.00	140.00
	2.20%	2.13%	0.85%
Planting material/seeds	800.00	680.00	180.00
	23.13%	19.09%	1.09%
Diesel fuel	666.00	666.00	1,115.00
	19.26%	18.70%	6.78%
Drying costs	240.00	340.00	1,060.00
	6.94%	9.55%	6.44%
Labour costs	1,300.00	1,450.00	13,500.00
	37.59%	40.71%	82.04%
Total variable costs	3,458.00	3,562.00	16,455.00

Source: Authors' calculation based on the data from ISMP

A sensitivity analysis was done for all three types of production, namely for the impact of price and yield on the gross margin, and the impact of the two highest variable costs in the production of the three medicinal herbs in question. Table 3 shows the sensitivity analysis of price and yield changes on the gross margin in mint production.

Table 3 shows the sensitivity analysis of the gross margin in mint production to price and yield changes. The analysis took into account changes in price and yield (10% and 20% increase and decrease), and calculating the gross margin in relation to the aforementioned changes. Based on the sensitivity analysis, it can be concluded that with 20% increase in price and yield, the gross margin of mint production would amount to EUR 6,910, which would be an increase of 84.66% compared to the estimated gross margin of EUR 3,742. Furthermore, if the price and yield were to decrease by 20% each, the gross margin of mint production would amount to only EUR 1,150, which would be a decrease of 69.27%.

Therefore, it can be concluded that mint production, despite a 20% reduction in price and yield, would still give a good economic result in the form of a positive gross margin.

Table 4 shows the sensitivity analysis of the impact of change in the two largest variable costs on the gross margin in mint production.

Table 3. Analysis of the sensitivity of price and yield changes on the gross margin

in mint production (per ha)

Yield (kg/ha)		Price (EUR/kg)				
		-20%	-10%	Real	10%	20%
		3.20	**3.60**	**4.00**	**4.40**	**4.80**
-20%	**1,440.00**	1,150.00	1,726.00	2,302.00	2,878.00	3,454.00
-10%	**1,620.00**	1,726.00	2,374.00	3,022.00	3,670.00	4,318.00
Real	**1,800.00**	2,302.00	3,022.00	**3,742.00**	4,462.00	5,182.00
10%	**1,980.00**	2,878.00	3,670.00	4,462.00	5,254.00	6,046.00
20%	**2,160.00**	3,454.00	4,318.00	5,182.00	6,046.00	6,910.00

Source: Authors' calculation based on the data from ISMP

Table 4 presents an analysis of the sensitivity of the gross margin in mint production to changes in the two most common variable costs (in this case the cost of labour and the cost of planting material). The analysis took into account changes in the amount of the costs (10% and 20% increase and decrease), and calculated the gross margin in relation to the aforementioned changes. Based on the sensitivity analysis of the impact of 20% increase in labour costs and planting material, it can be concluded that there is a decrease of 11.22% in the gross margin, while the 20% decrease of the costs would lead to almost the same percentage of increase in the gross margin.

Table 4. Analysis of the sensitivity of the change in the two largest variable costs on the gross margin in mint production (per ha)

Planting material (EUR/ha)		Labour costs (EUR/ha)				
		-20%	-10%	Real	10%	20%
		1,040.00	**1,170.00**	**1,300.00**	**1,430.00**	**1,560.00**
-20%	**640.00**	4,162.00	4,032.00	3,902.00	3,772.00	3,642.00
-10%	**720.00**	4,082.00	3,952.00	3,822.00	3,692.00	3,562.00
Real	**800.00**	4,002.00	3,872.00	**3,742.00**	3,612.00	3,482.00
10%	**880.00**	3,922.00	3,792.00	3,662.00	3,532.00	3,402.00
20%	**960.00**	3,842.00	3,712.00	3,582.00	3,452.00	3,322.00

Source: Authors' calculation based on the data from ISMP

Table 5 presents an analysis of the sensitivity of price and yield changes to the amount of gross margin in the production of lemon balm.

Table 5 shows the sensitivity analysis of the gross margin in the production of lemon balm to changes in price and yield. It can be concluded that if there was 20% increase in price and yield, the gross margin of lemon balm production would amount to EUR 7,238, which would be an increase of 83.80% compared to the estimated gross margin of EUR 3,938 per ha. Moreover, if the price and yield were to decrease by 20% each, the gross margin of lemon balm production would amount to only EUR 1,238 per ha, which would be a decrease of 68.56%. Based on this analysis, it can be concluded that the production of lemon balm, as well as the production of mint, despite the 20% reduction in price and yield, would still give a good economic result in the form of a positive gross margin.

Table 5. Analysis of the sensitivity of price and yield changes to the amount of gross margin in the production of lemon balm (per ha)

Yield (kg/ha)		Price (EUR/kg)				
		-20%	-10%	Real	10%	20%
		2.40	**2.70**	**3.00**	**3.30**	**3.60**
-20%	**2,000.00**	1,238.00	1,838.00	2,438.00	3,038.00	3,638.00
-10%	**2,250.00**	1,838.00	2,513.00	3,188.00	3,863.00	4,538.00
Real	**2,500.00**	2,438.00	3,188.00	**3,938.00**	4,688.00	5,438.00
10%	**2,750.00**	3,038.00	3,863.00	4,688.00	5,513.00	6,338.00
20%	**3,000.00**	3,638.00	4,538.00	5,438.00	6,338.00	7,238.00

Source: Authors' calculation based on the data from ISMP

Table 6 shows the analysis of the sensitivity of the impact of change in the two largest variable costs to the gross margin in the production of lemon balm.

Table 6. Analysis of the sensitivity of the change in the two largest variable costs to the gross margin in the production of lemon balm (per ha)

Planting material (EUR/ha)		Labour costs (EUR/ha)				
		-20%	-10%	Real	10%	20%
		1,160.00	**1,305.00**	**1,450.00**	**1,595.00**	**1,740.00**
-20%	**544.00**	4,364.00	4,219.00	4,074.00	3,929.00	3,784.00
-10%	**612.00**	4,296.00	4,151.00	4,006.00	3,861.00	3,716.00
Real	**680.00**	4,228.00	4,083.00	**3,938.00**	3,793.00	3,648.00
10%	**748.00**	4,160.00	4,015.00	3,870.00	3,725.00	3,580.00
20%	**816.00**	4,092.00	3,947.00	3,802.00	3,657.00	3,512.00

Source: Authors' calculation based on the data from ISMP

Table 6 presents an analysis of the sensitivity of the gross margin in the production of lemon balm to changes in the two most represented variable costs (in this case, as in the case of mint production, these were the cost of labour and cost of planting material). Based on the results of the sensitivity analysis, it can be concluded that 20% increase in the costs of labour and planting material would result in 10.82% decrease in gross margin, while 20% reduction of the costs

would lead to almost the same percentage of increase in the gross margin.
Table 7 presents an analysis of the sensitivity of change in price and yield to the gross margin in the production of marshmallow.

Table 7. Analysis of the sensitivity of price and yield changes to the gross margin in marshmallow production (per ha)

		Price (EUR/kg)				
		-20%	-10%	Real	10%	20%
Yield (kg/ha)		**9.60**	**10.80**	**12.00**	**13.20**	**14.40**
-20%	**1,600.00**	-1,095.00	825.00	2,745.00	4,665.00	6,585.00
-10%	**1,800.00**	825.00	2,985.00	5,145.00	7,305.00	9,465.00
Real	**2,000.00**	2,745.00	5,145.00	**7,545.00**	9,945.00	12,345.00
10%	**2,200.00**	4,665.00	7,305.00	9,945.00	12,585.00	15,225.00
20%	**2,400.00**	6,585.00	9,465.00	12,345.00	15,225.00	18,105.00

Source: Authors' calculation based on the data from ISMP

Table 7 shows the sensitivity analysis of the gross margin in marshmallow production to price and yield changes. Based on the results of the sensitivity analysis, it can be concluded that 20% increase in price and yield of marshmallow would result in the gross margin production of EUR 18,105 per ha, which would be an increase of 239.96% compared to the estimated gross margin of EUR 7,545 per ha. Nevertheless, 20% decrease in price and yield would result in a negative gross margin (EUR -1,095 per ha), which would be a decrease of 146.12%. Based on this analysis, it can be concluded that marshmallow production is very sensitive to changes in price and yield, which is also confirmed by the data on projected gross margin. The increase in price and yield of 20% would result in a large increase in the gross margin, almost 2.5 times higher, while the reduction of 20% of the parameters in question would make the gross margin negative.

Table 8 shows the analysis of the sensitivity of the change in the two largest variable costs to the gross margin in marshmallow production.

Table 8 presents the analysis of the sensitivity of the gross margin in marshmallow production to changes in the two most common variable costs (in this case, labour and fuel costs).

Based on the results of the sensitivity analysis, it can be concluded that the increase in labour and fuel costs of 20% would result in a decrease of 38.74% in a gross margin, while the reduction of 20% in the mentioned costs would lead to almost the same percentage of increase in the gross margin.

Based on this analysis, it can be determined that changes in the two most represented variable costs have a greater impact on changes in the gross margin in the marshmallow production compared to changes in the costs in the production of mint and lemon balm.

Table 8. Sensitivity analysis of the change in the two largest variable costs on the gross margin in marshmallow production (per ha)

Diesel fuel (EUR/ha)		Labour costs (EUR/ha)				
		-20%	-10%	Real	10%	20%
		10,800.00	**12,150.00**	**13,500.00**	**14,850.00**	**16,200.00**
-20%	**892.00**	10,468.00	9,118.00	7,768.00	6,418.00	5,068.00
-10%	**1,003.50**	10,356.50	9,006.50	7,656.50	6,306.50	4,956.50
Real	**1,115.00**	10,245.00	8,895.00	**7,545.00**	6,195.00	4,845.00
10%	**1,226.50**	10,133.50	8,783.50	7,433.50	6,083.50	4,733.50
20%	**1,338.00**	10,022.00	8,672.00	7,322.00	5,972.00	4,622.00

Source: Authors' calculation based on the data from ISMP

The results of many authors are in line with our results. The variety of soil and climate factors and production at different altitudes (even extremely high) are the objective basis of successful plantation production of medicinal plants in the Republic of Serbia. in Serbia, only between 1300 and 1800 ha of medicinal plants are planted annually. The European market shows an interest in high-quality medicinal plants originating from our area, which would provide significant financial results to business entities in the future. Incentive measures of the Ministry of Agriculture, Forestry and Water Management aimed at the cultivation of medicinal plants would significantly contribute to the development of hilly and mountainous regions, as well as the entire rural community of the Republic of Serbia (Ceranić *et al.*, 2005; Popović, 2008; Dajić, Dražić, 2003). The sustainable development of natural resources of medicinal and herbal plants directly depends on the implementation and improvement of legislation and standards that need to be harmonized with EU legislation and standards. Regardless of the great potential of the plant sector within the economic system of the country, many potentials, especially in export, higher stages of processing and cultivation of medicinal plants (especially on the principles of organic production) have not been used (Maletić *et al.*, 2010).

CONCLUSION

Serbia has excellent climatic and geographical conditions for growing medicinal and aromatic herbs. Growing medicinal and aromatic herbs on farms would create value-added products and be a source of additional farm income. In order to improve the production of medicinal and aromatic herbs, it is necessary to take a number of technological measures.

Based on all the aforementioned analyses, it can be concluded that the production of mint, lemon balm and marshmallow is very profitable, where around EUR 4,000 per ha and EUR 7,500 per ha of profit can be generated. A possible obstacle to the production on larger areas could be high labour requirements, which is confirmed by the fact that the cost of labour in all three lines of production was largest of all variable costs. When it comes to the sensitivity to price and yield changes, mint and lemon balm production achieved a positive financial result despite 20% decrease in both yield and price. Moreover, a positive financial result is achieved due to the increase of the two largest variable costs by 20% each, with other parameters unchanged.

Although marshmallow production can generate the highest financial result per hectare, with an increase of 20% in both price and yield the financial result of this production would be negative. Nevertheless, an increase in the two largest costs with other parameters unchanged would achieve a positive financial result. It can be concluded with a certainty that the introduction of profitable medicinal herbs into the sowing structure would generate an additional income on farms, and would increase the net profit of farms, as well as their economic sustainability.

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PECULIARITIES OF THE SODIUM AZIDE ACTION AS A FACTOR OF VARIABILITY ON WINTER WHEAT

SUMMARY

The study of new agents for indicating practical biodiversity in local varieties of winter wheat is a promising area in terms of obtaining both new commercial varieties and components for recombination selection. The purpose of the study was to demonstrate the possibilities in this regard as a sodium azide mutagen, primarily in terms of optimizing the yield of mutant forms based on local varietal resources. Winter wheat dry seeds of eight varieties were acted with water (control) and SA (sodium azide) action in concentrations of 0.01%, 0.025%, 0.05%, 0.1%. Successful use for all the varieties is primarily the effect of SA concentrations of 0.01%, 0.025%, for individual, less variable genotypes, the use of SA 0.05% can also be optimal in terms of the yield of valuable forms. Herewith, only three of the varieties demonstrated a significant genotype-mutagenic interaction in terms of significant changes in the parameters of variability, and only one of them in a positive sense. This agent is promising in obtaining short-stemmed, early-ripening, disease-resistant forms and lines with large grains and heads. Herewith, the number of negative changes is also quite high and their specific weight in the spectrum is higher in comparison with the previously studied factors of variability. Only one of the studied genotypes demonstrated the possibility of using it to create a mutation induction system with an increased yield of valuable forms. In the future, it is planned to study the variability in biochemical parameters of grain and resistance to abiotic environmental factors.

Keywords: winter wheat, chemical mutagenesis, sodium azide, mutation, plant improvement.

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INTRODUCTION

Bread winter wheat continues to be an extremely valuable grain food crop, especially for areas of risky farming, which include the entire territory of Ukraine (Nazarenko et al., 2021). Problems with global warming and climate change lead, on the one hand, to the advancement of more heat-loving crops to the north, mitigation of overwintering conditions, which is especially important for winter crops, but also with an increase in the likelihood and severity of droughts during critical periods of development of some crops (Hongjie, et al.,2019; Harkness, et al., 2020).

One of the actively used and promising ways is the genetic improvement of varieties to better match the growing conditions (Cann et al., 2022) or improve local forms and bring them up to the level of world samples in terms of yield and quality traits is the use of mutation induction, not least through chemical mutagenesis (Hassine, et al., 2023).

Chemical mutagenesis is of interest primarily in terms of a higher interaction with a specific genotype, site specificity with respect to native, original DNA, which is much less typical for physical mutagens (Yali & Mitiku, 2022). Herewith, several extremely promising opportunities open up at once for the genetic improvement of agricultural crops, which are less typical when using other factors of variability (Abdel-Hamed et al., 2021).

Firstly, the use of new factors on local material (in this case, sodium azide) allows us to hope for significant changes in terms of the spectrum and frequency of the changes obtained (Cann et al., 2022), a significant predominance of certain types of rare valuable mutations in the case of identifying a more susceptible genotype (Spencer-Lopes et al., 2018).

Secondly, the presence of complex small changes, without additional negative mutant traits, is more typical for chemical factors, primarily in terms of biochemical mutations, which can significantly improve the nutritional characteristics of traditional crops, which by no means always correspond in their compositions of biologically active substances and microelements to the needs of consumers (Kumar et al., 2018).

Last but not least, the use of a certain chemical, usually at a moderate concentration, can lead to an optimal source material-mutagen system in terms of mutation yield, which, under certain conditions can increase the yield of valuable forms, as well as the overall mutational variability, up to 60 – 80% and it is not a fact that such values can be limiting, especially for some classes of previously unused or limitedly used chemicals (Abdel-Hamed et al., 2021). In particular, the use of sodium azide on some cereal crops also demonstrates certain hopes in this area (Hassine et al., 2023).

Herewith, many chemical agents, especially those with a high damaging effect, can vary within one concentration in terms of the degree of inhibition of a trait up to 10-12% of its absolute value, which makes the study of the genotype-mutagenic component of the interaction even more important (Mangi et al., 2021).

Clearly, with an increase in the genetic activity of the factor of variability, this kind of interaction also decreases, but not always. It is possible to identify forms for which the rate of interaction decrease is significantly lower or even absent. (Chaudhary et al., 2019; Abdel-Hamed et al., 2021).

Qualitative differences in the genotype-mutagenic interaction led to the need to conduct appropriate studies on the frequency and spectrum of emerging changes in the widest possible range of genotypes in order to compare the source material in variability and the most diverse set of mutagens (Chakraborty et al., 2023). Herewith, the issue of limitation in the doses or concentrations used is raised each time, which is solved by monitoring studies in the first generation and by limiting the sample to establish the objective patterns of the mutation process, its key criteria (Chaudhary et al., 2019).

The purpose of the study was to demonstrate hereditary variability based on visual and biometric analysis of phenotypic changes in the second generation of mutant families, to identify new lines with inherited altered traits in the third and subsequent generations, to identify the boundaries of possible, primarily practically valuable, variability for the source material in comparison of individual varieties, including in connection with the already established depressive effects in the first generation, to identify possible optimal compositions in the system of source material – mutagen concentration.

MATERIAL AND METHODS

The experiments were carried out under the conditions of the research fields station of the Science-Education Center of the Dnipro State Agrarian Economic University during 2017–2021 (48°51′10″ n. l. 35°25′31″ e. l.).

Winter wheat seeds (1000 grains for each concentration and water) were acted with a SA (sodium azide) 0.01%, 0.025%, 0.05%, 0.1% (Sigma-Aldrich, Germany) at water solution. Seed material was acted with an exposition of 24 hours by the generally recommended protocols for chemical mutagens action. Concentrations were trivial for this type of mutagens. The control was soaked in water (Spencer-Lopes et al., 2018).

Seeds material has been sown by 40 variants (in total) (2-rows plots for second generation, 5-rows for third generations and 10-rows plots for next generations, initial variety as control, interrows were 0.15 m, 1.5 m length of row). Eight varieties with difference at ecotype were used (in brackets FS – forest-steppe ecotype, all for all zones, S – steppe by state examination classification) Balaton (FS for Ukraine, only one variety of West-Europe ecotype), Borovytsia (all), Zeleny Gai (S), Zoloto Ukrainy (FS), Kalancha (all), Niva Odeska (all), Polyanka (all), Pochayna (all). The genotypes were identified by general national breeding classification as for Steppe conditions semi-intensive Niva Odeska, Zoloto Ukrainy; intensive West-Europe ecotype Balaton, semi-intensive Forest-Steppe ecotype (all other) (Spencer-Lopes et al., 2018).

The crop cultivation is trivial for the Steppe zone. The control was non-treated initial and one were also grown after ten plots for each variant as comparison with the mutant families at second generation. The sowing was done by hand, at the end of September, at a depth of 4-5 cm and with a rate of 100 viable seeds to a row, 2 rows for sample with control-row of initial variety samples. Mutant lines were planted at three replications with control-rows of parent variety for each twenty-row plot (Mangi et al., 2021).

At M_2–M_3 generations mutations were identified though visual evaluation and biometrical analyze of yield structure. At second generation preliminary evolution by visible changes, at second identification as mutation by traits heredity. Estimation was conducted during 2018 – 2019 years for second-third generations and during for 2020 –2022 years for next generation in collection of genetic-value samples and lines grain production exam.

Level of changeability was calculated as $P\nu = \alpha * \gamma$, where $P\nu$ – level of changeability of variant; α – number of mutations for general number of families at variant; γ – number of types changed traits at variant.

Statistic analyze of data was performed by ANOVA-analysis, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSION

In total, for control and material after mutagen action 19400 families at second generation and 1692 mutant lines at third generation were investigated. Mutagen has been used in recommended concentrations for cereal breeding practice. The number of families at M2 generations on average is about 500 per variant, except extreme concentration SA 0.1% for six varieties (not for Polyanka and Pochayna).

Data about the general rates of change at second-third generation for each variety and concentration of mutagen factor are presented at tables 1 and 2 for two groups of genotypes respectively (with higher level of mutagen depression at first generation varieties for first group and with less depression effects at the same generation for second group (subsequently it will be shown by cluster analyze). As for previous investigations level of depression has significantly relation with mutation variability at next generation. Its main reason for this type classification of plant material.

However, when analyzing the first group, we do not find a significant high dependence, which is confirmed primarily due to the variability of the varieties of the first group. Thus, varieties of the first group showed the following mutation rates Balaton (general rate up to 21 %), Zoloto Ukrainy (up to 19,5 %), Zeleny Gai (up to 19.25 %), Niva Odeska (up to 20.5 %). This is approximately the same value as in the varieties of the second group. The exception was the varieties Polyanka and Pochayna, where the variability is much lower than in other genotypes. Thus, it makes sense to talk about the response of each genotype separately, but not by groups of mutagenic depression.

Table 1. General rate of hereditary changes for winter wheat samples at second – third generations. First group (more sensitive to mutagen action) (x ± SD, n = 400-500).

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Balaton	500	2	0.40 ± 0.10[a]
Balaton, SA 0.01%	500	34	6.80 ± 0.30[b]
Balaton, SA 0.025%	500	49	9.80± 0.43[c]
Balaton, SA 0.05%	500	69	13.80±0.55[d]
Balaton, SA 0.1%	400	84	21.00±0.62[e]
Zoloto Ukrainy	500	6	1.20 ± 0.24[a]
Zoloto Ukrainy, SA 0.01%	500	31	6.20± 0.28[b]
Zoloto Ukrainy, SA 0.025%	500	39	7.80±0.40[c]
Zoloto Ukrainy, SA 0.05%	500	64	12.80±0.44[d]
Zoloto Ukrainy, SA 0.1%	400	78	19.50±0.57[e]
Zeleny Gai	500	5	1.00 ± 0.20[a]
Zeleny Gai, SA 0.01%	500	27	5.40±0.32[b]
Zeleny Gai, SA 0.025%	500	41	8.20±0.37[c]
Zeleny Gai, SA 0.05%	500	58	11.60±0.51[d]
Zeleny Gai, SA 0.1%	400	77	19.25±0.65[e]
Niva Odeska	500	3	0.60 ± 0.18[a]
Niva Odeska, SA 0.01%	500	39	7.80±0.39[b]
Niva Odeska, SA 0.025%	500	53	10.60±0.44[c]
Niva Odeska, SA 0.05%	500	73	14.60±0.74[d]
Niva Odeska, SA 0.1%	400	82	20.50±0.69[e]

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

For first group mutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 146.23$; $F_{0.05} = 3.48$; $P = 3.15*10^{-9}$) and for genotype-mutagen interaction ($F = 6.10$; $F_{0.05} = 2.72$; $P = 0.01$), but not for genotypes ($F = 3.16$; $F_{0.05} = 3.86$; $P = 0.07$).

For second group mutagen action was statistically significant not only for the variance in the change in mutagen concentration ($F = 158.23$; $F_{0.05} = 3.48$; $P = 4.09*10^{-10}$) and for genotype-mutagen interaction ($F = 28.77$; $F_{0.05} = 2.72$; $P = 6.17*10^{-4}$), but for genotypes ($F = 9.90$; $F_{0.05} = 3.86$; $P = 0.003$) too.

Table 2. General rate of hereditary changes for winter wheat samples at second – third generations. Second group (more tolerance by genetic activity) (x ± SD, n = 400-500).

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Borovytsia	500	4	0,80 ± 0.08[a]
Borovytsia, SA 0.01%	500	29	5,80±0.27[b]
Borovytsia, SA 0.025%	500	40	8,00±0.41[c]
Borovytsia, SA 0.05%	500	60	12,00±0.52[d]
Borovytsia, SA 0.1%	400	79	19.75±0.65[e]
Kalancha	500	3	0,60 ± 0.06[a]
Kalancha, SA 0.01%	500	31	6,20±0.31[b]
Kalancha, SA 0.025%	500	39	7.80±0.42[c]
Kalancha, SA 0.05%	500	65	13.00±0.52[d]
Kalancha, SA 0.1%	400	85	21.25±0.68[e]
Polyanka	500	2	0,40 ± 0.12[a]
Polyanka, SA 0.01%	500	22	4,40±0.32[b]
Polyanka, SA 0.025%	500	33	6,60±0.51[c]
Polyanka, SA 0.05%	500	53	10,60±0.50[d]
Polyanka, SA 0.1%	500	62	12.40±0.63[e]
Pochayna	500	2	0,40 ± 0.14[a]
Pochayna, SA 0.01%	500	23	4,60±0.32[b]
Pochayna, SA 0.025%	500	34	6,80±0.41[c]
Pochayna, SA 0.05%	500	51	10,20±0.51[d]
Pochayna, SA 0.1%	500	61	12.20±0.66[e]

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

However, for all varieties in two groups supermutagen action was statistically significant in all cases for the variability in the change in mutagen concentration ($F = 131.23$; $F_{0.05} = 3.10$; $P = 1.92*10^{-8}$), by varieties ($F = 4.92$; $F_{0.05} = 3.59$; $P = 0.03$) and for genotype-mutagen interaction ($F = 11.98$; $F_{0.05} = 2.54$; $P = 0.002$).

For all cases, the differences were statistically significant for all concentrations of mutagens in all varieties, regardless of the group, both in relation to the control and in relation to the effect of the previous concentration (Tables 1 and 2, respectively).

To finally establish the differentiating ability of the overall mutation frequency as an indicator, a cluster analysis was carried out (Figure 1), which demonstrated that, in general, all the presented varieties are divided into three main groups when exposed to SA

Thus, at first group are varieties Balaton, Niva Odeska from the first group, which are characterized by somewhat different variability when acted to moderate concentrations of the mutagen. At second group varieties Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha with the similar variability as for the first group. At the third group are varieties Polyanka and Pochayna with lowest variability under SA action, first of all for higher concentrations (third and fourth). It is noticeable that SA in its action on individual varieties differs significantly from previously studied substances with a high damaging ability. It can also be concluded that the frequency of mutations increases progressively and sequentially with increasing concentration, especially high at the last, highest, concentration.

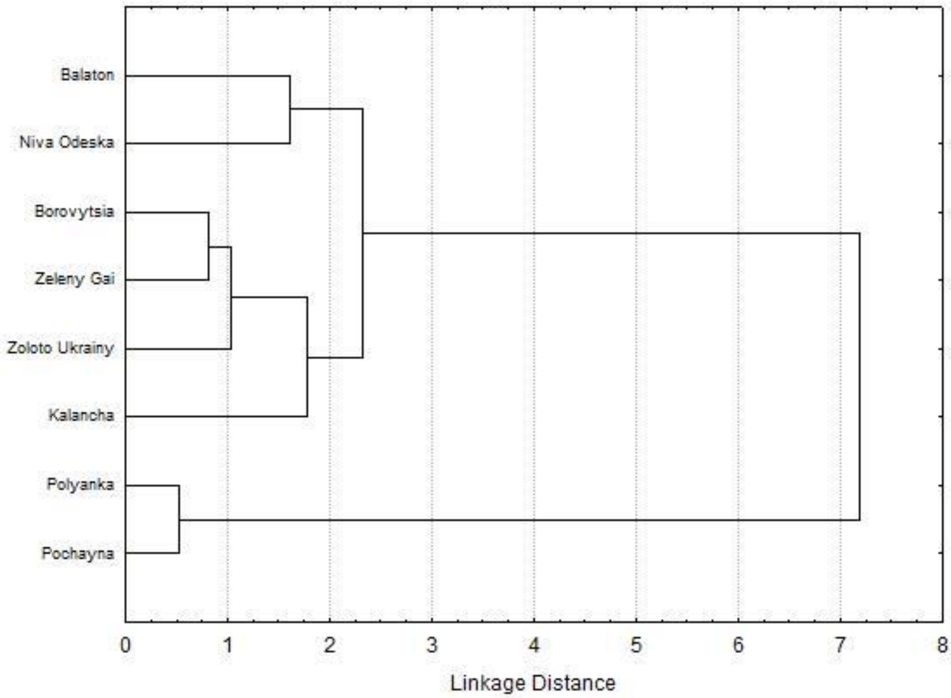

Figure 1. Results of cluster analysis by general mutation rate.

No less interesting, however, than variability in general is such a basic characteristic as the number of features that have undergone changes. Thus, an increase in the overall frequency does not always mean an increase in the diversity of the mutant material for selection, and, in some situations, it can, on the contrary, significantly reduce this indicator. Therefore, the level of variability is used, calculated as the ratio between the number of mutant cases and the number of traits that have undergone changes (Table 3 for the first group of varieties and Table 4 for the second group, the cluster analysis data for this parameter are presented in Figure 2).

Varieties of the first group showed the next level of changeability Balaton (level up to 6.72), Zoloto Ukrainy (up to 6.05), Zeleny Gai (up to 6.35), Niva Odeska (up to 6.15). Herewith, a significant decrease in the number of traits for which mutations have passed with an increase in concentration to the maximum is observed only in the Niva Odeska variety. And for this trait, in comparison with the varieties of the second group, only Polyanka and Pochayna differ in a low level, in which the dynamics in the number of traits, however, remains the same.

Table 3. Level of changeability, caused by mutation variability. First group (x ± SD, n = 400-500).

Variant	Level of changeability	Changed traits
Balaton	0.01±0.01[a]	2
Balaton, SA 0.01%	1.56±0.23[b]	23
Balaton, SA 0.025%	3.14±0.29[c]	32
Balaton, SA 0.05%	4.55±0.34[d]	33
Balaton, SA 0.1%	6.72±0.41[e]	32
Zoloto Ukrainy	0.07±0.01[a]	6
Zoloto Ukrainy, SA 0.01%	1.12±0.19[b]	18
Zoloto Ukrainy, SA 0.025%	1.56±0.21[c]	20
Zoloto Ukrainy, SA 0.05%	3.46±0.27[d]	27
Zoloto Ukrainy, SA 0.1%	6.05±0.34[e]	31
Zeleny Gai	0.02±0.02[a]	3
Zeleny Gai, SA 0.01%	1.03±0.21[b]	19
Zeleny Gai, SA 0.025%	2.05±0.31[c]	25
Zeleny Gai, SA 0.05%	3.83±0.34[d]	33
Zeleny Gai, SA 0.1%	6.35±0.41[e]	33
Niva Odeska	0.02±0.01[a]	3
Niva Odeska, SA 0.01%	1.95±0.01[b]	25
Niva Odeska, SA 0.025%	3.29±0.11[c]	31
Niva Odeska, SA 0.05%	4.96±0.23[d]	34
Niva Odeska, SA 0.1%	6.15±0.31[e]	30

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

For first group mutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 111.23$; $F_{0.05} = 3.48$; $P = 2.93*10^{-7}$) and for genotype-mutagen interaction ($F = 5.15$; $F_{0.05} = 2.72$; $P = 0.02$), but not for genotypes ($F = 3.01$; $F_{0.05} = 3.86$; $P = 0.08$).

For second group SA action was statistically significant not only for the variance in the change in mutagen concentration ($F = 142.93$; $F_{0.05} = 3.48$; $P = 1.22*10^{-9}$) and for genotype-mutagen interaction ($F = 19.34$; $F_{0.05} = 2.72$; $P = 2.98*10^{-3}$), but for genotypes ($F = 8.17$; $F_{0.05} = 3.86$; $P = 0.007$) too.

At Table 4 the level of changeability at the highest concentration were for the varieties of the second group Borovytsia (5.93), Kalancha (6.16), Polyanka (3.47) Pochayna (3.90), that is significantly lower for the varieties Polyanka and

Pochayna ($F = 22.17$; $F_{0.05} = 4.74$; $P = 5.15*10^{-5}$). The variability within the group is significantly higher than for first.

Table 4. Level of changeability, caused by mutation variability. Second group (x ± SD, n = 400-500).

Variant	Level of changeability	Changed traits
Borovytsia	0.03±0.01[a]	4
Borovytsia, SA 0.01%	1.16±0.08[b]	20
Borovytsia, SA 0.025%	2.40±0.18[c]	30
Borovytsia, SA 0.05%	3.48±0.28[d]	29
Borovytsia, SA 0.1%	5.93±0.37[e]	30
Kalancha	0.05±0.02[a]	5
Kalancha, SA 0.01%	1.36±0.15[b]	22
Kalancha, SA 0.025%	1.79±0.28[b]	23
Kalancha, SA 0.05%	3.90±0.43[c]	30
Kalancha, SA 0.1%	6.16±0.51[d]	29
Polyanka	0.01±0.01[a]	2
Polyanka, SA 0.01%	0.66±0.13[b]	15
Polyanka, SA 0.025%	1.45±0.21[c]	22
Polyanka, SA 0.05%	2.86±0.29[d]	27
Polyanka, SA 0.1%	3.47±0.37[e]	28
Pochayna	0.01±0.01[a]	2
Pochayna, SA 0.01%	0.83±0.13[b]	18
Pochayna, SA 0.025%	1.43±0.19[c]	21
Pochayna, SA 0.05%	2.96±0.25[d]	29
Pochayna, SA 0.1%	3.90±0.29[e]	32

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyze with Bonferroni amendment. Comparison in terms of one variety.

Although, for all genotypes in these two groups SA action was statistically significant in all variants for the changeability with the change in mutagen concentration ($F = 93.17$; $F_{0.05} = 3.10$; $P = 3.47*10^{-6}$), by genotypes ($F = 4.17$; $F_{0.05} = 3.59$; $P = 0.04$) and for genotype-mutagen interaction ($F = 10.02$; $F_{0.05} = 2.54$; $P = 0.004$).

For all cases, the differences were statistically significant for all concentrations of mutagens in all varieties, regardless of the group, both in relation to the control and in relation to the effect of the previous concentration with one exception. There was no difference between Kalancha, SA 0.01% and Kalancha, SA 0.025% ($F = 4.08$; $F_{0.05} = 4.82$; $P = 0.07$).

If the cluster analysis of other chemical mutagens demonstrated some differences in the classification of genotypes and a greater systemic nature of the second indicator, then in this case its results did not differ significantly(Fig. 2), again all the varieties presented under the influence of SA are divided into three of the same groups.

Thus, at first group are varieties Balaton, Niva Odeska from the first group, which are characterized by somewhat different variability when acted to moderate concentrations of the mutagen. At second group varieties Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha with the similar variability as for the first group. At the third group are varieties Polyanka and Pochayna with lowest variability under SA action, first of all for higher concentrations (third and fourth).

Cluster analysis for the level of changeability demonstrated a similar division into three groups like as previous parameter (Fig. 2). In spite of previous cases with other mutagens, it cannot be considered mathematically justified that the estimate is more accurate in terms of the level of changeability than in terms of the general rate of mutations. Only for one case, variety Niva Odeska, number of changed traits lower with statistically significance for SA 0.1% action.

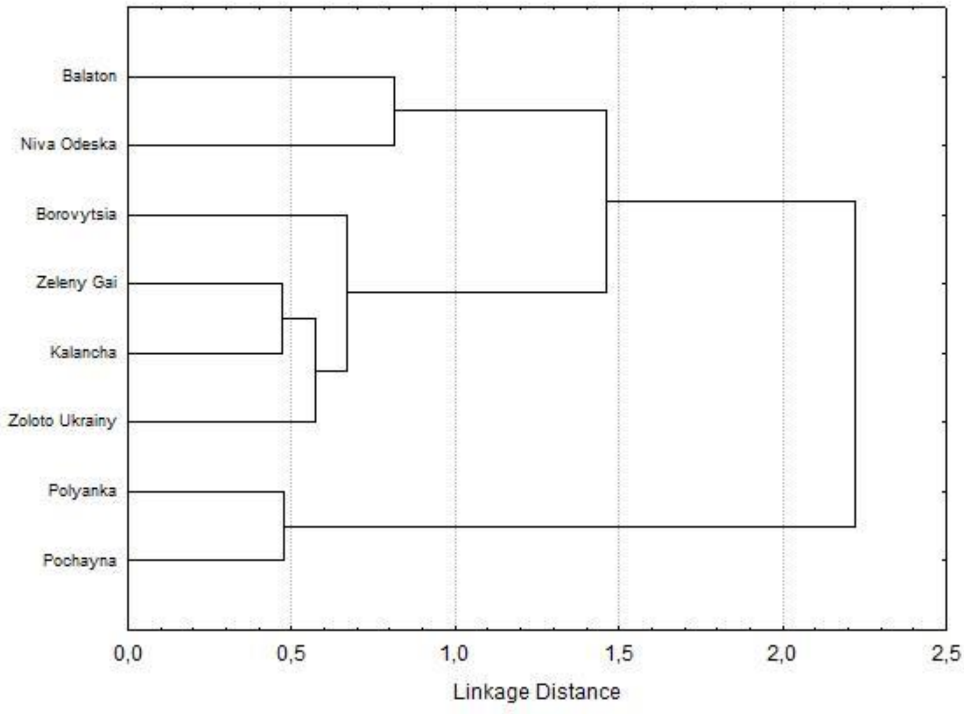

Figure 2. Results of cluster analysis by level of variability.

As for the spectrum of obtained changes, the traits can be divided into 6 groups due to the generally accepted classification for mutation breeding practice.

The first group was mutations by traits plant structure. Thise group includes such traits as thick, thin, high and short stem, semidwarf and dwarf forms, plants with intensive, weak and the presence or absence (regarding parent variety trait) of a waxy bloom. As for the group, new fact was the high probability for SA action induction rare mutation by stem thickness (up to 0.25 - 0.50%) and dwarf or semi-dwarf forms at high concentration (up to 0.75 – 1.00%). SA as a mutagen very effective in induction any type of mutations by first group for only one exception forms without waxy bloom. The highest (up to 1.50 -1.75%) probability of the appearance of high and short-stem forms, weak waxy bloom, which are present in every variant.

The second group consists of mutants by grains size and shape. Its traits like as barrel-shaped grain, coarse grain, large grain. Only the large grain mutations occur more or less probably (up to 0.20%, its probability decreases with increasing concentration for some varieties), other mutations are rare. Mutants with large grain were more often for varieties of first group ($F = 7.01$; $F_{0.05} = 4.32$; $P = 0.02$).

The third group consists of mutations by spike structure (the most numerous, 15 different types). The most part of these mutations tends to occur more frequently as the concentration increases, except large spike and some other seldom mutations. Varieties of first group and two more variable genotypes from second group Borovytsia and Kalancha are characterized by the presence of a greater number of such mutations as long and large spike, for less variable varieties are more inherent anthocyanin awns and a semi-awn spike. The forms with the changes in spike from awn to awnless is more frequent (almost four times) than from awnless to with awn. SA as a mutagen effective in induction awnless, long, small and dense spikes form (0.20 – 0.40% at average).

The fourth group consists of mutant forms with changes in the physiology of plant growth and development is one of the most variable, in spite of only four traits such as sterility, early-maturing, late-maturing, disease tolerance. More frequent (for all varieties, preferable for fist-second concentrations) are mutations by early maturity and disease tolerance (up to 1.75%). For some varieties (for third-fourth concentrations) late maturing may become more often (up to 1.75% for variety Zoloto Ukrainy at SA 0.1%, no more 1.0% for other genotypes). Sterility is more typical to for SA 0.05% - 0.1% concentrations and practically does not occur at low concentrations. In general, three (early-maturing, late-maturing, disease tolerance) traits for this group are in model for mutation process.

The fifth is the group of systemic mutations (extremely changes in the spike structure, going beyond the cultural form and leading to the phenotype of wild wheat relatives). Such traits are most probable under the action of SA 0.05% - SA 0.1% concentrations and low doses cannot act by the same way. More likely is the appearance of squareheads (up to 1.0% for some varieties) and sometimes speltoids (especially for Borovytsia and Zoloto Ukrainy at SA 0.1%), sometimes it can be form even at low concentrations. Other mutations are quite rare. This type of mutations isn’t interesting for breeding practice.

The sixth group includes agriculture-value forms with high grain yield or tillering ability. It occurs in most varieties, preferably for SA 0.01% and SA 0.025% concentrations. Its occurrence decreases with increasing concentration for all varieties. This type of mutations is not very common.

In addition to establishing the variability of individual parameters and their groups, it is quite important to demonstrate the model variability (especially for common parameters and a group of valuable mutations), which was done through the discriminant analysis of individual variables (Table 5, Fig. 3). The model was the general rate of mutations, the level of changeability, mutations in the first, third, fourth groups.

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F_{remove} (4,34)	p-level
Mutation rate	0.08	0.59	5.76	0.02
Level of changeability	0.07	0.47	9.34	0.01
First group	0.05	0.41	11.12	0.01
Second group	0.52	0.77	2.59	0.09
Third group	0.10	0,57	6,99	0,01
Fourth group	0.06	0.43	10.27	0.01
Fifth group	0.17	0.63	4.14	0.06
Sixth group	0.24	0.69	2.03	0.11

The data obtained demonstrate that the applied supermutagen is quite effective in terms of both the induction of general variability and the impact on individual traits. The range of variable features is wide, in fact, the maximum. Herewith, it should be noted that the effectiveness of this agent lies primarily in changes in plant height (induction of low-growing and semi-dwarf forms as a necessary part of an intensive ecotype), early maturity, and resistance to diseases. However, the sixth group is not in the model.

In addition, the discriminant analysis (Fig. 3) once again demonstrated a clear difference in the effects of individual concentrations of mutagens, while the effects of the first and second concentrations are mixed, however, the date cloud in this case is far from the case with water treatment. The second and third concentrations may partially associate, but not the first. Stands completely apart with a high value of the last concentration. The data obtained demonstrate that the first-third concentration may be more effective and partially (depending on the genotype) in action. Summing up with the previous analysis – the first and second, only for individual genotypes – the third (Polyanka and Pochayna).

Classification in the factor space of the obtained data demonstrated that such varieties as Niva Odeska, Polyanka and Pochayna demonstrated themselves most effectively in terms of genotype-mutagenic interaction. And the last two in terms of reducing variability (Table 6).

The data obtained demonstrate that the use of this substance as a mutagenic factor should be used primarily to obtain forms for use as components for the subsequent improvement of existing varieties through recombinant breeding (Ahumada-Flores et al., 2021; le Roux et al., 2021). It is much less likely to produce lines that can be used directly as commercial varieties (Shimelis et al., 2019).

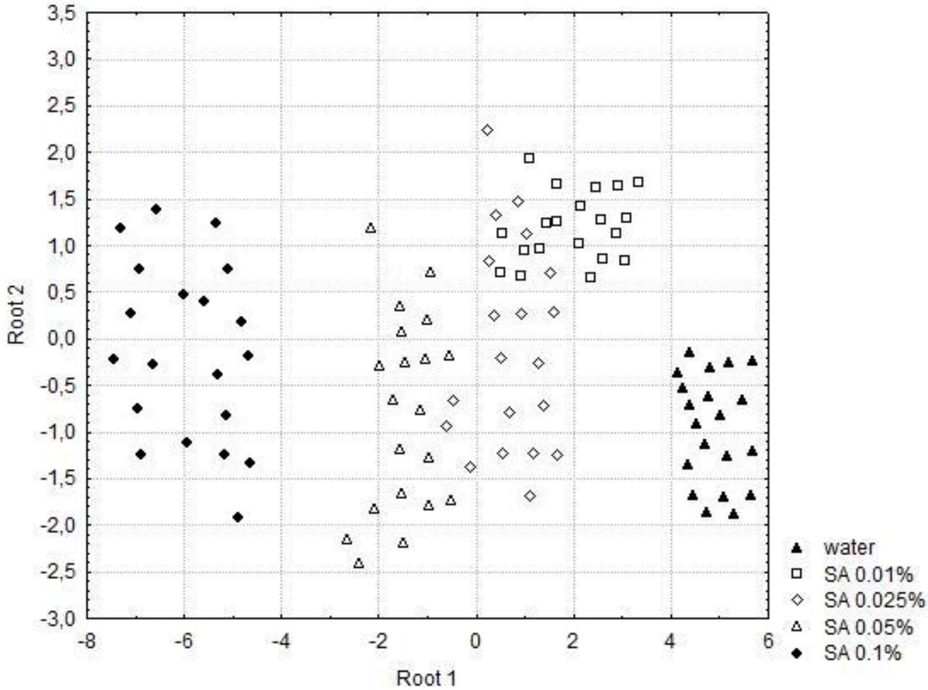

Figure 3. Results of discriminant analysis for model parameters

Table 6. Classification matrics - canonical roots.

Genotype	Percent of classification
Balaton	72.5
Zoloto Ukrainy	75.0
Kalancha	70.0
Niva Odeska	100.0
Borovytsia	72.5
Zeleny Gai	70.0
Polyanka	100.0
Pochayna	100.0
Total	86.3

It is more optimal to use this mutagen as a factor for the induction of short-stemmed forms with a long ear, early ripening lines, which, due to this feature, can avoid the typical May-June drought that is typical for our region (Nazarenko et al., 2019 Nazarenko et al., 2022). The use of sodium azide in selection for resistance to diseases is promising, however, in general, a fairly wide range of substances have demonstrated their effectiveness for this kind of improvement (Anter, 2021).

In contrast to previous studies (Shimelis et al., 2019; le Roux et al., 2021), although the given concentrations approached semi-lethal depression in the first generation, they did not demonstrate a significant decrease in mutational activity (OlaOlorun et al., 2020; Cann et al., 2022). Thus, even a higher concentration of the mutagen only led to a significant increase in biodiversity induction (OlaOlorun et al., 2021). Thus, the dose range used in principle is applicable without major problems to obtain genetically valuable forms when needed (Ariraman et al, 2018).

However, the shift towards greater complexity and an increase in the proportion of adverse changes leads to the conclusion that in this case, it is also more optimal to use optimal and low concentrations (Ahumada-Flores et al., 2021), primarily in the range of 0.01% - 0.05%. Herewith, the first and second (0.025%), according to the results of the analysis, should be used without taking into account the genotype of the initial material, the latter only for some initial forms (Yali & Mitiku, 2022)., especially those that demonstrated little variability (two varieties in our studies).

The genotype-mutagenic interaction of this substance is clearly significantly lower compared to the previous ones, especially in a positive way (Abdel-Hamed et al., 2021; Horshchar & Nazarenko, 2022). The low suitability of two varieties for the treatment of these agents was especially pronounced, and only one genotype can be recognized as promising.

Herewith, the use of higher concentrations is quite acceptable when obtaining genetically-valuable forms (Lal et al., 2020; Anter, 2021), especially for dwarfs and semi-dwarfs. The yield lines obtained under the action of the third or fourth concentration are late-ripening (Nazarenko et al., 2019), which is not very suitable for the country's agriculture and leads to increased vulnerability to droughts in the summer.

CONCLUSIONS

The studied chemical supermutagen demonstrated a fairly high overall level of variability and demonstrated extremely high activity in obtaining new forms for all essential features of winter wheat plants. However, the activity of this agent is predominantly focused on the induction of such types of mutations as changes in plant height, culm thickness, which sharply distinguishes the factor from a number of related ones, induction of changes in the length and width of the ear and grain, both positive and negative, changes in ripeness, before the entire production of a significant number of early ripening forms, the production of mutants with squarehead and speltoid head, disease-resistant mutants. Particular attention should be paid to the possibility of obtaining undersized, primarily semi-dwarf forms, mutants with a large ear and full grain, early maturing lines and disease-resistant lines. Only one of the studied varieties demonstrated a sufficiently high genotype-mutagenic interaction in a positive sense. It can be concluded that the chances of obtaining an optimal scheme for the release of valuable mutations under the action of this substance are significantly lower than those previously studied for these varieties. Further plans to study the effectiveness of this mutagen include an analysis of the variability in biochemical parameters of the obtained forms, in particular, the content of protein and gluten in grain, the quality of protein components and their ratio, the presence of biologically active substances and valuable trace elements. It will also be interesting to analyze the obtained forms for winter hardiness and drought resistance.

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QUALITY PROPERTIES OF SQUEEZED FRESH AND FROZEN POMEGRANATE JUICES

SUMMARY

The pomegranate fruit (*Punica granatum* L.) is characterized by a low energy value, but a high biological value and is desirable for both adults and children. Pomegranate fruits as a raw material is ideal for the production juices. Therefore, in this research were used two varieties of pomegranate fruits: *Karamustafa* and *Hicaz*, grown in Valandovo, R. North Macedonia. To produce the pomegranate juices with good quality properties it is important to have quality raw material. For that purpose, 30 fruits of each variety were taken to estimate the morphometric characteristics (mass, height and width). The production of the fresh pomegranate juices was with cold squeezing of both varieties of the pomegranate fruits, and then the juices were frozen, without using additives.

To determine the quality properties of squeezed fresh and frozen juices from the fruits of both pomegranate varieties, were applied different laboratory methods. Therefore, were analysed the following parameters: total dry matter, soluble dry matter, total sugars (sucrose, fructose and glucose), total acids (citric acid), vitamin C, anthocyanins, polyphenols and minerals. According to the

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performed analysis, the frozen juice of the *Karamustafa* variety had higher average values for: soluble dry matters (17.50 %) and polyphenols (131.86 mg/L). The estimation of the sensory properties was done descriptively and by using the scoring method (max. 20 points). The variety *Hicaz* had sweeter, more acceptable taste, and on the other hand the variety *Karamustafa* had more intensive color, due the higher contain of the anthocyanins.

Keywords: pomegranate juice, fresh, frozen, squeezed juice, quality properties

INTRODUCTION

Pomegranate as fruit is characterized by a low energetically, but a high biological value, due it is widely used in human nutrition. Pomegranate fruits represent an ideal raw material for the production of various types of processed products, among which juices have taken a special place. Fresh pomegranate fruits may be unavailable in certain periods during the year, while pomegranate processed products can be used throughout the year. For the production of juice, it is necessary to use pomegranate varieties with a higher content of dry matter - sugars, vitamins, mineral and coloured matters, with a pleasant taste and aroma. Pomegranate juice due a high biological value is desirable for both adults and children.

Pomegranate is one of the oldest fruit crops in the world. The pomegranate (*Punica granatum* L.,) belongs to the order *Mytreles*, *Lythraceae*, subfamily *Punicoideae*, genus *Punica* L., (known as pomegranate, pomegranate, rosehip). Two species are important for fruit growing: *Punica granatum* L. and *Punica nana*. It is widespread in countries where there are conditions for cultivation, such as: Chile, Argentina, the south of the United States and South Africa. The largest producers of pomegranate in the world are the countries of the Mediterranean Region (Milošević, 1997).

The attractiveness of the fruits, as well as their quality, largely depends on the morphometric characteristics of the fruits, such as: mass, height and width. On the other hand, these parameters represent the basis for the determination the type and varieties of the fruits. The values of the morphometric characteristics depend on the variety (genotype), the cultivation conditions (climatic and soil) and the applied agrotechnical and pomotechnical measures.

According to Teixeira da Silva et al., (2013), in their research were examined the more pomometric properties of pomegranate fruits, and found differences between the varieties in terms of: the size of the fruit, the colour of the skin, which is usually from yellow, purple to pink, i.e. red. The colour of the arils of the fruit is characterized from creamy whitish to red, and the fruits are characterized by different content of juice, containing various amounts of sugars, acids and present tannins.

The authors Markovski et al. (2017) made an examination of the more pomometric properties, such as the length, width and weight of the fruit as well as the colour of the arils in several varieties of pomegranate, including the varieties: *Lifanka*, LC, *Bejnarija*, *Karamustafa* and *Hicaz*. Among the examined varieties, the variety *Karamustafa* is characterized by an attractive and most attractive dark red colour of the arils. In the *Hicaz* variety, the lowest statistical value of the content of coloured matters on the arils was confirmed. The authors also indicate that the *Hicaz* variety is characterized by the dark red colour of the arils and therefore has found great use in the processing industry for the production of juice and vinegar.

The authors Pekmezi & Erkan (2000) made an examination of the quality properties of pomegranate fruits intended for consumption, indicating that the pomegranate fruit have a high quality if it has a smooth skin and if it is free of cracks and bruises. They also recommend using fruits with a greater amount of extracted juice and with a favourable ratio of sugars and acids. When using the whole pomegranate fruit, the content of separated juice is from 45 to 65 %, while when using separated arils from the pomegranate fruit, the content of separated juice is from 76 to 85 % in relation to its mass. Based on the size, a classification of pomegranate fruits was made. According to classes, there are: small, with a mass of 150 to 200 g and a diameter of 65 to 74 mm, medium, from 201 to 300 g and a diameter of 75 to 84 mm, large, from 301 to 400 g and a diameter of 85 to 94 mm and extra-large fruits with a mass of 401 to 500 g and a diameter of 94 to 104 mm.

The chemical composition of the fruit is characteristic for each species and variety. The average chemical composition characterizes each variety, and differences may occur due to the influence of different climatic, agrotechnical conditions of cultivation and the degree of maturity (Niketić – Aleksić, 1994).

In fresh pomegranate fruits, carbohydrates (glucose, fructose and sucrose) are the most represented, which are present in a relatively high amount, up to 20 %, and the water content ranges from 70 to 95 %. There is also a high content of mineral matters, ranging from 0.25 to 2 %, and as the most represented are: potassium, calcium, phosphorus, sodium, magnesium, iron, manganese, cobalt, sulphur, chlorine and iodine. The pomegranate abounds with a high content of organic acids. The most biologically valuable ingredients in pomegranate fruit are vitamins: vitamin C, vitamins from the B group (B-complex), vitamin K, carotenoids and other vitamins (Katalinić, 2010).

The authors Tehranifar et al., (2010) analysed the chemical composition of the fruits of several varieties of pomegranate, and found that the content of soluble dry matter ranges from 1.37 to 15.07 °Brix. The content of total sugars ranges from 13.23 to 21.72 g/100 g of fresh pomegranate fruit, the pH value from 3.16 to 4.09, total acids from 0.33 to 2.44 g/100 g, and the content of ascorbic acid from 9.91 to 20.92 mg/100 g in fresh pomegranate fruit. The content of total polyphenols ranges from 295.79 to 985.37 mg/100 g of fresh pomegranate, and the anthocyanins present range from 5.56 to 30.11 mg/100 g, in fresh pomegranate fruit. Based on the analysis, the authors confirm that the variety is an important factor and has a great influence on the physico-chemical properties of the pomegranate fruit.

Each plant species produces specific primary metabolites responsible for the growth, development and reproduction, as well as secondary metabolites which provides plants biotic and abiotic factors protection. Phenols are a group of secondary metabolites due to pronounced antioxidant, antimicrobial, antifungal and even anticancer activity. Many studies have confirmed that the peel and seeds of wild pomegranate, as its biowaste, are a valuable source of bioactive substances, as phenols, flavonoids and anthocyanins (Krivokapic et al., 2022).

Preservation means the application of different processes, i.e. technological procedures with an aim to preserve the quality properties of the product to a greater extent and for a longer period of time, and at the same time to prevent its spoilage and the degradation of some components (Markoviċ, 2018).

The juices, according to their physical characteristics, are a specific type of product, but according to their chemical composition, they are products that are closest to fresh fruit. To produce juice, the most important characteristic of the fruit is its juiciness, the content of sugars and acids, and the content of coloured and aromatic matters is of special importance. Also, the fruits should be healthy and with certain varietal characteristics (fruit shape, components proportion in the dry matters, etc.), and to be technologically mature, with a good mechanical and chemical composition. As significant factors are: the type of fruit, the variety, agro-ecological and agro-technical conditions of cultivation, the moment of harvesting, the conditions of transportation and storage of the raw material after harvesting until the processing period (Niketić – Aleksić, 1994).

From a technological point of view, the structure of the pomegranate is very specific and complicated because the fruits have a large number of lamellae that separate the arils. The peel and lamellae of the pomegranate fruits have a high content of tannins and their presence in the juices is undesirable. Therefore, for processing of the juices it is recommended to separate the arils from the lamellas and the peel. The pomegranate juice is valued because it has a specific taste, smell and colour due to the presence of anthocyanins, which have certain therapeutic and certain medicinal properties. The taste of the obtained juices is a little bitter, because it contains certain amounts of tannins matters. According to research, it was determined that in fresh pomegranate fruits, the energy value was 300 J/100 g, the total acidity was 1.5 % (expressed through the amount of citric acid) and the content of soluble dry matter was 20 % (Niketić – Aleksić, 1994).

Punicalagin is one of the main bioactive substances that contributes to the total antioxidant capacity of wild pomegranate fruit (*Punica granatum* L.). The wild pomegranate juice shows up to eight times stronger antioxidant activity than the juice of grapes, cranberries and oranges, and three times stronger than the activity of red wine and green tea (Krivokapic et al., 2022).

According to Dipakkumar (2007), it is better to perform the juice extraction with moderate pressure and thus to avoid releasing high quantities of tannins in the juice. With the extraction of juice from the pomegranate fruit cut into quarters, by using a hydraulic press, under moderate pressure, 36.41 % of juice is obtained from the total mass of the fruit.

The cold-pressing technique enables the transfer of most primary and secondary metabolites found in the original ripped fruit, from the fruit to the juice through the fruit crush at room temperatures, at a low speed. The extraction process generates almost no heat and preserves the juice's nutritional quality. Another critical factor associated with the juice nutritional value is the quality of storage conditions, mainly temperature and time (Llupa et al., 2022).

In practice, different equipment is used for squeezing pomegranate juice, but it has been established that the equipment has an impact on the amount of extracted juice, and thus on the amount of dry matter, pH-value and total acids. Also, the equipment affects the physico-chemical properties of the juice (Ismail et al., 2014).

Juices can be preserved by applying a low temperature. With this method of preservation are obtained final products with a quite high-quality, where both the sensory and the nutritional properties are preserved. Namely, preservation by freezing is a procedure where the temperature of the product is reduced to a state of freezing, during which practically all chemical and biochemical reactions are stopped. Microbiological activities are also stopped, part of the present microflora dies, biochemical process has slowing down, that is, the action of enzymes is prevented. In order to preserve the quality properties of the final product, it is recommended that the frozen product be stored at a temperature of -18 °C (Vračar, 2012),

The quality of the frozen product is greatly influenced by the storage temperature, as well as the storage period. Any increase in storage temperature has the effect of reducing the quality of the frozen product (Niketić – Aleksić, 1994).

The choice of the packaging material for the juices is of particular importance because it has its impact on the quality reduction of the juice during storage, in case if it is transparent and lets the light through, thus causing a change in the colour of the juice and in the nutritional properties (Dinesh and Ramasamy, 2016).

The authors Zaouay et al., (2014) emphasize that on the content of anthocyanins, a special influence has the content of acids. They determined a higher anthocyanin content of 47 mg/100 mL in juice obtained from the fruits of sour-tasting pomegranate varieties, and in the juices obtained from the fruits of sweet pomegranate varieties, they determined a lower anthocyanin content of 34.21 to 43.11 mg/100 mL.

Researches related to the qualitative and quantitative analysis of anthocyanins is of great significance, because the commercial value of fresh pomegranate fruits and their processed products depends on the type and amounts of anthocyanins. According to Gil et al., (1995), anthocyanins are a very significant component of the chemical composition of the juice obtained from the pomegranate fruit and are in correlation with the variety, the stage of maturity and the location where the fruit was grown. In juice obtained from pomegranate fruit harvested at the stage of full technological maturity, the content of anthocyanins ranges from 50 to 100 ηg/g of fresh aril.

The purpose of this paper is to compare the impact of a processing technology on the quality in terms of chemical and sensorial properties for obtained pomegranate juices, by using two varieties of pomegranate fruit, *Hicaz* and *Karamustafa*. For obtaining pomegranate juices, the pomegranate fruits have been squeezed with pressing, and afterwards have been used as fresh and frozen pomegranate juices.

MATERIAL AND METHODS

In this research were used two varieties of pomegranate fruit, *Hicaz* and *Karamustafa*, that are grown in Valandovo region, North Macedonia.

The variety *Karamustafa* originates from Asia Minor, where there are several varieties. It has been cultivated in our country for a long time and it is a domesticated variety, accepted in the List of domestic and foreign recognized or approved varieties of fruit crops (Official Gazette of RM, No. 41/2006). This variety is adapted to our climate and soil conditions and it is mostly grown in the area of Valandovo.

The variety *Karamustafa* (Figure 1) is an autochthonous variety whose fruits are characterized by a high mass, a yellow-green colour of the skin, and in the stage of technological maturity it is yellow-red. Regarding the content of total acids, this variety belongs to the group of sweet pomegranate variety. The colour of the juice obtained from the fresh fruits of the *Karamustafa* variety varies from light pink to pink (Dimovska et al., 2017).

Figure 1. Pomegranate fruits of the *Karamustafa* variety

The pomegranate fruits of *Hicaz* variety (Figure 2) originates from Turkey. It is characterized by a pronounced red skin colour, and the colour of the grains (arils) and the juice obtained from the fruits is characterized by a dark red colour (Polat et al., 2012).

The pomegranate fruits were harvested at full technological maturity. After the harvest, 30 fruits were taken of each variety of pomegranate, *Hicaz* and

Karamustafa, to analyse their pomometric properties. Afterwards were analysed the chemical composition and sensory properties of the fresh squeezed and frozen juices obtained from the *Hicaz* and *Karamustafa* varieties.

Figure 2. Pomegranate fruits of the *Hicaz* variety

From the morphometric properties of pomegranate varieties *Hicaz* and *Karamustafa*, the following measurements were performed: the height and width of the analysed pomegranate fruits varieties were determined with a caliper with an accuracy of ± 0.1 mm; the average mass of the fruits was determined by measuring with a digital analytical balance of the type “Sartorius” with an accuracy of ± 0.01 g. The results were expressed as the mean value of consecutive measurements of 30 pomegranate fruits.

The sensory evaluation of fresh pomegranate fruits was performed descriptively for the following parameters: colour, taste, smell and consistency. On the other hand, the sensory evaluation of the squeezed fresh and frozen juice was performed according to the scoring sensory method (point-system), with a maximum of 20 points (Karakashova and Babanovska–Milenkovska, 2012).

The chemical analyses were made in order to determine certain parameters, according of which the quality and nutritional properties was estimated of the squeezed fresh and frozen juice, obtained from the two pomegranate varieties. For this purpose, various standard laboratory methods had been applied to determine the following chemical parameters: total dry matter by drying the samples in an oven dryer, at 105 ºC to obtaining constant mass; soluble dry matters were determined with a digital refractometer, type – Mettler Toledo Refracto 30 GS, with previous calibration and automatic temperature regulation of 20 °C; total acidity by using titration solution 0.1 M of NaOH and 1 % solution of phenolphthalein as indicator; determination of sugars by applying HPLC-method with RI-detector; the mineral matters (total ash) was determined by a gravimetric method, by incineration and burning of samples in a Muffle oven, at a temperature of 525 ± 25 °C (Vračar, 2001); determination of Vitamin C was performed by using a solution of .0.1 N I_2 and 1 % starch solution as an indicator (Famakopeja, 1984); anthocyanins were determined by spectrophotometric method, where the measurements were performed on the absorption of light by the samples at a wavelength of 540 nm; polyphenols were determined by spectrophotometric method, where the measurements were performed on the absorption of light by the samples at a wavelength of 765 nm (Folin and Ciocalteu, 1927).

The obtained results from the research of the pomometric properties of the both pomegranate varieties and from the analysis of sensory and chemical properties of squeezed fresh and frozen juices, have been statistically calculated, with the statistical package R 3.5.1. An ANOVA analysis of variance was performed for all the obtained results, and the significance testing of the differences between the average values for each analysed property was performed using the LSD-test, at the 0.05 and 0.01 significance level.

Processing operation for obtaining fresh and frozen squeezed juices

The harvest of the pomegranate fruit was performed by hands, in the early morning hours. The fruits were in the stage of full technological maturity. The pomegranate fruits then were packed in cardboard boxes. After harvesting, they were stored in a room at a temperature of 4 °C, in order to preserve the quality of the raw material.

Before starting the processing of raw material, the inspection and selection were made, by visual control of the raw material in order to remove those pomegranate fruits where a rotting process was observed, as well as the pomegranate fruits with unacceptable mechanical damages. Afterwards, the following operations were performed on the raw material: washing, cutting into two halves by using a stainless steel knife, then the juices were squeezed by pressing the halves with a manual press.

The procedure of technological washing was carried out in order to remove mechanical impurities and the largest number of microorganisms from the surface of the fruit. The fruits were well washed, because they were not peeled. The washing process was performed with pure water, which is chemically and bacteriologically proper in accordance of the national regulation. The pomegranate fruits were cut in half, with a stainless steel knife, in order to avoid loss of vitamin C. The cut halves of pomegranate fruits were pressed by using a manual press, due the internal parts were released and fresh squeezed juice were extracted. In order to obtain juice with a more stable colour, this procedure should be performed in as short time interval as possible. The crushed mass is passed through a sieve, during which the small parts and seeds will pass through, and the large parts and lamellae remain on the sieve, after which they are gradually removed. The suspended particles in the pomegranate juice were separated by using sieves, made of stainless steel and with very small openings. The fresh squeezed juice, which still contains finely dispersed particles, was filtered through coarse filter paper (with larger pores). In this way, juice without sediment was obtained. By this operation, a larger amount of pectin matters was removed. The obtained filtrated fresh squeezed pomegranate juice was packed in appropriated packaging. This kind of juice, produced without any additives was kept at low temperature, up to 4 °C and used within 24 hours, due the possibility of losing its nutritional and biological values.

By intention to keep nutritional and biological values of fresh squeezed juice and to prolong the shelf life of the juice, it was frozen. The process of preservation with a low temperature was performed at a temperature below -18 °C, without any additives (as preservatives or sweeteners). After the process of freezing, the pomegranate juice packed in glass or plastic package, was stored in a freezer, at temperature of at least -18 °C or lower. All production phases for fresh and frozen squeezed pomegranate juice are presented in the scheme 1.

Scheme 1. Phases of production squeezed fresh and frozen pomegranate juices

RESULTS AND DISCUSSION

Before approaching the technology of fruit processing, it is necessary a more detailed acquaintance with its technological properties, above all with the mechanical and chemical composition of the fruits (Karakashova, 2011).

The morphometric characteristics of the fruits (mass, height and width) in both analyzed pomegranate varieties are represent in table 1.

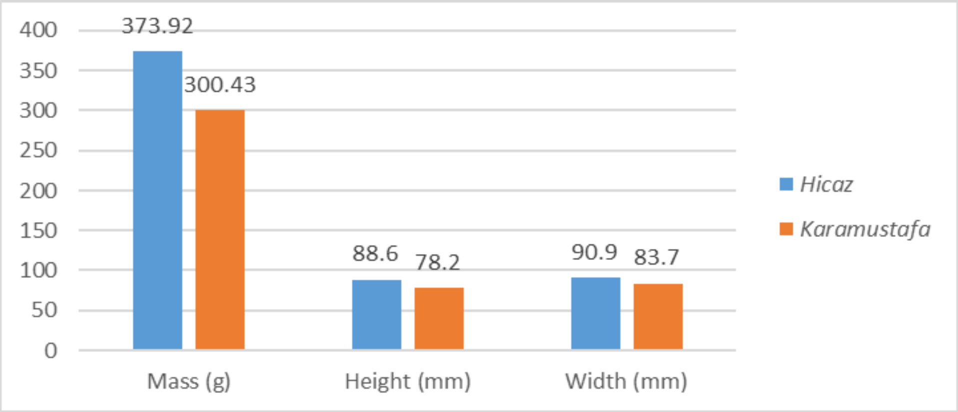
Table 1. Morphometric characteristics of pomegranate fruits, varieties *Hicaz* and *Karamustafa*

Variaty		Mass (g)			Width (mm)			Height (mm)		
	N	min	max	$\bar{x}$	min	max	$\bar{x}$	min	max	$\bar{x}$
Hicaz	30	288.32	773.05	373.92	81.70	114.10	90.90 b	81.00	108.50	88.60 b
Karamustafa	30	215.78	422.97	300.43	74.70	95.70	83.70 a	69.90	95.40	78.20 a
LSD 0.05				90.24			0.41			0.50

N - number of examined fruits;

$\bar{x}$ - mean value of each measurement of 30 fruits per pomegranate; the values for each morphometric characteristic of the fruits of the *Hicaz* and *Karamustafa* varieties, marked with different letters, are statistically significantly different from each other ($p < 0.05$).

The average value from the measurement of the morphometric characteristics of 30 pomegranate fruits, for each examined varieties, *Hicaz* and *Karamustafa* are presented in graph 1.



Graph 1. The average value of weight, height and width of pomegranate fruits, varieties *Hicaz* and *Karamustafa*

From the results in graph 1 and table 1, statistical analysis was performed. According to data it can be concluded that: the variety *Hicaz* was characterized by a higher average weight of the fruits (373.92 g) comparing to the variety *Karamustafa* (300.43 g), and no statistically significant difference was determined; the fruits of the variety *Karamustafa* were characterized by a lower average height of 78.20 mm compared to the fruits of the variety *Hicaz* (88.60 mm), and a statistically significant difference was found; the average value for the width of the fruits of the *Hicaz* variety was 90.90 mm, and the fruits of the *Karamustafa* variety had a smaller average width of 83.70 mm, and statistically significant difference was determined. The results obtained in this research for mass, height and width of the fruits of the two pomegranate varieties *Hicaz* and *Karamustafa*, comparing to the results obtained in research by other authors, it can be concluded that there are small deviations of the obtained values what is result from our climate and soil conditions.

The chemical composition of fruits means the content of all the ingredients of the product, including water. The components of the chemical composition, their quantity and mutual ratio are responsible for the sensorial, nutritional and biological properties of the product.

From the laboratory analysis in this research, the obtained average results presented in the table 2 are for comparison of the chemical composition of the fresh squeezed juice from the pomegranate fruits, varieties *Hicaz* and *Karamustafa*. According to the data that were statistically evaluated (table 2), the average values for each chemical property of fresh squeezed juice from the pomegranate fruits, varieties *Hicaz* and *Karamustafa*, that are marked with different letters, are significantly statistically different from each other ($p < 0.05$).

Table 2. Chemical composition of fresh squeezed juice from the pomegranate fruits, *Hicaz* and *Karamustafa* varieties

Variety	Total dry matters (%)	Soluble dry matters (%)	Sugars (%)		Total acids (%)	Vitamin C (mg/100 g)	Anthocyanins (mg/L)	Polyphenols (mg/L)	Mineral matters (%)
			Glucose	Fructose					
Hicaz	22.22	15.80	12.21a	7.69a	1.82b	17.00b	500.00b	43.96a	1.30b
Karamustafa	21.14	16.00	12.50	8.00	0.34a	15.00a	236.00a	46.50b	0.86a
LSD 0.05	**2.27**	**1.8**	**1.50**	**0.82**	**0.22**	**1.39**	**9.29**	**0.81**	**0.07**

The comparison of the average content of examined chemical parameters in squeezed fresh and frozen juices, from *Hicaz* and *Karamustafa* varieties pomegranate fruits are presented in the table 3.

Table 3. Comparison of examined chemical parameters in squeezed fresh and frozen juices, from *Hicaz* and *Karamustafa* varieties pomegranate fruits

	Fresh squiezed juice		Frozen squiezed juice	
Variety/parameters	*Hicaz*	*Karamustafa*	*Hicaz*	*Karamustafa*
Total dry matters (%)	22.22	21.14	15.04	16.33
Soluble dry matters (%)	15.80	16.00	16.08	17.50
Glucose (%)	12.21	12.50	7.15	8.78
Fructose (%)	7.69	8.00	5.60	6.61
Total acids (%)	1.36	0.84	2.06	0.58
Vitamin C (mg/100 g)	17.00	15.00	12.64	12.65
Anthocyanins (mg/L)	500.00	236.00	730.90	234.86
Polyphenols (mg/L)	43.96	46.50	115.71	131.86
Mineral matters (%)	1.30	0.86	0.22	0.24

From the table 3 it can be concluded that the average values for the content of total dry matters ranges from 22.22 %, in the squeezed fresh juice obtained from the fruits of the *Hicaz* variety, up to 21.14 % in the squeezed fresh juice obtained from the *Karamustafa* variety. For the squeezed frozen juice, the content of total dry matters for the *Karamustafa* variety was higher (16.33 %) in relation of the *Hicaz* variety (15.04 %). The average values for the content of total dry matters in the squeezed fresh juices obtained from the both varieties of pomegranate fruits, are statistically significantly different at the level of significance $p < 0.05$.

The average values for the content of soluble dry matter ranges from 16.00 % in the squeezed fresh juice obtained from the fruits of the *Karamustafa* variety, to 15.80 % in the squeezed fresh juice obtained from the fruits of the *Hicaz* variety. The content of soluble dry matter, as average values, were 16.08 % for the squeezed frozen juice of the *Hicaz* variety and 17.50 % for the squeezed frozen juice of the *Karamustafa* variety. The statistical analysis of the obtained average values for the content of soluble dry matter did not show statistically significant differences between the varieties.

In the squeezed fresh juice obtained from the fruits of the *Karamustafa* variety, higher average values for glucose content were determined, 12.50 %, in relation to the squeezed fresh juice obtained from the fruits of the *Hicaz* variety, 12.21 %. The higher average value for glucose content was determined (8.78 %), for the squeezed frozen juice of *Karamustafa* variety, and lower average value for glucose content (7.15 %) was determined for the squeezed frozen juice of *Hicaz* variety.

The higher average values were determined for the fructose content (8.00 %) in the squeezed fresh juice obtained from the fruits of the *Karamustafa* variety compared to the squeezed fresh juice obtained from the fruits of the *Hicaz* variety (7.69 %). The lower average value for fructose content was determined (5.60 %), for the squeezed frozen juice of *Hicaz* variety, and higher average value for fructose content (6.61 %) was determined for the squeezed frozen juice of *Karamustafa* variety. Regarding the statistical analysis of glucose and fructose content in the squeezed fresh juices obtained from the pomegranate fruits of the both examined varieties, no statistically significant differences were determined.

In terms of the average values for the content of total acids (expressed through citric acid) were within the range from 0.34 %, in the squeezed fresh juice obtained from the fruits of the *Karamustafa* variety, up to 1.82 % in the squeezed fresh juice obtained from the *Hicaz* variety. The average values for the content of total acids (expressed through citric acid) were within the range from 0.58 %, in the squeezed frozen juice obtained from the fruits of the *Karamustafa* variety, up to 2.06 % in the squeezed frozen juice obtained from the *Hicaz* variety. The statistical processing of the obtained data for this property showed statistically significant differences between the both examined varieties, at the significance level $p < 0.05$.

The average values for the content of vitamin C ranges from 15 mg/100 g in the squeezed fresh juice, up to 12.65 mg/100 g in the squeezed frozen juice, obtained from fruits of the *Karamustafa* variety and from 17 mg/100 g, in the squeezed fresh juice, up to 12.64 in the squeezed frozen juice, obtained from the *Hicaz* variety. Regarding the statistical data, statistically significant differences was found between the two varieties, at the level of significance $p < 0.05$.

According to the average values for anthocyanins, the content was within the range from 236 mg/L, in squeezed fresh juice obtained from fruits of the *Karamustafa* variety, up to 500 mg/L in squeezed fresh juice obtained from the *Hicaz* variety. The average values for anthocyanins, the content was within the range from 236.86 mg/L, in squeezed frozen juice obtained from fruits of the *Karamustafa* variety, up to 730.9 mg/L in squeezed frozen juice obtained from the *Hicaz* variety. Statistical processing of the obtained data for this property showed statistically significant differences between the two varieties, at the significance level $p < 0.05$.

The phenolic compounds presence is essential and directly influences the fruit juice quality by contributing into organoleptic characteristics, affecting the colour, astringency, and aroma (Llupa et al., 2022). According to the data from the table 3., it can be noted that the average content of total polyphenols ranges from 43.96 mg/L in squeezed fresh juice obtained from the *Hicaz* variety, up to 46.50 mg/L in squeezed fresh juice obtained from the fruits of the *Karamustafa* variety. The average values of the total polyphenols content in squeezed frozen juices were from 115.75 mg/L from the *Hicaz* variety and 131.86 mg/L from the *Karamustafa* variety. The statistical processing of the obtained data for this property showed statistically significant higher differences between the squeezed fresh juices obtained for both varieties, at the level of significance $p < 0.05$.

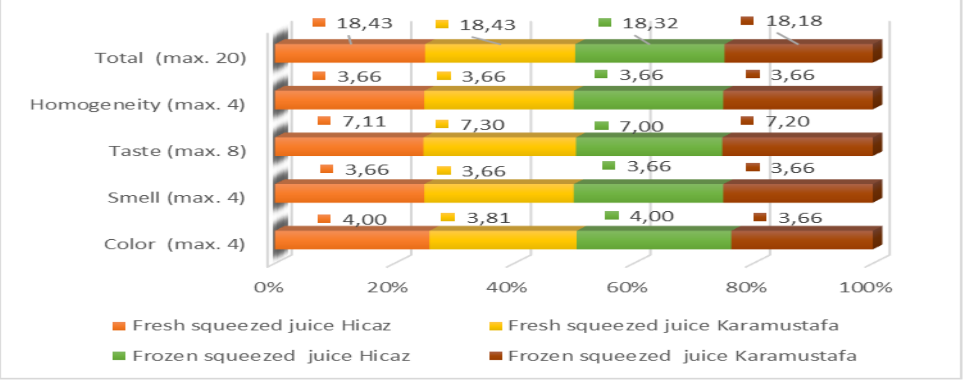
Comparing the presented results with the results of Radunić et al. (2017) paper which talks about the content of phenols, flavonoids and anthocyanins of wild pomegranate (*Punica granatum* L.) juice from the territory of the Mediterranean part of Croatia, which states that the content of total phenols is 679.6 mg/100 g dry matter (DM), total flavonoids 393.6 mg/100 g DM and total anthocyanins 81.06 mg/100 g DM.

In the research of Krivokapic et al. (2022), were determined the content of total phenols and anthocyanins in the samples of seed extract obtained from the fruits of wild pomegranate, collected from different location in Montenegro. The content of total phenols in seed extract were in range from 89.07 ± 2.52 up to 189.77 ± 7.81 as milligrams of gallic acid equivalent per 100 grams of dry matter (mg GAE/100 g DM). Also, the obtained results in the research of Gözlekçi et al. (2011) were in range from 125.3 to 177.4 mg GAE/100 g DM, while the amount of total phenols compared to the research of Peng et al. (2019) where the obtained values range from 62 to 68 mg GAE/100 g DM. The results for the total anthocyanins were presented as cyanidin-3-glucoside equivalents per gram (mg C3GE/g). The highest amount of total anthocyanins was recorded in the extract of the locality Škaljari, and the lowest in the extract of the locality Carev Laz and the results were in the range from 2.73 ± 0.56 to 4.34 ± 0.39 mg C3GE/g. In relation to the research of Parseh and Shahablavasani (2019) where the content of total anthocyanins of wild pomegranate seed extract was 28 mg C3GE/g, the obtained results of this research deviate and the content of total anthocyanins was lower.

For the content of mineral matters, it can be stated that the average values range from 0.86 % in the squeezed fresh juice obtained from fruits of the *Karamustafa* variety, up to 1.30 % in the squeezed fresh juice obtained from the *Hicaz* variety. The lower average value for mineral matters content was determined (0.22 %), for the squeezed frozen juice of *Hicaz* variety, and higher average value for mineral matters content (0.24 %) was determined for the squeezed frozen juice of *Karamustafa* variety. Statistical processing of the obtained results for this property showed statistically significant differences between the two varieties of pomegranate fruits, at the level of significance $p < 0.05$.

The estimation of the sensory properties of the fresh pomegranate fruits of the *Hicaz* and *Karamustafa* varieties, was performed descriptively for the following parameters: colour, taste, smell and consistency. There are differences in the crust colour of the fruit and in the taste, where the variety *Hicaz* has characteristic red colour, with acidic taste, and the variety *Karamustafa* had yellow-pink red colour, with sweet, astringent taste. The smell and consistency were characteristic for the both varieties.

The obtained results of sensory evaluation of fresh and frozen squeezed juices obtained from two varieties of *Hicaz* and *Karamustafa* pomegranates fruits are shown in the graph 2.



Graph 2. Comparison of the results from sensory evaluation (max. 20) of fresh and frozen squeezed pomegranate juice obtained from *Hicaz* and *Karamustafa* varieties

From the data presented in the graph 2, the variety *Hichaz*, had same average values for the colour (4.00 of max. 4.00), smell (3.66 of max. 4.00) and homogeneity (3.66 of max. 4.00) for fresh squeezed and frozen squeezed juices, while the taste had 7.11 for fresh and 7.00 for frozen squeezed juice (of max. 8). For the variety *Karamustafa*, the smell (3.66 of max. 4.00) and homogeneity (3.66 of max. 4.00) had same average values for fresh squeezed and frozen squeezed juices. The fresh squeezed juices had higher average values for colour (3.81 of max. 4) and for taste (7.30 of max. 8) than the same properties for frozen squeezed juices of the *Karamustafa* variety. Based on these data, the higher total average points (18.43 of max. 20) were obtained for fresh squeezed juices both varieties Hicaz and *Karamustafa*, while for the frozen squeezed juices the total average points 18.32 of max. 20, was for *Hicaz* and 18.18 of max. 20, was for *Karamustafa* variety.

CONCLUSIONS

According to the morphometric characteristics in terms of height, width and mass, higher values were determined in the *Hicaz* variety. In terms of the examined chemical ingredients, statistically significant differences were determined between the *Hicaz* and *Karamustafa* pomegranate varieties, for the content of sugars and acids, as well as for the content of polyphenols and anthocyanins. According to the results of the sensory examination of the squeezed fresh and frozen juice from the *Hicaz* and *Karamustafa* pomegranate variants, there were some differences in the colour and taste. The variety *Hicaz* contain more anthocyanins, and on the other hand the variety *Karamustafa* had sweeter, more acceptable taste.

Based on the previously examined properties, it can be concluded that the pomegranate fruits of the *Hicaz* and *Karamustafa* varieties are characterized by good quality properties and it can be used as an appropriate raw material for further processing. Also, generally can be said that the application of the freezing procedure does not have a significant impact on the quality characteristics of the obtained juice and the use of this procedure is recommended, so that, the pomegranate juice can be used throughout the year.

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MODELLING WINTER WHEAT SOIL WATER BALANCE IN CHANGING CLIMATIC CONDITIONS

SUMMARY

In the past decades, the climatic conditions in the region have changed, showing extreme weather events and gradual changes in precipitation and evapotranspiration. The historical climatic data (1961-1999) are calculated to average, but the extreme dry (2000) and wet (2014) years are also taken into consideration. The sediment's hydraulic conductivity is calculated using the Hydrus 1D model and measured on-site to establish the assessment model for various crops that will be easily simulated. The calculated vs. experimental values showed good agreement for the selected location of Srbovac village. Other parameters, such as soil moisture field capacity, soil moisture at the wilting point, maximum infiltration flux, and maximum drainage flux to the saturated zone, were calculated, too. Finding the adequate crop water requirement for changed soil water balance is done by using the FAO CROPWAT program. Three different soil textures were used for calculations: loam, sandy loam, and silty loam. Soil moisture at field capacity is found to be 220 mm/m, the maximum infiltration flux per day was 250 mm, and the maximum drainage flux to the saturated zone was 5 mm. The soil water balance was calculated by CROPWAT and presented for each month. In the period from 1961-1999, historical data, the total average precipitation was 911 mm, reference evapotranspiration (ETo) was 879 mm, and actual evapotranspiration (ETa) was 375 mm, but crop evapotranspiration of winter wheat (ETc-Crop) for the same period was 400 mm, that proves good climatic conditions for the selected crop. In the period from 2000-2023, the average climatic conditions were used for Crop-Water balance calculation, and the total precipitation was 712 mm, whereas ETo was 924 mm, ETa was 477 mm, and ETc-Crop of winter wheat for the same period was 641 mm, with 166 mm crop deficit in April, May, and June.

Keywords: Evapotranspiration, FAO CROPWAT 8.0, Effective Rainfall

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INTRODUCTION

Soil-water balance (SWB) represents the relationship between soil and water as its liquid phase. It is a key soil feature on ecological and economic parameters in agriculture and hydrology. This balance is determined by the entry of water into the soil, its surface runoff, the movement of water through the soil, either ascending, descending, or lateral, as well as loss from the soil, whether it is surface runoff, deep percolation, evaporation, or transpiration. Water in the soil has a decisive influence on agricultural crop growth, development, and yield. Water in the soil doesn't mean only the total water content but also presents the hydrological categories and, above all, the retention capacity and the wilting point. Between these two constants, water is available to plants, which plants can use for their growth and development. Soil-water balance depends on the quality of the soil, especially its physical properties (e.g., particle size distribution, porosity, capillarity of pores, etc.), but also on external conditions in the first raw climatic and topographic ones. Therefore, climatic changes have a high impact on SWB. Many authors have published research on this topic. Porporato and Daly (2004) present a simplified framework, analysing how hydroclimatic variability (mainly the frequency and amount of rainfall events) affects soil and plant relations in soil moisture dynamics and the impact on vegetation conditions. They have provided a general classification of the soil-water balance in global ecosystems based on two main dimensionless groups summarising climate, soil, and vegetation conditions. They concluded that fluctuations in evapotranspiration tend to increase the variance of soil moisture dynamics. Interestingly, it always reduces water losses compared to the case of constant potential evapotranspiration (PET). Ljusa *et al.* (2020) studied the perennial climatic parameters in the Mediterranean region of Bosnia and Herzegovina. They concluded that climate change is evident in B&H, which constituted the basis for this research into soil water balance and agricultural production. The predicted PET was based on an air temperature increase of 2°C and an expected decrease in precipitation by 25%. Touhami *et al.* (2015) conclude that forecasting climate change and groundwater recharge using three models is highly complex, especially in semiarid regions where recharge is reduced and associated with few yearly events. The analysed data suggest a transition from the semiarid conditions during the baseline period (1961-1990, 53% of the years with annual precipitation between 200-350 mm) to the arid condition at the end of the century (2071-2099; 62% of the years with annual precipitation <200 mm). Eitzinger *et al.* (2003) studied SWB and water stress on the winter wheat on two sites, without and with groundwater. Both agricultural sites with similar climatic conditions showed a simulated decrease in water stress, lower transpiration, and an increase in winter wheat yields under future climate scenarios. Groundwater in the rooting zone slightly increased the yield of wheat. Li *et al.* (2021) stated that the impacts of climate change on soil water balance mainly come from changes in spatial and temporal patterns of climatic variables such as rainfall and temperature. They have applied the HYDRUS-1D model to quantify the SWB components under multiple climate change scenarios and land use types. Their results show that considering the effect of climate change, the changes in precipitation variances dominated SWB variations. The availability of soil water and crop conditions are directly related and represent the key factors for agriculture sustainability. These factors are related to the crop type and its characteristics, such as root zone and plant physiology. Different crops have different demands for the quantity and dynamics of water. Water availability influences certain phases of plant growth, including growth, maturation, and development, as well as soil quality, tillage, and other agricultural techniques. The SWB also decisively affects water management and irrigation planning. The retention capacity of soil is a critical factor in water management. Soil texture, bulk density, and total and differential capacity influence water retention, migration, and supply to the plant. Retention capacity increases from sandy to silty and clayey soils. Water supply is crucial for the growth, differentiation, and yields of crops. Agro techniques, land use, crop rotation, and other measures are planned based on SWB, which is determined by precipitation, soil quality, and potential and actual evapotranspiration. This data provides information on the lack and surplus of water during the year, which is essential for crop selection and irrigation needs. Among others, the time and type of irrigation are determined based on water availability within the interval between retention capacity and wilting moisture. Climatic changes can significantly affect soil water balance, crop production, crop scheduling, and crop regionalisation. These changes are based on the altered conditions of precipitation, increased average annual temperature, and more frequent extreme events. Adapting agriculture production to the newly established soil-water-climatic conditions can prevent these issues. In a more comprehensive examination, Weng *et al.* (2008) delved into the heightened water cycle activity as a crucial element in safeguarding the photosynthetic apparatus. Knezevic *et al.* (2013) presented results of water balance simulation on winter wheat production in the area around Bijelo Polje (Montenegro) using CROPWAT and ISAREG models. These authors successfully used models to simulate soil water balance in similar agroecological conditions. The same authors (Knežević *et al.*, 2012) also studied soil-water balance with silage-maize using CROPWAT and ISAREG models. Qin *et al.* (2014) explored the interplay between natural and social facets of the water cycle, discussing four aspects of its dualistic evolution. Understanding water storage is pivotal for grasping global and local water cycles and monitoring climate and environmental shifts (Xu *et al.*, 2013). Actual evapotranspiration serves as a vital link between land surface water balance and energy balance, influencing hydrological simulations of climate change effects (Itier *et al.*, 1992; Gerla, 1992; Xu *et al.*, 2016; Blum and Gerig, 2006). Zhao *et al.* (2013) summarised the methods for estimating evapotranspiration applied in hydrological models. Buchtele and Tesar (2009) demonstrated the predominant role of transpiration within the vegetation's annual cycle. Gao *et al*. (2012) suggested that declining trends in annual precipitation and potential evapotranspiration may lead to reduced actual evapotranspiration in a given basin. The significance of estimating actual evapotranspiration has been acknowledged for some time (Rana *et al.,* 1997), with models tested in the field. Liu *et al*. (2014) evaluated how evapotranspiration and water availability changed under shifting climatic conditions in Northern

Eurasia. Dong *et al.* (2016) extensively investigated evapotranspiration estimation in water science. Gocic *et al.* (2016) conducted a long-term analysis of precipitation and the concentration of precipitation using support vector machine methods. Mupenzi *et al*. (2012) scrutinized evapotranspiration, evaporation, and seepage losses in arid and semi-arid regions to mitigate water losses. Morari and Giardini (2001) emphasized the need for better analysis of the water cycle and monthly average evapotranspiration in specific areas. Estimating evapotranspiration relies on various climatic parameters such as air temperature, vapor pressure, and humidity. Trajkovic and Kolaković (2010) analyzed the reliability of estimating reference evapotranspiration using a simplified pan-based approach that does not require data on relative humidity and wind speed, comparing the results with lysimeter measurements in the field. The term "crop water requirement" denotes the volume of water necessary to fulfill the evaporation demand of a crop. While crop evapotranspiration and crop water demand share similarities, the latter specifically pertains to the quantity of water needed for irrigation. In contrast, crop evapotranspiration refers also to the water lost during the evaporation process.

MATERIAL AND METHODS

Study site

The study was conducted in Zvečan municipality, which is located in the northern part of Kosovo. This particular part of Kosovo is specific for its geographic, social, and environmental features.

Figure 1 Location of the experimental site

The selected parcel is 0.45 ha, with the following coordinates:42.943 N, 20.843 E. Elevation is 565 m above sea level.

Soil analysis

Three samples were taken for the planned analysis of the soil of the selected location. The collection of samples for soil physical-mechanical analysis was carried out at different depths. The first sample is from a surface of 0-32 cm, the second from 32-64 cm, and the third from a depth of 64-100 cm. The samples were placed in plastic bags and taken to the laboratory, where they were prepared for analysis using standard procedures. Grain size distribution was performed using the ISSS method.

Modelling Soil-Water Balance by using CROPWAT 8.0

The term "crop water requirement" denotes the volume of water necessary to fulfill the water demand of a crop. While crop evapotranspiration and crop water demand share similarities, the latter specifically pertains to the quantity of water needed for irrigation, whereas crop evapotranspiration refers to the water lost during the evaporation process of the crop (Allen *et al*., 1998; FAO, 2005). CROPWAT, created by FAO's Land and Water Development Division, is a software tool designed to assist decision-making processes. Specifically, CROPWAT 8.0 for Windows is a computational tool used to determine crop water requirements and irrigation needs based on input data regarding soil, climate, and crop characteristics. It enables the formulation of irrigation schedules under diverse management conditions and facilitates the water supply assessment for different cropping patterns. FAO Penman-Monteith equation (Allen *et al*., 1998) is used in CROPWAT 8.0 to determine reference evapotranspiration (ETo). The reference evapotranspiration (ET_o) was multiplied by the crop coefficient (Kc) to obtain crop evapotranspiration (ETc), as shown below:

$$ETc = Kc \times ET_0$$

The meteorological data are taken from FAO Climate Estimation Tools for the selected location in the case of missing actual measurements for the north of Kosovo. There are historical data from 1961-1990 and the AGERA5 data for the period from 2000-2023. Three different data sets are used for calculation: dry 2000, wet 2014, and average 2019 years.

The hydraulic properties of the analysed soil were calculated by the HYDRUS program after the soil analysis concerning the particle size distribution was done. Based on the obtained experimental results, water flow was observed through loam, sandy loam, and silty loam textured soils. The program sets the parameters characteristic of all three soil textures. The cumulative flux of the given soil profile, i.e. the upper and lower boundary flow of water in the soil. Water infiltrates on the sample's surface, which conditions a positive (Pressure Head) pressure, which represents the upper boundary flow, thanks to the selected free drainage (Free Drainage), representing free drainage under the influence of gravity.

RESULTS AND DISCUSSION

SWB and crop condition are directly connected and represent the critical factors for agriculture sustainability. They are related to the crop type and its habitus, such as the root zone and plant physiology. Various crops have various demands for the quantity and dynamics of water. Water influences certain phenophases, such as growth, maturation, and fragmentation. It also influences soil quality and the type of tillage and agro-techniques. On top of that, SWM decisively affects water management and irrigation planning. Retention capacity (0.33 kPa), soil texture, specific density total, and differential capacity decisively influence water retention, water migration, and water supply to the plant. Retention capacity rises from sandy to silty to clayey soils. Water supply is crucial for crop growth, differentiation, and yields.

Based on this, agro techniques, land use, crop rotation, and other measures are planned. To determine these measures, SWB must be determined based on precipitation, soil quality, and potential and actual evapotranspiration. This way, we get data on the annual lack and surplus of water. This data is crucial for crop selection and irrigation requirements. This can significantly influence crop selection, the time and type of planting, and crop rotation. Since water is available within the interval between retention capacity and wilting moisture, it determines the time and type of irrigation.

Climatic changes can significantly affect soil water balance, crop production, crop scheduling, and crop regionalization based on changed precipitation conditions, increased average annual temperature, and, more often, extreme occasions. This can be prevented by adjusting agricultural production to the newly established soil-water-climatic conditions. Based on the obtained experimental results with the HYDRUS model, the water flow was observed through loam, sandy loam, and silty loam.

Water content in relation to time in the observed nodes/depths (N1 - 40 cm, N2 - 80cm, N3 - 120cm, N4 - 160 cm, and N5 - 200 cm) is presented in Fig. 2. The initial water content in the observed nodes is different. In the first node (40 cm), it is 0.452 cm^3/cm^3; in the second node (80 cm), it is 0.448 cm^3/cm^3; in the third node (120 cm), the initial water content is 0.446 cm^3/cm^3, in the fourth (160 cm) 0.442 $cm^3/cm^3$and in the fifth node (200 cm) 0.44 cm^3/cm^3. The water content in all nodes reaches a maximum value of 0.45 cm^3/cm^3 (saturated water content) for similar time intervals.

The saturated water content for the loam texture is 0.43, sandy loam is 0.41, and silty loam is 0.45. The average saturated water content for the examined profile coincides with the fractions of three materials in the investigated sample, as shown in Fig. 3. The further calculation by HYDRUS 1D presented the values for Soil moisture at field capacity 220 mm/m, maximum infiltration flux 250 mm/day, and maximum drainage flux to saturated zone 5 mm/day, as seen in Fig. 4.

The cumulative flux of the given soil profile, i.e. the upper and lower boundary flow of water in the soil, are shown in Figure 4. Water infiltrates on the surface of the sample, which conditions a positive (Pressure Head) pressure, which represents the upper boundary flow (green), and maximum drainage flux (red), representing free drainage under the influence of gravity. The graph shows that the inflow and outflow of water are equal after 22 hours (the upper and lower limit flow lines are then parallel), which makes the soil stable about the water. The following input parameters for winter wheat are used for simulations (Table 1), and the following soil hydraulic properties are calculated by the HYDRUS model (Table 2).

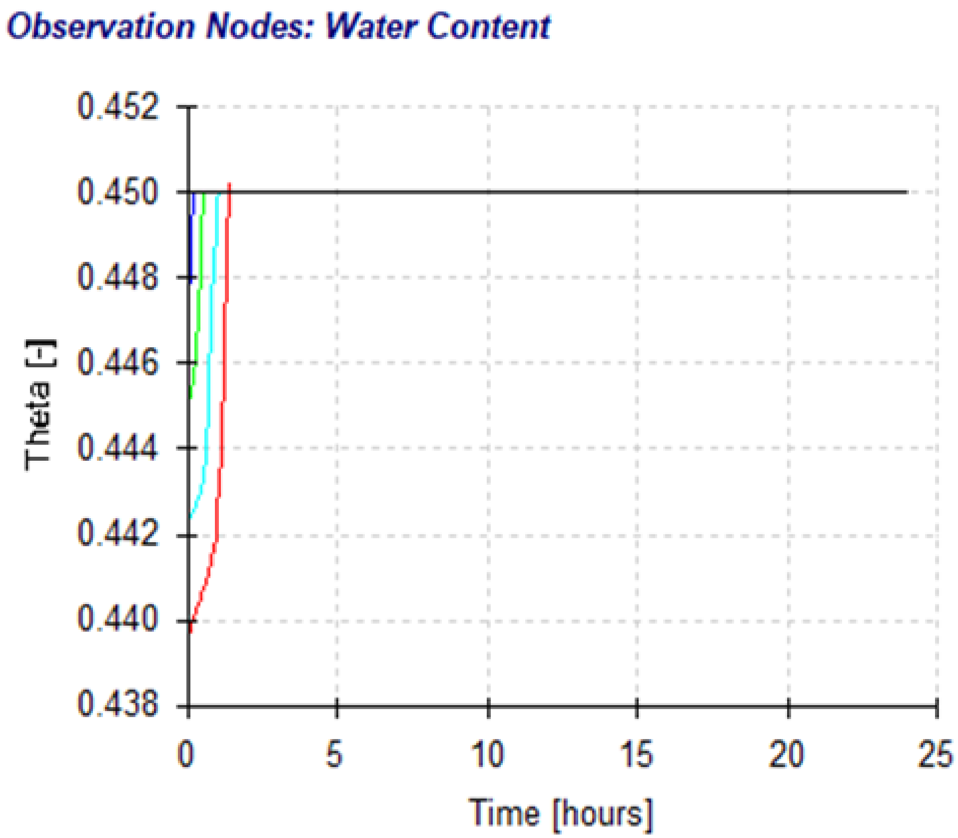

Figure 2. Soil water content (Theta) by depths vs time

Table 1. Crop data

Crop	Winter_wheat
Planting/Sowing Date	Nov-01
Depletion factor	0.55
Rooting depth [m]	0.7
Crop factor (outside growing season)	0.5
Depletion factor (outside growing season)	0.5
Rooting depth (outside growing season)	0.5

Table 2. Calculated soil hydraulic properties

Soil	sandy_loam
Soil moisture at field capacity [mm/m]	220
Soil moisture at wilting point [mm/m]	80
Maximum infiltration flux [mm/day]	250
Maximum drainage flux to saturated zone [mm/day]	5

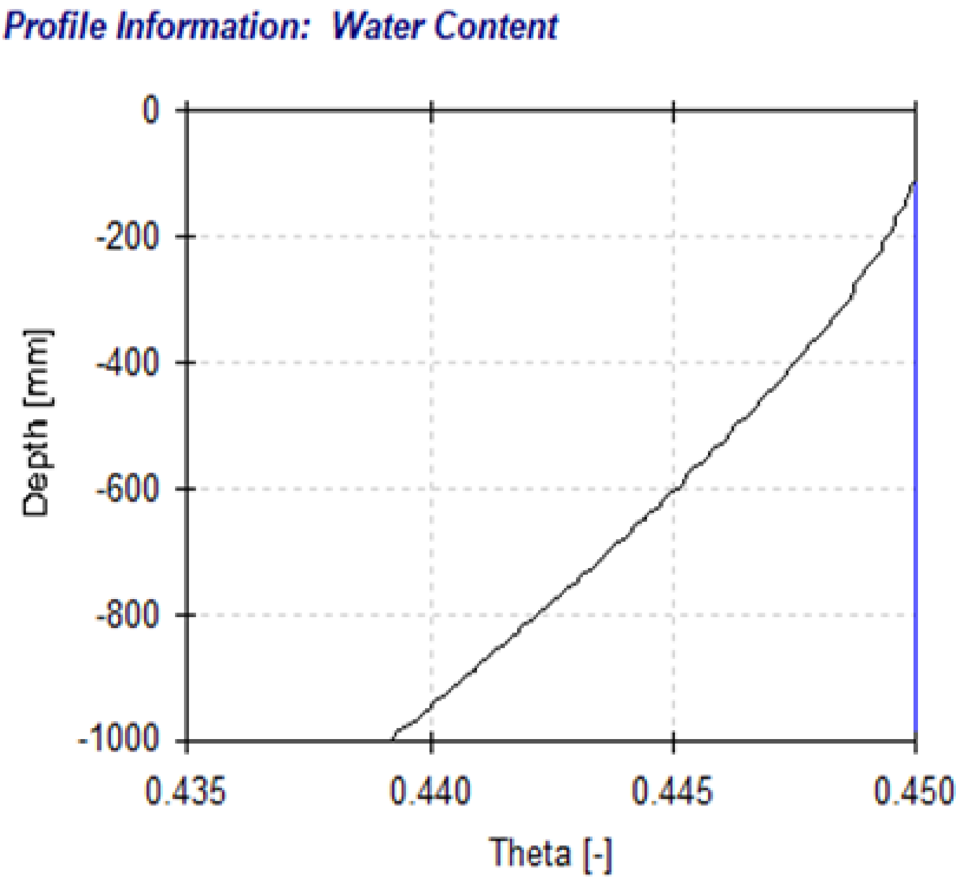

Fig. 3 Profile information - Depth vs Water Content

Table 3 presents the results of the soil water balance and crop response to water for the historical average 1961-1999.

The same crop and soil data are used to calculate the soil water balance for the average climatic conditions from 2000 to 2023. The results are presented in Table 4.

The ratio between precipitation and effective precipitation is presented in the Fig. 5.

The trends in changing climatic conditions are presented in Figure 6. In the period from 1961-1999, historical data, the total precipitation was 911 mm, and the reference evapotranspiration (ETo) was 879 mm, actual evapotranspiration (ETa) was 375 mm, but Crop Evapotranspiration of winter wheat (ETc-Crop) for the same historical period was 400 mm, that proves good climatic conditions for the selected crop. In the period from 2000-2023, the average climatic conditions were used for Crop-Water balance calculation, and the total precipitation was 712 mm, reference Evapotranspiration was 924 mm, actual evapotranspiration was 477 mm, and Crop Evapotranspiration of winter wheat for the same period was 641 mm.

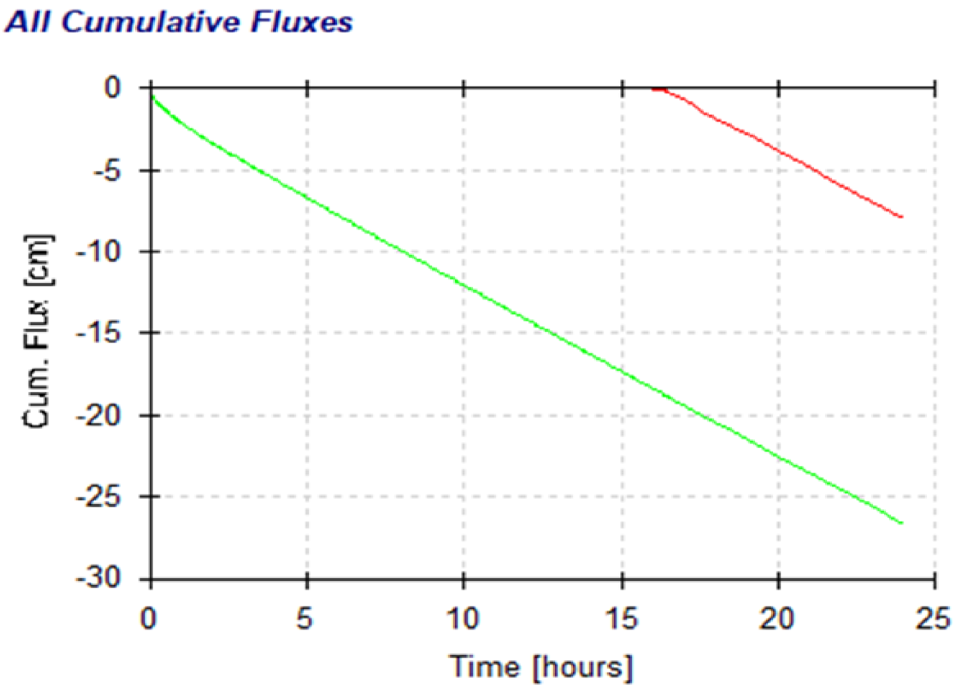

Fig. 4. Boundary Water Fluxes and Pressure Heads vs Time

Table 3. Soil water balance and crop response to water for the historical average (1961–1999)

Month	Prc.	Wet	ETo	Crop	ETcCrop	ETa	CropDef	Drain	Soil Water
	mm/m	days	mm/m	days	mm/m	mm/m	mm/m	mm/m	mm
Jan	79	15	18	31	13	13	0	0	226
Feb	69	13	27	28	23	23	0	0	225
Mar	67	9	52	31	52	52	0	0	200
Apr	113	15	78	30	88	88	0	0	193
May	56	6	106	31	122	102	20	0	135
Jun	57	8	123	28	75	70	5	0	120
Jul	73	7	145	0	0	0	0	0	0
Aug	109	16	132	0	0	0	0	0	0
Sep	93	13	91	0	0	0	0	0	0
Oct	51	10	57	0	0	0	0	0	0
Nov	76	14	31	30	16	16	0	0	220
Dec	69	15	19	31	11	11	0	0	219
Total	911	141	879	240	400	375	20	0	

Where: Prc – Precipitation in mm/month; ETo – reference crop evapotranspiration; ETcCrop - evapotranspiration under standard conditions; ETa – crop evapotranspiration under non-standard conditions; CropDef – Crop water deficit; Drain – Drainage loss; Soil Water – Soil water content in a root zone.

Table 4. Soil water balance and crop response to water (2000-2023)

Month	Prc.	Wet Days	ETo	Crop Days	ETc-Crop	ETa	Crop Deficit	Drain	Soil Water
	mm/m	days	mm/d	days	mm/m	mm/m	mm/m	mm/m	mm
Jan	24	11	15	31	51	51	0	0	157
Feb	75	25	31	28	67	67	0	0	160
Mar	107	29	68	31	98	98	0	0	168
Apr	96	27	84	30	148	136	12	0	116
May	62	23	98	31	164	69	95	0	108
Jun	22	14	131	28	86	29	59	0	100
Jul	33	18	143	0	0	0	0	0	0
Aug	17	8	145	0	0	0	0	0	0
Sep	68	24	96	0	0	0	0	0	0
Oct	72	24	62	0	0	0	0	0	0
Nov	92	27	31	30	7	7	0	0	231
Dec	43	25	19	31	20	20	0	0	201
Total	712	255	924	240	641	477	164	0	

Where: Prc – Precipitation in mm/month; ETo – reference crop evapotranspiration; ETc – Crop evapotranspiration under standard conditions; ETa – crop evapotranspiration under non-standard conditions; CropDef – Crop water deficit; GW – Groundwater recharge; Soil Water – Soil water content in a root zone.

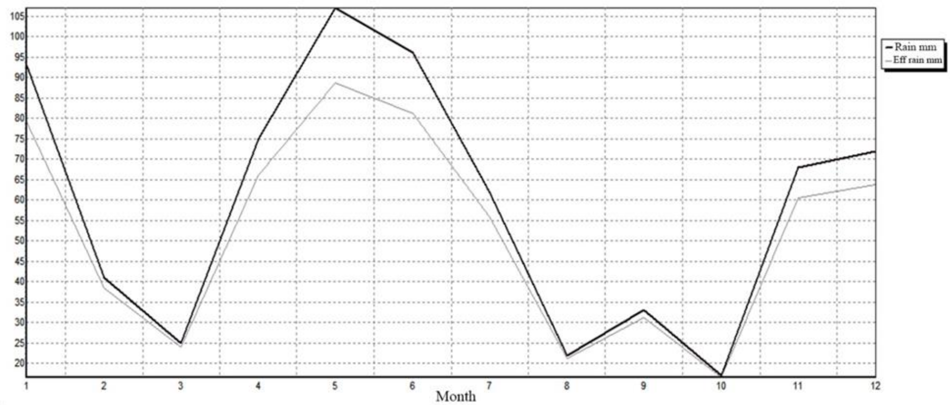

Fig.5. Rain (bold line)/Effective rain (dashed line)

Based on the presented figure, it is visible that the content of soil water in the soil profile decreased in the first decades of the 21st century. For the particular crop (winter wheat), the decrease affects the crop, especially in May and June. In June, the crop is in the phase of maturation, and the critical month is May. In the early phenophases of winter wheat lack of soil water is not affecting the crop significantly.

Čadro *et al.* (2023) stated that for the conditions of Bosnia and Herzegovina and Croatia, the key characteristic of the 1991-2020 period compared to 1961-1990 is the greater variation of all components of the water balance.

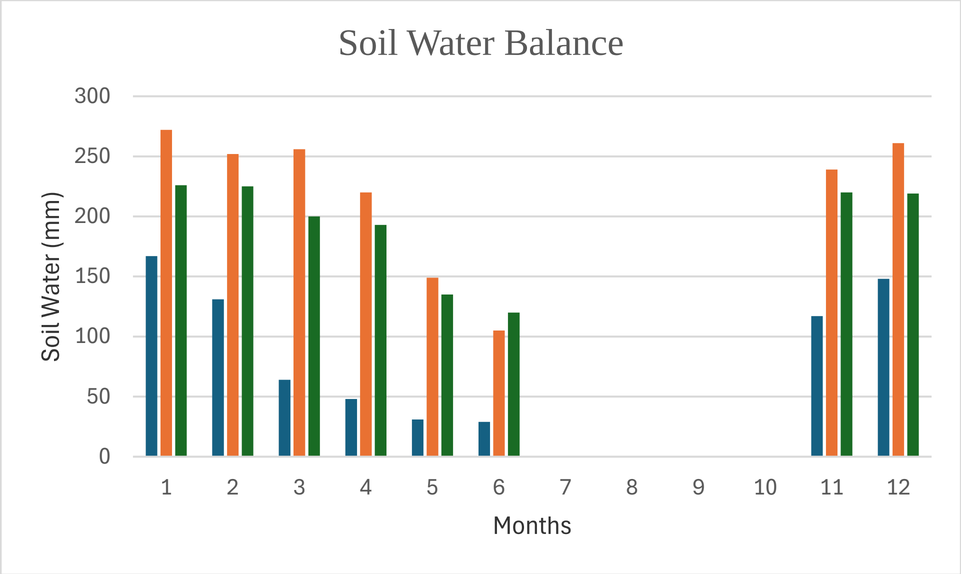

Fig. 6. Difference in Soil/Water Balance: Dry 2000(blue), Average AGERA 5 (red), Historical (green)

The obtained results can be compared with the other authors' results obtained in similar climatic conditions. Knezevic *et al.* (2013) studied Soil-water balance in the area of Bijelo Polje, Montenegro. The results show that ETa ranges from 345.5–463.3 mm. ETc ranges from 539.3–598.6 mm. Our results for winter wheat are in the range of Knezevic *et al.* (2013). The ratio ETa/ETc for the historical average is 93.8%, whereas for the AGERA5 average dataset amounts to 74.4%. In conditions of Montenegro, a 70–80% reduction in ETa resulted in a 70–80% reduction in winter wheat yield. Therefore, our results indicate that the changes in soil water balance are going to cause a reduction of more than 20% in winter wheat yield. Winter wheat is not a cash crop, and this yield decrease cannot be fixed with irrigation because the investment can hardly be paid. The extreme dry year in the period 2000-2023 was 2000, with a water deficit of 293 mm in April, May, and June. In 2000, Eta was 328mm, ETc was 684 mm. The ratio Eta/ETc was 0.48. Based on this research, the irrigation of winter wheat is not required in average conditions, but in the case of drought, as reported in 2000, irrigation is necessary.

CONCLUSIONS

The study shows that there is a change in SWB as a consequence of changed climatic conditions. Comparing the historical data (1961-1999) with recent climatic parameters (2000-2023), it can be concluded that there is a significant change in soil-water balance. This change is mainly expressed during the spring and summer months. Since winter wheat decreases its need for water by June, it suffers less than crops whose key phenophases are ongoing during summer. The obtained data show the shift in climatic conditions in the last four decades, the average climatic conditions have changed, showing higher values of Actual Evapotranspiration and Crop Evapotranspiration in the period 2000-2023. The extreme dry year in the period 2000-2023 was 2000, with a water deficit of 293 mm in April, May, and June. Based on this research, the irrigation of winter wheat is not required in average conditions, but in the case of drought, as reported for 2000, the cost-benefit analysis has to be carried out to see whether the irrigation is feasible. Further investigations will reveal the details of the climate change effects on the Soil/water balance in the region.

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QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF SELECTED LETTUCE HYBRIDS (*LACTUCA SATIVA* L.) IN A PROTECTED AGROSPACE

SUMMARY

In order to solve the mentioned problem, two-factor experimental research was conducted with eight lettuce hybrids: Tonale, Donertie, Hetti, Limeria, Nolanie, Australiae, Cencibel and Saturdai. Lettuce was grown in the winter period in a protected agricultural area without a heating system, and the quantitative and qualitative component of the yield was monitored with an emphasis on the vitamin C content, and the amount of accumulated nitrates and nitrites depending on the hybrid, the technological maturity of the plant and the position of the leaf in the head. Based on the results of the analysis (ANOVA), a significant influence of the tested parameters (hybrid) on the quantitative characteristics of the plant was determined, the Hettie hybrid with a head weight of 633 g was rated as the best hybrid. Different time periods of plant development, as well as the type of hybrid, showed a statistically significant influence on the average nitrate content in the fresh mass of the plant, as well as the amount of nitrate depending on the part of the plant (outer rosette leaves, middle leaves and inner leaves), and the highest amount of nitrate recorded in all hybrids in the outer leaves of the leaf rosette and increased in relation to the extension of the plant's development time, ranging from 524,212 mg/kg hybrid Tonale to 2562,323 hybrid Hetti. In all tested hybrids, a drop in vitamin C concentration was recorded after 60 days of development.

Key words: nitrates, nitrites, *Lactuca sativa*, quality, vitamin C.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an annual herbaceous plant, a widespread type of leafy vegetable from the *Asteraceae* family. The leaf is consumed fresh, low in calories, rich in minerals and vitamins: C, B1, B2, carotene, etc., and mineral

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substances such as potassium, iron and phosphorus salts (Parađiković, 2009). Since lettuce is characterized by a high production of leaves in a short period of time, it is one of the leading vegetable species worldwide, and the growing demand for it on the market requires more intensive agricultural practices and abundant application of fertilizers. However, lettuce has the ability to accumulate and accumulate nitrates, which can potentially become harmful to human health. Nitrites oxidize the iron in hemoglobin, thus preventing the normal supply of blood with oxygen. As a consequence, poisoning occurs, which is especially dangerous for cardiovascular patients (Stephan, 2017). Numerous studies have confirmed that nitrates are the cause of methemoglobinemia in children (Addiscott & Benjamin, 2004). Methemoglobinemia is a disease in which hemoglobin is in an oxidized form and cannot bind oxygen. The amount of nitrate in vegetables is greatly influenced by environmental factors (atmospheric humidity, water content in the substrate, temperature, radiation and photoperiod), as well as agricultural factors (nitrogen doses and chemical forms, availability of other nutrients), (Santamaria, 2006). Light intensity is a key factor in the amount of nitrate in leafy vegetables as a result of nitrogen assimilation and electron transport during the process of photosynthesis in the leaves. Winter crops generally contain a higher amount of nitrates than summer crops grown in the same environment, and plants grown in the area of northern Europe contain higher levels of nitrates compared to areas of southern Europe (Weightman et al., 2006). The mentioned differences can be explained by the fact that greater lighting in summer reduces the nitrate content in favor of increasing plant growth (Kanaan & Economakis, 1992). According to the World Health Organization (WHO) and numerous epidemiological studies, a healthy diet with a daily intake of 400 to 500 g of fruit is recommended and vegetables. This can promote good health, reduce the risk of various diseases and strengthen immunity (FAO 2020; Leenders et al., 2013; Boffeta et al., 2010). The legislation of the European Union in Regulation 1258/2011 prescribes maximum permissible concentrations only for nitrates, while the legal regulation is limited only for three types of vegetables (spinach, lettuce and rocket), (European Commission 2011).

On the basis of Art. 16 and 72 of the Law on Food ("Official Gazette of BiH", No. 50/04) and Article 17 of the Law on the Council of Ministers of Bosnia and Herzegovina ("Official Gazette of BiH", No. 30/03, 42/03, 81/ 06, 76/07, 81/07, 94/07 and 24/08), the Council of Ministers of Bosnia and Herzegovina, on the proposal of the Agency for Food Safety of Bosnia and Herzegovina, in cooperation with the competent authorities of the entity and Brcko District of Bosnia and Herzegovina, passed regulation according to which the maximum allowed amount of nitrates for fresh lettuce (*Lactuca sativa* L.), harvested from October 1 to March 31, from greenhouses is 5000 mg or grown outdoors 4000 mg NO_3/kg, for lettuce harvested from April 1 to On September 30, from the greenhouse 4000 mg or grown outdoors 3000 mg NO_3/kg.

The purpose of the conducted research is to determine the differences in the quantitative and qualitative characteristics of the selected hybrids grown in a protected agricultural area according to the principles of conventional agricultural production, and after the research to provide information about the most favorable harvest time, parts of consumption and the most adequate lettuce hybrid with regard to the investigated parameters.

MATERIAL AND METHODS

A two-factorial one-year study was carried out according to a random block arrangement in four repetitions, where factor A = hybrid, factor B = technological maturity. The experiment was done in a greenhouse in the northwestern part of Bosnia and Herzegovina, location Bihac (figure 1).

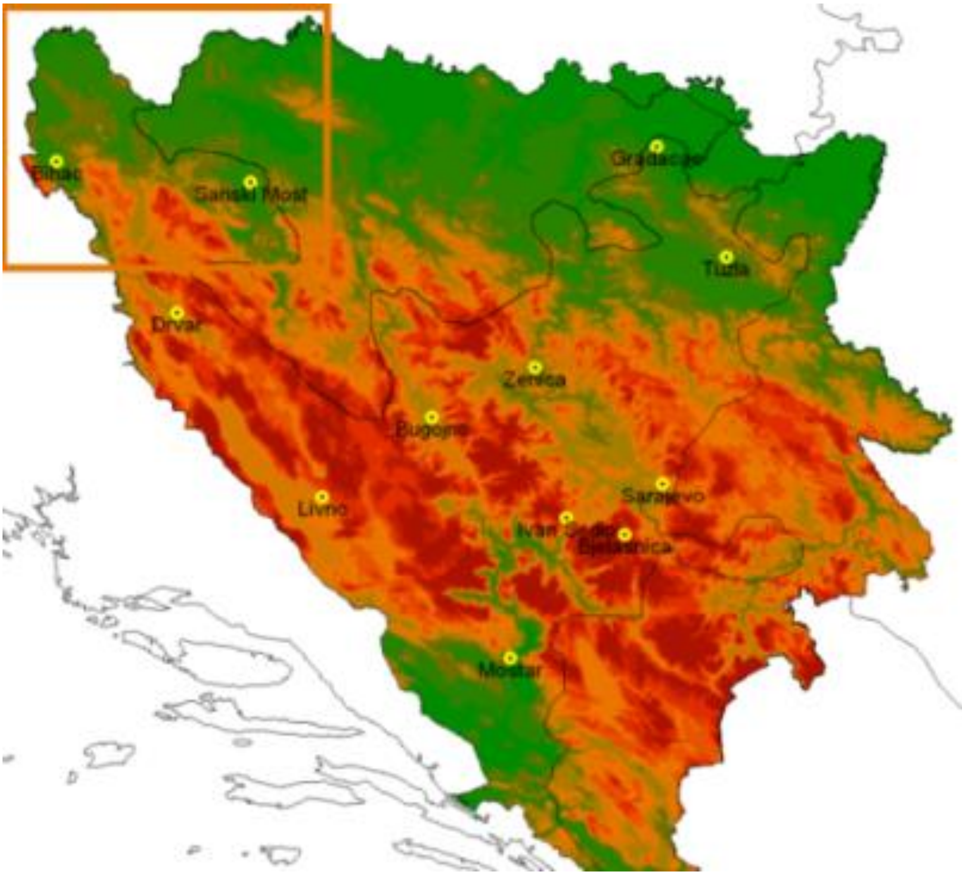


Figure 1. Map of Bosnia and Herzegovina, source: maps.google.com

Lettuce is grown according to the principles of conventional agriculture The total yield (biological and marketable mass for each tested hybrid) was determined, followed by the determination of mineral substances in the salad (Fe, P, K, Ca, Mg, content of dry matter, fiber, fat, protein, ash and UH, as well as water content, sugar and vitamin C), as well as nitrate and nitrite values, sampling was carried out over a period of 3 months, depending on the technological development of the plant. Special emphasis is placed on determining the amount of nitrates in the plant material depending on the technological development of the plant (baby lettuce, medium-developed lettuce and lettuce at full technological maturity), as well as the hybrid used. The analysis were carried out in the laboratories of the Agricultural Institute of the Una Sana Canton, as well as the laboratory of the Faculty of Biotechnology of the University of Bihac (BiH). In all samples, dry matter was determined by drying at 105°C until constant weighing, amount of water, protein concentration according to Kjeldahl's principle, determination of vitamin C using the iodometric titration method, P concentration (by photometry with ammonium molybdate) and reading on a spectrophotometer by the International standard - Water quality - Spectrometric determination of phosphorus using ammonium molybdate -ISO 6878 (1998) method, and K, Ca and Mg by the flame photometry method according to International standard determination of sodium and potassium by flame emission - ISO 9964-3 (1996), total sugars by the Luff-Schoorl method, and the application of ion chromatography (eng. Ion chromatography, IC) in the analysis of nitrates and nitrites in leafy vegetables. All results were statistically processed (PAST 4.0) and SAS 9.4 (SAS, 2012).

RESULTS WITH DISCUSSION

Given that a greenhouse without an additional heating system and additional lighting was used for the experimental research, two parameters (temperature and light) that directly affect the quantitative and qualitative characteristics of the investigated species were processed in the further part of the paper for the time period October. 2022 until March. in 2023. Average monthly temperatures for the vegetation period for the Bihac locality were: 13,9 °C, 10,7 °C, 6,2 °C, 1,3 °C, 5,4 °C and 10,0 °C (min. -1,8° C, max. 20,9° C), while the temperature in the greenhouse is 7 °C higher on average. The determined air temperature is significantly above the standard normal value, and according to the percentile distribution of temperature conditions in the year of conducting the experiment, we classify autumn and the month of March in the extremely warm category, with only 4 cold days and no extremely cold days compared to an average of 22 cold days and 8 extremely cold days cold ones. The most pronounced monthly negative deviation of sunshine was recorded in February and March at the meteorological station Bihac thirty eight (38) hours of sunshine were recorded in Bihać, which is about 51% lower than the average. Comparing the research results with Cometti et al., (2013); Anusiya & Sivachandiran (2019), who investigated the influence of the amount, spectrum of light and the influence of shade on the quality of lettuce, proved that light plays a very important role in the life of the plant, and that light directly affects the amount of vitamin C in lettuce leaves.

Examining the total weight of the head per plant, and the yield per unit area, significant statistical differences were determined depending on the lettuce hybrid grown, but also the sampling time shown in table 1.

Statistically significant differences ($P<0,01$) were found in the average total mass of plants between lettuce hybrids, as well as the time of harvest. Namely, the highest yield was achieved by Hetti with an average weight of piece of 633 g (90 days), 381 g (60 days) and 190 g (30 days) from sowing, while the lowest yield was recorded with the hybrid Saturdai 299 g (90 days), 163 g (60 days) and only 98 g (30 days) from sowing. Alisson Franco et al., (2017) studied the reaction of the plant and the influence of the organic fertilizer and variety on plant growth and development and yield. The experiment was conducted in an open field using the split plot method, with a combination of organic fertilizer of 0, 20, 40, 60 and 80 t/ha and three lettuce varieties Delícia, Babá de Verão and Itapuã 401. The influence of fertilization on yield was determined, but continuously good yields were achieved by the Delícia and Babá de Verãovarieties in all treatments and in 50% shade, which proves that the yield components are largely varietal characteristics. Therefore, the stated results are in accordance with our research.

Table 1. Yield per plant based on hybrids and technological maturity

tonale	donertie	hettie	limeria	nolanie	Ostralie	concibel	saturdai
173±1,26^{a}	131±1,86^{b}	190±1,17^{a}	132±1,86^{b}	113±2,11^{c}	100±2,43^{b}	125±1,93^{b}	98±2,96^{d}
245±4,98^{c}	361±5,81^{a}	381±5,71^{a}	248±4,96^{c}	380±5,71^{a}	357±5,81^{a}	252±4,91^{c}	163±3,96^{d}
460±7,89^{b}	496±7,12^{b}	633±6,49^{a}	396±8,67^{bc}	612±6,43^{a}	468±7,24^{b}	358±8,19^{bc}	299±12,02^{c}
$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$	$p \leq 0,01$

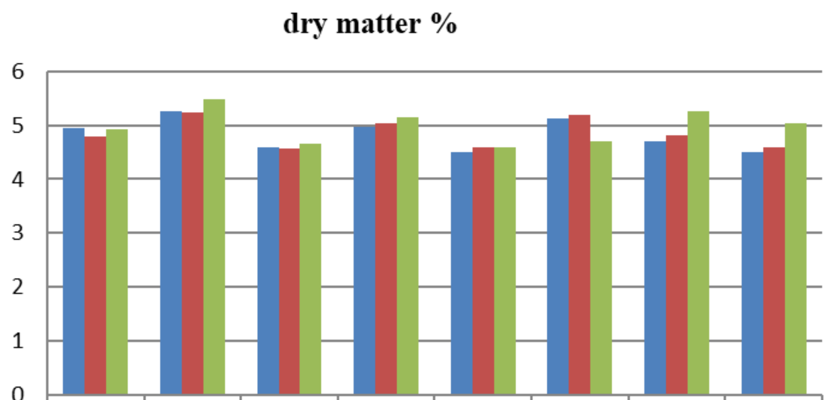
ANOVA	Suma	df	Average value	F	$p \leq 0,01$
Hybrid	442227	2	221114	31,1	0,0338*
Tec. maturity	148937	21	7092,23	8	0,02961**

Kruskal - Walis test: H(ch^2):17,8 H_c (tie corrected):17,8 p ($\leq$0,01):0,0001367
There is a statistically significant difference between the samples

Drăghici et al. (2016) investigating the effect of fertilizers on the yield components of lettuce, conducted experimental research with the use of four types of fertilizers: three organic fertilizers and a chemical one. The experimental fertilization variants were: V1 - organic Grow; V2 - Bio Leafez; V3 - Formulex; V4 - chemical fertilizer. The total cultivated area was 160 m^2 with 17,5 plants per m^2. Three varieties of lettuce were used: Markies, Lollo Bionda and Lollo Rosa.

The analysis of the results and their statistical processing showed that, regardless of the type of applied fertilizer, the cultivated varieties of lettuce and the genetic specificity were superior when all the investigated parameters, including the plant weight, were in question, hich is also in line with our research.

In addition to the examined yield parameters, analyzes of qualitative characteristics were also carried out on salad samples, and the content was determined: % protein, % fat, % water, % vitamin C mg/100, % ash, % UH, % fiber % and % sugar. Chemical analyzes were carried out in three repetitions (30, 60 and 90 days of development from the day of sowing) for all eight tested lettuce hybrids. The absolute highest percentage of dry matter (graph 1) was measured in the Donertie hybrid: 5,26% (30 days), 5,25% (60 days) and 5,49% (90 days) from the day of sowing, while the lowest content recorded in the hybrid Hetti of only 4,59% (30 days), 4,56% (60 days) and 4,65 (90 days). Statistical processing of the data revealed a small statistical significance in the values of dry matter between different hybrids, but not in dependence on the technological age of the plant.



	Tonale	Donertie	Hettie	Limeria	Nolanie	Ostralie	Concibel	Saturdai
30 days	4.94	5.26	4.59	4.97	4.49	5.12	4.7	4.49
60 days	4.79	5.24	4.56	5.04	4.59	5.2	4.82	4.59
90 days	4.92	5.49	4.65	5.14	4.59	4.69	5.25	5.03

Graph 1. Dry matter content depending on the hybrid and harvest time

Slamet et al., (2017) determined the content of dry matter in the range of 5,56 to 6,98% when applying different organic fertilizers in the production of lettuce, which is a significantly higher content compared to our research. Similar research was carried out by Alaeddin et al., (2010) who, investigating the application of five types of organic fertilizer and the cultivation of lettuce according to the principles of conventional production, came to the conclusion that the content of dry matter in conventional production was around 5,6%, which is in accordance with our research, while when growing with compost, the content of dry matter increased up to 8,02%

Few researchers have analyzed the properties of raw proteins in lettuce, but Sularz et al., (2020) investigating the impact of iodine biofortification (potassium iodate/KIO_3/, 5-iodosalicylic acid /5-ISA/ and 3,5-diiodosalicylic acid /3, 5-diISA/) in the chemical composition of lettuce (*Lactuca sativa* L. *capitata*) state that the application of iodine compounds had an effect on the yield and content of dry matter, and thus the chemical composition of lettuce leaves. The recorded values of protein in our research, depending on the hybrid and the time of harvest, are from 1,48% to 2,10%, they are significantly lower than the values achieved in the research of Sularza et al., (2020) which ranged from 1,1 % control up to 5,1%.

Lettuce (*Lactuca sativa* L.) is a highly valued vegetable in the human diet not only because of its wealth of minerals and vitamins, but also because of the fact that today it is produced throughout the year and consumed fresh, so that all ingredients remain intact. Even if lettuce contains nutrients that promote health, according to research by Kim et al., (2016) the biosynthesis of such phytochemicals varies depending on the variety, leaf color and growing conditions. The average values of the concentration of vitamin C and the statistical processing of these values are shown in table 2. The highest concentration of vitamin C was recorded in the hybrid Nolania of 30,24% (30 days), 35,88% (60 days) and 34,33% (90 days), in terms of values, the Tonale hybrid is in second place at 32,75%, while the lowest amount of vitamin C was recorded in the Hetti hybrid at 22,1%. The specifics of the vitamin C content are reflected in the fact that in all hybrids the time period of plant development had an effect on the vitamin C content, whereby a statistically significant difference in values was determined at a time period of 60 days of development, after which a decrease in the amount of vitamin C was recorded.

Table 2. Vitamin C content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	54,449	2	27,2245	0,758	0,7545
Tehn. maturity	2036,54	21	96,9569		0,02874*

Kruskal - Walis test: H(ch^2): 2,555 H_c(tie corrected):2,555 p(≤0,01): 0,2787
There is a statistically significant difference between the samples

Similar experimental research carried out by Aćamović-Djoković et al., (2011) investigating the content of vitamin C concluded changes in the value of vitamin C depending on the variety of cultivation, which is in accordance with our research and the research of other researchers. The research of Lozano et al., (2021) makes interesting conclusions, stating that until now lettuce breeding was mainly aimed at obtaining more fertile crops and more resistant to biotic stresses, but little attention was paid to its nutritional quality. By comparing the vitamin C content between wild and commercial lettuce varieties, they came to the conclusion that the same up to eight times higher vitamin C content is found in wild lettuce varieties compared to commercial varieties.

Koudela & Petříková (2008), studying the content and yield of nutrients in selected varieties of lettuce leaves (*Lactuca sativa* L. var. *crispa*), conducted experimental research with five varieties of lettuce, and after two years of experimental research determined a significant influence of the variety in the case of K content, Na and Ca, as well as the amount of dry matter and the weight of the leaf rosette, fiber is an exception.

Research by Bajwa & Kwatra (2013) states that dietary potassium is usually limited to 2000 to 3000 mg per day-1 for patients suffering from chronic kidney disease. In lettuce, the potassium content can be partially reduced by boiling or soaking in water. Unfortunately, other nutrients, such as ascorbic acids and minerals, also lose their nutritional value during these processes. Thanks to the rapid increase in kidney disease patients Zhang et al., (2017); Zhang & Rothenbacher (2008) increased the demand for fresh lettuce with a low potassium content. In Japan, some companies are dedicated to the production of low-potassium salad. The accumulation of potassium in our research did not significantly depend on the time of harvest, and no statistically significant differences were found even in dependence on the hybrid, so the value of K ranged from 2,517 mg/100 g to 4,165 mg/kg.

The closing of the leaves and the formation of the head leads to a reduced concentration of Ca, which is often manifested by visually visible injuries of the tips of the leaves that look like a burn injury. Barta et al., (2000) studied the concentration of Ca in leaves depending on different nutrition systems, cultivars, as well as the rate of plant development, that is, the formation of the head. The first injuries on the tips of the leaves were noticed on the 22nd day of plant development from the time of emergence, when the leaves were about 3 cm long, however, in the phase of head formation, 83% of the leaves had visible injuries due to Ca deficiency. With the measured amounts of Ca from 261 mg/100 g to 449 mg/100 g, no differences were observed between the hybrids, but a decrease in Ca concentration depending on the time of harvest. Plants with deficiency of P form shorter leaves and deficiencies are visible after 2 weeks, and after four weeks, necrotic spots and the presence of anthocyanins are observed on the edges of the leaves. Since the land on which the experiment was built was rated as extremely well supplied with phosphorus in the amount of 72,43 mg/100 g of land, no deficiencies of P were observed in the plants. The recorded values were uniform based on both tested treatments and ranged from 31 mg/100 g to 48 mg/100 g.

Matson et al., (2015) conducted experimental research with one variety of Fladnria lettuce using the system of hydroponics and growing on rock wool, where they monitored the plant's development with different concentrations of nutrients. According to their research, approximately 10 days after Mg deficiency, the leaves show a slight chlorosis, and not long after, marginal necrosis becomes visible. Recorded Mg values ranged from 122 mg/100 g to 146 mg/100 g, and no significant statistical differences were found based on the tested treatments, as well as visible symptoms on the plant. Based on the recommended daily intake of lettuce, the most important bioactive components are: iron, folates, vitamin C, β-carotene, lutein, total phenol content, which play a prominent role in the prevention of many chronic diseases (Kim et al., 2016).

There were no statistically significant differences in Fe content depending on the tested treatment, and the recorded values ranged from Fe 1,3 mg/100 g to 3,2 mg/100 g.

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables in the world, but it is often considered to have a low nutritional value. However, lettuce contains health-promoting nutrients, and the biosynthesis of such phytochemicals varies depending on variety, leaf color, and growing conditions. Earlier research by numerous scientists showed that the content of nutritional components can change depending on growing conditions, but the fiber content in lettuce is mainly a varietal characteristic, which is confirmed by these studies (tab. 4).

Table 3. Nutrient content mg/100 g based on hybrids and technological maturity

Hybrid	**K**	**Ca**			**P**	**Mg**	**Fe**
		I	II	III			
TONALE	**2,600**	**483**	469	**443**	40	142	2,9
DONERTIE	2,971	423	420	415	39	122	2,7
NOLANIE	2,770	442	439	432	41	139	3,2
CENCIBEL	3,062	312	309	289	**36**	146	2,5
OSTRALIE	**4,165**	475	467	412	**36**	141	2,4
LIMERIA	3,727	390	389	367	38	137	1,9
HETTIE	3,771	440	435	410	46	130	1,3
SATURDAI	3,888	**298**	287	**261**	**48**	133	1,5

Table 4. Fiber content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	0,0384111	2	0,0192056	0,1607	0,0545*
Tehn. maturity	1,69463	15	0,112976		0,9537

Kruskal - Walis test: H(ch^2): 2,775 H_c (tie corrected): 2,783 p (≤0,01):0,2485
There is a statistically significant difference between the samples.

The results of the research showed that there are significant differences in the fat content depending on the hybrid and statistical differences of less significance when the question is about the fat content depending on the harvest time (tab. 5).

Table 5. Fat content % based on hybrids and technological maturity.

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	0,0056333	2	0,000281667	8,349	0,003352**
Tehn. maturity	0,00501667	15	0,00033444		0,077658*

Kruskal - Walis test: H(ch2):9,026 Hc(tie corrected):9,207 p(≤0,01) 0,01002
There is a statistically significant difference between the samples.

The highest fat overestimation was recorded in the hybrid Hettie after 90 days of development (0,18 %) compared to the hybrid Limeria with only 0.09 % fat (30 days) and 0,13 % (90 days) of development. Most of the research is related to the research of the fat content in lettuce seeds, so Afsharypuor et al., (2018) in research on the analysis of the composition of fatty acids in the crude oil of *Lactuca sativa* L. GC-MS and GC methods identified ingredients that represented 98,20% of of total eluates were methyl esters of linoleic (52,38%), oleic (34,42%), palmitic

(7,25%), stearic (2,66%), arachidic (1,32%) and myristic (0, 17%) acid. The total percentage of saturated methyl esters and unsaturated fatty acids identified in the examined oil was 11,4 and 86,80%, respectively. At the end of the research, they conclude that the seeds and oil from the seeds of *Lactuca sativa* L., as well as many other vegetable fats, are rich in unsaturated fatty acids and as such should be included in the diet of the population of our regions, which was not the case until now.

Among the primary metabolites found in vegetables, soluble sugars and organic acids are important components and both contribute to flavor and nutritional value. Soluble sugars found in lettuce are glucose, fructose and sucrose in different concentrations. Research by Lopez (2014) was conducted in order to assess the difference in the chemical composition and sugar concentration of three varieties of lettuce (Romaine, Little Gem and Mini Romaine). All tested varieties were grown in a greenhouse according to conventional principles, but significant differences in sugar concentration were found depending on the variety, which is also confirmed by our research.

Similar experimental research was conducted by Shwerif et al., (2018) where they monitored the influence of different temperature regimes on the sugar content of two types of lettuce grown in a controlled environment. The results of the research proved that the temperature regimes had a very significant influence on the sugar content of both tested varieties of lettuce (Dixter and Exbury). The highest sugar content was recorded at low temperature (12/8 °C), and it decreased with increasing temperature, while Dixter lettuce had a higher concentration of sugar than Exbury lettuce.

We obtained similar results in our research, where it was found that the amount of sugar was statistically significant depending on the variety, and the interaction between temperature and amount of sugar was lower, because the lettuce was grown in the winter period, when the optimal temperatures for the development of lettuce were determined. The results of the research are shown in table 6.

Table 6. Sugar content mg/100 g based on hybrid and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	12,261	2	6,13052	0,002401	0,09954*
Tehn. maturity	53627,8	15	2553,71		0,9963

Kruskal - Walis test: H(ch^2): 0,285 H_c (tie corrected): 0,285 p (≤0,01) 0,0867
There is a statistically significant difference between the samples.

No statistically significant difference was found in the concentration of sugar in the tested lettuce hybrids depending on the technological maturity at the level of significance (p≤0.01), while a smaller statistical difference was found depending on the cultivated hybrid, the highest sugar content was recorded in the hybrid Cencibel 5,26 mg/100 g, and the lowest in Limeria at a value of 4,14 mg/100 g.

Leafy vegetables occupy a very important place in the human diet, but unfortunately, they form a group of foods that contribute to the intake of nitrates through food in the human body.

Under the excessive application of nitrogen fertilizers or in the case of a lack of light, these vegetables can accumulate a high level of nitrates and, when used in the diet, represent a serious health hazard. Shaid & Umar (2007) in their research on the impact of leafy vegetables on human health state that vegetables are the main source of nitrate intake in the human diet and nitrates and that people unknowingly consume more harmful and less useful substances for human health. Burns et al., (2004) investigating the accumulation of nitrates in lettuce under the influence of various factors, they state that nitrates tend to accumulate in lettuce shoots when the supply of nitrates exceeds the required amount of N crops, especially during the summer. It also accumulates when there is a reduction in solar radiation or when parts of the head of lettuce become susceptible to shading due to increased density and reduced vegetation space of the plant.

In winter, more nitrate accumulates because: the growth rate tends to decrease faster than the nitrate uptake rate; there is less light energy for the reduction of nitrates into organic forms of N; winter crops seem to use N more efficiently for dry matter production; and yields are often lower, so relatively more nitrates are available in the crops. Research also confirms that nitrate concentrations in heads of lettuce are more variable, depending on the variety and stage of development, which is in line with our research. As we assumed, we found a statistically significant difference, depending on the hybrid ($P<0,01$) and the time of harvest ($P<0,01$), in the average nitrate concentration (tab. 7).

Table 7. Nitrate content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	$p \leq 0,01$
Hybrid	3,08355	2	1,547706	5,22	0,0001093**
Tehn. maturity	6,20015	15	295245		0,0001482**

Kruskal - Walis test: H(ch^2): 7,505 H_c (tie corrected):7,505 p (≤0,01): 0,02346
There is a statistically significant difference between the samples.

The number of hours of sunshine in the months of research(October, November and December, as well as January) was higher in all analyzed stations compared to the thirty-year average (1961-1990), however, the most pronounced monthly negative deviation of sunshine was recorded at the end of February and March at the weather station Bihac 38 hours of sunshine were recorded in Bihac, which is about lower than 51% of the average value. As expected, the highest nitrate values (NO_2 mg/kg) were recorded in the month of March, ranging from 883,483 mg/kg in the Tonale hybrid to an enormously high 2540,138 mg/kg in the Hettie hybrid. Koudela & Petříková (2008) investigated 5 hybrids of lettuce: Lollo

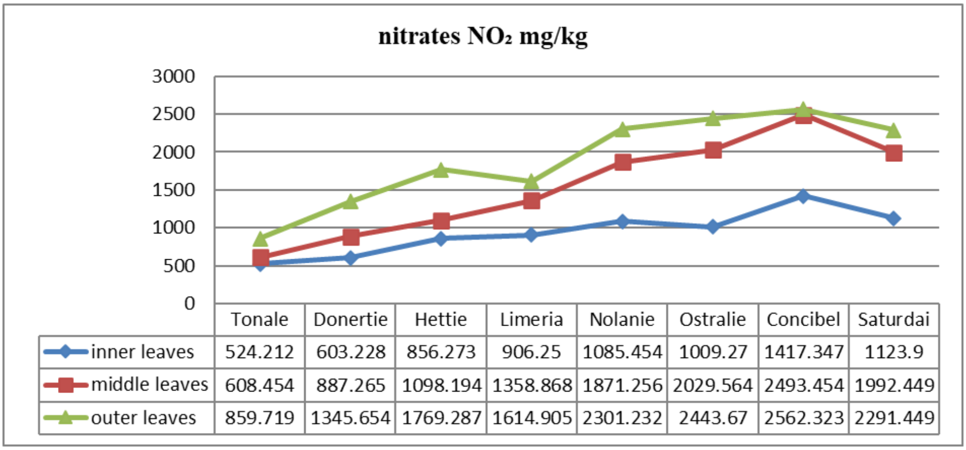
Rossa, Redin, Bergamo, Dubacek and Frisby, through two years of research and growing lettuce in three seasons, as expected, they recorded the highest nitrate content in autumn/winter cultivation in the hybrid Lollo Rossa with a value of 3817 mg/kg in autumn, 1993 mg/kg in spring/summer cultivation and 1193 mg/kg in summer/autumn cultivation, thus confirming the ditrect amount of nitrates in lettuce depending on the amount of light, but also on the variety. Tosun & Utson (2004) studied the nitrate content of lettuce grown in a greenhouse, they examined 20 varieties and concluded that in addition to the nitrate content depending on the lettuce variety, the amount of available light also plays a major role, so the nitrate content ranged from 425 mg/kg to 4040 mg/kg. Jernej & Osvald (2004). They conducted research with four varieties of lettuce, radish and spinach in greenhouse conditions and fertilized with different concentrations of N fertilizer. The experiment was carried out in the winter cultivation of lettuce, lower temperatures and less light. The highest concentration of nitrates was recorded in radish roots, then in lettuce leaves and finally in spinach (higher amount in the stem, lower in the stem).

As expected, the amount of nitrates in lettuce differed significantly from the variety itself, so the highest amount of nitrates was found in the Grand Rapids variety, and significantly lower in the Domineer and Kolrekt varieties.

Azmi Abu-Rayyan et al., (2004) conducted field research in two locations (Jordan Valley and Al-Jubeiha), which differ based on altitude, rainfall, and temperature range. The experiment was set up to evaluate the optimal planting density, form of nitrogen and level of irrigation in order to achieve the best quality of the lettuce crop in terms of minimum nitrate content and minimize the impact on the environment. Three forms of N fertilizer; ($Ca(NO_3)_2$, $(NH_4)_2SO_4$ and $CO(NH_2)_2$) were applied three times in the amount of 100 kg N ha/ha. Three row spacings (15, 20 and 25 cm) and two levels of irrigation were applied. Level one the usual amount of water m^2 and level two twice the amount of water than usual. The results showed that there was an increase in the amount of nitrate depending on the applied fertilization, but also that the outer lettuce leaves had a 5 times higher concentration of nitrate than the inner leaves, which proves to us that the plant loses the amount of vitamin C as it ages, and the nitrate content increases. which is consistent with our research.

The obtained results of this research proved that all varieties after 90 days of development from the time of sprouting have up to three times the amount of nitrates in the leaves, as well as the fact that the outer leaves have a higher content of nitrates compared to the inner younger ones, (graph 2).

The concentration of nitrates in lettuce leaves was statistically significantly affected by the hybrid, as well as the time of harvest, so statistically significant differences were found, as the average lowest amount of nitrates recorded in the Tonale hybrid was 613,954 mg/kg (30 days), 680,005 mg/kg (60 days) and 883,468 mg/kg (90 days), while the highest amount of nitrates was recorded in hybrid Saturdai 1834, 389 mg/kg (30 days), 2014, 389 mg/kg (60 days) and 2285, 562 mg/kg (90 days).



Graph 2. Nitrate content mg/kg depending on the position of the leaf in the head

At the time of conducting the research, the highest NDK of nitrates were prescribed by the Ordinance on the maximum allowed amounts of contaminants in food (OG 154/2008. OG 78/2019), and for lettuce (*Lactuca sativa* L.) (grown in a greenhouse) and harvested up to 1. March, the permitted amount of nitrates was up to 4000 mg/kg, while in the open field that amount was 2500 mg/kg.
Nitrates and nitrites are on the list of human carcinogens. In 2012, the Scientific Committee on Food (SCF) determined the acceptable daily intake for nitrates (ADI) which is from 0 to 3,7 mg/kg of body weight/day, which is equivalent to the intake of 222 mg of nitrate for an adult a 60 kg person. The ADI for nitrites is 0,07 mg/kg/day. If an adult consumes only 400 g of different vegetables per day, the average nitrate intake is 157 mg/kg.

If we add fruits that contain significantly less, the intake of nitrates ranges from 81 to 106 mg/day for the majority of Europeans, which is within the acceptable daily intake. Exceptions are vegetarians and people who eat significantly more green leafy vegetables and may exceed these values. Recorded nitrite values are presented in table 9 and graph 3.

Table 9. Nitrite content % based on hybrids and technological maturity

Hybrid	0,823812	2	0,411906	5,837	0,000946**
Tehn. maturity	1,48198	21	0,0705707		0,00231**

Kruskal - Walis test: H(ch^2): 8,725 H_c (tie corrected): 8,728 p (same):0,01273
There is a statistically significant difference between the samples.

Based on the data from table 15, a statistically significant difference was found in the nitrite content in lettuce, depending on the hybrid, but also the time of harvest. Namely, it turned out that the hybrid factor had a greater influence on the nitrite content, so the highest average amount measured in the Limeria hybrid was 1,105 mg/kg, and the lowest recorded value in the Donertie hybrid was 0,657 mg/kg.

The amount of nitrites in vegetables is also affected by the storage method and the time that passes from the moment of harvesting to consumption, so research by Silalahi et al., (2016) on the topic of the influence of temperature and storage time on the amount of nitrates and nitrites in lettuce leaves proves the stated claim. Namely, the experimental research was conducted on freshly picked lettuce that was stored at two temperature values of 1 °C and 22 °C for 48 hours, and the nitrate and nitrite content was analyzed every 4 hours. The results of this research show that during storage for 48 hours at room temperature, the nitrate level increased from the initial value of 6,07 mg/kg to 70,83 mg/kg, and the nitrite level increased from 22,63 mg/kg to 48,14 mg/kg. The nitrate and nitrite levels in salads stored in the refrigerator increased from the initial level of 3,06 mg/kg to 64,42 mg/kg, and the nitrite level increased from 21,89 mg/kg to 40,08 mg/kg. Research recommends storing salad for no longer than 3 days in the refrigerator, because any longer storage leads to a deterioration in the quality of the food and a negative effect on human health when consuming it.

CONCLUSIONS

In the research, the most favorable harvest date was determined, as well as the type of hybrid for growing larger and better quality plants with the ultimate goal of a positive impact on human health. On the basis of the conducted study, it is evident that the greater amount of natural light resulted in positive effects on the growth and development of the plant in all measured parameters, but also the lack thereof in the increased amount of harmful substances in the leaves. The positive and negative influence of the harvest time was observed for most of the tested parameters when the question is the quantity and quality of the product. So we conclude that the hybrids Nolanie and Hetti are the hybrids with the most favorable characteristics for growing in the winter period, because the ratio of quantitative and qualitative characteristics is approximately the same. But it is recommended harvest after 60 days, because with a later harvest there is a sudden increase in nitrates and nitrites.

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BIOFORTIFICATION OF PEA MICROGREENS THROUGH ZINC-SOLUBILIZING BACTERIA INOCULATION WITH FOLIAR IODINE APPLICATION

SUMMARY

Biofortification of plant food products including microgreens is one of the most promising approaches to combat with the problem of balanced nutrition for the human population. This study introduces the microbiological biofortification using plant growth-promoting (PGP) bacteria, specifically three strains of zinc-solubilizing *Pseudomonas* sp. (STF10, STF14, STF16) isolated from the rhizosphere of *Tussilago farfara* L. The research explores their impact on growth parameters and photosynthetic pigments in pea (*Pisum sativum* L.) microgreens following iodine spraying, providing groundbreaking insights. The experimental results indicate that inoculation of pea seeds with these rhizobacteria significantly increased the fresh and dry biomass of 14-day-old microgreens (by 22 and 15%, respectively, on average) compared to non-inoculated plants. At the same time the content of chlorophylls and carotenoids in seedlings increased on average by 18 and 36%, respectively. Spraying with one form of iodine (0.01% KI or KIO_3) slightly increased fresh and dry biomass (by 8% on average) and did not alter the content of photosynthetic pigments. Wherein iodine content in pea microgreens increased by 4.4 and 3.8 times, after KI and KIO_3 spraying, respectively. By conducting experiments in a hydroponic nutrient solution, this study provides a robust platform for evaluating the biofortification potential of the three *Pseudomonas* strains along with foliar iodine feeding, marking a significant stride towards enhancing the nutritional profile of pea microgreens.

Keywords: *Pisum sativum* L., *Pseudomonas* sp., biofortification, micronutrient deficiency, plant morphophysiological traits, iodine accumulation

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INTRODUCTION

The problem of micronutrient deficiency in the dietary habits of human populations holds significant importance for both developing and developed countries (Medrano-Macías *et al.,* 2016; Gharibzahedi and Jafari, 2017; Van Der Straeten *et al.,* 2020; Golubkina *et al.,* 2021). One of the most promising approaches to reduce global malnutrition is biofortification, or the enrichment of plant raw materials and crop products, including microgreens (White and Broadley, 2009; Eliseeva *et al.,* 2022; Sarwar *et al.,* 2022; Ahmad *et al.,* 2023). Currently, zinc (Zn), iron (Fe), and iodine (I) stand out as the three most crucial micronutrients from a global public health perspective, with their deficiency posing a serious threat to public health worldwide (Medrano-Macias *et al.,* 2016; Gharibzahedi and Jafari, 2017; Van Der Straeten *et al.,* 2020; Şahin, 2020; Singh and Prasanna, 2020; Ahmad *et al.,* 2023).

Biofortification, which was carried out by feeding crops with various micronutrients, became prevalent (Voogt *et al.,* 2010; Smoleń *et al.,* 2016; Jerse *et al.,* 2017). The experiments on the enrichment of various crops with micronutrients by adding them to the soil or folia spraying were carried out by many authors. Some of scientists studied the effect of biofortification using one element (Voogt *et al.,* 2010; Weng *et al.,* 2013), whereas others used two elements (Smoleń *et al.,* 2016; Jerše *et al.,* 2017; Golubkina *et al.,* 2021). Data on the use of the three elements is fragmentary. For example, Şahin (2020) determined the effect of combined I-Fe-Zn treatments of tomato and reported that biofortification is an important way to eliminate the deficiency of these three elements in plants.

In recent years, microbial biofortification has garnered considerable interest, focusing on using various microorganisms to enrich crops with essential elements (Sunithakumari *et al.,* 2016; Ahmad *et al.,* 2023). Microbe-assisted biofortification attracted much attention recently due to its sustainable and eco-friendly nature for improving nutrient content (Sun *et al.,* 2021). Plant growth-promoting rhizobacteria (PGPR) emerge as a promising technique particularly biofortification, possessing capabilities such as nitrogen fixation, solubilization of phosphorus, potassium, iron, zinc, etc. that are inaccessible to plants, and production of siderophores and phytohormones (Saravanan *et al*., 2003; Kumar *et al*., 2021; Aloo *et al.,* 2022; Saleem *et al*., 2022; Yadav *et al.,* 2022).

Recent studies have reported the use of microorganisms for micronutrient biofortification of crops, but most authors have studied fortification of only one element. Hussain *et al.* (2015) studied prospects of zinc-solubilizing bacteria for enhancing growth of maize. Yadav *et al.* (2022) fortified wheat with zinc using Zn-solubilizing bacteria. Sarwar *et al.* (2021) reported the feasibility of iron biofortification using siderophore producing rhizobacteria strains to improve growth, yield and quality of groundnut. Daliran *et al.* (2022) found *Thiobacillus* sp. bacteria that could enhance iron biofortification of soybeans in limestone soil with elevated levels of ferrous sulfate. In recent years, biofortification with two or more microelements using PGP-bacteria has gained particular interest, however, positive results of "combined" biofortification with all studied micronutrients were reported only in a few studies. For instance, Ahmad *et al.* (2023) isolated the strains of Zn-solubilizing and siderophore producing bacteria from the genera *Bacillus* and *Paenibacillus* and studied their biofortification potential for maize.

The challenge of enriching plant materials and food products, including microgreens, with essential micronutrients is relevant globally. However, despite its potential, microbial biofortification has not seen widespread application, and the exploration of combined biofortification possibilities remains limited, motivating the objectives of our research.

Thus, the study aims to assess the zinc-solubilizing potential and PGP attributes of three rhizobacteria strains (genus *Pseudomonas*), as well as their effect on growth parameters and the content of photosynthetic pigments in pea microgreens after spraying with iodine.

MATERIAL AND METHODS

To isolate the rhizosphere bacteria, one of the most dominant plant species *Tussilago farfara* L. (Asteraceae family) growing on the disturbed territory close to Safyanovsky copper mine (Rezh, Sverdlovsk region, Russia) was selected. The rhizosphere soil adhered to root strands of a random selection of three flowering plants (at the end of May 2023) was collected by shaking them gently in a zip-lock bags and transferred to the laboratory. About 10 g of rhizosphere soil was mixed with 90 mL of phosphate buffer (pH 6.5) and shaken at 180 rpm for 2 h at 28 °C. A series of dilutions of each sample were made and 100 μL was added to a Petri plate with Luria-Bertani (LB) agar supplemented with 100 mg/L Zn (sulfate form). A total of 16 cultures were isolated from soil and subjected on MSM agar (Bhakat *et al.*, 2021) supplemented with 0.1% of $ZnCO_3$, $Zn_3(PO_4)_2$ and ZnO for 2 days at 28 °C. The halo zone around the bacterial colony confirms the Zn-solubilizing property. The Zn solubility ratio was calculated as diameter of solubilized halo zone (i.e. halo + colony) to the diameter of the colony (Eshaghi *et al.*, 2019).

Siderophore production was assessed as described earlier (Kumar *et al.*, 2021) after interaction of rhizobacteria culture (10^8 cfu/mL) with Fe-CAS (chromazurol S) indicator solution and was identified by orange to yellow halo zones around bacterial colonies. Siderophore production ratio was calculated as diameter of solubilized halo zone to the diameter of the colony.

The iodine test was performed to understand the strain resistance level. The iodine rich LB agar plates were prepared, and all isolates were subjected to an increasing concentration of two form of iodine (KI or KIO_3): 0%, 0.001%, 0.01%, 0.1%. Following incubation at 27 °C for 3 days, colonies displaying unrestricted growth on both KI-LB and KIO_3-LB agar plates were identified as resistant to the corresponding concentration of iodine.

Following the screening of bacterial isolates for their zinc solubilization, siderophore production, and iodine resistance, three strains were singled out for in-depth analysis through morphological, physiological, and molecular genetic identification. The selected strains underwent Gram staining to determine their Gram-positive or Gram-negative nature, and their morphological properties such as shape, size, texture, etc. were assessed. For genus identification, molecular genetic analysis was conducted using 16S rRNA (rDNA) genome sequencing (Voropaeva *et al.*, 2022). Genomic DNA from liquid cultures of rhizobacteria was isolated utilizing spin columns, following the guidelines provided by the manufacturer (DiaGene kit 3318.0250, Dia-M LLC, Russia). The total DNA (10 ng) was used as a template for the amplification of 16S rRNA genes using 16S Barcoding Kit (SQK-16S 024, Oxford Nanopore Technologies, UK) and LongAmp Hot Start Taq 2 × Master Mix (New England Biolabs, USA). The PCR product was purified using AMPure XP (Beckman Coulter, USA) and used to prepare a sequencing library (SQK-16S024, Oxford Nanopore Technologies, UK). Sequencing was performed on a GridION™ sequencer in an R9.4 flow cell (Oxford Nanopore Technologies, UK). The computer programs of Oxford Nanopore Technologies were used for data analysis. Primary data processing was carried out in MINKNOW software ver. 21.05.8. The raw FAST5 files were base called using Guppy version 5.0.11 to generate FASTQ files. The EPI2ME Fastq 16S ver. 3.3.0 were used for systematic classification of 16S rRNA gene.

The selected isolates were tested for phosphate solubilization, indol-3-acetic acid (IAA), and ammonia production as described previously (Kumar *et al.*, 2021). Phosphate solubilization was confirmed by appearance of yellow color after reacting of freshly grown (10^8 cfu/mL) bacteria with vanado-molybdic reagent and measured at 420 nm using UV-Vis spectrophotometer (Teccan, Thermo Scientific, USA). The ability to solubilize phosphates was assessed by preparing a calibration curve based on the standard solution of potassium dihydrogen orthophosphate and expressed in mg PO_4^{3-}/L. The IAA production was checked by appearance of pink color after adding Salkowski's reagent to freshly prepared bacteria cultures (10^8 cfu/mL) and measured at 530 nm. Commercially available IAA (Sigma-Aldrich) was used to prepare a calibration curve. The ammonia production was determined by the color change from yellow to reddish-brown after incubation of the freshly prepared bacterial inoculum (10^8 cfu/mL) with Nessler's reagent.

Antibiotic resistance of the selected strains was checked by disc diffusion method (Rajkumar *et al.*, 2013). The concentrations of the antibiotic discs used were 10 μg erythromycin, 30 μg kanamycin, 30 μg streptomycin, 30 μg tetracycline, 10 μg ampicillin, 30 μg chloramphenicol, and 6 μg penicillin. The zone of inhibition around the disc was measured and categorized under as resistant, intermediate, and susceptible on the basis of company guidelines (NICF, Russia).

The influence of selective strains on the germination of pea seeds (*Pisum sativum* L., Madras var.) was assessed in Petri plate experiment. The mature seeds similar in size and shape were surface sterilized (by 70% ethanol for 30 s, then 2 min with 4% sodium hypochlorite), and finally multiple washing using sterile Millipore water (Millipore, USA). The seeds were soaked overnight and were inoculated for 2 hours with the selective bacterial cultures (10^8 cfu/mL) pre-grown on LB medium, while in the control (CS) it was only sterile LB medium. A total of 36 seeds were placed to each Petri plate (10 plates in each treatment), equipped with moistened sterile filter paper at the bottom, and seed germination was checked daily until full sprouting. After that, the seedlings were transferred to the plastic sprouters (“The home AeroGARDEN”, SmartGidroCompany, LLC, Russia) with hydroponic nutrient solution (calcium nitrate: 0.868 g/L; potassium nitrate: 0.426 g/L; magnesium sulfate: 0.378 g/L; monopotassium phosphate: 0.284 g/L; ferrous sulfate: 0.02 g/L; ammonium sulfate: 0.01 g/L; borax: 0.01 g/L; manganese sulfate: 5 mg/L, zinc sulfate: 0.5 mg/L; copper sulfate: 0.5 mg/L) under the following controlled environmental conditions: photosynthetic photon flux density of 180 ± 20 μmol/m^2 s provided by phytolamps (ULI-P10-18W/SPFR IP40); day/night regime of 14/10 at 23 ± 3 °C. Upon the emergence of the first leaves, the seedlings were sprayed with 0.01% KI (SI1) or KIO_3 (SI2) or just sterile water without iodine (NS). Every treatment included 3 independent boxes with 40 pea seedlings in each.

Germination characteristics were calculated according to Kumar *et al.* (2012). Germination percentage (%) was calculated as total number of seeds germinated at the end of counting days/total number of seeds × 100%; seedling vigor index was calculated as germination percentage (%) × mean seedling dry biomass (g). Growth parameters such as length, fresh biomass (FW) and dry biomass (DW), and photosynthetic pigment content were measured in 14-day-old pea seedlings. Photosynthetic pigments such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) were extracted in 80% cold acetone, measured at 470, 647, and 663 nm and finally calculated per one gram of DW according to Lichtenthaler (1987). The iodine content in dry pea seedling biomass (shoot) was determined according to GOST 28458-90 (2006) and expressed in mg/kg DW.

The results were presented as mean values (Means) with standard errors (SE). The normality and homogeneity of variances were verified using the Shapiro-Wilk’s and the Levene’s test, respectively and significant difference between treatments were determined by analysis of variance (ANOVA) followed by Tukey’s test. Different alphabetical letters indicate significant difference between treatments at $p < 0.05$.

RESULTS AND DISCUSSION

Three bacterial cultures, namely STF10, STF14, and STF16, isolated from the rhizosphere of *T. farfara*, were assessed for their capacity to solubilize various insoluble forms of zinc (Table 1). The best Zn solubilization was noted for the strain STF14, while STF10 and STF16 better solubilized zinc oxide or zinc carbonate. Furthermore, an examination of siderophore production revealed that, after a 3-day incubation period, all isolates demonstrated approximately the same level of siderophore production (Table 1).

The iodine test with KI and KIO_3 revealed no adverse effects on the growth of the studied strains, qualifying them for potential use in plant biofortification (Table 1). The Gram staining test showed that all three isolates were negative. Morphologically, STF14 and STF16 exhibited similarities in colony characteristics, while STF10 differed in traits such as shape, size, and pigmentation (Table 2). In addition, all strains showed fast growing properties which are very useful for conducting the experiment.

Table 1. Zinc solubilization, siderophore production and iodine resistance of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Zn solubilization ratio*			Siderophore production ratio*	Iodine resistance	
	ZnO	$ZnCO_3$	$Zn_3(PO_4)_2$		KI	KIO_3
STF10	3.0	2.8	2.0	1.4	High	High
STF14	3.8	3.0	2.8	1.4	High	High
STF16	1.2	2.5	2.2	1.5	High	High

*Ratio of halo zone + colony to colony diameter

Table 2. Morphological properties of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Morphological characteristics of colonies (on solid LB medium)					
	Shape	Margin	Elevation	Size	Texture	Pigmentation
STF10	Irregular	Entire	Raised	Large	Smooth	Pale brown
STF14	Circular	Entire	Raised	Moderate	Smooth	Brown
STF16	Circular	Entire	Raised	Moderate	Smooth	Brown

The isolates were identified with 16S rRNA sequencing as *Pseudomonas* sp. with 93.6–94.7% similarity (Table 3). It was previously noted that most zinc solubilizing bacteria belongs to genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Xanthomonas*, and *Stenotrophomonas* (Saravanan *et al.*, 2003; Hussain *et al.*, 2015; Sunithakumari *et al.*, 2016).

Further, the studied strains were tested for additional PGP attributes (Table 3). All strains were able to solubilize insoluble phosphates and produce IAA. The maximum values of these PGP traits were found in strain STF14. The ammonia production was also noted for all studied *Pseudomonas* sp. strains (Table 3). Moreover, these strains demonstrated high or moderate resistance to erythromycin, tetracycline, ampicillin, chloramphenicol as well as penicillin. However, they turned out to be susceptible (highly or moderate) to kanamycin and streptomycin.

Table 3. Bacterial identification and plant growth promoting attributes of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Closest relative sequence	[1]Percentage of similarity/ Number of reads	[2]Phosphate solubilization, mg PO_4^{3-}/L	[2]IAA production, mg/L	NH_3 production
STF10	*Pseudomonas* sp.	94.7/35098	10.4 ± 0.5b	3.9 ± 0.8c	+
STF14	*Pseudomonas* sp.	94.4/83713	34.8 ± 0.9a	31.8 ± 0.8a	+
STF16	*Pseudomonas* sp.	93.6/26200	5.5 ± 0.5c	12.4 ± 0.6b	+

[1]Similarity with the strains of NCBI database. [2]Data are presented as Means ± SE (n = 3). IAA – indole-3-acetic acid

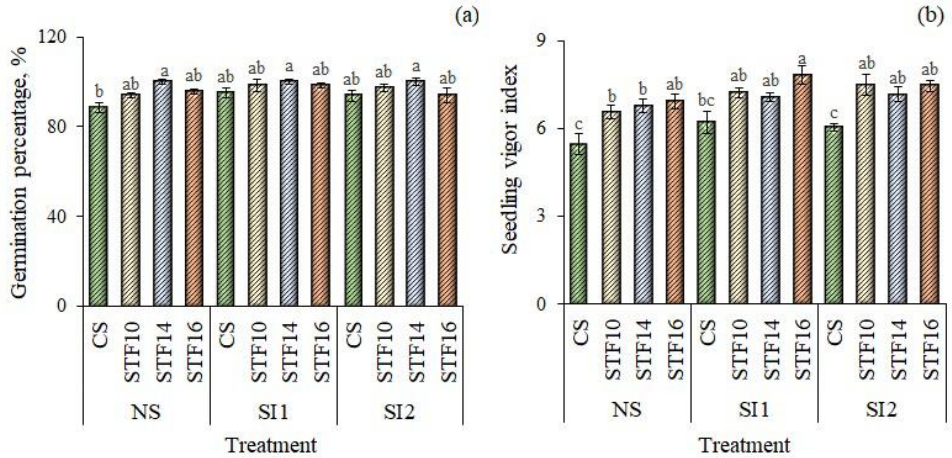

Fig. 1. The germination percentage (a) and vigor index (b) of *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 10). CS – control seedlings; NS – no spraying

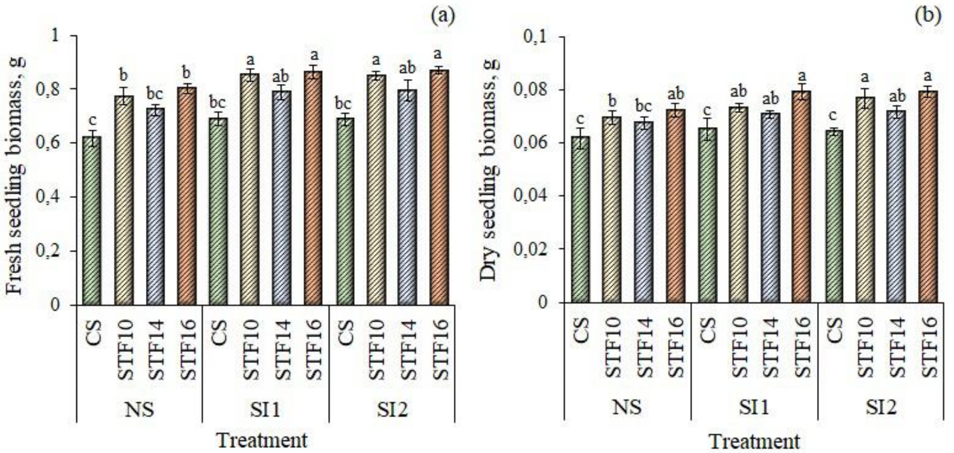

Fig. 2. The fresh (a) and dry (b) biomass of 14-day-old *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 20). CS – control seedlings; NS – no spraying

The percentage of seed germination is one of the most important characteristics of the crops. Only those seeds that germinate rapidly and vigorously under favorable conditions in the laboratory are likely to produce vigorous seedlings in the field (Kumar *et al.*, 2012). The percentage of seed germination in all treatments varied from 88.3% (in control without iodine spraying) to 100% (in pea seeds inoculated STF14) (Fig. 1a). Moreover, in all inoculated plants the seedlings vigor index significantly increased (by 20% on average) in comparison with non-inoculated control (Fig. 1b). Seed vigor is the sum total of those properties of the seed that determine the level of activity and performance of the seeds during germination and seedling emergence (Kumar *et al*., 2012).

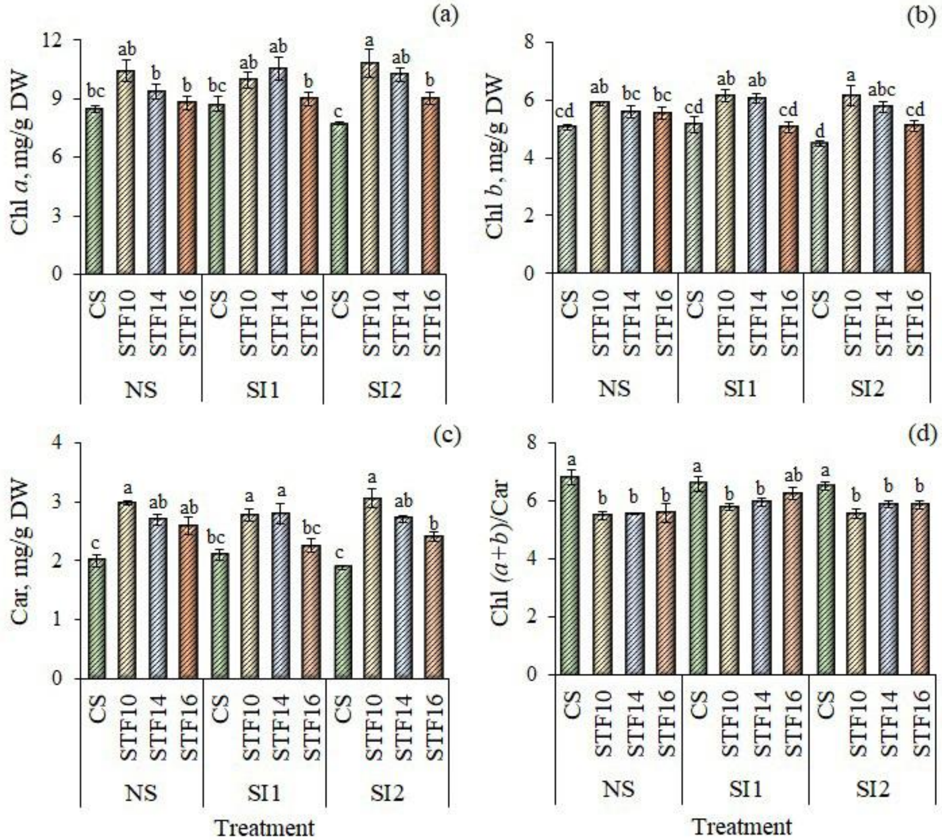

Fig. 3. The content of chlorophyll *a* (a), chlorophyll *b* (b), carotenoids (c) and ratio of total chlorophyll to carotenoids in leaves of 14-day-old *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 4). CS – control seedlings; NS – no spraying

No significant differences among all treatments were found in the length of 14-day-old seedlings (averaged 10.7 cm), while fresh and dry biomass of inoculated seedlings increased on average by 22 and 15%, respectively, compared to control (Fig. 2 a,b). The revealed effect is consistent with the data of other authors (Hussain *et al.,* 2015; Ahmad *et al*., 2023), reported that the zinc solubilizing bacteria significantly improved the growth of maize seedlings.

Spraying *P. sativum* seedlings with either potassium iodide (SI1) or potassium iodate (SI2) did not significantly affect the biomass of non-inoculated plants (Fig. 2 a,b). However, spraying with iodine slightly increased the biomass of inoculated seedlings (by 8% on average).

Inoculation of pea with bacteria increased the content of Chl *a* and Chl *b* in seedlings by 18% on average (Fig. 3 a,b), and Car by 36% (Fig. 3 c). Moreover, in terms of their effect on photosynthetic pigments, the studied PGPR strains followed the descending sequence: STF10 > STF14 >STF16. The strain STF10 had the best positive effect on the content of chlorophylls (25% on average) and carotenoids (48% on average). The positive effect of zinc solubilizing bacteria from the other genus (*Bacillus*) on the chlorophyll content in maize seedlings was also noted in other study (Hussain *et al.*, 2015).

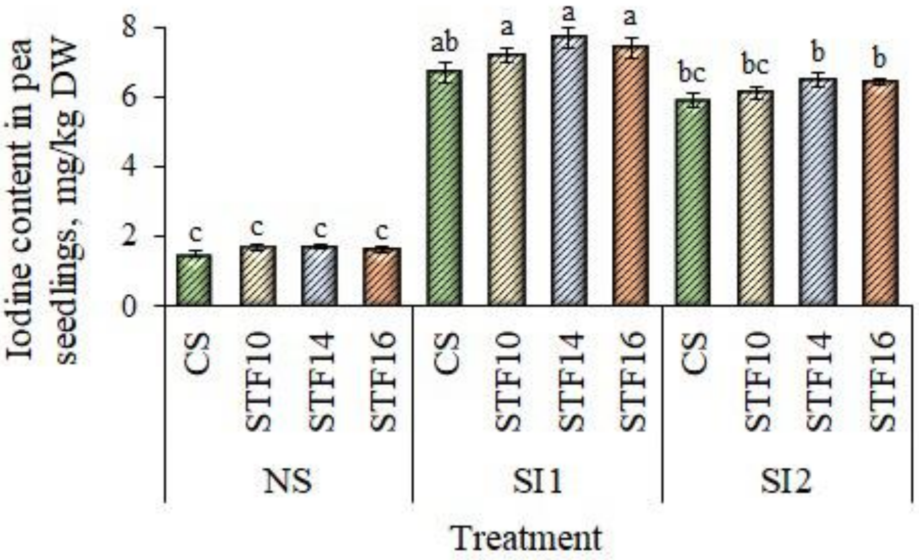

Fig. 4. Iodine accumulation in the 14-day-old *P. sativum* seedlings (shoot) after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 3). CS – control seedlings; NS – no spraying

At the same time, the ratio of Chl *a* to Chl *b* in all treatments remained at a constant level (about 3 on average), and the ratio of Chl (*a*+*b*) to Car in inoculated plants decreased significantly due to a considerable increase in carotenoids (Fig. 3 d). It is known that Car are auxiliary photosynthetic pigments and important photoprotectors that defend chlorophyll molecules from oxidation. In addition, they play an important role in reducing the negative effect of reactive oxygen species not only in plants, but also in humans. Thus, Car are bioactive substances in human diet with powerful antioxidant activity (Dymova *et al.*, 2014).

Spraying the leaves of *P. sativum* seedlings with iodine did not affect the pigment content (Fig. 3 a,b,c). This observation aligns with earlier findings by Jerše *et al.* (2017), who also reported no positive effects of iodine on the biomass and photosynthetic pigment content of pea seedlings. Notably, our experiments revealed that the pivotal factor influencing these parameters was the inoculation of pea seeds with Zn-solubilizing rhizobacteria, rather than spraying with iodine, which was confirmed by a two-way ANOVA (F_{FW} = 30.1; F_{DW} = 12.3; $p < 0.001$ and F_{Chl} = 5.9; F_{Car} = 12.7; $p < 0.001$).

The iodine content in pea biomass treated with potassium iodide and iodate increased by 4.4 and 3.8 times respectively, compared to the NS-treatment (Fig. 4). At the same time, the iodine accumulation in microgreens didn't depend on type of rhizobacteria strain, however, differ in dependence on the form of iodine: SI1 enhanced iodine content by 16% higher compared to SI2. Previously Voogt *et al.* (2010) also reported that the iodine content in lettuce leaves in a hydroponic growing system using potassium iodide was significantly higher compared to potassium iodate.

CONCLUSIONS

The zinc-solubilization potential and other PGP attributes of three rhizobacteria strains *Pseudomonas* sp. (STF10, STF14, STF16) had been reported for the first time in this study. The experimental results indicate that inoculation of pea seeds with these PGP-rhizobacteria significantly enhanced the fresh and dry biomass of *Pisum sativum* microgreens as well as the content of chlorophylls and especially carotenoids compared to non-inoculated control seedlings. Among the strains, inoculation with the STF10 strain resulted in the most substantial increase in the content of photosynthetic pigments in pea seedlings. The folia spraying of inoculated *P. sativum* seedlings with iodine slightly increased fresh and dry biomass and did not change the photosynthetic pigment content. The seedlings spraying with KI or KIO_3 increased its content in pea microgreens by 4.4 and 3.8 times, respectively, as compared to the non-spraying treatment. Thus, experiments in hydroponic nutrient solution made it possible to evaluate the biofortification potential of three isolated *Pseudomonas* strains along with foliar iodine feeding. However, it is essential to conduct further investigations, particularly in pot-scale experiments, to validate the positive effects observed with the studied strains and iodine spraying.

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FORMATION OF PRODUCTIVITY AND GRAIN QUALITY OF PEAS DEPENDING ON PLANT GROWTH REGULATOR

SUMMARY

The formation of high and stable yields is the main value of sowing peas (Pisum sativum L.) as a leguminous crop. The aim of the research was to study the influence of plant growth regulators on the processes of growth and development, formation of productivity, and grain quality of peas. The study of the influence of plant growth regulators on the productivity and quality indicators of peas was conducted according to the following scheme: control (without treatment with preparations), at the budding stage treatment with Humifield VR-18 w.s. (0.4 L ha^{-1}), at the pod formation stage treatment with Fulvigrin Bor (0.5 L ha^{-1}), at the budding stage treatment with Humifield VR-18 w.s. (0.4 L ha^{-1}) + at the pod formation stage treatment with Fulvigrin Bor (0.5 L ha^{-1}). The maximum yield was obtained in the variant with treatment of pea plants at the budding stage with Humifield VR-18 + at the pod formation stage with Fulvigrin Bor, amounting to 3.81 t.ha^{-1}. According to the research results, the highest protein content was obtained from treatment at the budding stage with Humifield VR-18 + at the pod formation stage with Fulvigrin Bor – 23.2%. Based on the research results, it was established that to obtain a pea yield of 3.81 t.ha^{-1} with a protein content of 23.2%, it is proposed to use plant growth regulators at the budding stage with Humifield VR-18 + at the pod formation stage with Fulvigrin Bor.

Keywords: plant growth regulator, plant density, productivity, yield, protein.

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INTRODUCTION

The growth and development of agricultural crops are among the most significant agrobiological features that reflect the interaction of the plant's genetic potential with a complex of technological practices and agroclimatic resources of the cultivation zone (Pylypenko *et al.,* 2016; Radchenko *et al.,* 2021). Obtaining high yields of agricultural crops in general, and peas in particular, significantly depends on the timely passage of their development phases, which are determined by both the genetic characteristics of the crop and the weather and climatic conditions of the years. Recent research indicates that the yield of peas depends on many elements of cultivation technology (Rudnichenko, 2019).

Scientific observations have identified that the main problem encountered by farmers in pea cultivation is the failure to adhere to scientifically based cultivation technology, the use of outdated cultivation techniques, and low-productivity, disease- and pest-prone, lodging-prone varieties resulting in grain shedding (Musatov *et al.,* 2010; Zając *et al.,* 2013; Kahlon *et al.,* 2018).

The formation of high and stable yields is the main value of sowing peas as a leguminous crop. Compared to other leguminous crops, peas have good grain quality indicators and a short vegetative period. They are one of the best precursors for winter grain crops. Cultivating legumes is more complex than cultivating grain crops due to the lodging of plants under conditions of excess moisture, their lodging, pod cracking, and seed shedding during ripening (Bushulian and Koblai, 2019). The basis for forming the most important economically valuable quantitative traits such as productivity, yield, and quality is the morphotype of plants. The results of assessing the potential of agricultural plants, including sowing peas, depend on the biometric indicators of the studied varieties (Avramenko *et al.,* 2011; Lemishko *et al.,* 2023).

The indicator of seed productivity of sowing peas is one of the main elements of the crop structure, determined by the interaction of many factors under the influence of soil fertility, resistance to diseases and pests, drought, low temperatures, lodging, etc. To increase the production of high-protein and quality food products with a balanced protein content, essential amino acids, and digestibility, among leguminous crops, each of the listed traits is quite important (Nebaba, 2020; Karpenko *et al.,* 2021).

The implementation of modern technologies aimed at maximizing the utilization of plant biological potential is one of the directions for increasing the quality and yield of cultivated crops (Karpenko *et al.,* 2020; Tanchyk *et al.,* 2021). In the conditions of the need for the development of intensive crop production technologies and the necessity of maintaining the normal state of soils, their microbiological background, and the ecological balance of agricultural production, plant growth regulators become an important element in improving yield (Vasylenko *et al.,* 2018; Khodanitska *et al.,* 2018). Of particular interest in the study of growth regulators in the last 10–15 years is establishing the specificity of their anti-stress effect. To date, significant data has been accumulated on the positive effect of various plant growth regulators on cereals, legumes, vegetables, and other agricultural crops under stress conditions such as drought, low temperatures, waterlogging, salinity, etc (Marchuk *et al.,* 2018; Pervachuk *et al.,* 2018).

Main features of plant growth regulators functioning – high specificity, manifested both in their influence on physiological processes and in the interaction of simultaneous or strictly sequential implementation of stimulators and inhibitors of metabolism in the general system of hormonal regulation, ensuring coordination and functional integrity of the plant organism (Kuryata *et al.,* 2019). As peas are a promising crop in Ukraine, the development of effective cultivation techniques using growth regulators is of practical interest (Musiienko and Kapinos, 2018). The relevance of using growth regulators is also determined by the fact that similar preparations, when applied in low doses, enhance plant resistance and decompose into natural metabolites (Strydhorst *et al.,* 2019; Shepilova *et al.,* 2021). The aim of the research is to study the influence of plant growth regulators on the processes of growth and development, formation of productivity, and grain quality of peas.

MATERIAL AND METHODS

Research on the study of plant growth regulators on pea crops was conducted in the conditions of field stationary experiments at the territory of the Institute of Agriculture of the Northeast, National Academy of Agrarian Sciences of Ukraine. The research field is located in Sad village, Sumy district, Sumy region, Ukraine, with the following geographical coordinates: latitude 50°53'03", longitude 34°42'50", 167.0 m above sea level (Map date ©2023 Google). The research was carried out during 2021–2023. The study of the influence of plant growth regulators on the productivity and quality indicators of peas was conducted according to the following scheme: control (without treatment with preparations), at the budding stage treatment with Humifield VR-18 w.s. (0.4 L ha^{-1}), at the pod formation stage treatment with Fulvigrin Bor (0.5 L ha^{-1}), at the budding stage treatment with Humifield VR-18 w.s. (0.4 L ha^{-1}) + at the pod formation stage treatment with Fulvigrin Bor(0.5 L ha^{-1}). Humifield VR-18 w.s. is a universal anti-stress agent based on potassium humate, which is used for foliar application. It contains the following components: fulvic acid salts 20 g per L; humic acid salts 180 g per L including: amino acids – 25 g per L, potassium – 30 g per L, microelements – 5 g per L. pH 10–11. Formulation: aqueous suspension. Fulvigrin Bor is developed to compensate for boron deficiency in agricultural crops. It contains: fulvic acids – 100 g per L, boron – 100 g per L. pH 7.5–8.5. Formulation: aqueous suspension. The object of the research was the pea variety Oplot. The cultivation technology was generally accepted for the research area. The predecessor was winter wheat. After harvesting the predecessor, primary tillage was carried out, which included discing to a depth of 8–10 cm followed by plowing to a depth of 22–25 cm. In spring, early spring moisture closure and pre-sowing tillage were carried out, which included cultivation to a depth of 6–8 cm with rolling to provide optimal sowing conditions at the specified depth.

Seed sowing for peas was carried out using a solid method with the SS-16 seeder at a seeding rate of 1.4 million sprouted seeds per hectare, at a soil temperature of 6–8°C. Prior to sowing (20 days before), pea seeds were treated with the fungicide Maxim Star 025 FS (1 L t.). On the day of sowing, the seeds were treated with Ryzogumin with a rate of 2 kg t. Experimental treatments were laid out systematically, with repetitions placed in the same strip, each plot area measuring 100 m^2 and the effective area being 70 m^2.

For a significant assessment of field experiments, phenological observations were conducted according to the "Methodology of State Variety Testing of Agricultural Crops" and the "Methods of conducting research in fodder production". Growth stages and plant development phases were noted. The beginning of the phase was determined when it occurred in 10% of the plants, and the full phase was determined when it occurred in 75% of the plants (Yeshenko *et al.*, 2005; Volkodav, 2021). Additionally, plant density was determined at the full emergence stage and before harvest in triplicate. To determine the quantity and mass of rhizobial formations, soil monoliths measuring 25×25×30 cm were sampled. After washing the roots, 5 plants were selected from each repetition, nodules were separated from the roots, their average number per plant was calculated, then they were dried and weighed.

Before harvest, a sample sheaf was taken from each treatment to determine the individual productivity of pea plants. Pea grain was harvested using a Massey Ferguson combine at the full maturity stage, with moisture content of 14–15%, determined by a Wile 55 moisture meter with yield metering and sample selection for further analysis.

The weight of 1000 seeds was determined according to DSTU (State Standards of Ukraine) – 2949. The quality indicator of the yield, protein content, was determined by the Kjeldahl method.

The mathematical and statistical processing of experimental data and the establishment of the reliability of the obtained results were carried out according to Dospekhov (1985) using Microsoft Excel.

The soils of the research plots are typical chernozem, low-humus, weakly leached, coarse-loamy to medium-loamy on forest, with the following main indicators in the plowed layer: humus content – 4.6%, salt pH – 5.5, sum of absorbed bases – 35.6 mg-equiv., content of mobile forms of phosphorus – 19.3 mg per 100 g of soil, exchangeable potassium – 8.1 mg per 100 g of soil, content of easily hydrolyzable nitrogen according to the Kornfield method – 14.2 mg per 100 g of soil.

The average daily annual air temperature in 2021 was 9.4°C, which was 2.0°C higher than the long-term average of 7.4°C. The absolute maximum of 35.0°C was recorded in June in the third decade, and the minimum was -24.0°C in the second decade of January. The total precipitation for the reporting agricultural year 2020–2021 was 453 mm, which was 140 mm less than the long-term average (593 mm).

The average annual air temperature in 2022 was 8.7°C, which was 1.3°C higher than the long-term average of 7.4°C. The absolute maximum of 36.0°C was recorded in June in the third decade, and the minimum was -18.0°C in January in the second decade. The total precipitation for the reporting agricultural year 2021–2022 was 604 mm, which was 11 mm more than the long-term average (593 mm).

The average annual air temperature in 2023 was 9.0°C, which was 1.6°C higher than the long-term average of 7.4°C. The absolute maximum of 36.0°C was recorded in August in the first decade, and the minimum was -19.0°C in January in the first decade. The total precipitation for the reporting agricultural year 2022–2023 was 634 mm, which was 41 mm more than the long-term average (593 mm).

Overall, the most favorable years for crop yield formation were 2022 and 2023. Drought conditions occurred in 2021, characterized by low precipitation and extreme deviations in air temperature throughout the growing season.

RESULTS AND DISCUSSION

Important characteristics of seedling quality and uniformity are seed similarity and germination energy since the potential harvest depends on how successfully mass, full, and uniform seedlings are obtained while adhering to sowing norms and avoiding crop crowding (Khodanitska *et al.,* 2021; Radchenko *et al.,* 2022). In particular, research by many authors has shown that a decrease in seed similarity indicators in field conditions by each percentage point reduces the harvest by 1.5–2%. According to reference data, normal legume seeds should have minimum field similarity indicators of at least 70–80% (Maksimović *et al.,* 2020). In the studies conducted by the Institute of Agriculture of the Northeast, the field similarity of peas ranged from 89.3% to 91.4%, and the number of similar plants ranged from 1.25 to 1.28 million plants ha^{-1} (Table 1). It is worth noting that foliar fertilization did not affect seed similarity, as growth regulators were applied during vegetation.

The yield of agricultural crops depends on the productivity of each plant and their density per unit area. Therefore, determining the number of plants or the density of their stand has direct practical significance in assessing the quality of the crop (Trotsenko *et al.,* 2023). Many scientists in Ukraine in their works argue that the plant density of peas per unit area is a very important factor that significantly affects both the growth and development of the crop (Hamaiunova and Tuz, 2016; Andrushko, 2019). According to the research results of the Institute of Agriculture of the Northeast, the plant survival rate of peas during the vegetation period ranged from 73.6% to 76.4% (LSD_{05} (Least Significant Difference) = 0.7). The maximum number of surviving plants was obtained in the variant with the application of growth regulators in the budding phase, Humifield VR-18 + in the pod formation phase Fulvigrin Bor – 76.4% (1.07 million plants ha^{-1}), while the minimum was observed in the control (without treatment with preparations) – 73.6% (1.03 million plants ha^{-1}) and for treatments in the pod formation phase Fulvigrin Bor – 73.6% (1.03 million plants ha^{-1}). For treatments in the budding phase Humifield VR-18, the plant survival rate was 74.3% (1.04 million plants ha^{-1}) (Table 1).

Table 1. Plant density and survival rate of pea plants under the influence of a growth regulator (average for 2021–2023)

Treatment during vegetation	Plant growth phase			
	full emergence		full ripeness	
	plant count, million plants ha^{-1}	field germination, %	plant count, million plants ha^{-1}	preservation of plants during the vegetation period, %
Control (no treatment)	1.25	89.3	1.03	73.6
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1})	1.27	90.7	1.04	74.3
In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	1.26	90.0	1.03	73.6
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1}) + In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	1.28	91.4	1.07	76.4
LSD_{05}	0.02	0.6	0.02	0.7

One of the measures to increase plant resistance and enhance the efficiency of legume-rhizobial symbiosis and increase yield is the application of plant growth regulators (Petrychenko and Kots, 2014; Backer *et al.*, 2018). When using inoculant and plant growth regulator in combination, the number of active nodules on plant roots increased by 44.2% (5–6 leaf formation stage), 74.8% (flowering stage), and 35.2% (flowering phase) compared to the control (Didur and Shevchuk, 2020). Dynamics of the number of nodules on pea roots in the conditions of the Institute of Agriculture of the Northeast varied from 17.4 to 19.3 units per plant during the budding phase (LSD_{05} = 0.6). Thus, the maximum number of nodules was noted for the treatment of pea plants during budding with Humifield VR-18 + during pod formation with Fulvigrin Bor, amounting to 19.3 units per plant, while the minimum was observed in the control group at 17.4

units per plant. With the application of the growth regulator during budding with Humifield VR-18, the number of nodules was 18.8 units per plant, and with application during pod formation with Fulvigrin Bor, it was 17.6 units per plant. During the flowering phase, the number of nodules on pea roots increased compared to the budding phase and ranged from 27.4 to 30.8 units per plant (LSD_{05} = 0.4). Maximum values were observed for the treatment with Humifield VR-18 during budding + Fulvigrin Bor during pod formation, reaching 30.8 units per plant. The lowest number of nodules was observed in the control group, at 27.4 units per plant. With the application of the growth regulator during budding with Humifield VR-18, the number of nodules was 29.6 units per plant, and with application during pod formation with Fulvigrin Bor, it was 27.7 units per plant. The highest number of nodules on pea roots was observed during pod formation and ranged from 35.7 to 44.4 units per plant (LSD_{05} = 0.6). Thus, the maximum number of nodules was noted for the treatment during budding with Humifield VR-18 + during pod formation with Fulvigrin Bor, amounting to 44.4 units per plant, while the minimum was observed in the control group at 35.7 units per plant (table 2).

Table 2. Dynamics of the number (units per plant) of nodules on pea roots under the application of plant growth regulator (average for 2021–2023)

Treatment during vegetation	Nodule count, units per plant		
	Budding phase	Flowering phase	Pod formation phase
Control (no treatment)	17.4	27.4	35.7
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1})	18.8	29.6	40.1
In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	17.6	27.7	36.0
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1}) + In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	19.3	30.8	44.4
LSD_{05}	0.6	0.4	0.6

It is known that the weight of nodules depends on the stage of plant development and growing conditions. The formation and activity of the symbiotic apparatus are directly related to various environmental factors, including soil moisture (Kots *et al.,* 2010). In the conditions of the Institute of Agriculture of the Northeast, during the budding phase, the weight of nodules varied depending on the growth regulator from 0.087 to 0.097 g per plant (LSD_{05} = 0.005). The maximum weight of nodules on pea roots was observed in the treatment with Humifield VR-18 during budding phase combined with Fulvigrin Bor during pod formation phase, reaching 0.097 g per plant, while slightly lower weights were recorded for treatments with Humifield VR-18 during budding phase (0.094 g per plant), Fulvigrin Bor during pod formation phase (0.088 g per plant), and in the control group (0.087 g per plant). During the flowering phase, an increase in nodule weight on pea roots was observed, ranging from 0.137 to 0.154 g per plant across different treatments (LSD_{05} = 0.004). The maximum weight of nodules was recorded in the treatment with Humifield VR-18 during budding phase combined with Fulvigrin Bor during pod formation phase, reaching 0.154 g per plant, while slightly lower weights were observed for treatments with Humifield VR-18 during budding phase (0.148 g per plant), Fulvigrin Bor during pod formation phase (0.139 g per plant), and in the control group (0.137 g per plant). The highest nodule weight on pea roots was observed during pod formation phase, ranging from 0.179 to 0.222 g per plant (LSD_{05} = 0.005). The maximum weight of nodules was recorded in the treatment with Humifield VR-18 during budding phase combined with Fulvigrin Bor during pod formation phase, reaching 0.222 g per plant, while the lowest weight was observed in the control group, with 0.179 g per plant (see Table 3).

Table 3. Dynamics of nodule weight (g per plant) on pea roots under the influence of plant growth regulator (average for 2021–2023)

Treatment during vegetation	Nodule weight, g per plant		
	Budding phase	Flowerin g phase	Pod formation phase
Control (no treatment)	0.087	0.137	0.179
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1})	0.094	0.148	0.201
In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	0.088	0.139	0.180
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1}) + In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	0.097	0.154	0.222
LSD_{05}	0.005	0.004	0.005

Increasing the number of pods per plant and preserving the maximum number of seeds in them is the most effective way to increase the seed productivity of leguminous crops. In the research by Yeremenko and Kapinos (2020), the highest number of pods was formed with the application of growth regulators – 3.43 pods per plant, which exceeded the control indicator by 1.7 to 8.5%. According to the results of research, the number of pods per plant varied from 3.24 to 3.44 pods (LSD_{05} = 0.07). The highest number of pods was observed with treatment in the budding phase with Humifield VR-18 + in the pod formation phase with Fulvigrin Bor – 3.44 pods per plant, while the lowest number of pods was observed in the control – 3.24 pods per plant. With the application of growth regulators in the budding phase with Humifield VR-18, the number of pods was 3.40 pods per plant, and with application in the pod formation phase with Fulvigrin Bor – 3.32 pods per plant (Table 4).

It has been established that the use of growth regulators contributed to an increase in the number of seeds per plant regardless of the pea variety under study. In the research by Yeremenko and Kapinos (2020), for pea cultivation, treatment with growth regulators contributed to the formation of 12.58 seeds per plant, which was higher than the control indicators by 1.1–3.5%. In studies conducted at the Institute of Agriculture of the Northeast, the number of seeds per pea plant varied from 12.8 to 13.4 seeds (LSD_{05} = 0.3). The maximum number of seeds was recorded with treatment in the budding phase with Humifield VR-18 + in the pod formation phase with Fulvigrin Bor – 13.4 seeds per plant, while the lowest seed indicators per plant were obtained in the variant without treatment with growth regulators (control) – 12.8 seeds per plant (Table 4).

One of the main indicators of plant productivity is grain weight per plant. Over the years of research, the weight of seeding peas per plant (g) depending on the growth regulators ranged from 2.43 to 2.65 g. An increase in grain weight per plant from 3.3 to 9.1% compared to the control was observed (Kapinos, 2019). The maximum grain weight per plant in the studies conducted by the Institute of Agriculture of the Northeast was noted in the variant treated in the budding phase with Humifield VR-18 + in the pod formation phase with Fulvigrin Bor – 3.57 g, slightly lower grain weight per plant was obtained with treatment in the budding phase with Humifield VR-18 – 3.48 g, in the pod formation phase with Fulvigrin Bor – 3.40 g, and in the control – 3.36 g (LSD_{05} = 0.11) (Table 4).

Table 4. Elements of pea crop structure depending on the influence of plant growth regulator (average for 2021–2023)

Treatment during vegetation	Number per plant, pcs.		Grain weight per plant, g	Weight of 1000 grains, g
	Pods	Seeds		
Control (no treatment)	3.24	12.8	3.36	262.5
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1})	3.40	13.1	3.48	265.6
In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	3.32	12.9	3.40	263.6
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1}) + In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	3.44	13.4	3.57	266.4
LSD_{05}	0.07	0.3	0.11	1.1

Researchers have concluded that the most realistic approach is to create new pea varieties with 7–8 pods, 3–5 productive nodes per plant, and 5–6 seeds per pod, which would allow increasing the yield to 250–280 g per 1000 seeds. At the same time, the weight of 1000 seeds should be maintained at 250–280 g, as varieties with larger seeds have a greater need for moisture during germination, and the sowing rate also increases (Popov *et al.*, 2018). According to the research results of the Institute of Agriculture of the Northeast, the weight of 1000 seeds varies depending on the growth regulator within the range of 262.5–266.4 g (LSD_{05} = 1.1). The highest weight of 1000 seeds was recorded in the variant treated in the budding phase with Humifield VR-18 + in the pod formation phase with Fulvigrin Bor – 266.4 g, which is higher by 0.3% (265.6 g) compared to treatment in the budding phase with Humifield VR-18, by 1.06% (263.6 g) compared to treatment in the pod formation phase with Fulvigrin Bor, and by 1.47% (262.5 g) compared to the control (Table 4).

Pea yield depends largely on the photosynthetic activity of crops, therefore, to achieve optimal yields, it is necessary to create the most favorable conditions. One of the elements of technology for improving photosynthetic activity is the use of growth regulators. With the use of growth regulators, yield increases on average from 0.15 to 0.24 $t.ha^{-1}$ (Vuiko, 2023). In the conditions of the Institute of Agriculture of the Northeast, pea yield ranged from 3.46 to 3.81 $t.ha^{-1}$ (LSD_{05} = 0.12). The maximum yield was obtained with treatment during the budding phase with Humifield VR-18 + during the pod formation phase with Fulvigrin Bor, reaching 3.81 $t.ha^{-1}$. Slightly lower yields were obtained with treatment during the budding phase with Humifield VR-18 – 3.61 $t.ha^{-1}$, during the pod formation phase with Fulvigrin Bor – 3.50 $t.ha^{-1}$, and in the control – 3.46 $t.ha^{-1}$ (Table 5).

Table 5. Grain yield and protein content in pea grain depending on the effect of plant growth regulator (average for 2021–2023)

Treatment during vegetation	Yield, $t.ha^{-1}$	Protein content, %
Control (no treatment)	3.46	22.2
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1})	3.61	22.8
In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	3.50	22.5
In the budding phase, Humifield VR-18 w.s. (0.5 L ha^{-1}) + In the pod formation phase, Fulvigrin Bor w.s. (0.5 L ha^{-1})	3.81	23.2
LSD_{05}	0.12	0.4

Quality of the harvest is a complex indicator formed during the cultivation of the crop. It depends on the variety, soil type, agronomy practices, meteorological conditions, and the nature of their interaction. The developers of new intensive varieties of field peas are particularly interested in obtaining grain with a high protein content. Pea seeds may contain 2.0–2.5 times more protein than grains of cereal crops (Lykhochvor and Andrushko, 2020). Depending on the application of growth regulators, the protein content in pea grain varies within the range of 22.2–22.8% (Tsyliuryk and Izhboldin, 2022). In the experiments of the Institute of Agriculture of the Northeast, the maximum protein content was observed for treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, reaching 23.2%. Treatment during the budding phase with Humifield VR-18 showed a lower value of 22.8%, while treatment during pod formation with Fulvigrin Bor resulted in 22.5%. The lowest protein content in pea grain was recorded for the variant without the application of growth regulators (control) – 22.2% (Table 5).

CONCLUSIONS

According to the results of the conducted research, it was found that the maximum plant survival during the vegetation period was achieved for the treatment with growth regulators during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, reaching 76.4% (1.07 million plants per ha^{-1}). The highest number of nodules on pea plant roots during pod formation was observed with the maximum number of nodules for the treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, reaching 44.4 nodules per plant, while the lowest number was observed in the control group with 35.7 nodules per plant. The maximum grain yield per plant was noted for the treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, at 3.57 g, followed by slightly lower values for the treatment during the budding phase with Humifield VR-18 at 3.48 g, during pod formation with Fulvigrin Bor at 3.40 g, and in the control at 3.36 g. The highest weight of 1000 grains was recorded for the treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, at 266.4 g. The maximum yield was obtained for the treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, reaching 3.81 $t.ha^{-1}$, followed by slightly lower yields for the treatment during the budding phase with Humifield VR-18 at 3.61 $t.ha^{-1}$, during pod formation with Fulvigrin Bor at 3.50 $t.ha^{-1}$, and in the control at 3.46 $t.ha^{-1}$. According to the research results, the highest protein content was achieved for the treatment during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor, reaching 23.2%.

In the natural-climatic conditions of the Sumy region (northeastern Forest-Steppe of Ukraine), for obtaining a pea yield at the level of 3.81 $t.ha^{-1}$ with a protein content of 23.2%, it is recommended to apply growth regulators during the budding phase with Humifield VR-18 + during pod formation with Fulvigrin Bor.

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ANALYSIS OF THE EFFICIENCY OF FOREST RESTORATION IN THE TERRITORY OF ALTAI

SUMMARY

In this paper, the trend of changes in the surface of forests created by natural succession, artificial establishment and combined in the area of Altai (Russian Federation) was investigated. The method of time series analysis for the period 2019-2023 was used. The obtained dispersion measures show that the linear trend model is representative. A trend of increasing the area under forest by an average of 368.5 hectares per year was determined as a result of afforestation and natural progressive succession of vegetation. The area of actually established forests in the analyzed period was larger than planned each year (7237 ha/year), and individual growth rates show an increase in new forest areas. The success of afforestation with white pine seedlings expressed by area in all years of the analyzed period was above 50%. However, in these areas, the success of afforestation with white pine seedlings shows a negative trend, i.e. a decrease of 58.2 ha/year.

Keywords: *Forest restoration, afforestation, Altai, Pinus sylvestris.*

INTRODUCTION

The area of forests in the world is decreasing, which affects the reduction of the effects and functions of forests (FAO 2020), and therefore forest restoration is a priority for most countries and forestry development strategies. The main task today is to ensure a balance between biological productivity and the use of forest resources. This balance is enshrined in the concept of sustainable development of forest ecosystems and is a fundamental principle of global forest policy (UNDP, 2016; Baumgartner, 2019). Numerous challenges in forest management such as fires, conversion of forest land into industrial land, deforestation, climate change, diseases and pests lead to a reduction in forest area. Although the risks and

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uncertainties in forest management are increasingly pronounced, the strategic goals of forestry development envisage an increase in the area under forests. The European Union's Forestry Development Strategy envisages the planting of 3 billion seedlings by 2030 (EC, 2013). Comparing this strategy with pan-European criteria and indicators for sustainable forest management, it was noted that the New European Forest Strategy includes boundaries (thresholds and ranges) for sustainable management (Lier et al., 2022), within which the establishment of new forests or increasing forest cover is of great importance.

International definitions (FAO, 2020) in terms of increasing forest cover refer to the expansion of forests on areas that previously had some other purpose. The establishment of new forests is usually done in a natural, artificial and combined way. The establishment of forests naturally on areas where there was no forest before implies a natural progressive succession of vegetation. Artificial establishment of forests (afforestation by planting seedlings) is carried out mainly on areas where previously, in the foreseeable future, there was no forest, and the goal is to preserve the economic, ecological and social functions of forests. Objects favorable for afforestation are most often clearings, forest clearings, areas damaged by fires and other areas that are not covered with forest vegetation but are suitable for afforestation. There are areas of forest land on which it is necessary to carry out a combined natural and artificial establishment of forests, most often as a result of very heterogeneous habitat conditions or efforts to establish mixed stands in the same habitat. Combined afforestation is carried out by planting and sowing in forest areas where natural afforestation of forest plantations of valuable species is not fully ensured forests trees (Chzhan and Puzanova, 2012). One of the directions for increasing the productivity of forests is artificial afforestation on lands that are not covered with forest vegetation (Zalesov and Lugansky, 2002; Khairetdinov 2011). The latter is explained by the fact that artificial plantations created with standard planting material with timely and high-quality agrotechnological and sylvicultural care are superior in productivity to natural plantations of the same age (Yusupov et al. 1999; Zalesov et al. 2015). Artificial afforestation is particularly relevant in areas that experience intense recreational activities (Dancheva et al. 2014) and other anthropogenic influences (Uzhgin et al. 2012), as well as in areas with difficult soil and climatic conditions (Danilik et al. 2001; Freiberg et al. 2012).

Artificial afforestation in the Altai region is associated with a number of difficulties. Especially, due to the rare fruiting of high-quality seeds of the main tree species for growing planting material. In addition, tight deadlines for sylvicultural work often lead to their failure, since planting seedlings with an open root system in dry warm weather in conditions of the steppe climate sharply reduces the survival rate of seedlings. In order to minimize these shortcomings in the Altai Territory, in 2009 it was decided to establish a Forest Seed Selection Center as part of a Russian project to improve the quality of forest restoration. The Altai Territory was chosen as the only area beyond the Urals where such a center was built. The purpose of the creation of this Center was to organize the processing and storage of up to 2,000 kg of seeds and the cultivation of 7 million seedlings of white pine with a closed root system with forest seeds of improved genetic characteristics. This provided not only an increase in the quality of planting material, but also created the possibility of a sharp increase in the period of formation of forest crops with a significantly lower risk to their survival. Otherwise, the need for intensive development of nursery production and afforestation developed in the sixties of the last century, in the period of intensification of forest protection and afforestation of virgin and pasture lands. Also, the large forest fires that engulfed forests in the Altai region in the 1990s, as well as the need to restore lost forests, gave impetus to the development of a base of forest nurseries. In the Altai Region, up to 40 million seedlings of various tree species are grown annually in 30 nurseries, eight of which nurseries are classified as "High Culture Forest Nurseries".

Since 2019, the regional project "Forest Conservation" has been launched in the Altai Territory within the framework of the national project "Ecology". The main tasks are to ensure a balance between biological forest production and use, an annual increase of 7,200 hectares of forest area, including afforestation of 3,500 hectares, equipping forest holdings with specialized firefighting equipment and forming a stock of seeds for afforestation (Dobrynin and Mishchenko, 2021).

MATERIAL AND METHODS

The Altai Territory occupies the southern part of Western Siberia (Fig. 1) and includes four natural zones: the steppe, the forest-steppe, the low mountain taiga of Salaira and the foothills of the Altai taiga. The land of the Forest Fund of the Russian Federation, located on the territory of the Altai Territory, occupies 4,433,300 hectares, which is 98.4 percent of the forests in the region and 26.4 percent of all land in the region. There are 31 forestry areas in the Altay territory. The climate of the Altai region according to the Köppen-Geiger system is classified as a Warm-summer humid continental climate (Dfb) where there is no significant difference in the amount of precipitation between the seasons (He et al. 2021). The average annual temperature is 0,5-2,1^{o}C, and the average annual rainfall is 250-350 mm. It can be magnified up to 700 mm. The dominant land is mountain brown forest soils lighter granulometric composition. In addition to these lands, there are also sub-sill soils on which afforestation was carried out. A comparison of soils belonging to the same type occurring in the northwestern, central and southeastern regions of the Altai Mountains in terms of the ratio of carbon in humic acids to carbon in fulvic acids (Cha/Cfa) as one of the integral indices of humus composition, showed that there are no significant differences between these soils (Dergacheva et al).

Data collection was carried out on the basis of available records on the number of seedlings, area, tree species and survival rate in the period from 2019 to 2023 (Forest Seed Selection Center, 2024). The survival rate of seedlings was determined (Pilipko, 2013):

$$A_{p(\%)} = \frac{A+\frac{1}{2}A_d}{A_S} \quad (1)$$

$A_{p(\%)}$ - Survival rate
A - Area with live plants (ha)
A_d - Area with dubious plants (ha)
A_s - Total area of planting or seeding places (ha)

Figure 1. The location of the Altai territory (red color) on the map of Russia

For the purpose of statistical data processing, time series analysis was applied using linear trend (2), trend standard deviation (3) and coefficient of variation (4) (Velicer et al. 2003; Hadživuković, 1991).

$$\hat{y}t = a + bx \quad (2)$$

$$\sigma_{\hat{y}} = \sqrt{\frac{\sum_{t=1}^{N} y^2 - a\sum_{t=1}^{N} y_t - b\sum_{t=1}^{N} x_t y_t}{N}} \quad (3)$$

$$V\hat{y} = \frac{\sigma_{\hat{y}}}{\bar{y}} 100 \quad (4)$$

The analysis includes the following features:

- Ps – the area actually restored planting of seedlings and natural regeneration,
- R – artificially restored area (afforestation),
- Rk – combined renewal,
- Pps – The area is successfully afforested with white pine seedlings.

RESULTS AND DISCUSIONS

The total forested area in the Altai region in the last 10 years amounted to 120,000 hectares. In the period from 2013 to 2017, the area of forests that was naturally regenerated amounted to 33,100 ha, while the share of artificial afforestation was 26,900 ha. The largest area within the artificial establishment of forests was treated with agrotechnical measures (9,800 ha) as well as planting seedlings on the area of 2,010 ha (Table 1). Sowing of seeds in regional forest nurseries was carried out on a total area of 18.8 ha.

Table 1. Silvicultural treatments in the artificial establishment of forests of the Altai Region (2013-2017) (Source: Department of Forests of the Altai Region, 2018)

Silviculture treatmens	Area (ha)	(%)
Planting seedlings	2.010	13,4
Combined afforestation	910	6,1
Promoting natural regeneration	490	3,3
Agrotechnical measures	9.800	65,3
Land preparation	1.800	12,0

According to the forest management plans of the Altai Region in the period 2019 to 2023, an equal area for forest restoration by natural, artificial and combined methods is planned, which annually amounts to 7,237 ha.

The area of forests actually established in the analyzed period was larger than planned each year (Table 2). Individual growth rates determined on the basis of the chain index show an increase in new forest area (Ps) and area created by combined regeneration (Rk), except for the period between 2019 and 2020.

Table 2. Planned and actual afforestation in the Altai Region (2019-2023)

Year	Pp (ha)	Ps (ha)	S_P	R (ha)	S_R	Rk (ha)	S_{Rk}	$R_{\%}$
2019	7237	7981		3720		362,0		46,6
2020	7237	7734	-3,1	3782	1,7	258,9	-28,5	48,9
2021	7237	7994	3,4	4003	5,8	321,0	24,0	50,1
2022	7237	8129	1,7	3511	-12,3	332,8	3,7	43,2
2023	7237	9626	18,4	3715	5,8	344,8	3,6	38,6

Pp – Planned area for the establishment of new forests; Sp, S_R, S_{Rk} – rates of change in the characteristics analysed; $R_{\%}$ - Participation in artificial restoration

The analysis of trends shows that the realized (actual) increase in forest cover is conditioned by the planting of seedlings and natural regeneration (progressive succession of vegetation) on average by about 368.5 ha per year (Table 3). A slight increase is also shown by combined regeneration (3.95 ha), which is the result of the lack of areas that could be renovated in this way (Fig. 2).

In the Altai region, due to the specific conditions of habitats and the way of management on relatively large areas, mainly natural regeneration is applied by progressive succession of vegetation, and artificial afforestation by planting seedlings is used in conditions of difficult natural regeneration or failure of restoration. The artificial method of reforestation is not very applicable due to the low survival rate of plantings of forest crops and their death (Puzanova, 2019). The problems of artificial intelligence are indicative on the basis of the negative value of the individual rate in the middle of the analyzed period (-12.3).

Table 3. Measures of the Representativeness of the Linear Trend of Analyzed Parameters

Parameters (ha)	$\hat{y}t = a + bx$						
	Ȳ (ha)	a	b	r^2	σ (ha)	$V\hat{y}$ (%)	e (ha)
Ps	8292,8	7198,3	368,5	0,58	434,8	5,2	408,6
R	3746,2	3830,5	-28,1	0,06	152,5	4,0	115,8
Rk	323,9	312,0	3,95	0,02	34,8	10,7	25,6
Pps	2139,2	2313,2	-58,2	0,46	88,65	4,1	82,12

Ȳ - Average value of the analysed characteristic; r^2 – The coefficient of determination.; σ – The standard deviation.; $V\hat{y}$ – Coefficient of Variation; e – residuals (average);

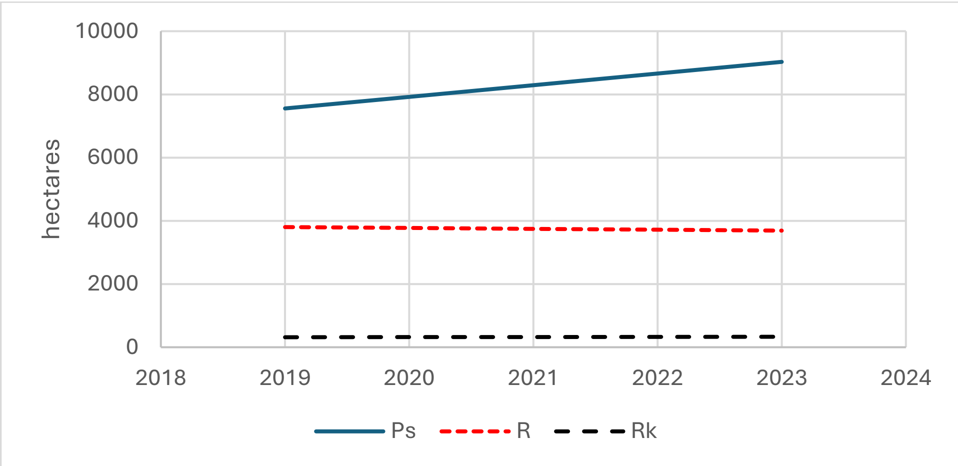

Figure 2. Trend of analyzed characteristics in the period 2019-2023

The main species for afforestation in the Altai region is white pine (*Pinus sylvestris* L), followed by Siberian spruce (*Picea obovata* Ledeb.). When establishing new forests artificially, individual selection instead of mass selection is very important. Thus, studies of 46 different provenances of *Pinus brutia* Ten. in Turkey showed a better current increment of the two basic taxiation elements (tree diameter and height) in individual selection within the same provenance (Ozben and Bilir, 2022). Such principles of selection are also used when establishing plantations in the Altai region at the Seed Selection Center. The total area covered by forests in the Altai Territory is 3.825 million hectares. The average forest cover along the edge is 32.8%. Forests cover the territory of the region unevenly, in spots. These are mostly even-aged stands formed as a result of habitat conditions that are suitable for the bioecological characteristics of white pine. Due to favorable natural conditions and a very wide range of this species, artificial regeneration is an advantage in the establishment of new forests. It is characteristic that in the same time periods of the analyzed period, the absolute change in the areas that were successfully afforested with white pine based on the coefficient of linear trend direction shows a decreasing flow by about 58.2 ha on an annual basis (Fig. 3).

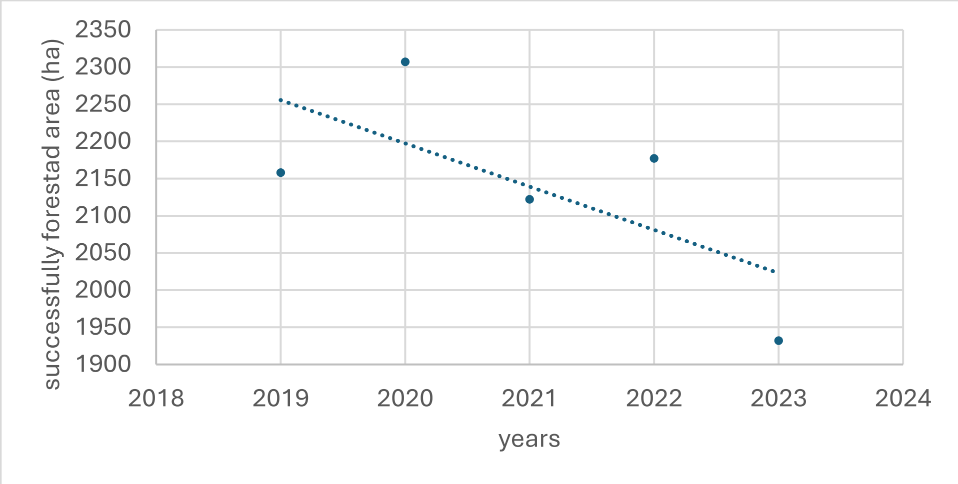

Figure 3. The trend of changing successfully afforested areas with white pine seedlings

The standard deviation of the trend of successfully afforested areas with white pine seedlings is 88.6 ha, and the coefficient of variation is 4.1%. The obtained dispersion measures show that the linear trend model is representative. In relation to the plan, the actual establishment of forests with white pine seedlings every year in the period 2019-2023 is planned to afforest the same area (3,720.69 ha). A successfully afforested area with a percentage of surviving seedlings of more than 50% was noted every year, and the most successful was 2022 with a seedling survival rate on an area of 2,177 ha (62%). By the way, white pine is a very useful species for establishing new forests in degraded habitats. For this reason, it is also used for afforestation of ore waste dumps for the purpose of land reclamation (Vacek et al. 2021). The productivity of white pine expressed by volume increase in habitats after mining is lower by 9 to 32% compared to the original natural native habitats in the Hradec Králové region in the Czech Republic. Such results can be expected in the Altai region, especially on poor soils that lack water. However, new research is also needed in terms of the quality of newly established stands and monitoring of their growth and development due to the trend of constant changes in risk factors caused by climate warming.

CONCLUSION

Throughout the analyzed period from 2019 to 2023, the afforestation plan in the Altai region was regularly exceeded. Thus, based on the above data, the main way of successful forest restoration in the Siberian region is to promote natural regeneration, since this method makes it possible in such natural and climatic conditions to increase the reproductive potential of coniferous forests, as well as to reduce the period of silviculture, reduce labor and costs compared to artificial afforestation. The artificial method of afforestation is not very applicable due to the low survival rate of forest seedlings, especially white pine, and their death, due to the problem of limited financial and technical capabilities of forestry enterprises, low technological level of production and lack of flexible adaptation to local conditions. That is why artificial afforestation is used here when natural regeneration is difficult for conifers. Combined afforestation is used where natural and artificial methods of forest restoration need to be combined in a single forest area. The trend of increasing the area under forest in the Altai region is in line with the promoted global Sustainable Development Goals (SDGs) and the efforts of forestry to contribute to the fight against key environmental endangering factors.

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BUNCH LOAD AS A FACTOR ON THE QUALITY OF THE GRAPEVINE VARIETIES 'RIBIER' AND 'ITALIA'

SUMMARY

The application of ampelotechnical measures in table grape production profoundly affects grape yield and quality. This study aims to assess the influence of the ampelotechnical practice of bunch thinning on the yield and quality of Ribier and Muscat Italia grape varieties. The research was conducted over three consecutive years on production plantations of Ribier and Muscat Italia varieties grown on pergola training system with a planting distance of 2.5 x 2.5 m. The plantations are in full fertility, aged between 12-15 years, located in the Gevgelija-Valandovo vineyard, renowned as one of the most suitable sites for cultivating table grapes in the Republic of Macedonia. Three different bunch thinning variants were applied in both varieties: V1 (thinning to 4 bunches/m^2), V2 (thinning to 5 bunches/m^2), and V3 (thinning to 6 bunches/m^2). These were compared with the standard variant where no bunch thinning was performed.

Our research findings demonstrate that the applied ampelotechnical measure significantly impacts the average bunch and berry mass, transportability, must chemical composition, packed grape quantity, and organoleptic evaluation of the grapes. The average bunch mass of the Ribier variety ranges from 381,3 g in the standard (St) variant to 445,7 g in the V1 variant. Similarly, in the Italia variety, the bunch mass fluctuates between 607.3 g (St variant) and 703,0 g in the V1 variant, while the berry mass ranges from 7,5 g (St) to 8,5 g (V1). Moreover, the packed grape quantities range from 1,9 kg/m^2 in the standard variant to 2,31 kg/m^2 in the V2 variant for the Ribier variety, whereas for the Italia variety, the lowest amount of packed grapes was recorded in the V1 variant (2,65 kg/m^2), and the highest in the

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V3 variant (3,27 kg/m^2). Notably, the organoleptic evaluation of the grapes was influenced by bunch thinning. The V1 and V2 variants in both varieties received better ratings compared to the other variants, with the Ribier variety rated as excellent quality and the Italia variety as extra quality.

Keywords: table grapes, bunch thinning, bunch and berry mass, transportability, packaged grape quantity, organoleptic evaluation

INTRODUCTION

Modern table grape production extensively relies on the intensive implementation of ampelotechnical measures, which has garnered increasing attention, particularly among major table grape producers like Italy, California, South Africa, and Chile. Successful production of table grapes depends significantly on the meticulous and timely application of these measures (Prculovski, 2019).

Summer pruning, a technique ensuring quality yield and vegetative balance of the vine, enables the enhancement of the microclimate and facilitates timely and better ripening of the grapes (Di Lorenzo et al., 2011). Canopy management techniques, involving practices such as altering the position or number of shoots, leaves, or bunches, facilitate the achievement of the desired spatial arrangement, allowing for improved lighting and aeration (Dry, 2000).

Each ampelotechnical measure during green pruning directly impacts the production and technological characteristics of grape varieties. For instance, shoot and bunch pinching affect the distribution of photoassimilates (Mota et al., 2010), while defoliation affects air temperature, solar radiation frequency, and aeration in the bunch zone (Mandelli and Miele, 2003). Pinching and the reduction of bunches not only ensure a more uniform and faster ripening of the grapes but also result in a higher packing percentage of extra and first class grapes (Prculovski, 2021).

Optimizing crop (bunch) density of the vine is one of the fundamental factors for successful table grape production. An excessive number of bunches on the vine may reduce berry diameter and total sugar content (Somkuwar and Ramteke, 2010). Conversely, reduced vine density can lead to a decrease in yield, an increase in grain mass, and an improvement in grape chemical composition (Ezzahouani and Williams, 2003), including phenolic components and vegetative vigor (potential) (Kavoosi et al., 2009). Therefore, our research aimed to determine the ideal yield potential of Ribier and Italia varieties, as one of the most important late table grape varieties cultivated in the Republic of Macedonia.

MATERIAL AND METHODS

Our research was conducted on production plantations of Ribier and Italia varieties in the Gevgelija-Valandovo vineyard. These plantations, aged between 12 and 15 years, utilized a pergola training system with a planting distance of 2.5 x 2.5 m. Three variants were employed for each variety:

1. V1 – bunch thinning to 4 bunches/m^2
2. V2 – bunch thinning to 5 bunches/m^2
3. V3 – bunch thinning to 6 bunches/m^2

Comparisons were made with the standard (St) variant, where no thinning was applied. Other ampelotechnical measures such as pinching and defoliation were implemented simultaneously across all variants. Proper pruning, with leaving four canes with eight buds and two buds per wine, was implemented uniformely for each of the variants including the standard, on both variety. Bunch load was reduced 7-14 days after blooming phenophase. The harvest took place when the bunches exeeded between 16 and 17 ^{o} Brix.

Averige bunch and berry mass, as well as bunch and berry mechanical properties (berry firmness and berry adherence strength) were exeminated. Total yield was classified in two categories (packeg grape and residue). The organoleptic evaluation was carried out by using ten-point system where the expers evaluated tast quality, consistency and external appearance.

Mean values, standard deviation and coefficient of variation were calculate based on the results. Our hypotheses was tested by using analysis of variance (ANOVA) while the mean values were compared using the LSD test.

RESULTS AND DISCUSSION

We assessed the impact of the ampelotechnical measure of bunch thinning by examining the mechanical composition and properties of bunches and berries, grape chemical composition, harvested grape quantity, and organoleptic evaluation. Detailed results can be found in Tables 1 and 2, presenting the average bunch and berry mass across each variant of the Ribier and Italia varieties.

Table 1 Ribier variety bunch and berry mass

	Bunch mass (g) Variant				**Berry mass (g)** Variant			
Year	St	V_1	V_2	V_3	St	V_1	V_2	V_3
2014	335,0	428,0	416,0	382,0	6,5	7,8	7,0	6,7
2015	400,0	462,0	431,0	402,0	6,5	8,2	7,5	6,9
2016	409,0	447,0	451,0	418,0	6,2	6,3	6,1	6,2
Mean x	381,3a	445,7b**	432,7b**	400,7a	6,4a	7,4b	6,9ab	6,6a
+ - ø		64,3	51,3	19,3		1,0	0,5	0,2
Index	100	117	113	105	100	116	107	103
SD	40,4	17,0	17,6	18,0	0,2	1,0	0,7	0,3
CV	10,6	3,8	4,1	4,5	3,0	13,4	10,7	5,2
LSD 0,05	29,35				0,74			
0,01	44,48				1,11			

The lowest recorded average bunch mass was 381,3 g in the standard variant, whereas the V1 variant exhibited the highest average mass of 445,7 g. In the case of the V2 variant, the average bunch mass was 432,7 g, and for the V3 variant, it was 400,7 g.

Regarding the Ribier variety, the average grain mass ranged from 6,4 g for the standard variety to 7,4 g for the V1 variety. Additionally, the V2 and V3 variants demonstrated berry masses of 6,9 g and 6,6 g, respectively.

Table 2 Italia variety bunch and berry mass

	Bunch mass (g) Variant				**Berry mass (g)** Variant			
Year	St	V_1	V_2	V_3	St	V_1	V_2	V_3
2014	611,0	674,0	690,0	641,0	7,1	8,1	7,4	7,2
2015	616,0	724,0	665,0	614,0	8,2	9,4	8,7	8,3
2016	595,0	711,0	684,0	659,0	7,2	8,1	7,9	7,7
Mean x	607,3a	703,0c	679,7bc	638,0ab	7,5a	8,5c	8,0b	7,7ab
+ - ø		95,7	72,3	30,7		1,0	0,5	0,2
Index	100	116	112	105	100	114	107	103
SD	11,0	25,9	13,1	22,6	0,6	0,8	0,7	0,6
CV	1,8	3,7	1,9	3,6	8,1	8,8	8,1	7,4
LSD 0,05	43,06				0,32			
0,01	65,23				0,49			

The average bunch mass for the observed years in the Italia variety ranged from 607,3 g in the St variant to 703 g in the V1 variant. The V2 variant demonstrated an average mass of 679,7 g, while the V3 u variant exhibited an average bunch mass of 638,0 g. Additionally, the average berry mass ranged from 7,5 g for the standard variant to 8,5 g for the V1 variant.

In both studied varieties, the impact of bunch thinning on the average bunch and berry mass was evident. The V1 variant in both varieties notably showed a statistically significant increase in average bunch and berry mass compared to the St and V3 variants, where the load on the vine was the highest. Various studies have addressed the application of this ampelotechnical measure and its influence on the production and technological characteristics of different varieties. Berkey et al. (2011), in their investigation of the impact of bunch thinning on Seyval Blanc variety production, observed variations in the effects of this measure across different years, attributing these differences to the management in previous production cycles. Several studies of different varieties have shown that the application of this measure leads to an increase in both bunches and berries when there are fewer bunches on the vine (Kavoosi et al., 2009; Somkuwar and Ramteke, 2010; Gil et al., 2013; Prculovski et al., 2021). They attribute this phenomenon to the increased concentration of photoassimilates directed towards the remaining bunches when there are fewer bunches per vine. The intensity of thinning must align with the cultivation conditions and varietal characteristics, as excessive thinning can result in a substantial yield reduction without enhancing quality (Prculovski et al., 2021; Avizcuri-Inac et al., 2013).

Bunch and berry mechanical properties show the resistance of bunches and berries to various mechanical forces. Berry firmness and adherence strength (the attachment strength of the berry to the stem) were examined, considering their significance for table varieties, particularly in relation to grape transportability and refrigerated storage capabilities. Tables 3 and 4 present the results of the investigation into the mechanical properties of Ribier and Italia varieties.

Table 3 Bunch and berry mechanical properties of the studied variants of Ribier variety

		Variant				Index			
	Year	St	V_1	V_2	V_3	St	V_1	V_2	V_3
Berry firmness	2014	1 550	1 710	1 700	1 620				
	2015	1 530	1 730	1 610	1 560				
	2016	1 530	1 700	1 600	1 570				
	x	**1536a**	**1713c**	**1636b**	**1583ab**	**100**	**111**	**107**	**103**
	+ - ø		177	100	47				
	SD	11,5	15,3	55,1	32,1				
	CV	0,8	0,9	3,4	2,0				
Berry adherence strength	2014	683	691	655	613				
	2015	600	760	752	640				
	2016	620	714	680	658				
	x	**634a**	**722b**	**696ab**	**637a**	**100**	**114**	**103**	**100**
	+ - ø		88	62	3				
	SD	43,3	35,1	50,4	22,6				
	CV	6,8	4,9	7,2	3,6				

The berry firmness of the Ribier variety ranges from 1,536 g/cm^2 in the St variant to 1,713 g/cm^2 in the V1 variant. The V2 and V3 variants exhibit an average berry firmness of 1,636 g/cm^2 and 1,583 g/cm^2, respectively.

Additionally, the St variant is associated with the lowest berry adherence strength of 634 g/grain. A similar value was obtained for the V3 variant. The V2 variant demonstrated a 3% higher berry adherence strength compared to the standard variant, while the highest berry adherence strength was recorded in the V1 variant, exhibiting a 14% increase compared to the standard.

The berry firmness of the Italia variety (Table 4) ranges from 1,543 g/cm^2 in St variant up to 1,627 g/cm^2 in the V1 variant. Berry adherence strength on the Italia variety ranges from 690 g/grain (St variant) to 770 g/grain (V1 variant).

A statistically significant difference in the bunch and berry mechanical properties within the studied variants of the Italia variety was only observed in the V1 variant, particularly in terms of berry firmness. Similar significance was noted for this variant in terms of berry adherence strength. The coefficient of variation and the standard deviation, concerning the berry firmness property and berry adherence strength, did not exhibit significant differences among the variants. Detailed results of the tests conducted on the mechanical properties of this variety are presented in Table 4.

Table 4 Bunch and berry mechanical properties of the studied variants of Muskat Italia variety

		Variant				Index			
	Year	St	V_1	V_2	V_3	St	V_1	V_2	V_3
Berry firmness	2014	1 570	1 610	1 590	1 580				
	2015	1 520	1 600	1 570	1 540				
	2016	1 540	1 670	1 620	1 590				
	x	**1543a**	**1627b**	**1593ab**	**1570a**	**100**	**105**	**103**	**102**
	+ - ø		84	50	27				
	SD	25,2	37,9	25,2	26,5				
	CV	1,6	2,3	1,6	1,7				
Berry adherence strength	2014	690	760	745	710				
	2015	700	785	740	715				
	2016	680	765	710	690				
	x	**690a**	**770c**	**732bc**	**702ab**	**100**	**112**	**106**	**102**
	+ - ø		80	42	12				
	SD	10,0	13,2	18,9	13,2				
	CV	1,4	1,7	2,6	1,9				

The berry firmness and adherence strength of the studied varieties fall within the parameters of the varietal characteristics, a finding corroborated by the research of several authors in the region (Božinović, 2010; Roičev, 2012; Colapietra, 2006). A lower number of bunches per vine leads to an increase in berry firmness. This phenomenon is attributable to the enhanced availability of carbohydrates and other molecules, which, when integrated into the cell wall of the berries, contribute to their heightened firmness. Similar principles apply to minerals, such as calcium, which can be more effectively distributed among the bunches when there are fewer bunches per vine (Perez et al., 1998). Conversely, De Sousa Leao and Coelho De Lima (2017) did not establish a correlation between bunch thinning and berry firmness in their research.

Table grape varieties intended for fresh consumption are classified into two categories: packed grapes and waste. Packed grapes conform to the standards for packing and are divided into three categories: extra quality, first quality, and second quality. The waste comprises grapes that fail to meet the minimum standards for packed grapes and are typically utilized for various processing purposes. Production characteristics of table grapes, as indicated by the quantity of harvested grapes, are not assessed based on the total quantity of grapes harvested but rather on the quantity of packed grapes. The results confirm a close correlation between the amount of total harvested grapes and the number of bunches retained on the vine per square meter. A larger number of retained bunches leads to a higher yield. However, the quantity of packed grapes increases up to a certain threshold of load, beyond which it begins to decline. Consequently, the quantity of waste starts to increase. The specific load threshold is primarily contingent upon the biological characteristics of the respective variety.

Our tests revealed notable disparities in the load threshold between the two tested varieties. Our objective was to determine the influence of vine load on the quantity and quality of table varieties and to ascertain the most suitable degree of vine load. Based on the yield results highlighted in Tables 5 and 6, it can be deduced that the highest quantity of total harvested grapes in the Ribier variety was observed in the St and V3 variants. However, these variants exhibited the largest quantity of waste and the lowest percentage of packed grapes compared to the other variants. The quantity of grapes harvested in the Ribier variety ranged from 2.34 kg/m^2 in the V1 variant to 2.79 kg/m^2 in the V3 variant, whereas the quantity of packed grapes ranged from 1.9 kg/m^2 in the standard variant to 2.31 kg/m^2 in the V2 variant. In the Ribier variety, bunch thinning demonstrated a statistically significant difference in the quantity of packed grapes in the V1 variant, particularly in the V2 variant compared to the standard, while the packed grapes of the V3 variant did not exhibit a statistically significant difference compared to the standard variant and the V1 variant.

Table 5. Influence of the number of bunches on the amount of grapes harvested in the studied variants of Ribier variety

Type	Year	Variant				Percentage (%)			
		St	V_1	V_2	V_3	St	V_1	V_2	V_3
Packed grapes kg/m^2	2014	1,84	2,02	2,21	2	67,2	87,1	81,5	70,9
	2015	1,96	2,08	2,27	2,06	71	91,2	85,7	72,5
	2016	1,9	2,1	2,45	1,9	68,8	87,5	86	70,4
	x	**1,9a**	**2,07b**	**2,31c**	**1,99ab**	**69,0**	**88,6**	**84,4**	**71,3**
	+ - ø		1,7	4,1	0,09				
	SD	0,6	0,4	1,2	0,8				
	CV	3,2	2,0	5,4	4,1				
Waste	2014	0,9	0,3	0,5	0,82	32,8	12,9	18,5	29,1
	2015	0,82	0,2	0,38	0,78	29	8,8	14,3	27,5
	2016	0,86	0,3	0,4	0,78	31,2	12,5	14	29,6
	x	**0,86d**	**0,27a**	**0,43b**	**0,79c**	**31,0**	**11,4**	**15,6**	**28,7**
	+ - ø		-0,59	-0,43	-0,07				
	SD	0,04	0,06	0,06	0,02				
	CV	4,7	21,7	15,1	2,5				
Total	2014	2,74	2,32	2,71	2,82				
	2015	2,76	2,28	2,65	2,84				
	2016	2,76	2,4	2,85	2,7				
	x	**2,76b**	**2,34a**	**2,74b**	**2,79b**	100	100	100	100
	+ - ø		-0,42	-0,02	0,03				
	SD	0,01	0,06	0,10	0,08				
	CV	0,4	2,6	3,8	2,7				

In the Italia variety, the V1 variant is characterized by the lowest content of harvested and packed grapes, as well as the lowest percentage of waste. Of the total quantity of grapes harvested, which amounts to 2.71 kg/m^2 in the case of the V1 variant, 2.65 kg/m^2 or 97.6% belongs to the category of packed grapes. The waste category accounts for only 0.06 kg/m^2 or 2.4% of the total amount. Conversely, the V3 variant demonstrates the highest content of harvested and packed grapes, with a total quantity of packed grapes amounting to 3.58 kg/m^2. Among these, 3.27 kg/m^2 or 91.4% belongs to the category of packed grapes, while 0.31 kg/m^2 or 8.6% falls into the waste category. Statistical analysis within this variety revealed a significant difference concerning the quantity of packed grapes, particularly in the V2 variant and, notably, in the V3 variant when compared to the standard.

Table 6. Influence of the number of bunches on the quantity of harvested grapes in the studied variants of Muscat Italia variety

Type	Year	Variant				Percentage (%)			
		St	V_1	V_2	V_3	St	V_1	V_2	V_3
Packed grapes kg/m^2	2014	2,55	2,55	3,05	3,25	87,0	96,2	92,4	89,0
	2015	2,69	2,68	3,01	3,26	87,3	97,1	95,0	92,6
	2016	2,89	2,73	3,29	3,31	87,8	99,6	99,4	92,7
	x	**2,71a**	**2,65a**	**3,12b**	**3,27c**	**87,4**	**97,6**	**95,6**	**91,4**
	+ - ø		-0,6	4,1	5,6				
	SD	1,7	0,9	1,5	0,3				
	CV	6,3	3,5	4,9	1,0				
Waste kg/m^2	2014	0,38	0,10	0,25	0,40	13,0	3,8	7,6	11,0
	2015	0,39	0,08	0,16	0,26	12,7	2,9	5,0	7,4
	2016	0,40	0,01	0,02	0,26	12,2	0,4	0,6	7,3
	x	**0,39b**	**0,06a**	**0,14a**	**0,31b**	**12,6**	**2,4**	**4,4**	**8,6**
	+ - ø		-0,33	-0,25	-0,08				
	SD	0,01	0,05	0,12	0,08				
	CV	2,56	74,62	80,86	26,36				
Total kg/m^2	2014	2,93	2,65	3,30	3,65				
	2015	3,08	2,76	3,17	3,52				
	2016	3,29	2,74	3,31	3,57				
	$\bar{x}$	**3,10b**	**2,71a**	**3,26b**	**3,58c**	100	100	100	100
	+ - ø		-0,39	0,16	0,48				
	SD	0,18	0,06	0,08	0,07				
	CV	5,83	2,16	2,40	1,83				

Several authors, such as Kavoosi et al. (2009) and Prculovski et al. (2021), studying the effects of bunch thinning observed a reduction in total yield but an increase in grape quality and quantity of packed grapes. Conversely, intensive thinning can cause a notable reduction in yield (Avizuri-Inac et al., 2013; Fanzone et al., 2011). The yields observed in our tests are within the parameters of the varietal characteristics studied by various authors in the region, including Božinović (2010), Avramov and Žunić (2001), and Žunić and Garić (2017).

The assessment of grape quality was conducted using standard organoleptic evaluation techniques. Our tests employed the 10-point system. The expert tasting committee evaluated the submitted samples of each variant based on external appearance, consistency, taste, as well as the typicity and authenticity of the variety, utilizing their senses of sight, taste, and smell. Additionally, table grape standards were defined for the studied varieties and variants, as outlined by the Codex Alimentarius Commission (2007).

The results of the tasting evaluation for the Ribier and Italia varieties are depicted in Charts 1 and 2. The results for the Ribier variety illustrate variations in the external appearance of the grapes, consistency, taste qualities, and the overall tasting score across the studied variants. The total tasting score for the variants in this variety ranges from 7.6 points for the St variant to 8.4 points for the V1 variant. Grapes from the V1 and V2 variants are classified as excellent quality, while those from the St and V3 variants as very good quality.

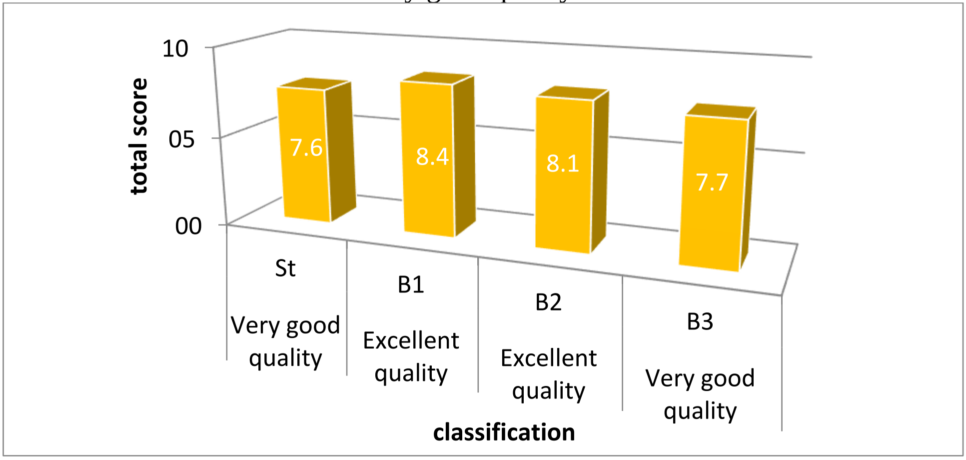

Chart 1. Total tasting score of the studied variants of Ribier variety

For the Italia variety, grapes from the V1 variant display the best external appearance, scoring 2.8 points, while grapes from the St variant exhibit the weakest external appearance, scoring 2.3 points.

The highest total score of 9.2 points was found in the V1 and V2 variants, whereas the lowest score of 7.9 points was observed in the St variant (Chart 2).

Grapes from the V1 and V2 variants are classified as exceptional quality, those from the V3 variant as excellent quality, and those from the St variant as very good quality.

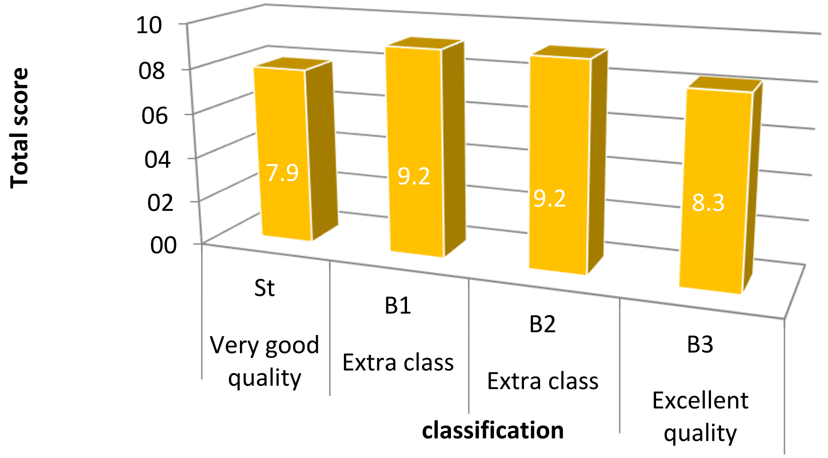

Chart 2. Total tasting score of the studied variants of Muscat Italia variety

CONCLUSIONS

The implementation of bunch thinning in the Ribier and Italia varieties, within the 7-14 day period after flowering, resulted in a significant increase in the average bunch and berry mass, enhanced transportability, increased quantity of packed grapes, and improved organoleptic evaluation. For the Ribier variety, thinning to 5 bunches/m^2 is recommended to attain the best quality grapes, while in the Italia variety, the optimal balance between yield and quality is achieved through thinning to 6 bunches/m^2.

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RESEARCH OF THE CHARACTERISTICS OF AN ADULT WOODY ALBINO MESIAN BEECH (*FAGUS MOESIACA, ASINE PIGMENTE*) AS A TREE FORM IN CENTRAL SERBIA

SUMMARY

The paper presents the beginning of the research on the characteristics of albino beech in the Central Serbia region. The studied beech is perennial, of sprout origin, fruitful, and most likely parasitic on the parent tree, considering that its lack of chlorophyll renders it unable to feed and grow on its own. By measuring the physical characteristics of common and albino beeches that share the same root system, and by comparing the length and width of their leaves, the obtained results show that the leaves of albino beech are noticeably smaller. The average length of leaves of common beech is 7.19 cm, while the average length of leaves of albino beech is 6.08 cm. The average width of leaves of common beech is 3.83 cm, whereas the average width of leaves of albino beech is 3.35 cm. To achieve transpiration as a basic physiological function of any tree form, albinism causes significant weakening of the maximum strength and sap during water transport. Additionally, since the leaf is deprived of any of the essential pigments - chlorophyll (a, b, c, d), anthocyanin, carotene - this results in dwarf growth or the formation of bush-like forms of trees. For genome sequencing, it is planned to take samples of leaves with and without pigment and preserve them in liquid nitrogen at a temperature of -195°C in special canister freezers with liquid nitrogen. The view on the phenomenon and the potentially still unrecognized mechanisms of functioning of the plant itself, as well as the plants around it, fungi associated with its root system, or even the discovery of a completely new network or system of connections, remains to be uncovered. What enables the nutrition and influx of basic sugars into this plant may be revealed through the sequencing of its entire genome, which is a basic, logical, and one of the first steps to be taken. The exact location of the plant is kept confidential at this time.

Keywords: beech, albinism, chlorophyll, parasitism, mycorrhiza, Serbia

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INTRODUCTION

Albinism (from Latin *albus*: white) occurs in both animals and plants and is defined as a congenital or hereditary inability of an organism to produce pigment or coloration. Albinism is a recessive gene and usually results in reduced chances for survival of such organisms. The probability of the occurrence of albinism is 1 in 20,000, but the occurrence of albinism in plants means that due to the lack of chlorophyll pigment (a, b, c, or d), they are unable to transform solar energy into food. As a result, after seed germination, they usually do not live more than a few days or weeks, making the presence of adult albino individuals extremely rare worldwide.

The snow-white color of leaf tissue is due to the partial or complete loss of chlorophyll pigments and the incomplete differentiation of chloroplast membranes, which hinders or completely prevents photosynthesis, a process that plays a crucial role in the growth and development of plants (Kumari *et al,* 2009). Therefore, if chlorophyll is absent or deficient for any reason (primarily due to mutations), or if the chloroplast as an organelle is not functioning properly, it leads to a highly probable fatal outcome for the plant organism.

It is important to note that just as an albino person must take protective measures, a plant must do the same, although it is far more complex in its case. At the beginning of the development of an albino plant, there are certain mechanisms that allow it to nourish itself and develop its first leaves, enabling it to begin continuous and reliable self-care (seed reserves or direct connection with a neighbor). These are nutrients in the form of fats contained in the tissue within the cotyledons. By breaking down these molecules, the plant obtains more than enough energy to sprout into the beginnings of a stem and root and to produce its first white leaves. Once these nutrients are completely used up, the plant without chlorophyll depletes all its reserves, after which it will not be able to survive on its own and will soon wither, thus ending its life cycle. Given that the albino plant lacks the ability to develop reproductive organs, it prevents the possible error from spreading to its offspring, as anticipated by evolution. However, this phenomenon has also been observed later on in the development, allowing some plants to mature to a level of fertility and thus leave offspring that inherit these traits. Naturally, the development of these albino plants is not due to chlorophyll (any type from a to d), but possibly to a range of factors: genetic mutations, extremely complex interactions between spatially close living organisms, perhaps a pigment that doesn't behave like the others – some new and unique environmental conditions...

One of them is a specific phenomenon involving fungi - mycorrhiza. Characterized as a type of interspecies relationship between two or more organisms, mycorrhiza conditions or facilitates their survival by participating in the transport of molecules between the fungal mycelium and the plant's root system. If such a mutated albino specimen occurs, it manages to survive and develop into an adult exclusively by parasitizing the fungus (more as a symbiont than a kleptoparasite, as the fungus also stores nitrogen from the air in this manner). Such individuals are known as mycoheterotrophs. The most well-known example of a mycoheterotrophic plant is *Monotropa uniflora* (Yang and Pfister, 2006). As a mycoparasite, it draws from the mycelium in the existing mycorrhizal network between fungi (which were determined to be mostly from the genus *Russula*) and conifers, obtaining sugars originating from the conifers that were originally destined for the fungus. This makes it a true, adapted albino individual, enabling it to live a full lifespan despite a trait that would in nature almost certainly result in its shortened life expectancy.

Another exception is found in the albino sequoias (*Ghost sequoia*; for normal trees with half plant organ *pigmente chlorophille* possession it is *S. giganteum*, (Lindl.) J.Buchh., 1939), where specimens over 100 years old and up to 20 meters in height have been found. These trees propagate through propagules - underground stems from which new individuals emerge. They feed on roots, thus behaving as parasites. The tree parasitized by the albino specimen does not reject it because concentrations of toxic metals within the white needles (nickel, cadmium, copper, etc.) are deposited far from the photosynthetic leaves, thus not hindering their function, giving these mutated individuals an adaptive advantage.

The albinism of the specimen described herein - the beech from the Central Serbia area, which is surviving with all vital organs intact, has so far never been recorded anywhere, nor have similar specimens been documented or described in the entire global flora. Since this is a sprout stand and an ancient, decayed, shared root / root flare (conditionally speaking), the discussion chapter mentions the surrounding plants as being directly or indirectly linked to this system. The potential connections could include proximity, links to the same decayed branch in the ground, or something that is yet to be explained but cannot even be surmised at this time. This truly unique research on the albino beech as a flowering plant from the family Fagaceae and as a specific discovery could offer an entirely new perspective, possibly even shedding light on phenomenological aspects and perhaps even unrecognized mechanisms of the plant's functioning, the plants around it, fungi associated with its root system, or even the discovery of a completely conditionally termed new network or system of connections that enable the nutrition and supply of basic sugars to this plant. Considering that it evidently does not produce them on its own, or it does so by a yet unknown method, this should be elucidated as soon as possible - even during this vegetation season, phenology allowing. As science would suffer a significant loss if this specimen were to perish by tragic circumstance, the related work should be highly prioritized and expedited, precisely because of the value of this singular specimen.

MATERIAL AND METHODS

Samples for analysis were taken from a beech stand on a private property, in the area of the Municipality of Aleksinac, Golešnica cadastral municipality. The albino beech, several years old, is of sprouting origin (growing out of a stump). The terrain is inaccessible and very steeply sloped, at the elevation of 600.049 meters above sea level and the eastern exposure.

On April 18, 2024, soil samples were collected using the standard method at depths of 0-10 cm, 10-20 cm, and 20-30 cm for the following pedological analyses:

active and exchangeable acidity; accessible forms of P_2O_5 i K_2O; total humus and nitrogen; soil texture composition. If the pH in KCl is less than 7, hydrolytic acidity and base sum will be examined, and if the pH is greater than 7, the content of free carbonates will be assessed.

Samples of the roots of the albino beech were collected using a random sampling method for potential examination of the presence of mycorrhizal fungi. At the same time, samples of leaves with and without pigment were collected in order to measure their physical characteristics (leaf length and width) and chemical composition (content of toxic metals within albino leaves - nickel, cadmium, and copper).

For the purposes of genome sequencing, samples of leaves with and without pigment will be collected and preserved in liquid nitrogen at a temperature of -195°C using special canister freezers.

RESULTS AND DISCUSSION

However unfavorable albinism may be viewed as a characteristic in nature, it is evident that it manages to find a way to survive and contribute to the already rich diversity of the plant world.

The examined albino beech specimen is surrounded by several beech trees without any signs of mutations (with green leaves) that originate from the same stump (picture 1), suggesting that it feeds and develops at the expense of the parent root system. The albino beech is spherical in shape, about 50cm tall, with the presence of fruit cupules observed on it (picture 2).

Picture 1. Appearance of the albino beech specimen

Picture 2. Fruit of the albino beech

Picture 3. Leaf of the albino beech

Picture 4. Geometridae on an albino leaf

Picture 5. Albino leaf damage

Table 1. Dimensions of leaves from albino and green beech collected from the same root by random sampling

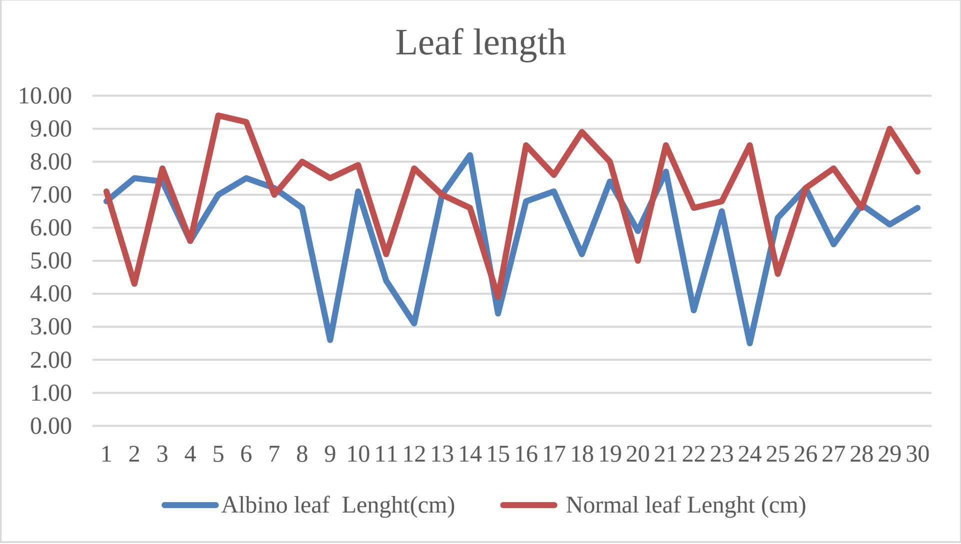
No.	Leaf length (cm)		Leaf width (cm)	
	Albino	Green	Albino	Green
1	6.8	3.7	7.1	4.5
2	7.5	4.3	4.3	2.5
3	7.4	4.4	7.8	4.3
4	5.6	3.5	5.6	3.4
5	7.0	4.0	9.4	5.2
6	7.5	3.6	9.2	4.7
7	7.2	3.5	7.0	3.6
8	6.6	3.5	8.0	3.8
9	2.6	1.2	7.5	3.9
10	7.1	3.6	7.9	3.9
11	4.4	2.3	5.2	2.7
12	3.1	1.4	7.8	4.3
13	7.0	3.8	7.0	3.5
14	8.2	4.8	6.6	3.5
15	3.4	1.5	3.9	1.8
16	6.8	3.6	8.5	4.5
17	7.1	4.0	7.6	3.7
18	5.2	2.4	8.9	4.9
19	7.4	4.3	8.0	4.1
20	5.9	3.5	5.0	2.7
21	7.7	4.4	8.5	4.5
22	3.5	1.5	6.6	3.3
23	6.5	3.7	6.8	3.6
24	2.5	1.2	8.5	5.0
25	6.3	3.6	4.6	2.4
26	7.2	4.1	7.2	3.6
27	5.5	3.4	7.8	4.1
28	6.7	4.3	6.6	3.8
29	6.1	3.5	9.0	4.7
30	6.6	3.9	7.7	4.3

The occurrence of a beech with snow-white leaves was first noticed in the fall of 2022 by the owner of the forest. The leaves of this beech are of pure white color (Image 3), very thin and silky compared to the green leaves of the surrounding trees. During the control inspection in April 2024, a noticeably higher presence of defoliators –winter moths (Geometridae) (Picture 4) was recorded on these leaves, as well as damage to the leaf mass - chew marks (Picture 5), which is a consequence of the extremely soft leaves that caterpillars can chew more easily. The goals and main purpose of the research is precisely the importance of further examining the importance of beech forests in Europe and further studying them as an ecosystems.

Physical characteristics of the examined leaves of *Fagus moesiaca, asine pigmente* - albino beech

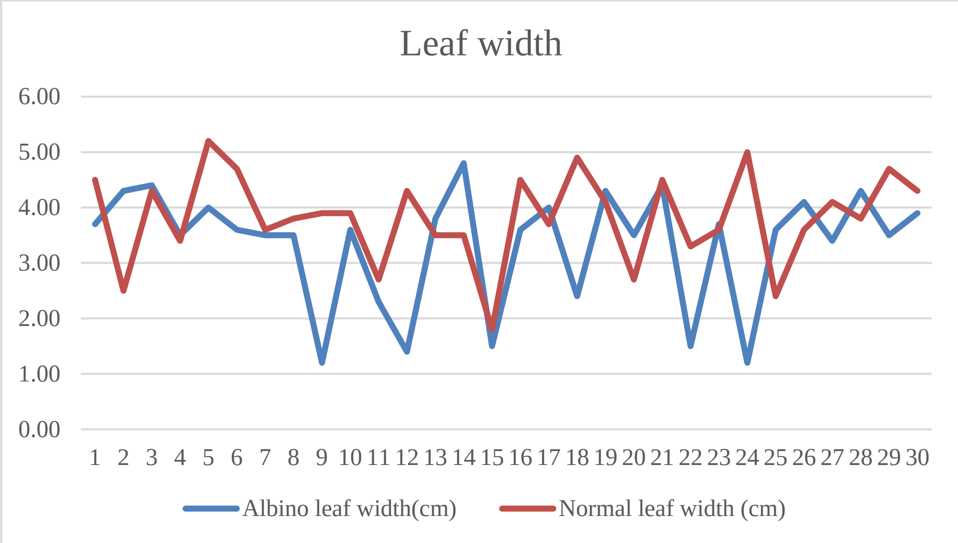
The leaves of the albino beech are of regular shape, somewhat smaller in size than the green leaves (Table 1), while this year's twigs are light, pale pink in color.

According to the literature sources (Silva *et al.*, 2020), albino plants exhibit reduced growth, which is why a comparison of the leaf sizes of white and green beech was performed. Graph 1 shows the leaf length of the albino beech and the common beech from the same site. The parameter used for the graph is leaf length. For the purposes of comparison, 30 leaves of each type were measured. It is apparent that the leaves of the common beech are somewhat larger, with the maximum length being 9.4 cm, while the longest leaf of the albino beech is 8.2 cm. The average leaf length of the common beech is 7.19 cm, while the average length of the albino beech leaves is 6.08 cm. It can also be observed that in the selected sample of 30 measured leaves, 24 leaves of the common beech were longer than those of the albino beech. In other words, 80% of the albino beech leaves in the sample recorded smaller dimensions when length is used as the parameter.



Graph 1. Leaf length of the albino and common beech

Graph 2 shows the comparison of the leaf width of the albino beech and the common beech from the same site. The parameter used for this graph is leaf width. It is apparent that once again, in this case, the leaves of the common beech are somewhat larger, with the maximum width being 5.2 cm, while the widest leaf of the albino beech has a width of 4.8 cm. The average width of the common beech leaves is 3.83 cm, while the average width of the albino beech leaves is 3.35 cm. It can also be observed that in the selected sample of 30 measured leaves, 24 leaves of the common beech were wider than those of the albino beech. In other words, 60% of the albino beech leaves in the sample recorded smaller dimensions when width is used as the parameter.



Graph 2. Leaf width of the albino and common beech

Diagram 1. Difference between the average size of albino and green leaves

Diagram 1 shows the difference between albino and green leaves, with the note that average values were used for the illustration. The larger, dark green leaf represents the common beech and has a width of 7.19 cm and a length of 3.83 cm, while the smaller, light leaf represents the albino beech and has a width of 6.08 cm and a length of 3.35 cm.

Height and thickness characteristics and causes of the dwarf and bushy form of the specimen *Fagus moesiaca, asine pigmente* - albino beech

In plants with chloroplasts without pigment, the absorption through the root and the pressure necessary for the transport of water from the root to the leaf would be too weak for heights over 1-1.5 meters (Wheeler-Dubas 2017, taken from https://simbioza.bio.bg.ac.rs/albino-biljke/). To achieve transpiration as the basic physiological function of any tree form, this would be the maximum power and reach for water transport when the leaf is devoid of any of the essential pigments: chlorophyll (a, b, c, d), anthocyanin, carotene... Anything contrary in terms of trunk length and height, due to the basic laws of physics, would be an insurmountable problem and could even kill the plant, especially during the period when dormancy ends - that is, when vegetation survives the winter. The sap flow, i.e., the strong movement of plant juices through the vessels of the xylem and phloem from the root hairs to the stomata and vice versa (but with assimilation solutions) where transpiration and water release occur, would be impossible. The existence of another plant and its connection to it would be, hypothetically speaking, sufficient to sustain a specimen with dwarf characteristics, but only those that are related to and connected with the morphological traits of a specimen with such chloroplast structure - organelles with such pigmentation, and even without any pigment. In short, the existence of a connection with another specimen explains the height, i.e., the lack of potential for this beech to achieve even the average height and thickness growth typical for the species over the years. Albinism brings with it a dwarf form, limited growth, and the expected formation of multiple shoots similar to bushy forms in tree forms (taken from http://bioloska.blogspot.com/2012/12/albino-biljke-bez-zelenog-hlorofila-u.html).

The peculiarity of entomological damage on *Fagus moesiaca, asine pigmente* - albino beech

The coloration or mimicry of early oak defoliators from the family Geometridae - winter moths varies from light brown and reddish-brown to green in different shades. Insect species that generally live in Central Serbia, due to the dominant acting agents (adaptation to the environment and coloration derived from food), are mainly brown, green, or yellow-green in color. In the case of darker colors, they originate from tannins proved to be secreted by all attacked plants, or from other pigments when these pigments are the main component of their diet. This is especially true for specimens whose assimilation organs are the main source of nutrients. It is important to note that on-site (on the leaves of the described beech specimen), there were two larvae of the large winter moth found: one almost completely white, and the other beige in color (Picture 4), which specifically indicates and correlates with the coloration of the branches, which are reddish-pink, and the snow-white leaves. Just as it is characteristic of albinism in the animal kingdom, systematically categorized into the classes of reptiles, birds, and mammals, albinism is marked by the absence of melanin pigment and the reddish-pink coloration of the irises. It appears that our (human) vision is capable of recognizing colors of this spectrum in the same way.

Albinism as a phenomenon in plants of different systematic affiliations in *Fagus moesiaca, asine pigmente* - albino beech

Similar to parasitic plants and mycoheterotrophs, chlorophyll (i.e., photosynthetic capacity) may be lacking, but such plants are forced to live in symbiosis with fungi that obtain food from autotrophic plants (mycorrhiza). Although this relationship can even lead to the death of the host, mycoheterotrophic plants are not classified as parasites because they do not harm the host, which is the case with the examined beech specimen. All specimens of the same species in the immediate vicinity are in optimal health and vitality, without visible changes in defoliation or chlorosis, and even healthier with regard to the presence of damage. Mycelium, or the tissue or hyphae of fungi, is present, connecting root systems through a mechanism called mycorrhiza. Mycorrhiza is generally associated with the Fabaceae family in nature, so these fungi (among beeches) need to be thoroughly investigated (Yang & Pfister, 2006). Since these are not semi-parasitic plants (i.e., specifically the analyzed albino specimen), it is logical that they also lack the basic organs typical for them –haustoria, and nothing resembling such a connection has been observed. This separation in this sense is clearly visible.

Due to the exclusion of this phenomenon, haustoria (from Latin *haurire* - to draw, absorb) or so-called suckers are a specific type of organ in parasitic and semi-parasitic flowering plants that grow into the body of the host plant, from where they extract nutrients. The widely known orchids are a typical example (Wheeler-Dubas, 2017; accessed on April 29, 2024).

Another option that was taken into consideration is the so-called Beechdrops (‘beech tears”) event. Plants exhibiting this phenomenon show an intriguing characteristic of producing two types of flowers and the way in which pollination occurs. The first type has open corollas (chasmogamous inflorescences), where cross-pollination typically occurs, usually at the top of the inflorescence. In the second case, with closed (cleistogamous) flowers, self-pollination is evident, most often at the base of the inflorescence. This is particularly noticeable during mass flowering in the spring, as seen in Picture 6, where these flowers are quite large and prominent (http://dept.ca.uky.edu/PLS220/Formparasite.pdf).

Regressive evolution in *Fagus moesiaca*, *asine pigmente* - albino beech

Albino flowering plants (like the beech described here) are often the result of regressive evolution, which, along with the absence of chlorophyll in holophytes, is a phenomenon and deviation that merits detailed and thorough investigation of individual genes, groups of genes, or entire sequences within the genome. This can be done using the latest methodology which currently being developed. In typical and state-of-the-art research of this phenomenon, called DNA sequencing, the 3D spatial structure of the chlorophyll molecule from the organelle – chloroplast - is shown in Diagram 2, while the material for these studies is taken from the nuclear content.

The next step would be a fully focused examination of the moot sequence or gene group, but it is possible that due to the uniqueness, intriguing nature, and exceptional rarity of this phenomenon that is referred to as pigment-less (a, b, c, d) phenomenologically explainable occurrence, the work must entail a well-organized verification of all physiological and vital characteristics of the living plant. Upcoming examinations will involve the entire chromosomal content—the entire mentioned genome of this specimen - precisely because of the relatively new characteristics of chlorophyll that are still being studied, but also for all the reasons mentioned above. On this basis, it is expected that findings will be made and that conclusions will be drawn, which are either partially known or entirely unknown, similar to the results of previous studies conducted worldwide. This is further evidenced by the limited scope in the search for the existing references on albino flowering plants (Angiospermae). Albinism and these plants - the beech from the Central Serbia region, which survives with all its vital organs –do not exist at this time, and only those indirectly related to this phenomenon are mentioned here. According to Candeias M. (2016), among flowering plants or the clade of higher plants, angiosperms [Angiospermae, flowering plants] - here specifically tree forms - this phenomenon is more frequent and linked to the group of semi-parasitic flowering plants—orchids, in which the mechanism of so-called pseudo-kleptoparasitism in feeding has already been explained and studied (retrieved November 14th, 2022).

Picture 6. The appearance of inflorescence of *Fagus moesiaca*, February 20, 2024. Site Kraljeva stolica, Fruška Gora ***Original***

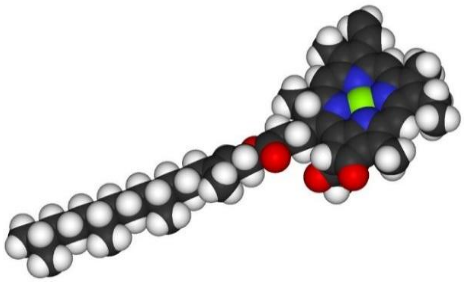


Diagram 2. Spatial model of a chlorophyll molecule, absorbing light in blue (430-490 nm) and red (630-760 nm) parts of the spectrum –where is the green color coming from, ref. [*Monotropa uniflora*, Retrieved November 14, 2022]

However, the study of the beech as a flowering plant from the family Fagaceae may contain entirely new insights into this phenomenon and possibly yet unrecognized mechanisms of the plant's functioning, the plants around it, fungi associated with the root system, or even the discovery of a completely new network or system of connections that enables the nutrition and supply of basic sugars to this plant, considering that it evidently does not produce them itself [(https://www.enciklopedija.hr/clanak/regresivna-evolucija.;Virtanen *et al.*, 2020)].

CONCLUSIONS

By measuring the physical characteristics of common and albino beeches that share the same root system, and by comparing the length and width of their leaves, the obtained results show that the leaves of albino beech are noticeably smaller. The average length of leaves of common beech is 7.19 cm, while the average length of leaves of albino beech is 6.08 cm. The average width of leaves of common beech is 3.83 cm, whereas the average leaf width of albino beech is 3.35 cm.

A pedological analysis is currently under way to perform the following examinations: active and exchangeable acidity; accessible forms of P_2O_5 i K_2O; total humus and nitrogen; soil texture composition. If the pH in KCl is less than 7, hydrolytic acidity and base sum is examined, and if the pH is greater than 7, the content of free carbonates is assessed.

The examination of the chemical composition of leaves (in particular concentration of toxic metals within albino leaves – nickel, cadmium, and copper) is also in progress.

The sequencing of the genome of albino and green leaves is planned. Continued research is undertaken in order to determine the cause of this mutation and the formation of this extremely rare adult albino beech specimen, but in particular in order to determine the conditions under which it survives in nature and successfully bears fruit.

The conclusions determined by systematic affiliation about albinism as a phenomenon are that this occurrence is more common in lower plants—ferns, Pteridophyta (Pallardy, 2018), then gymnosperms, with the most well-known example being the sequoia [*Sequoia sempervirens* (D. Don) Endl. Gymnospermae]. This occurrence can be caused by both cross-pollination and self-pollination, as seen in this exceptional and very rare specimen - *Fagus moesiaca asine pigmente chlorophyll* (*a*, *b*, *c*, *d*), found right here in Serbia.

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HYDROCLIMATIC DYNAMICS AND WATER RESOURCE MANAGEMENT IN THE LOUKKOS BASIN (1950-2000): INSIGHTS FOR SUSTAINABLE DEVELOPMENT IN MOROCCO

SUMMARY

Morocco's sustainable water resource management is critical for its development, particularly in the water-rich Loukkos Hydraulic Basin. However, climate change projections indicate impending impacts, necessitating integrated planning. This study analyzes precipitation, temperature, and runoff data from 1950-2000 to establish comprehensive datasets, including mean areal precipitation (MAP) and mean areal temperature (MAT). Seasonal and annual regression equations linking runoff to MAP and MAT were developed, yielding historical runoff series (Qbaseline). Insights gained are pivotal for adaptive water management amidst climate change.

Keywords: Water Resources, Climate Change, MAP, MAT, Qbaseline, Multiple Regression, Management, Sustainable Development.

INTRODUCTION

Water is an essential and freely available natural resource that supports life on Earth and is also a vital factor for the economic and social development of society (Halli et al., 2023). Morocco's commitment to sustainable water resource management is paramount to its development agenda (Ouallali et al., 2024), particularly in regions like the Loukkos Hydraulic Basin, known for its abundant water resources. However, projections indicate that climate change will

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significantly impact this region, posing challenges to its water security and management strategies (IPCC, 2021).

Integrating climate change considerations into water resource planning processes is thus imperative (Badraoui & Berdai, 2011; LHBA, 2012; MEMSD, 2022; GIZ, 2013; Hahn & Fröde, 2011; WB, 2011).

This study aims to address these challenges by analyzing comprehensive datasets of precipitation, temperature, and runoff. These datasets include mean areal precipitation (MAP) and mean areal temperature (MAT), and involve the development of seasonal and annual multiple regression equations linking runoff volumes to MAP and MAT. These equations are crucial for calculating seasonal historical runoff series (Qbaseline), essential for effective water resource management and adaptation strategies in the face of climate change (LHBA, 2012).

The Loukkos Hydraulic Basin Agency's Master Plan for Water Resources (IMPWR) forms a foundational document in this context, outlining current water resource assessments, identifying challenges, proposing solutions, and setting the stage for sustainable management practices (LHBA, 2012). However, the plan did not foresee the impacts of climate change on water resources, underscoring the need for this study's integrated approach.

This introduction sets the stage for a methodological examination that systematically analyzes hydroclimatic data to enhance understanding of water resource dynamics in the Loukkos hydraulic basin. The methodology section outlines steps involving data collection, processing, and analysis, crucial for deriving meaningful insights into the impacts of climate change on water resources in Morocco's northwestern region.

The goal of research is analyze the hydroclimatic dynamics in the Loukkos Hydraulic Basin, focusing on the period from 1950 to 2000. The study aims to provide insights that are essential for sustainable water resource management in Morocco, particularly in light of projected impacts from climate change.

Key objectives of the research include: (1) Analyzing historical data on precipitation, temperature, and runoff to develop comprehensive datasets. (2) Establishing mean areal precipitation (MAP) and mean areal temperature (MAT) series. (3) Developing seasonal and annual regression equations linking runoff volumes to MAP and MAT. (4) Calculating seasonal historical runoff series (Qbaseline) to understand past hydrological trends and patterns. (5) Providing essential insights and recommendations for effective water resource management and adaptation strategies in response to climate change.

The research also aims to integrate climate change considerations into the water resource planning processes, highlighting the importance of adapting management strategies in response to projected climate impacts. By leveraging historical data and statistical analyses, the study seeks to contribute valuable knowledge to enhance sustainable development practices in the Loukkos Basin and similar regions facing similar challenges globally.

MATERIAL AND METHODS

Study area: North Morocco is vulnerable to water erosion risk (Bammou et al., 2024; Sadkaoui et al., 2024; Ouallali et al., 2024). The Loukkos hydraulic basin, located in this region, covers a total area of 13,000 km² and is known as one of the wettest regions in the country. This basin exhibits a diverse climate ranging from humid to semi-arid, contributing to a substantial renewable water potential estimated at approximately 4 billion m³/year (3,600 Mm³/year from surface water and 460 Mm³/year from groundwater, LHBA, 2012).

According to Morocco's fourth national communication to the United Nations Framework Convention on Climate Change (UNFCCC), the Loukkos basin is already vulnerable to climate-related hazards (MEMSD, 2022). Therefore, a major challenge in the coming decades will be adapting to the new context and challenges imposed by climate change (CC), while simultaneously managing these resources carefully and establishing effective and transparent mechanisms for their allocation.

The Loukkos Hydraulic Basin Agency's Master Plan for Water Resources (IMPWR), adopted in 2012, assesses the current state of water resources, evaluates water demands, identifies and prioritizes challenges to be addressed, proposes potential solutions to ensure water security in the region, and outlines the necessary means to achieve these goals (LHBA, 2012). However, this plan did not incorporate future climate change impacts on water resources.

Methodology: The methodology employed in this study follows a systematic approach aimed at comprehensively analyzing and processing hydroclimatic data to assess water resource dynamics in the Loukkos hydraulic basin. The methodological steps undertaken are outlined as follows:

***Data Collection*:** Initial data collection involved gathering monthly and annual precipitation, temperature, and runoff data from relevant sources.

Preliminary Processing and Formatting: Data underwent preliminary processing to ensure uniform formatting and consistency across the time series.

Identification and Correction of Outliers: Outliers and potential data errors were identified and corrected to enhance the reliability of the dataset.

Calculation of Monthly, Seasonal, and Annual Data: Monthly, seasonal, and annual averages were calculated from the processed data to capture seasonal variations and long-term trends.

Evaluation of Series Homogeneity: The homogeneity of the data series was assessed to verify consistency and reliability throughout the study period.

Data Processing: This phase involved several key analyses:

MAP and MAT Series Development: Mean Areal Precipitation (MAP) and Mean Areal Temperature (MAT) series were developed using the Thiessen polygon method to spatially represent climate variables across the basin.

Multiple Regression Analysis: Statistical relationships between historical runoff volumes and the MAP and MAT series were established using multiple regression analysis.

Calculation of Seasonal Historical Runoff Series (Qbaseline): Historical runoff series (Qbaseline) were calculated based on the developed regression equations, providing insights into past hydrological trends and patterns.

By employing these methodological steps, this study aims to enhance understanding of hydroclimatic variability in the Loukkos hydraulic basin and support informed decision-making for sustainable water resource management in the face of climate change impacts.

Equations of the following form have been developed:

$$Q = a * MAP + b * MAT + c \quad (1)$$

Where Q is the runoff volume in Mm3, MAP is the seasonal mean spatial precipitation in mm, MAT is the seasonal mean spatial temperature in °C and c is the intercept.

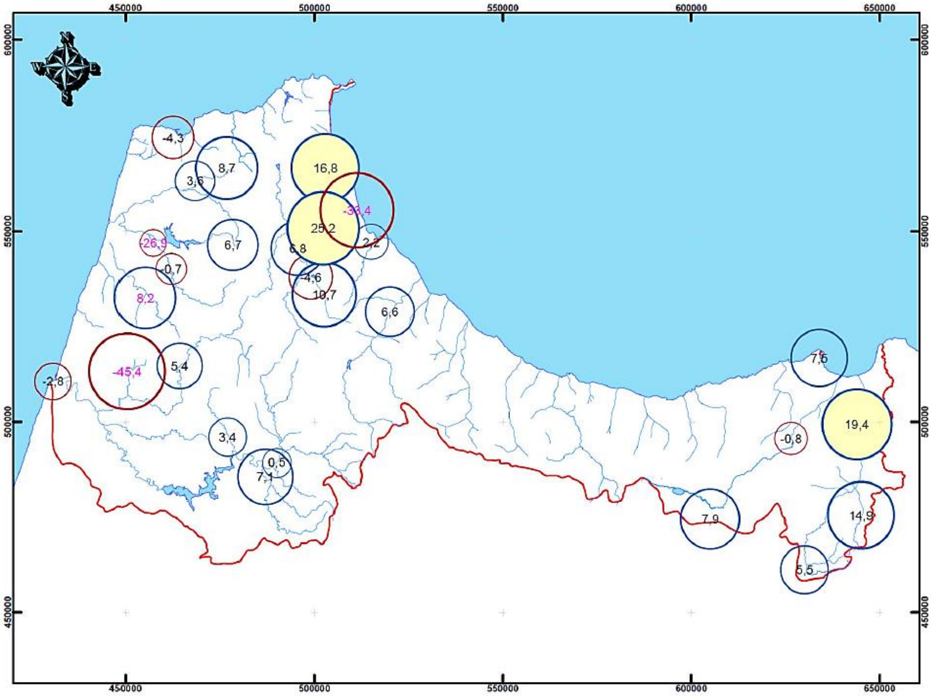

Fig. 1. Map showing observed trends in annual rainfall at LHBA.
Red circles represent a negative trend; blue circles represent a positive trend; the size of the circle represents the importance of the trend; the color of the text represents the number of years of data available (Magenta writing: Number of years <30 and Black writing: Number of years >30).

RESULTS AND DISCUSSION

For each region within the study area, specific sub-basins were selected as focal points for climate change planning, based on the availability of data. These areas are situated between the Tangier hydrological unit and the Western Mediterranean hydrological unit. The selected sub-basins were further categorized into sub-regions labelled as Q1 to Q15 and Q16 to Q20.

This version maintains your original intent while improving readability by ensuring the information flows logically.

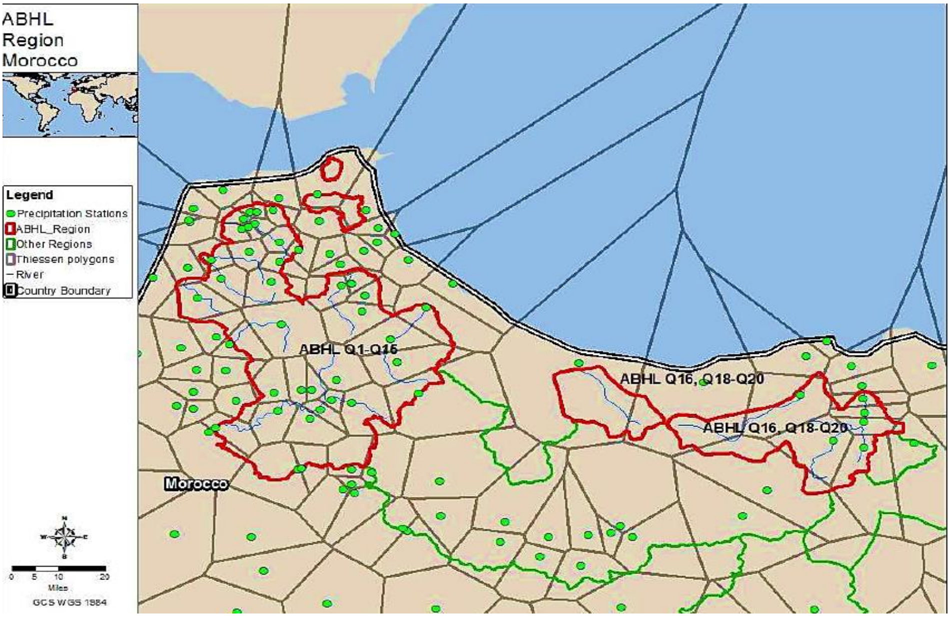

Fig. 2. Thiessen polygons of LHBA pluviometric stations for calculating the MAP series.

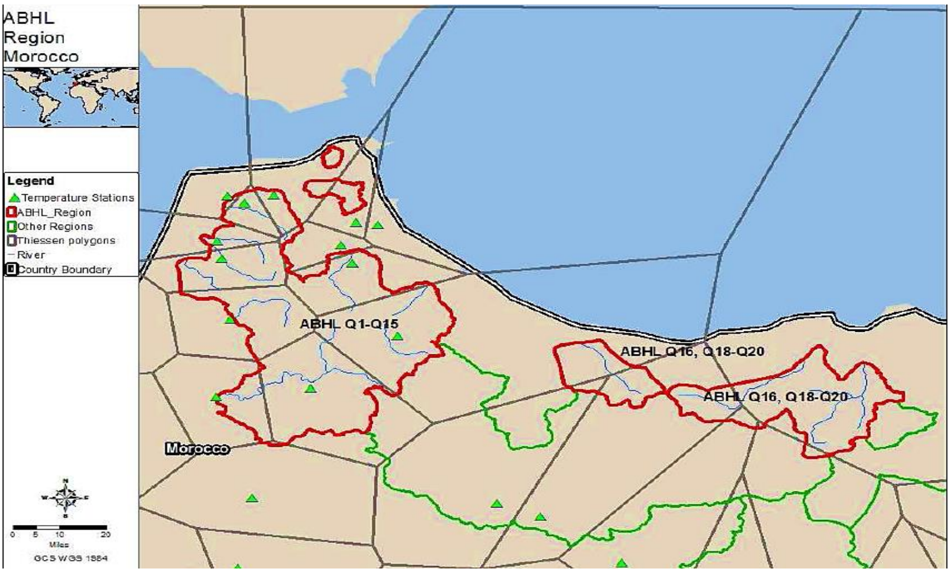

Fig. 3. Thiessen polygons of LHBA temperature stations for calculating the MAT series.

Table 1. Summary of regression equations for LHBA (Q1 - Q15).

Season	Equation	R^2
Annual	Q = -339.025 + 0.908 * MAP	0.834
1 (Dec-Feb)	Q = -124.105 + 0.939 * MAP	0.828
2 (Mar-May)	Q = 22.325 + 0.640 * MAP – 2.110 * MAT	0.627
3 (Jun-Aug)	Q = 78.728 + 0.081 * MAP – 2.868* MAT	0.133
4 (Sep-Nov)	Q = -21.586 + 0.303 * MAP	0.522

Table 2. Summary of regression equations for LHBA (Q16 – Q20).

Season	Equation	R^2
Annual	Q = 0.781 + 0.200 * MAP – 0.919 * MAT	0.660
1 (Dec-Feb)	Q = -5.261 + 0.183 * MAP	0.732
2 (Mar-May)	Q = -1.587 + 0.197 * MAP	0.703
3 (Jun-Aug)	Q = 2.632 + 0.072 * MAP	0.119
4 (Sep-Nov)	Q = -2.501 + 0.132 * MAP	0.787

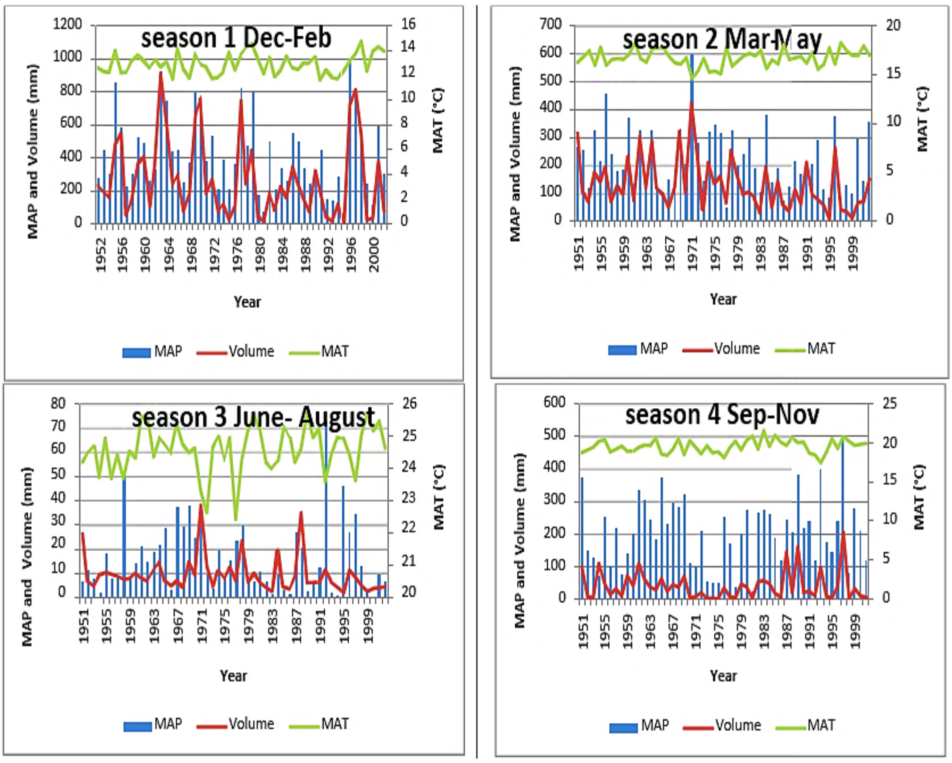

Fig. 4. Seasonal MAP, MAT and runoff for LHBA (Q1 to Q15).

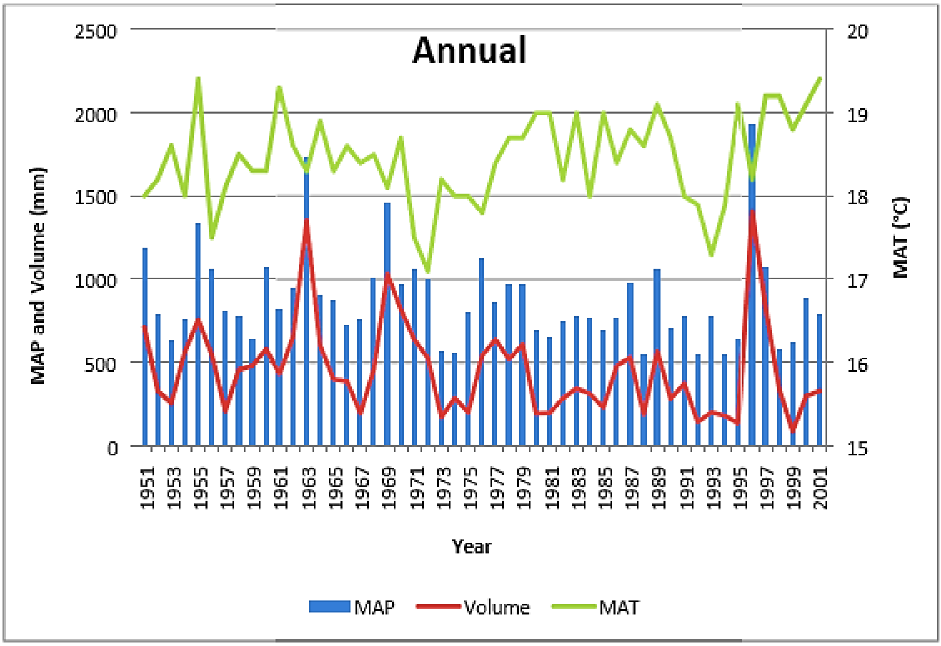

Fig. 5. Seasonal MAP, MAT and runoff for LHBA (Q16 to Q20).

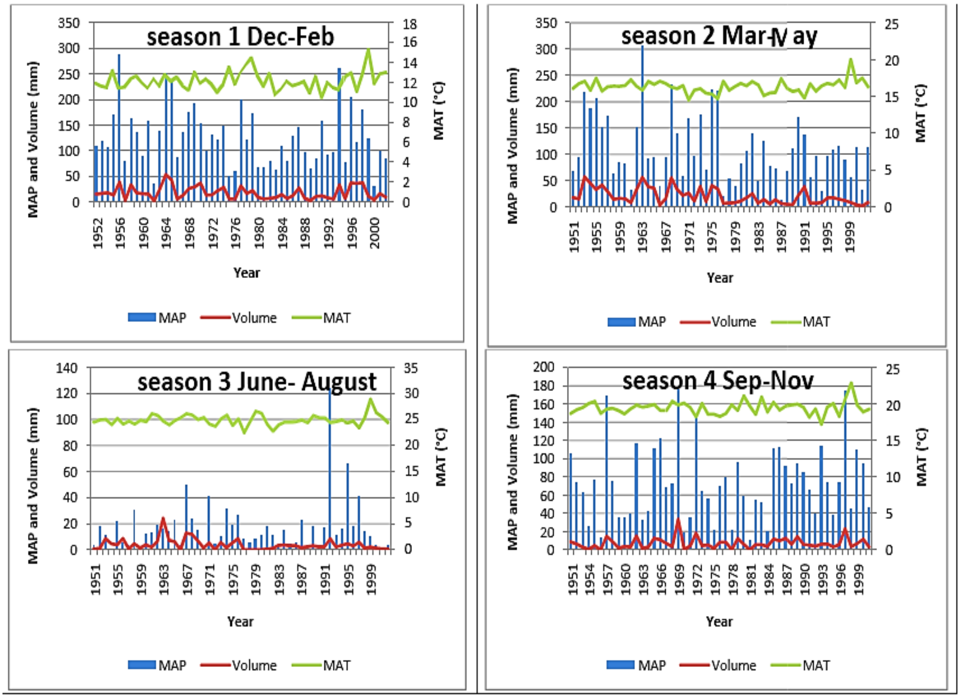

Fig. 6. Annual MAP, MAT and runoff for LHBA (Q1 to Q15).

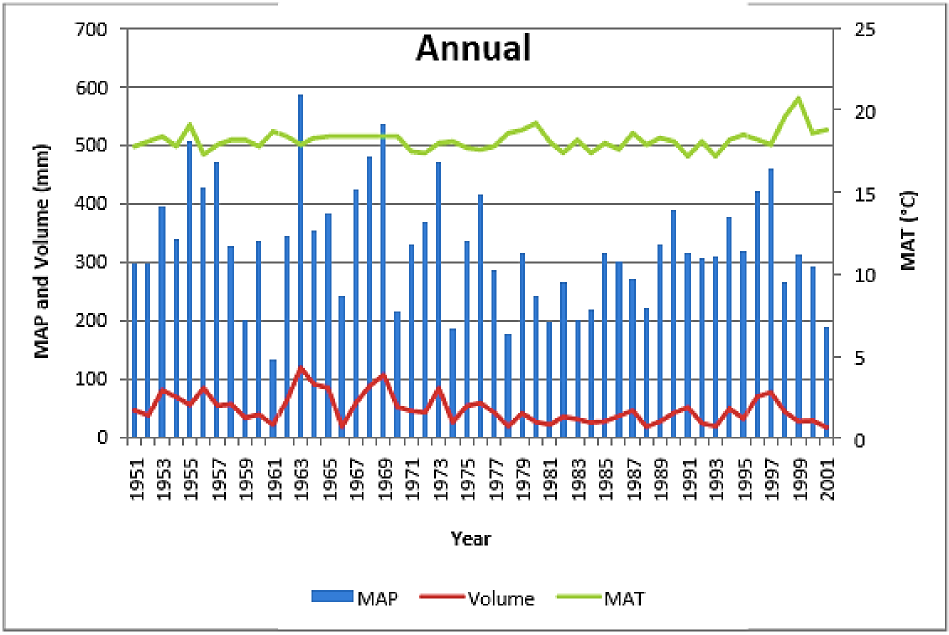

Fig. 7. Annual MAP, MAT and runoff for LHBA (Q16 to Q20).

The examination of monthly precipitation and temperature series revealed several data issues critical to understanding the hydroclimatic dynamics of the Loukkos hydraulic basin. These issues included multiple gaps and anomalies such as stations reporting consecutive years of zero precipitation across all months, specific months with recurring zero precipitation over consecutive years, discrepancies in data reported from different stations at the same location, and extended periods of missing data both in precipitation and temperature records. Additionally, outliers were identified in temperature data, further complicating the dataset's reliability for analysis.

To address these challenges, daily rainfall data from 85 LHBA stations spanning 1939 to 2012 were retrieved and analyzed. Through rigorous data validation and quality control processes, 27 stations were identified with the most complete and representative rainfall records for further detailed study. Among these, 11 rainfall stations from LHBA were selected based on their extensive data coverage from 1960 to 2011, ensuring robustness in analyzing temporal trends in cumulative annual and seasonal precipitation across the study area.

Despite efforts to secure comprehensive rainfall data, temperature data from LHBA stations were excluded from the analysis due to their limited availability and insufficient digitization. This limitation underscores the need for enhanced data management and digital infrastructure to support future hydroclimatic studies effectively.

The development of annual and seasonal Mean Areal Precipitation (MAP) and Mean Areal Temperature (MAT) series, using the Thiessen polygon method, facilitated spatial representation of climate variables across the basin. These series were pivotal in establishing statistical relationships through multiple regression analysis, linking historical runoff volumes with MAP and MAT. The regression equations provided insights into hydrological patterns and trends, crucial for assessing water resource availability and variability under changing climatic conditions.

Statistical tests conducted on the coefficients of the regression equations revealed significant relationships between runoff volumes and MAP/MAT, as indicated by high coefficients of determination (R^2) approaching 1 in several seasonal and annual models. These findings underscore the robustness of the regression models in capturing variations in runoff volumes attributable to changes in precipitation and temperature patterns.

However, challenges arose in establishing clear relationships between Mean Areal Temperature (MAT) and runoff due to MAT's inherent variability. This variability necessitates further investigation and refinement of modeling approaches to enhance predictive accuracy and reliability in future hydroclimatic assessments.

The study's findings provide a foundational understanding of hydroclimatic dynamics in the Loukkos hydraulic basin and offer critical insights into water resource management strategies amid climate change impacts. The identified data quality issues highlight the importance of continuous monitoring, data validation, and technological advancements in ensuring the reliability and accuracy of hydroclimatic datasets for informed decision-making and sustainable development planning.

Overall, this study contributes to ongoing efforts in Morocco and similar regions to strengthen resilience against climate change through integrated water resource management practices, emphasizing the need for adaptive strategies tailored to local hydroclimatic conditions.

Messages to local decision-makers in Morocco, based on the study's findings:

Highlight the pressing need to integrate climate change considerations into water resource management policies. Emphasize that the Loukkos Basin, despite its current water abundance, is vulnerable to climate hazards, necessitating proactive adaptation measures.

Stress the importance of using comprehensive and reliable hydroclimatic data for informed decision-making. The study's methodology and results provide a framework for understanding historical trends and projecting future impacts on water resources.

Advocate for sustainable practices in water management. Encourage the adoption of strategies that enhance water security, minimize vulnerabilities to climate change, and ensure equitable distribution of resources among stakeholders.

Recommend investments in infrastructure and technology that support efficient water use and management. This could include modernizing data collection systems, enhancing forecasting capabilities, and implementing adaptive measures in response to changing hydrological conditions.

Promote collaborative governance frameworks that involve local communities, government agencies, and stakeholders in decision-making processes. Emphasize the importance of transparency, accountability, and stakeholder engagement in achieving effective water resource management.

Advocate for capacity building initiatives to enhance local expertise in climate resilience and water management. Foster awareness among decision-makers about the potential impacts of climate change on water resources and the benefits of proactive adaptation.

Encourage the development of long-term planning strategies that consider climate projections and prioritize adaptive measures. Highlight the benefits of early action in mitigating risks and maximizing opportunities for sustainable development.

These messages aim to empower local decision-makers with actionable insights and recommendations derived from the study's findings, fostering a proactive approach to addressing water resource challenges in the face of climate change in Morocco.

Based on the experiences from Morocco between 1950 and 2000 regarding water resources and climate variability, several key lessons and messages can be derived for the global research society:

Morocco's experience underscores the importance of integrated water management approaches that consider both surface and groundwater resources. Research should focus on developing holistic water management strategies that balance water availability, demand, and environmental sustainability (Halli et al., 2023).

The period highlighted the vulnerability of Morocco's water resources to climate variability and change. Research efforts globally should prioritize understanding regional climate impacts on water availability and develop adaptive strategies that can be tailored to local contexts (Sreeshna et al., 2024).

Data collection and monitoring systems are critical for effective water resource management. Global research should emphasize improving data quality, accessibility, and spatial coverage to enhance predictive modeling and decision-making capabilities (Ahmad & Ureeb, 2024; Bashir et al., 2024).

Morocco's experiences underscore the importance of engaging local communities in water management practices. Research should explore participatory approaches that empower local stakeholders and integrate traditional knowledge with scientific advancements.

Advances in technology can significantly enhance water management practices. Research efforts should focus on developing and deploying innovative technologies such as remote sensing, GIS, and hydrological modeling to improve water resource assessments and monitoring (Shinde et al., 2023; Sestras et al., 2023).

Effective water governance frameworks are essential for sustainable water management. Research should investigate policy instruments, institutional arrangements, and governance structures that promote equitable access to water resources while ensuring environmental sustainability (Holland , 2011).

Enhancing technical and institutional capacity is crucial for implementing sustainable water management practices. Global research should prioritize capacity building initiatives, training programs, and educational outreach to empower water professionals and decision-makers.

Building resilience to climate change impacts requires proactive adaptation planning. Research should focus on developing robust adaptation strategies that integrate climate projections, risk assessments, and scenario planning to anticipate and mitigate water-related risks.

Given the transboundary nature of many water resources, international cooperation and diplomacy are vital. Global research should advocate for collaborative frameworks that promote dialogue, cooperation, and shared benefits in managing transboundary water bodies (Choudhary & Purushothaman, 2023).

Research should advocate for increased investment in sustainable water infrastructure, ecosystem-based approaches, and nature-based solutions that enhance water security while conserving biodiversity and ecosystem services.

All of the listed provide insights into the challenges and opportunities associated with managing water resources in the context of climate variability and change. Global research efforts should leverage these lessons to inform policy, practice, and innovation for achieving sustainable water management globally.

CONCLUSION

In conclusion, the examination of monthly precipitation and temperature series revealed significant data issues that impacted the analysis. These issues included stations with multiple consecutive years of zero precipitation across all months, such as from January to December, as well as periods with zero precipitation in specific months over consecutive years, notably continuous zeros in July. Furthermore, discrepancies were found with different stations reporting identical data despite their diverse geographical locations, and inconsistencies in data reported from the same station over time were observed. Extended periods of missing precipitation records and outliers in temperature data also posed challenges, as did prolonged periods of missing temperature records, collectively highlighting the complexities and limitations in the dataset's reliability for thorough hydroclimatic analysis.

Daily rainfall data from 85 stations operated by LHBA were retrieved spanning from 1939 to 2012. Analysis of missing data enabled the selection of 27 stations with the most complete and representative rainfall records for further study. Temporal trends in cumulative annual and seasonal precipitation across these 27 stations were analyzed to identify patterns.

Out of these, 11 rainfall stations from LHBA, with over 30 years of continuous data (1960-2011), were selected for their completeness and reliability. However, temperature data from LHBA stations were not considered due to limited availability (less than 10 years of non-digitized data).

Annual and seasonal series of Mean Areal Precipitation (MAP), Mean Areal Temperature (MAT), and runoff were plotted together to assess data quality for multiple regression analysis. These plots revealed relationships among variables and highlighted significant data shifts. MAT, being highly variable, presented challenges in establishing clear relationships with runoff.

Statistical tests were conducted to evaluate the significance of coefficients in the multiple regression equations, measured by their p-values. Regression coefficients (R^2) approaching 1 indicated a good fit of observed data to the regression model.

Efforts were made to develop complete monthly series wherever possible. In cases where base ratios were significantly wide and alternative data sources were unavailable, seasonal data filling was employed. Although base ratios ideally should be less than 2, some series exceeded this criterion (less than 10) to ensure comprehensive data coverage.

It is crucial to acknowledge that the identified inadequacies in precipitation and temperature data quality could potentially impact the outcomes of subsequent stages of this research, particularly in analyzing the impacts of climate change on water resources within the study area.

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