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PARAMETRIC AND NON-PARAMETRIC METHODS FOR THE EVALUATION OF WINTER RYE GENOTYPES BY GRAIN YIELD STABILITY

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Abstract: The analysis of yield stability has become increasingly relevant in recent years due to changing climatic conditions that negatively affect crop yields. It is of utmost importance for farmers to cultivate widely adaptable and yield-stable varieties with high yield potential, as this determines economic predictability and mitigates their risk. Stability is also crucial for plant breeders to create genotypes adapted to a wide range of diverse environments. The aim of this study was to identify high-yielding, stable and adaptive rye landraces and cultivars for commercial and breeding purposes. The trial was carried out from 2014 to 2022 and included 16 cultivars and 7 Bulgarian landraces. The experiment was conducted in a block design with the randomized design of the variants in four replications. Sixteen parametric and non-parametric parameters of grain yield stability were determined. The average of the sum ranks (ASR), the AMMI stability value (ASV), the yield stability index (YSI) and the genotype selection index (GSI) were also calculated. Year had the highest statistically significant effect on the grain yield per hectare. Parametric and non-parametric stability parameters estimated G13, G12, G20 and G21 genotypes as the most stable. ASV identified G13, G18, G12 and G9 as the most stable genotypes, while YSI identified G13, G19, G12 and G18, respectively. GSI classified G13, G12, G18, and G19 as genotypes with the broadest adaptability to adverse climatic conditions. They could serve as source material for rye breeding programs.

Key words: rye, yield, stability analysis, adaptability.

Introduction

The analysis of yield stability has become increasingly relevant in recent years due to changing climatic conditions that negatively affect crop yields. It is of utmost importance for farmers to cultivate widely adaptable and yield-stable varieties with high yield potential, as this determines economic predictability and mitigates their risk. Stability is also crucial for plant breeders to create genotypes

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adapted to a wide range of diverse environments. Human food security also depends on crop yield stability (Ahrends et al., 2021; Reckling et al., 2021).

Yield stability is a complex indicator of a genotype's ability to fully utilize the available environmental factors and achieve its maximum potential, and is closely linked to its adaptability (Hassani et al., 2023). A number of statistical methods have been devised to quantify the stability of genotypes. Stability analyses (parametric and non-parametric) simultaneously estimate the relative ranking of genotypes tested in a series of environments. The most commonly used parametric estimates of stability are: regression coefficient, deviation from regression, coefficient of determination, coefficient of variance, Wricke's ecovariance, Shukla's stability variance, mean variance component, GE variance component, Kang's rank-sum, yield stability index, AMMI stability value (Sabaghnia, 2010; Karimizadeh et al., 2012; Fasahat et al., 2015; Pour-Aboughadareh et al., 2022). Non-parametric measures of phenotypic stability are based on ranking the genotypes in each environment. Genotypes with close rank values in different environments are referred to as stable (Sabaghnia, 2015).

Rye is a crop mainly used for the production of dietary bread and as animal feed. It is grown on infertile and sandy soils (Klimek-Kopyra et al., 2023). The crop possesses a great number of advantages such as a unique nutritional value, winter hardiness and tolerance to environmental stresses such as low temperatures, drought and poor soil conditions (Laidig et al., 2017; Desheva and Valchinova, 2023). Interest in culture has grown in recent years due to changes in consumption needs (Podolska and Aleksandrowicz, 2019). The development of new rye varieties with improved performance and persistence is fundamental to the resurgence of the crop as a uniform and cultivated commodity in Europe (Haffke et al., 2015; Hackauf et al., 2022). Therefore, it is crucial to have information on the yield stability and variability of winter rye cultivars before releasing them for commercial cultivation and to enhance the effectiveness of cultivar improvement programs.

The aim of this study was to identify high-yielding, stable and adaptive rye landraces and cultivars for commercial and breeding purposes.

Material and Methods

The studies were carried out from 2015 to 2022 in the experimental area of the IPGR "K. Malkov" in Sadovo, Bulgaria, on clay soil. It involved 23 accessions from the National Seed Gene Bank, including 16 cultivars and 7 Bulgarian landraces (Table 1). Sowing was conducted between 20 and 30 October, after the predecessor pea, at the favorable timing for the region. The experiment was arranged in a block scheme with the randomized design of the variants in four replications. The size of the experimental plot was 10 m^2. The necessary agronomic practices were employed during the whole vegetation period to assure equal plant development.

The grain yield data (t/ha) from seven-year genotype testing were statistically analyzed using one-way and two-way ANOVA. To measure specific differences between pairs of means, the Duncan's multiple range test was employed. Statistical processing of the data was carried out using the IBM SPSS Statistics 22 for Windows program.

Table 1. A list of the rye accessions involved in the trial.

Genotype code	Species	Name of accessions	Biological status	Origin
G1	Secale cereale L.	Milenium	cultivar	BGR
G2	Secale cereale L.	Danae	cultivar	DEU
G3	Secale cereale L.	A9E0050	landrace	BGR
G4	Secale cereale L.	A9E0053	landrace	BGR
G5	Secale cereale L.	A9E1386	landrace	BGR
G6	Secale cereale L.	A9E1390	landrace	BGR
G7	Secale cereale L.	A9E1387	landrace	BGR
G8	Secale cereale L.	A9E1388	landrace	BGR
G9	Secale cereale L.	A9E1389	landrace	BGR
G10	Secale cereale L.	Igusinskaja	cultivar	BLR
G11	Secale cereale L.	Sjabrouka	cultivar	BLR
G12	Secale cereale L.	Albedo	cultivar	CZE
G13	Secale cereale L.	Aventino	cultivar	CZE
G14	Secale cereale L.	Matador	cultivar	CZE
G15	Secale cereale L.	Selgo	cultivar	CZE
G16	Secale cereale L.	Visello	cultivar	CZE
G17	Secale cereale L.	Veronika	cultivar	UKR
G18	Secale cereale L.	Harkovskaya 88	cultivar	UKR
G19	Secale cereale L.	Harkovskaya 95	cultivar	UKR
G20	Secale cereale L.	Harkovskaya 98	cultivar	UKR
G21	Secale cereale L.	Prima	cultivar	CAN
G22	Secale cereale L.	AC Rifle	cultivar	CAN
G23	Secale cereale L.	AC Remington	cultivar	CAN

The *Stabilitysoft* statistical program was used to calculate sixteen stability parameters for grain yield. These parameters included: mean variance component (θ_i) (Plaisted and Peterson, 1959), GE variance component ($\theta_{(i)}$) (Plaisted, 1960), Wricke's ecovariance (W_i^2) (Wricke, 1962), regression coefficient (b_i) (Finlay and Wilkinson, 1963), deviation from regression (S^2d_i) (Eberhart and Russel, 1966), Shukla's stability variance (σ^2_i) (Shukla, 1972), coefficient of variance (CV_i) (Francis and Kannenberg, 1978), parameters of Nassar and Huehn (1987) ($S^{(1)}$ – the mean of the absolute rank differences of the genotype across all environments tested, $S^{(2)}$ – the variance between ranks across all environments tested, $S^{(3)}$ – the sum of the absolute deviations for each genotype relative to the mean of the ranks, $S^{(6)}$ – the sum of the squares of the ranks for each genotype relative to the mean of the ranks), Thennarasu parameters (1995) ($NP^{(1)}$, $NP^{(2)}$, $NP^{(3)}$, and $NP^{(4)}$), and Kang's rank-sum (KR) (Kang, 1988).

The additive main effect and multiplicative interaction (AMMI) analysis and genotype plus genotype by environment interaction (GGE) biplot analysis were performed using the PBTools software. The AMMI stability value (ASV) was calculated based on the interaction principal component axis of the AMMI model (IPCA1 and IPCA2 values) for each genotype and each environment, as suggested by Purchase (1997), using the PBTools Software and Microsoft Excel 2010.

The study utilized the yield stability index (YSI) to assess genotype stability based on the mean yield rank across environments and the ASV rank (Farshadfar et al., 2011).

Additionally, the genotype selection index (GSI) was calculated as the sum of the ASV and YSI ranking positions (Köse, 2022).

Results and Discussion

Over the seven-year study period, the rye genotypes had an average grain yield ranging from 3.61 t ha^{-1} to 5.22 t ha^{-1}, with an overall average of 4.51 t ha^{-1}. The highest yield was reported for G13 and the lowest for G4. The Duncan's multiple range test revealed significant differences between the means, indicating considerable variation between genotypes for the trait studied (Figure 1).

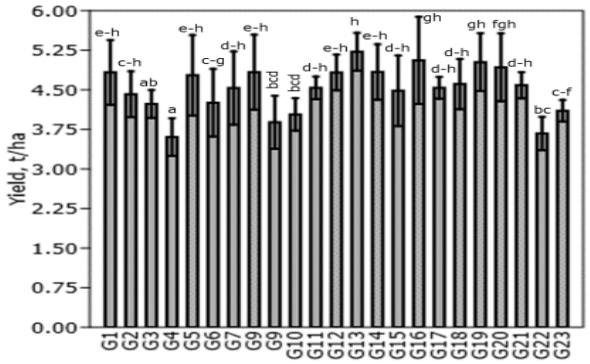

Figure 1. Mean yield (t ha^{-1}) for 23 winter rye genotypes for the period of 7 years (data are presented as mean ± standard error, different letters indicate the statistically significant differences between the means at $p \leq 0.05$).

Genotype, environment, and genotype x environment interaction (GEI) all contribute to grain yield (Đekić et al., 2018; Kartina et al., 2019; Egea-Gilabert et al., 2021). GEI studies help in selecting genotypes for breeding, based on their suitability for general or specific purposes, and provide information on how the environment affects crop performance (Amelework et al., 2023; de Filho et al., 2023).

In this study, the analysis of variance showed that there were highly significant differences in grain yield in regard to the genotype and the years investigated. This indicates the presence of both environmental and genetic variability. Year had the highest statistically significant effect on the trait (η^2=58.50%), followed by year x genotype interaction (η^2= 30.25%) (Table 2). Siekmann et al. (2021) also found that the GxE interaction variances of grain yield were larger than the variances of the genotype.

Table 2. Two-way ANOVA and the degree of influence of the sources of variation (η^2, %) on yield in 23 rye genotypes.

SV	df	MS	η^2, %
G	22.00	53546.08***	10.45
Y	6.00	1099084.01***	58.50
GYI	132.00	25835.39***	30.25
Error	483.00	186.93	0.80
Total	643.00		

SV – sources of variation; G – genotype; Y – year; GYI – genotype x year interaction; df – degree of freedom; MS – mean squared; η^2 – strength of influence of sources of variation – genotype, year and genotype x year interaction; *** – statistically significant values at $p \leq 0.001$.

Several studies emphasize the significance of stability parameters in identifying genotypes that are widely adapted (Hadasch et al., 2020; de Souza et al., 2020; Dias et al., 2022; Matova et al., 2022; Amelework et al., 2023; de Filho et al., 2023; Matongera et al., 2023; Mengistu and Abu, 2023).

To ensure a dependable evaluation of the genotype in terms of grain yield stability and adaptability, a proven effect of the genotype x environment interaction on the expression of the trait under study is essential. The impact of G×E interaction is demonstrated by the varying response of genotypes in different environments. To identify yield-stable genotypes in our study, we used both parametric and non-parametric stability parameters. Based on the ecovalence method of Wricke and Shukla's stability variance (σ^2_i), genotypes G13, G12, and G18 were identified as the most stable. With the exception of G13, which was ranked as the highest yielding, it should be noted that the other two were not the top performers in terms of mean yield, but ranked 8th and 10th, although their yield was above the mean. Conversely, the most unstable genotypes were G16, G22, and G10, which ranked 2nd, 22nd, and 20th respectively for mean yield (Table 3).

The most desirable genotypes are those with above-average productivity and low CV_i (Pour-Aboughadareh et al., 2022), as demonstrated by G17 and G11 in this study. Conversely, G16, G5, and G7 had the highest CV_i values, indicating lower stability and variability in their yield over the years of the study (Table 3).

Based on the lower mean variance component (θ_i) of Plaisted and Peterson (1959) and the higher GE variance component $\theta_{(i)}$ of Plaisted (1960), G13, G12 and G18 were found to be more stable (Table 3).

The results of the stability analysis, presented as linear regression for each cultivar using the Finley-Wilkinson (1963) model, indicate that 11 genotypes (G1, G5, G6, G7, G8, G14, G15, G16, G18, G19, G20) had a b_i value greater than 1 (Table 3). This suggests that these genotypes are more sensitive to changing environmental conditions and have a greater specific adaptability to high-diversity environments. G9 was found to be the most adaptive to all environments with a b_i value of 1. Genotypes G2, G3, G4, G10, G11, G12, G13, G17, G21, G22, and G23 were found to have a b_i value less than 1 (Table 3). This indicates that these genotypes are more resistant to environmental changes, making them more adaptable to low-yielding environments (Shojaei et al., 2022; Pour-Aboughadareh et al., 2022; Kebede et al., 2023). The b_i-value variation confirms that the genotypes studied respond differently to environmental changes (Akbar et al., 2021).

Table 3. Yield parametric stability parameters for 23 rye genotypes and their ranking.

G	Y	R	W_i^2	R	σ^2_i	R	CV_i	R	$\theta_{(i)}$	R	θ_i	R	b_i	s^2d_i	R
G1	4.83	7	2.22	6	0.37	6	33.57	15	0.66	6	0.53	18	1.45	0.11	8
G2	4.42	16	3.71	15	0.65	15	25.94	10	0.65	15	0.66	9	0.79	0.48	20
G3	4.23	18	2.22	5	0.37	5	16.41	5	0.66	5	0.53	19	0.55	0.11	7
G4	3.61	23	3.30	10	0.57	10	26.12	11	0.65	10	0.63	14	0.64	0.34	16
G5	4.78	9	5.30	20	0.94	20	42.25	22	0.63	20	0.80	4	1.83	0.04	2
G6	4.25	17	3.81	16	0.67	16	39.80	20	0.65	16	0.67	8	1.43	0.35	17
G7	4.53	14	3.96	17	0.69	17	40.57	21	0.64	17	0.68	7	1.64	0.15	10
G8	4.83	6	4.83	18	0.85	18	38.78	18	0.64	18	0.76	6	1.63	0.28	14
G9	3.88	21	3.42	11	0.59	11	34.18	16	0.65	11	0.64	13	1.00	0.49	21
G10	4.03	20	6.33	21	1.12	21	20.12	8	0.62	21	0.89	3	0.33	0.45	19
G11	4.54	12	3.57	13	0.62	13	12.44	2	0.65	13	0.65	11	0.38	0.12	9
G12	4.83	8	1.11	2	0.17	2	18.44	7	0.67	2	0.43	22	0.75	0.10	5
G13	5.22	1	0.33	1	0.03	1	18.11	6	0.67	1	0.37	23	0.85	0.02	1
G14	4.84	5	2.65	9	0.45	9	28.66	13	0.65	9	0.57	15	1.12	0.36	18
G15	4.48	15	3.61	14	0.63	14	39.51	19	0.65	14	0.65	10	1.56	0.19	13
G16	5.06	2	9.20	23	1.65	23	43.20	23	0.60	23	1.14	1	1.86	0.56	22
G17	4.54	13	3.49	12	0.61	12	12.04	1	0.65	12	0.64	12	0.38	0.11	6
G18	4.61	10	1.16	3	0.18	3	27.29	12	0.67	3	0.44	21	1.08	0.16	11
G19	5.02	3	2.57	8	0.44	8	28.85	14	0.66	8	0.56	16	1.20	0.33	15
G20	4.93	4	2.54	7	0.43	7	34.46	17	0.66	7	0.56	17	1.53	0.08	3
G21	4.59	11	2.11	4	0.35	4	14.34	4	0.66	4	0.52	20	0.53	0.08	4
G22	3.67	22	8.91	22	1.59	22	22.74	9	0.60	22	1.11	2	0.17	0.57	23
G23	4.10	19	4.91	19	0.87	19	13.04	3	0.64	19	0.77	5	0.28	0.17	12
Mean	4.51		3.71		0.65		27.43		0.65		0.66		1.00	0.25	

G – genotype code; R – rank; W_i^2 – Wricke's ecovariance; σ^2_i – Shukla's stability variance; CV_i – coefficient of variation; $\theta_{(i)}$ – GE variance component; θ_i – mean variance component; b_i – regression coefficient; S^2d_i – regression variance.

Based on the yield stability metrics of Becker and Leon (1988), Finlay and Wilkinson (1963), and Eberhart and Russell (1966), the perfect genotype should have a higher mean yield, a b_i value equal to or closer to one, and an S^2d_i value close to or equal to zero. Among the variants tested, G13 proved to be the ideal genotype. G23, G16 and G9 had the highest S^2d_i value, indicating the lower stability across all environments (Table 3) (Pour-Aboughadareh et al., 2023).

Table 4. Non-parametric yield stability parameters of Nassar and Hühn (1987) for 23 rye genotypes.

Genotype code	$S^{(1)}$	R	$S^{(2)}$	R	$S^{(3)}$	R	$S^{(6)}$	R
G1	5.62	7	22.29	5	9.18	5	1.69	4
G2	8.38	21	59.81	21	30.27	19	3.76	16
G3	5.71	8	28.00	8	21.00	14	3.00	15
G4	5.52	6	27.33	7	32.80	20	6.00	22
G5	8.29	19	48.67	19	19.47	12	2.80	12
G6	6.57	10	31.62	10	22.13	15	4.03	18
G7	5.90	9	29.14	9	14.75	8	2.31	9
G8	6.67	11	42.14	16	15.95	9	1.86	5
G9	6.76	12	38.81	13	28.60	18	4.35	20
G10	9.90	22	75.95	22	48.33	22	5.88	21
G11	7.05	13	37.67	11	18.83	11	2.83	13
G12	4.67	3	15.81	3	6.26	2	1.38	2
G13	3.33	1	7.90	1	2.53	1	0.87	1
G14	7.33	15	39.57	15	17.31	10	2.35	10
G15	7.62	18	44.48	18	23.95	16	2.77	11
G16	10.19	23	77.48	23	33.55	21	3.96	17
G17	7.14	14	39.48	14	19.98	13	2.92	14
G18	4.29	2	15.57	2	9.08	4	2.11	7
G19	7.33	15	38.48	12	14.56	7	2.22	8
G20	5.24	4	20.48	4	7.29	3	1.47	3
G21	5.33	5	25.48	6	13.72	6	1.92	6
G22	8.29	19	56.90	20	50.85	23	6.43	23
G23	7.52	17	42.48	17	27.88	17	4.28	19
Mean	6.72		37.63		21.23		3.10	

G – genotype code; R – rank; $S^{(1)}$ – the mean of absolute rank differences of the genotype across all environments tested; $S^{(2)}$ – the variance between ranks across all environments tested; $S^{(3)}$ – sum of the absolute deviations for each genotype relative to the mean of the ranks; and $S^{(6)}$ – sum of the squares of the ranks for each genotype relative to the mean of the ranks.

Tables 4 and 5 show the non-parametric yield stability parameters. In this study, the seven non-parametric estimates of phenotypic stability, $S^{(1)}$, $S^{(2)}$, $S^{(3)}$ and $S^{(6)}$ of Nassar and Hühn (1987), and $NP^{(1)}$, $NP^{(3)}$ and $NP^{(4)}$ parameters of Thennarasu (1995) identified G13 as the most stable, while $NP^{(2)}$ ranked G20 as the most stable. According to KR (Kang's rank-sum) (Kang, 1988), which uses both

yield and σ^2_i as selection criteria, G13 was also ranked as the most stable genotype followed by G12, G19 and G20, which were characterized by higher yields than the average in the set of genotypes studied. The undesirable genotypes with the highest rank sums were G22, G23 and G10. $S^{(1)}$, $S^{(2)}$ and $NP^{(1)}$ classified G16 as the most unstable, while $S^{(3)}$, $S^{(6)}$, $NP^{(3)}$ and $NP^{(4)}$ ranked G22 as the most unstable. According to $NP^{(2)}$, G4 was considered as the most unstable.

Table 5. Non-parametric yield stability parameters of Thennarasu (1995) for 23 rye genotypes.

Genotype code	$NP^{(1)}$	R	$NP^{(2)}$	R	$NP^{(3)}$	R	$NP^{(4)}$	R	KR	R
G1	4.71	9	0.22	5	0.35	4	0.39	4	13	5
G2	6.86	16	0.77	19	0.61	16	0.71	15	31	16
G3	3.29	4	0.75	18	0.52	13	0.71	16	23	9
G4	6.43	12	3.67	23	1.24	22	1.10	22	33	19
G5	6.86	16	0.28	10	0.51	10	0.55	11	29	14
G6	7.29	20	0.82	20	0.81	19	0.77	18	33	19
G7	4.00	5	0.25	7	0.47	7	0.50	9	31	16
G8	4.43	7	0.20	2	0.38	6	0.42	6	24	10
G9	6.57	13	1.69	21	0.77	18	0.83	20	32	18
G10	9.57	22	0.59	16	0.91	21	1.05	21	41	22
G11	6.14	11	0.55	15	0.52	14	0.59	12	25	11
G12	3.00	3	0.20	3	0.24	2	0.31	2	10	2
G13	1.71	1	0.24	6	0.09	1	0.18	1	2	1
G14	6.71	14	0.28	9	0.52	12	0.53	10	14	7
G15	4.43	7	0.39	12	0.54	15	0.68	14	29	14
G16	11.71	23	0.40	13	0.73	17	0.74	17	25	11
G17	5.57	10	0.49	14	0.52	11	0.60	13	25	11
G18	4.00	5	0.21	4	0.49	9	0.42	5	13	5
G19	6.86	16	0.29	11	0.48	8	0.46	7	11	3
G20	6.71	14	0.18	1	0.36	5	0.31	3	11	3
G21	2.57	2	0.26	8	0.35	3	0.48	8	15	8
G22	8.71	21	2.26	22	1.43	23	1.23	23	44	23
G23	6.86	16	0.67	17	0.85	20	0.82	19	38	21
Mean	5.87		0.68		0.60		0.63		24	

R – rank; $NP^{(1)}$; $NP^{(2)}$, $NP^{(3)}$, and $NP^{(4)}$ – Thennarasu's non-parametric estimates; KR – Kang's rank sum.

To select potentially better stable genotypes, the average of the sum ranks (ASR) was calculated for all statistics. The genotype with a low ASR value is considered the most superior stable genotype, as explained by Pour-Aboughadareh et al. (2019). The results indicated that G13 (ASR = 3) was the most stable genotype among the studied genotypes, followed by G12 (ASR = 4.38), G20 (ASR = 6.38), G21 (ASR = 6.44), G18 (ASR = 6.63), G1 (ASR = 9.94) and G19 (Table 6).

Table 6. Sum of ranks (SR), mean sum of ranks (ASR) and standard deviation (SD) calculated on the base of parametric and non-parametric stability estimates.

Genotype code	SR	ASR	SD
G1	114	7.13	3.96
G2	259	16.19	3.39
G3	169	10.56	5.44
G4	247	15.44	6.11
G5	220	13.75	6.02
G6	259	16.19	3.76
G7	182	11.38	4.83
G8	170	10.63	5.57
G9	257	16.06	3.97
G10	302	18.88	5.51
G11	184	11.50	2.92
G12	70	4.38	5.06
G13	48	3.00	5.60
G14	180	11.25	3.47
G15	224	14.00	3.08
G16	282	17.63	7.39
G17	182	11.38	3.42
G18	106	6.63	5.00
G19	159	9.94	4.31
G20	102	6.38	5.10
G21	103	6.44	4.29
G22	319	19.94	5.89
G23	259	16.19	5.19

The AMMI stability value (ASV) is a suitable statistic in situations where the first two principal component interactions explain a significant G x E interaction (Ghazvini et al., 2018). It has been used to measure and classify stable genotypes from the smallest value range (Karuniawan et al., 2021). Using the ASV parameter, all genotypes were ranked by stability. Four genotypes, namely Aventino (G13), Harkovskaya 88 (G18), Albedo (G12), and A9E1389 (G9), were identified as stable with values below 1 (Table 7).

Poudel et al. (2020) noted that stability should not be the only parameter considered when selecting genotypes. This is because the most stable genotypes may not necessarily provide the best yield performance. Therefore, it is important to use approaches that take into account both mean yield and stability in a single index. The YSI provides a more effective means of evaluating the potential of a genotype in various environments. The genotype with the lowest YSI is considered the most stable (Thiam et al., 2023). In our study, according to this index, the most stable genotype was G13, followed by G19, G12 and G18. The genotype selection index (GSI) allows for the ranking and clear classification of the breeding value of genotypes. It is important to note that genotypes with the lowest GSI coefficient

exhibit broad adaptation (Wodebo et al., 2023). In our study, these were genotypes G13 (GSI=2), G12 (GSI=6), G18 (GSI=6) and G19 (GSI=9) (Table 7).

Table 7. Mean performance of rye genotypes based on AMMI stability value (ASV), yield stability index (YSI) and genotype selection index (GSI).

G	Y, t ha^{-1}	R	ASV	R	YSI	R	GSI	R
G1	4.83	7	1.617	11	18	7	18	8
G2	4.42	16	1.162	5	21	9	14	5
G3	4.23	18	1.407	10	28	13	23	10
G4	3.60	23	1.917	12	35	17	29	14
G5	4.78	9	2.659	20	29	14	34	16
G6	4.25	17	2.236	16	33	16	32	15
G7	4.53	14	2.258	17	31	15	32	15
G8	4.83	6	2.345	18	24	10	28	13
G9	3.88	21	0.933	4	25	11	15	6
G10	4.03	20	2.926	21	41	19	40	18
G11	4.54	12	2.205	15	27	12	27	12
G12	4.83	8	0.810	3	11	3	6	2
G13	5.22	1	0.489	1	2	1	2	1
G14	4.84	5	1.362	8	13	5	13	4
G15	4.48	15	1.177	6	21	9	15	6
G16	5.06	2	3.572	22	24	10	32	15
G17	4.54	13	2.019	14	27	12	26	11
G18	4.61	10	0.652	2	12	4	6	2
G19	5.02	3	1.180	7	10	2	9	3
G20	4.93	4	1.985	13	17	6	19	9
G21	4.59	11	1.378	9	20	8	17	7
G22	3.67	22	3.644	23	45	20	43	19
G23	4.10	19	2.588	19	38	18	37	17

G – genotype code, Y – yield (t ha^{-1}); ASV – AMMI stability value; YSI – yield stability index; GSI – genotype selection index; R – rank of genotype.

The AMMI1 biplot was instrumental in facilitating the interpretation of the interaction effects among genotypes and environments, as well as in assessing the adaptability of genotypes. Al-Naggar et al. (2020) have noted that the genotypes on the right side of the axis have higher yields than those on the left side. A high average yield and a high interaction score, both pointing, characterize the optimally adapted genotype in environments in the same direction (Sitaresmi et al., 2019; Bishwas et al., 2021). Genotypes G18, G12, G13 and G19 exhibited mean values that exceeded the overall mean and showed PC1 scores close to zero. Consequently, they were regarded as exhibiting broad adaptability across diverse environmental contexts. On the other hand, G16, which had high mean values and a high score for PC1 but far from the origin showed specific adaptability to the environment (Figure 2 left) (Khan et al., 2021; Gerema et al., 2024). In accordance with the findings of the AMMI2-based analysis, genotypes and environments

exhibiting lower PC1 and PC2 values that are closer to the origin are deemed to be the most stable (Bishwas et al., 2021). In the present study, genotypes G13 and G12 were identified as the most stable (Figure 2 right).

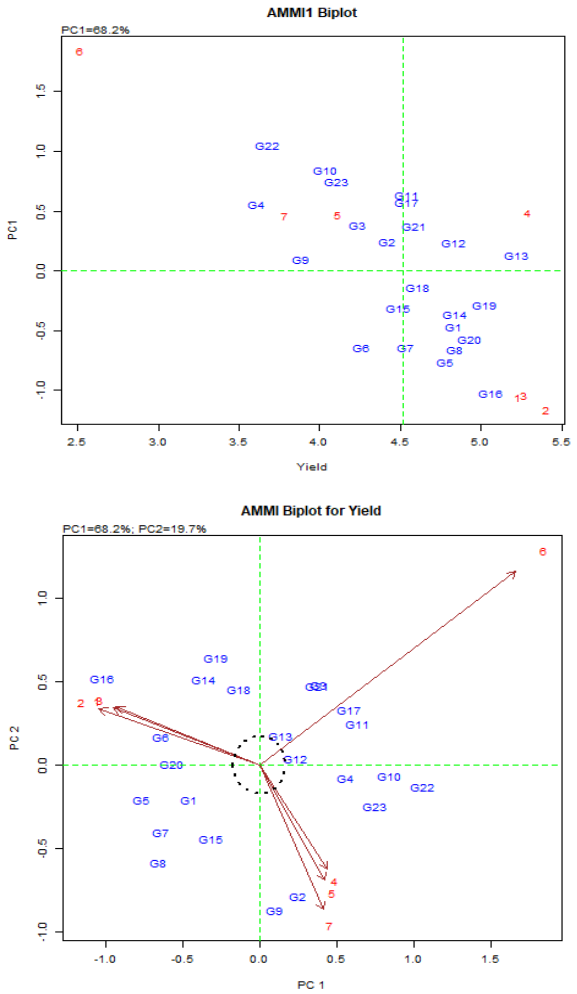

Figure 2. The AMMI1 biplot of PC1 for yield (left) and the AMMI2 biplot of PC1 and PC2 for grain yield (right).

The identification of mega-environments has the potential to facilitate the management of genotype-by-environment interactions, with the subsequent possibility of generalizing results to analogous agroclimatic locations (Fekadu et al., 2023). The best way to sum up the genotype and genotype-environment interaction of the dataset is the polygon-view of the GGE biplot, which visualizes

the "which-won-where" pattern of a multi-environment dataset. German et al. (2024) observed that the GGE biplot possessed a more comprehensive and versatile nature, offering a more profound comprehension of the GGE interaction compared to alternative methods. In this study, the GGE biplot analysis revealed that the total GGE variation for grain yield of the genotypes evaluated at seven growing seasons was 83.9%, of which 64.2% was explained by PC1 and 19.7% by PC2. The "which-won-where" model of the GGE biplot polygonal view showed the main effect of genotype plus the G × E interaction effect of the 23 genotypes studied in seven seasons for grain yield. According to the polygon view, the investigated 23 genotypes fell under 4 sectors, while 7 environments fell under 3 sectors in the polygon. Genotype G22, G11, G17 and G21 were recorded as high-yielding and stable for environment E6 (growing season 2020–2021), while genotypes G7 and G8, respectively for environments E4 (growing season 2018–2019) and E7 (growing season 2021–2022). G13, G19, G16 and G5 were recorded as the most stable and high-yielding for environments E1 (2015–2016), E2 (2016–2017), E3 (2017–2018), E5 (2019–2020). Genotype G9, which was located at the top of the polygon in a section of the biplot where no environmental indicator was present, exhibited the poorest performance in all environments tested (Figure 3).

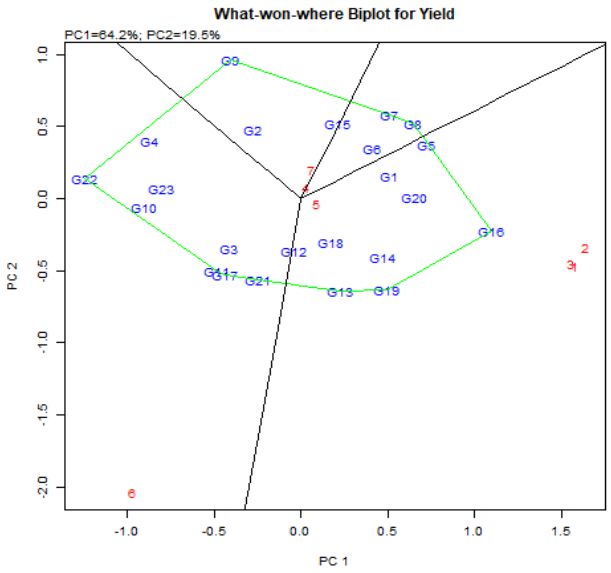

Figure 3. GGE biplot of the polygon view displaying 23 genotypes and 7 environments for grain yield.

The identification of an ideal genotype is of paramount importance in the selection of genotypes that perform well in a range of environments. This in turn

can result in enhanced crop yields and more stable agricultural production. The utilization of the ideal genotype concept as a reference within breeding programs has been observed in numerous studies (Omrani et al., 2022; Ahakpaz et al., 2023; Mullualem et al., 2024; Pramanik et al., 2024). Wardofa et al. (2019) noted that genotypes deviating from the ideal genotype can be eliminated in early selection cycles, while those that are closer to the ideal genotype can be subjected to further testing. Mullualem et al. (2024) observed that genotypes located in the inner circle are considered to be more desirable than those in the outer circle. In this study, G16 was identified as a superior genotype. It was located in the center of the first concentric circle in the biplot, therefore close to the ideal genotype, followed by the genotypes in the following order G19, G14, G13, G20, G18, while G22, G4 and G9 were the most unstable and unfavorable genotypes, because they were located far from the ideal genotypes (Figure 4). The GGE biplot for genotypes also shows the stability of genotypes as a function of environments. The stable genotypes are those located closest to the AEA axis. Genotypes G19, G14, G18, G13, G12 were the most stable genotypes, because they showed the shortest distance from the average environment abscissa. However, taking into account the stability of the yield with the average yield and the location of the genotypes relative to the ideal genotype, genotypes G19, G12, G13 and G18 could be considered as the most favorable and adaptable to a wide range of environments (Figure 5).

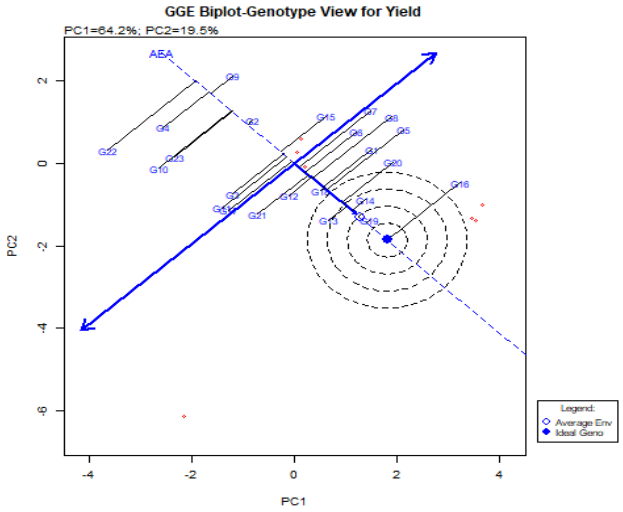

Figure 4. GGE-biplot based on genotype-focused scaling for comparing the genotypes with the ideal genotype.

The findings of the study indicate the presence of substantial disparities between genotypes, environments, and the interplay between environment and

genotype. Consequently, stability analyses have been conducted to determine which genotypes possess the capacity to adapt to diverse environments. These analyses reveal that certain genotypes are stable and have the ability to adapt to various environments, while others are unstable and can only adapt to specific environments. The most stable genotypes identified by the univariate (parametric and non-parametric) models, which had the lowest ASR values were G13, G12, G20, G21, G18 and G19. The AMMI stability value (ASV) identified G13, G18, G12 and G9 as stable, while the most stable genotypes with high yield mean according to YSI were G13, G19, G12 and G18. The last genotypes were also classified as genotypes with broad adaptation to adverse climatic conditions according to GSI, AMMI biplot and GGE biplot analyses. The "which-won-where" model of the GGE biplot polygonal view, which shows the main effect of genotype plus the G × E interaction effect, which shows the main effect of genotype plus the effect of a G × E interaction, identified genotypes G16, G8, G9, G22, G17, and G19 as corner genotypes suitable for certain environments.

Conclusion

The study revealed that rye productivity (t/ha) fluctuated significantly depending on year, genotype and the interaction between year and genotype. Year exerted the largest effect on trait performance, followed by the interaction between genotype and year. Genotypes G13, G12, G19 and G18 were identified as high-yielding and stable, with the broadest adaptability to unfavorable environmental conditions. These genotypes are suitable for growth under various climatic conditions and can be a suitable source material in rye breeding programs. Further research on genotypes at diverse locations is required to enhance the validity of these results.

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PARAMETARSKE I NEPARAMETARSKE METODE ZA EVALUACIJU GENOTIPOVA OZIME RAŽI PREMA STABILNOSTI PRINOSA ZRNA

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R e z i m e

Analiza stabilnosti prinosa postala je sve relevantnija poslednjih godina zbog promenljivih klimatskih uslova koji negativno utiču na prinose useva. Od suštinskog je značaja da poljoprivrednici uzgajaju vrlo prilagodljive i stabilne sorte sa visokim potencijalom prinosa, jer to određuje ekonomsku predvidljivost i smanjuje njihov rizik. Stabilnost je takođe od ključnog značaja za oplemenjivače biljaka, kako bi stvorili genotipove prilagođene širokom spektru različitih okruženja. Cilj ovog istraživanja bio je da se identifikuju visokoprinosne, stabilne i prilagodljive lokalne populacije i sorte raži za komercijalne i oplemenjivačke svrhe. Ogled je sproveden u periodu od 2014. do 2022. godine i obuhvatio je 16 sorti i 7 lokalnih populacija poreklom iz Bugarske. Ogled je sproveden u slučajnom blok rasporedu u četiri ponavljanja. Određeno je šesnaest parametarskih i neparametarskih pokazatelja stabilnosti prinosa zrna. Takođe su izračunati: prosek zbira rangova (engl. *average of the sum ranks* – ASR), vrednost stabilnosti AMMI (engl. *AMMI stability value* – ASV), indeks stabilnosti prinosa (engl. *yield stability index* – YSI) i indeks selekcije genotipova (engl. *genotype selection index* – GSI). Godina je imala najznačajniji statistički uticaj na prinos zrna po hektaru. Parametarskim i neparametarskim pokazateljima stabilnosti ocenjeno je da su genotipovi G13, G12, G20 i G21 najstabilniji. ASV je pokazao da su genotipovi G13, G18, G12 i G9 najstabilniji, dok je YSI izdvojio G13, G19, G12 odnosno G18. GSI je kao najprilagodljivije genotipove na nepovoljne klimatske uslove identifikovao G13, G12, G18 i G19. Oni mogu poslužiti kao izvorni materijal za programe oplemenjivanja raži.

Ključne reči: raž, prinos, analiza stabilnosti, prilagodljivost.

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VARIATION IN THE DEVELOPMENT OF RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Abstract: Red clover is an important perennial fodder plant for the production of quality fodder. Since it can be successfully produced even on soils of poorer quality, it is increasingly present in the sowing structure, primarily in hilly and mountainous areas. Trials were carried out on eight genotypes of red clover over several years. There were no significant differences between the average values of stem thickness and plant height of the two first cuttings from the two experimental years. The first cutting of the second year had the highest height (80.0 cm). Genotype 1 had the highest average height (78.0 cm) and leaflet length (45.52 mm). Genotype 6 had the lowest average plant height (74.0 cm), number of stems (7.15), stem thickness (3.31 mm) and leaflet length (36.68 mm). Genotypes 6 and 8 had the widest leaflet in the first cut of the second year of testing (28.94 and 28.93 mm). Aside from plant height, no significant differences were found between the genotypes. Nevertheless, all other examined variables showed statistically significant differences across clover cuttings and genotypes. The average values of the number of stems per plant, the length and width of leaflet in the first clover cut of the first year, and second clover cut of the second year were not statistically significantly different. Red clover is most productive in the first cutting of the second year of life, as confirmed by these investigations, which yielded the greatest values for the majority of the investigated features.

Key words: red clover, cut, genotype, morphological traits, variability.

Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes alongside alfalfa. It is grown on about 20 million hectares worldwide. In addition to the great importance of clover, a clear trend towards a decrease in areas

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sown with red clover, white clover and trefoil can be observed in the Republic of Srpska, while alfalfa maintains a consistent trend in the sowing structure (Gatarić et al., 2014). In addition to growing red clover in monoculture, it adapts well and is combined in the system of growing grass-clover mixtures (Cnops et al., 2010). Due to the good quality of the forage, red clover favors the nutrition of ruminants (Lee et al., 2009). When grown in grass-clover mixtures and used in the buttoning phase, cheap nutrients are obtained (Karagić et al., 2016). The plant height of red clover is mainly determined by the genetic characteristics of the variety and environmental factors. Leto et al. (1998) determined the highest plant height in the cut that had enough moisture – for the Nada variety of 94.9 cm at the Maksimir locality and 92.44 cm at the Medvednica locality. When measuring the plant height of red clover, it was found that the average height of all tested genotypes was 64.48 cm (Asci, 2011).

Genotype G42 had the lowest plant height (46.20 cm), while genotype G3 had the highest plant height (92.20 cm). When examining the morphological characteristics of diploid and tetraploid varieties of red clover, Muntean (2008) found that in the first year the average plant height of the diploid varieties was 22.96 cm and in the second year it was 49.84 cm, while the tetraploid varieties had an average plant height of 32.6 cm in the first year and 50.3 cm in the second year. In the Republic of Serbia, the average height of a red clover plant is 42.8 cm (Radinović et al., 2022a); in India – 52.0 cm (Verma and Ahmad, 2017).

Popović et al. (2007) tested 16 populations/varieties of red clover and obtained plant heights between 48.97 and 73.99 cm. The phenotypic characterization of 12 populations of red clover resulted in a stem thickness of 4.15–5.04 mm in the second year of life (Primorac et al., 2008). When examining the morphological characteristics of 46 genotypes of red clover, Radinović et al. (2022a) determined an average stem thickness of 3.85 mm. Gatarić et al. (2010) analyzed the number of stems per red clover plant and found that the plants had an average of 8.30 stems per plant in the second year of life, and 9.83 stems per plant in the third year of life. A red clover crop with an area of 1 ha develops leaves with an area of 25 ha (Tatić and Petković, 1998). Red clover produces high yields of green mass and hay, with the first cutting in the second year of life being the most productive (Petković et al., 2021). A higher crude protein content is obtained from the hay of the first cut compared to the second cut from the same year (Petković et al., 2020). In the Republic of Serbia, research was conducted on the correlation between morphological traits, yield and quality of red clover. In the second year, there was the highest correlation between the yield of green mass and the yield of dry matter (Radinović et al., 2022b).

In hilly and mountainous areas and on soils with poor chemical composition, forage is mainly produced from red and white clover, birdsfoot trefoil and sainfoin (Radović et al., 2010). In the examination of eight genotypes of red clover in Banja

Luka, the average annual yield of green mass depended on the genotype and was between 44 and 59 t ha^{-1}, and of hay between 8.6 and 13.3 t ha^{-1} (Petković et al., 2021). In Banja Luka, in the second and third year of using red clover, genotype G1 had the highest yield of hay – 13.7 t ha^{-1} in the first year, and the lowest genotype G2 – 8.3 t ha^{-1} in the second year of testing (Gatarić et al., 2010). In the research of Stevović et al. (2012) in Serbia, the lowest average yield of green mass and hay of red clover was given by the Viola variety, and the highest average yield of green mass of 39.6 t ha^{-1} and hay of 7.18 t ha^{-1} was achieved by the Una variety. The dry matter yield of red clover in the second year of life in Bulgarian conditions is in the interval 16.9–19.7 t ha^{-1} (Mihovsky and Naydenova, 2017). The optimal time for mowing red clover is at the stage when 20% of the inflorescence appears (Katić et al., 2004). Recently, the demand for red clover has increased, which is partly a result of new contemporary trends in plant production, but also the increasing importance of red clover in the production of animal feed and partly due to its use in the pharmaceutical industry. This research aimed to study the morphological characteristics of red clover in the conditions of the mountainous area of the Banja Luka region.

Material and Methods

The experiment was carried out on 13.05.2010 southwest of Banja Luka, in the locality of Dobrnja (N 44°39' E 17°00', 527 m altitude). The morphological characteristics of eight genotypes of red clover were studied in the first cutting of the first year and the first two cuttings of the second year of life. Four prospective genotypes of red clover of domestic origin (G-1, G-2, G-3 and G-4) and four varieties of red clover (G-5 Nike, G-6 Viola, G-7 Kolubara and G-8 Start) were included in the experimental trials. The sowing rate was 17 kg ha^{-1}. Sowing was carried out in four replicates (by hand) at a depth of 1.5–2 cm, and the distance between the rows was 20 cm. The size of the trial plot was 1 x 2 m. Seeds of red clover genotypes from the breeding program of the Agricultural Institute of the Republic of Srpska were used for sowing (G-1, G-2, G-3 and G-4), while the seeds of G-5, G-6, G-7 and G-8 were purchased at retail. The basic soil tillage (plowing) was done in the fall of 2009. The seedbed was prepared on the day of sowing. Pre-sowing fertilization was carried out with 250 kg ha^{-1} NPK 8:20:30. Chemical protection measures against weeds, diseases and harmful insects were not carried out.

Plant height (cm), the number of stems per plant, stem thickness (mm), and length and width of leaflets (mm) were recorded from five plants in the central part of each subplot. The samples for all tests were taken from the middle rows of the plots one day before mowing the green mass in the phenological phase – the beginning of flowering. The results of the biometric measurements were processed

with PC applications for Windows: Statistical Package for Social Sciences and Excel. The results of the studied traits were processed by analysis of variance (ANOVA) using a computer program and the GLM procedure. The Duncan's multiple range test (DMRT) was used to determine the significance of the differences between the examined cuttings and genotypes, and their ranking for the significance level R=0.01. According to this test, average values with the same letter are not statistically different.

Agroecological conditions

The soil in the experimental plot was well supplied with humus (5.2%) and with potassium (30.0 mg K2O in 100 g of soil), whereas the phosphorus content was low (7.0 mg P_2O_5 in 100 g of soil). Soil acidity (pH) in H_2O and KCl, the humus content (%), the phosphorus content (mg P_2O_5/100 g soil) and the potassium content (mg K_2O/100 g soil) were determined by chemical soil analysis. The analyzed parameters were determined using the following methods: total nitrogen in the soil, using the semi-micro-Kjeldahl method, modification according to Bremner (1960), easily accessible phosphorus and potassium in the soil, using the AL-method according to Egner et al. (1960) soil reaction (pH), using the potentiometric method.

Meteorological data: Mean monthly air temperatures and precipitation amounts were taken from the Hydrometeorological Institute of the Republic of Srpska, from the nearest measuring station in Banja Luka, which is located in the village of Lazarevo ($44^0$80'806" N, $17^0$21'278" E and 150 m altitude), about 30 km from the location of the experiment. In both years of testing, mean monthly temperatures were higher than the multi-year average (Table 1).

Table 1. Monthly and annual total precipitation sums (mm), mean monthly and annual air temperatures (ºC) in 2010 and 2011 and multi-year averages of precipitation sums and mean temperatures (1961–2010) for the location of Banja Luka (Republican Hydrometeorological Institute).

	Month												Total/ average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
P10	132.2	101.6	113.8	71.1	148	234.6	66.3	87.0	196	83.8	73.8	87.6	1395.8
P11	51.6	29.3	34.2	37.7	62.6	37.0	112.7	8.9	26.3	62.1	5.1	120.7	588.2
AP	70.4	62.8	80.1	88.7	94.4	113.2	91.8	86.1	94.5	78.4	94.6	90.9	1044.3
T10	0.2	2.4	7.5	12.0	16.5	20.3	23.1	21.8	15.7	9.4	8.9	1.5	11.6
T11	1.9	1.7	7.1	13.0	16.0	21.2	23.1	23.7	20.2	11.0	3.1	3.9	12.2
AT	0.1	2.0	6.5	11.1	16.1	19.5	21.2	20.7	16.1	11.2	6.3	1.4	11.0

P10 – monthly and annual sum of precipitation for 2010; T10 – average monthly and annual temperature for 2010; P11 – monthly and annual sum of precipitation for 2011; T11 – average monthly and annual temperature for 2011; AP – multi-year average of precipitation sums (1961–2010); AT – multi-year averages of mean temperatures (1961–2010).

In both years of testing, the mean monthly temperatures were higher than the multi-year average (Table 1). In both years, the mean monthly temperatures in June, July and August were higher than the long-term average for these months. The first year of testing was characterized by 351.5 mm more and the second with 456.1 mm less precipitation compared to the multi-year average (1961–2010). In the first year compared to the second year, there was a higher amount of precipitation in the amount of 807.6 mm (Table 1). The amount of precipitation in the first year affected the production of tall plants with thicker stems in the first cutting of the first year. During the vegetation period, the highest amount of precipitation in 2010 was in the month of June, 234.6 mm, while in the vegetation period of 2011, the highest amount of precipitation was in July 112.7 mm, however, a small amount of precipitation was recorded in June – 37.0 mm. The low amount of precipitation and high average monthly temperatures in June 2011 caused a lack of moisture and less favorable conditions for the growth and development of the red clover plants of the second crop in that year.

Results and Discussion

The cut had a statistically highly significant influence on all investigated traits (Table 2). Genotype had a statistically significant effect on stem thickness and leaf width, and a highly significant influence on the number of stems per plant and length of leaf. The interaction of cut x genotype had a statistically significant effect on leaflet length.

Table 2. Analysis of variance for plant height, number of stems per plant, stem thickness, leaf length, leaf width of the eight tested genotypes of red clover.

Source of variation	Traits				
	Plant height	Number of stems per plant	Stem thickness	Leaf length	Leaf width
Cut	**	**	**	**	**
Genotype	ns	**	*	**	*
Cut x genotype	ns	ns	ns	*	ns
Error	1460	37.5	4,30	937	354
Total	565093	6471.7	1228.9	181688	56185

* $p<0.05$; ** $p<0.01$; ns – not significant.

The average values of the investigated traits for the first cutting of the second year were statistically significantly higher than the values obtained for the second cutting of the same year (Table 3). There were no significant differences between the average values of all genotypes for plant height and stem thickness between the

first cuttings of the two years. Still, the average values of these two traits were significantly higher in the first cutting of the first year than in the second cutting of the second year. Except for plant height, significant differences were found between the mean values of the genotypes for all investigated traits.

The average values of traits by cut showed that the lowest average plant height, number of stems, stem thickness and leaflet width were obtained in the second cut of the second year of research and the life span of the red clover. Except for leaflet width, genotype 6 had the lowest average values of the tested traits. Genotype 7 obtained the highest average number of stems per plant (8.52), but also the leaflet with the smallest width (21.88 mm). Genotype 1 had the highest average plant height (78.0 cm) and leaflet length (45.52 mm), and genotype 4 had the highest average leaflet width (25.19 mm). In these studies, the average plant height by genotype ranged from 74.0 to 78.0 cm. Popović et al. (2007) examined 16 populations/varieties of red clover when they determined plant height in an interval from 48.97 to 73.99 cm. In these experiments, the plant height was determined in a larger interval (46.20–92.20 cm), but with a smaller average value (64.50) (Asci, 2011). The average plant height of red clover collections from the northwestern Himalayas is 52.0 cm (Verma and Ahmad, 2017).

The average plant height was 77.0 cm. A lower average height was determined by Leto et al. (1998), in whose tests the average plant height at a site in a lowland area was 58.93 cm, and at a site at a higher altitude – 64.0 cm. Tucak et al. (2016) determined the plant height of red clover in an interval of 59.38–78.66 cm in the second year of the experiment. Radinović et al. (2022a) presented the results of their studies on the morphological characteristics of red clover, in which the average plant height was 42.8 cm.

Statistically significant differences were found between cuts and genotypes for the trait number of stems per plant. The highest average number of stems per plant (10.0) was obtained in the first cutting of the second year, and the lowest (7.04) in the second cutting of the same year. The reason for the lower number of stems per plant in the second cutting is the tendency of red clover to produce higher yields of green mass and hay in the first cutting compared to subsequent cuttings in the same year. The differences in the average values of stem thickness between genotypes and cut were statistically significant. In addition to the genetic predisposition of red clover, sufficient moisture during the vegetation period of this section also affected the achievement of a high average stem thickness value in the first section of the second year.

Table 3. Average values of plant height (cm), number of stems, stem thickness (mm), leaf length (mm) and leaf width (mm) in eight genotypes of red clover in a two-year period.

	Cut				
Genotype	First 2010	First 2011	Second 2011	Average by genotypes	CV (%)
			Plant height (cm)		
G-1	80	82	73	78[a]	6.18
G-2	79	81	70	77[a]	8.01
G-3	80	82	71	77[a]	7.48
G-4	80	81	70	77[a]	10.19
G-5	80	77	70	76[a]	8.69
G-6	75	78	69	74[a]	6.19
G-7	78	80	72	76[a]	8.06
G-8	78	79	72	76[a]	6.85
Average per cut	79[a]	80[a]	71[b]	77	
			Number of stems per plant		
G-1	7.15	10.35	7.35	8.28[a]	20.21
G-2	7.45	10.00	7.35	8.27[a]	17.82
G-3	7.55	10.40	6.55	8.17[a]	23.44
G-4	7.45	10.20	6.80	8.15[a]	20.16
G-5	7.00	10.30	7.25	8.18[a]	20.30
G-6	6.20	8.90	6.35	7.15[b]	19.38
G-7	7.90	10.10	7.55	8.52[a]	15.56
G-8	6.30	9.75	7.15	7.73[ab]	21.45
Average per cut	7.13[b]	10,00[a]	7.04[b]	8.06	
			Stem thickness (mm)		
G-1	3.87	3.79	3.24	3.63[a]	9.72
G-2	3.89	3.71	3.17	3.59[ab]	11.60
G-3	3.91	3.67	3.16	3.58[ab]	11.33
G-4	3.78	3.60	3.55	3.65[a]	4.91
G-5	3.75	3.77	3.50	3.67[a]	4.61
G-6	3.44	3.39	3.10	3.31[b]	9.03
G-7	3.67	3.65	3.16	3.49[ab]	10.26
G-8	3.88	3.65	3.17	3.57[ab]	10.99
Average per cut	3.77[a]	3.65[a]	3.26[b]	3.56	
			Leaf length (mm)		
G-1	46.60	48.20	41.77	45.52[a]	2.35
G-2	45.67	44.72	38.08	42.83[a]	2.24
G-3	43.75	46.78	43.75	44.76[a]	1.65
G-4	44.45	48.03	42.91	45.13[a]	1.61
G-5	43.26	46.85	42.70	44.27[a]	1.87
G-6	31.26	45.47	33.30	36.68[b]	3.34
G-7	40.35	47.93	40.88	43.05[a]	2.17
G-8	40.20	46.22	43.35	43.26[a]	1.61
Average per cut	41.94[b]	46.78[a]	40.84[b]	43.19	
			Leaf width (mm)		
G-1	23.11	27.55	22.29	24.32[ab]	11.89
G-2	21.73	25.29	22.61	23.21[ab]	10.25
G-3	22.98	25.66	23.14	23.93[ab]	9.36
G-4	22.82	28.28	24.46	25.19[a]	12.93
G-5	23.09	26.60	25.55	25.08[a]	11.34
G-6	22.21	28.94	20.18	23.77[ab]	18.82
G-7	19.98	24.22	21.44	21.88[b]	10.10
G-8	21.76	28.93	22.74	24.47[ab]	16.99
Average per cut	22.21[b]	26.93[a]	22.80[b]	23.98	

CV = coefficients of variation; Values marked with the same letter are not statistically significantly different at the p=0.01 level (Duncan's multiple range test).

Jakešova et al. (2011) determined a stem thickness of 4.53 mm when investigating the morphological characteristics of red clover. Radinović et al. (2022a) obtained an average thickness of red clover stem of 3.85 mm. Primorac et al. (2008) measured the mean leaflet in the second year of life in three cuttings of 12 populations of red clover, and found it to be between 42.31 and 48.51 mm. In our study, the average length of leaflets (average in three sections) was in the indicated interval in all other genotypes except genotype 6, so it can be said that the data obtained in this work are consistent with the above results. When examining the variability of leaflet length of three genotypes of red clover in the Manjača region, an average length of 4.10 cm in the second year of use and 4.03 cm in the third year of use was determined (Gatarić et al., 2010). Compared to the data presented in this paper, all genotypes, except genotype 6, showed a higher average leaflet length.

When describing the morphological and biological properties of the red clover variety Viva, Popović et al. (2011) state that the leaf is trifoliate, and the leaflets have an elongated shape with a length in the interval 4.6–5.5 cm. Primorac et al. (2008) examined the middle leaflet in the second year of use of 12 populations of red clover, and determined its average width from 21.62 to 27.74 mm. Comparing the average leaflet width obtained in this work, it can be seen that all genotypes had average leaflet widths in the specified interval. Compared to the data of Gatarić et al. (2010), who found that the average width of the leaflets in two years was 2.10 and 2.21 cm, in this work, all genotypes showed a larger average width of the leaflet. Popović et al. (2011) state that the leaf width of the red clover variety Viva is 1.6–2.6 cm. The average value of the length and width of the leaflet in this paper was higher compared to the data for the average leaflet of the red clover presented by Radinović et al. (2022a). In this paper, the data for the width and length of the leaflets had smaller values compared to the data for the middle leaflet of red clover determined by Grljušić et al. (2006), in whose study the average length was 5.30 cm and the width was 2.94 cm.

Conclusion

The studied red clover genotypes showed significant variability for all traits. In the first year (the first cutting in the year of sowing), high values of the tested traits were obtained. For good growth and development in that cutting, a large contribution was made by the available sufficient amount of moisture. The values of the height of the plant, the number of stems per plant, the thickness of the stem, the length and the width of the leaf were smaller in the second cut compared to the first cut in the same year, thereby confirming the biological characteristic of red clover that it gives lower values in that cut. Unfavorable weather conditions in June also had an impact on obtaining lower values of the tested traits in the second

section of the second year. The researched genotypes represent good material for growing red clover with high values of the examined traits in the locality of the research, as well as in other localities characterized by similar conditions.

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VARIRANJE RAZVIĆA CRVENE DJETELINE (*TRIFOLIUM PRATENSE* L.)

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R e z i m e

Crvena djetelina je značajna višegodišnja krmna biljka za proizvodnju kvalitetne stočne hrane. Zbog mogućnosti uspijevanja na zemljištima lošijeg kvaliteta, sve više je zastupljena u setvenoj strukturi prvenstveno u brdsko-planinskim područjima. Eksperimentalna ispitivanja su realizovana na osam genotipova crvene djeteline u višegodišnjem periodu. Između prosječnih vrijednosti debljine stabla i visine biljke dva prva otkosa iz dvije godine ispitivanja nije bilo značajnih razlika, a najveću visinu je imao prvi otkos druge godine (80,0 cm). Genotip 1 je imalo najveću prosječnu visinu (78,0 cm) i dužinu liske (45,52 mm). Genotip 6 je imao najmanju prosječnu visinu biljke (74,0 cm), broj stabala (7,15), debljinu stabla (3,31 mm) i dužinu liske (36,68 mm). Genotipovi 6 i 8 su imali najširu lisku u prvom otkosu druge godine ispitivanja (28,94 mm i 28,93 mm). Izuzev osobine visina biljke kod koje nisu dobijene značajne razlike između genotipova, za sve ostale ispitivane osobine između otkosa i genotipova dobijene su statistički značajne razlike. Prosečne vrednosti broja stabala po biljci, dužine i širine lista u prvom otkosu prve i drugom otkosu druge godine nisu se statistički značajno razlikovale. Crvena djetelina je najproduktivnija u prvom otkosu druge godine života, i to se potvrdilo i u ovim istraživanjima u kojima su najveće vrijednosti većine ispitivanih osobina dobijene u tom otkosu.

Ključne reči: crvena djetelina, otkos, genotip, morfološke osobine, varijabilnost.

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ECOLOGICAL AND BIOLOGICAL ROLE OF LIGHTING IN THE DEVELOPMENT AND SEASONAL DYNAMICS OF NORTHERN WHITE-CEDAR (*THUJA OCCIDENTALIS* L.) NEEDLES AND ITS ORNAMENTAL FORMS IN LVIV

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Abstract: For the research, we selected urban green spaces with a simple and complex planting rhythm, where we examined the effects of light on the results of competition between individuals, according to the nature of the light flow: from the top to the bottom of the crown and towards the open space. The differentiation of the columnar *Thuja occidentalis* L. in terms of tree thickness distribution was revealed, and a forecast was made for the further development of plants that have lagged behind in growth and should be removed from the row in a timely manner so as not to reduce the aesthetic compositional integrity of the green walls. In order to clarify the role of light in the competition individuals for its intensity, the relationship between the level of lighting of the crown of *Thuja occidenatlis* (Th.o.) "Fastigiata" in summer and autumn and their growth was examined. The role of lighting in changing the colour of coloured cultivars and the place of chlorophylls a and b, as well as carotenoids in this process were studied. In the shaded crowns of Th.o. "Aureaspicata" and Th.o. "Aureovarigiata", the golden colour changed to light green due to a decrease in carotenoid content. At the same time, the winter brown-green colour was a consequence of an increase in the concentration of carotenoids in the cells. Understanding of the statics and dynamics of the colour of decorative forms of northern white-cedar makes it possible to select cultivars with the most decorative colour for various garden and park compositions.

Key words: green wall, planting rhythm, insolation, tiering, photosynthesis, chloroplasts, pigments, growth, intraspecific competition.

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Introduction

Introduced by French importers at the end of the 17th century, *Thuja occidentalis* (Th.o.) spread very quickly in Europe, and its numerous ornamental cultivars can be found today in different parts of the world with a mild, humid climate. Northern white-cedar is used in various phytomeliorative areas of urban life: sanitary and hygienic (improves the quality of the urban environment by enriching the air with oxygen, phytoncides, absorbing and settling air pollutants, creating microclimatic comfort, etc.), engineering protection (wind, gas, snow, noise protection strips), architectural and planning (alley plantings on streets, squares, parks), recreational (creation of green "corners", places on streets and squares), and aesthetic protection (decoration of the landscape, especially urban, by using beautiful cultivars in terms of crown shape and colour). Many domestic and foreign references pay considerable attention to these phytomeliorative factors (Ahmida Saleh and Işınkaralar, 2022; Kycheryavyj, 2020; Ogunkunle et al., 2019; Shlapak et al., 2014; Yerezhepova et al., 2024).

In the conditions of Lviv, 43 ornamental forms have been acclimatised, 26 of which have been introduced, showing a fairly high winter and frost resistance, while the rest continue the adaptation process and are under the observation of Lviv experts. The widespread use of northern white-cedar and its decorative forms in the city of Lviv can be explained by two reasons: firstly, by a rather high level of vitality in the urban edaphic climate (native mesophytes *Picea abies* L., *Abies alba* M., *Larix deciduea* Mill. do not tolerate dry microclimate of streets and squares), and secondly, by the chromatic properties, especially in autumn and winter (Bouslimi et al., 2014; Chang et al., 2000; Henyk et al., 2023; Kramarets et al., 2023; Kycheryavyj, 2022).

The process of growth and formation of light shoots largely depends on the crown geometry of northern white-cedar and its cultivars, which are described in numerous publications (Bouslimi et al., 2022; Hofmeyer et al., 2009; Krynytskyy et al., 2022; Zheng et al., 2021).

The study of the ecological and biological role of lighting of decorative forms of northern white-cedar, which is characterised by three aspects: duration, intensity and character (i.e., wavelength of light flux), is necessary for the creation of durable and effective plantations in terms of phytemelioration (Binkley et al., 2013; Dhont, 2021; Paul et al., 2014; Seidel and Ammer, 2023).

It is well known that the intensity and lighting change significantly under the influence of local natural and climatic factors, which mainly affect the meso- and microclimate. In large cities, the light flux is often influenced by the building stock, street width and the azimuth of its direction. Therefore, theoretical findings on lighting often need to take into account its change as the sun moves across the sky,

as well as the outlines of buildings (Kowarik et al., 2020; Kycheryavyj et al., 2019; Nestoriak, 2015).

As for the interaction between plants and tree plantations, their tiering is taken into account, which ensures a stratification of the light flux and a regulation of the photosynthesis process (Kincaid, 2016; Kharachko and Skolskyi, 2017). Solar radiation is retained by the vegetation in a young oak forest by 96.8%, and in a pure spruce forest by 99%. With a horizontal crown closure of 1.0, less than 10% of the solar radiation that falls on the open surface reaches the tree canopy (Kycheryavyj et al., 2018; Hnativ, 2014).

According to the lighting level, the forest and woodland park phytocoenoses are divided into the following groups: "dark", which transmits 2.0–3.4% of the light flux (associations of hornbeam, red oak, and Norway maple), "medium illumination" – 3.3–4.8% (associations of common oak, common oak with European larch, Scots pine, common ash, and green ash); "light" – 8–6.2% (associations of *Robinia pseudoacacia,* silver birch, European larch, and ash-leaved maple).

Accordingly, the percentage of light reflection (albedo) from the tree crown varies in green spaces, ranging from 8% – to 46%. The total albedo of the entire tree crown differs by almost 12–18% from the albedo of a single leaf blade. The albedo of conifers is much lower than that of deciduous plants. This takes into account that sunlight mainly falls on the leaves and needles of the upper part of the crown, which is reflected in the differences in annual growth and the efficiency of photosynthesis, in numerous reactions in which pigments play a major role, affecting the synthesis of growth substances and the movement of assimilants, depending on external factors, primarily light (Holubets, 2010; Johnson et al., 2022; Weber et al., 2017).

Within this work, we visually observed changes in the colour of needles of *Thuja occidentalis* L. cultivars growing in the conditions of street gardening in Lviv (Ukraine). It was necessary to find out which factor has a significant impact on these changes; it was necessary to confirm the existing scientific statement about the influence of light on the change in seasonal colour of needles, which is confirmed by the conducted research.

Material and Methods

The programme was supposed to investigate: the influence of light on the growth of Th.o. "Columna" in row plantings; the effect of intensive insolation on the growth of apical shoots in cultivars of *Thuja occidentalis*; the role of the pigment composition of chloroplasts in the colour of the needles of northern white-cedar cultivars. The research was carried out in 2012. The soil type was light loam.

The northern white-cedar was brought to Lviv from the world-famous Polish nursery Kurnik, where many of its ornamental forms were introduced. The first

specimens, grown from cuttings by S. Vilchynskyi, a professor at the Medical University, were planted at the Department of Pharmacognosy. Later, he created a small garden of decorative (mainly columnar and pyramidal) forms of this species nearby. It may be claimed that 43 ornamental forms have undergone the adaptation period in Lviv, including 26 cultivars that have shown high frost resistance and are now widely represented in the urban environment of the city. The rest of the cultivars are currently being monitored.

The assessment of the studied parameters was carried out in the process of visual observations during the year. The planting of *Thuja occidentalis* "Fastigiata" cultivars in the study areas took the form of row plantings with a distance between plants that promotes the growth and development of plant specimens and minimises competition for space and environmental resources between plants. The study of the collected needles was carried out in the laboratory (study of the ratio of chlorophylls a and b to carotenoids by pigment separation). The research was conducted once in January in the study area.

The northern white-cedars were planted in a herbaceous border, and fertile soil was used for planting. The trees were 30 years old, with an average height of 7.2 m. The length of the green wall was 38 m, there were 58 trees in a row, the average distance between the trees was 67 cm, and the average trunk diameter was 14.2 cm.

Various instruments were used in the research. To measure the height of trees and growth of apical shoots, an optical height meter (Anuchin height meter) was used, and the thickness of trees was determined using a centimetre tape. The light flux was measured with a Benetech GM1030 luxmeter using a retractable ladder. The intensity of the insolation flux was measured using a luxmeter on certain days of the months of the study period. The measurement was carried out at noon (13:00–14:00 hours), when the contrast of the insolation indicators was most pronounced.

The concentration of chlorophylls in the needles was determined by the photometric method using a KFK-3 spectrophotometer. To determine the influence of pigments on the seasonal colour of the needles, we used the adsorption chromatography method, which is based on passing a liquid obtained from needles ground in acetone through a sorbent (starch and talc). This made it possible to identify the layers in which the chlorophylls a and b and the carotenoids were quite clearly distinguished. The research was carried out with two cultivars of northern white-cedar – "Fastigiata" and "Globosa" – in early January, when the brownish colour of the needles was clearly visible.

The scientific observations were carried out systematically over the 12 months of the study period. During this period, a seasonal change in colour was recorded. A distinct browning of the needles of *Thuja occidentalis* L. cultivars was detected in January.

The methodological basis of the study is the ecologicalcomparative method, which is widely used in environmental science. This aspect is fully disclosed in

Results and Discussion. The studies conducted primarily took into account the influence of the natural insolation regime, and did not study the heat factor.

Results and Discussion

The city of Lviv is located on the western border of the forest-steppe and has a mild, humid climate: the Martonne aridity index is 41.3 (for Kiev, for example, it is 34.2, for Warsaw – 40.3). In general, the climatic conditions of Lviv, as a region of the introduction of northern white-cedar and its cultivars, are close to the climate of the natural range of this species – North America. Due to its geographical location, the city of Lviv is simultaneously influenced by the air masses of Eurasia and the Atlantic Ocean. The climate of Lviv is characterised by mildness, which is reflected in small differences in summer and winter temperatures, and high humidity, which is reflected in different amounts of precipitation. The study area is characterised by frequent thaws in winter, significant cloud cover and rainfall. The area is exposed to a variety of air masses. The dominant air is temperate or polar air. At all times of the year, there is humid polar air, which brings cloudy weather with fogs in winter and causes thaws, and unstable cool weather with showers and thunderstorms in summer.

As is known, sufficient climate humidification conditions are the result of the influence of the radiation situation, which is regulated by the weather conditions: in Lviv, there are only 50 days of direct sunshine and almost 150 cloudy days throughout the year, with the rest being characterised by variable cloudiness.

According to the data, the city receives an average of 163.3 k cal/cm^2 of total solar radiation per year. However, its actual value is much lower and amounts to 92.4 k cal/cm^2. In this regard, the insolation regime is also different: in summer it is within 36–25 thousand lux, in winter – 7–6 thousand lux, which affects the development and seasonal colouring of the decorative forms of northern white-cedar.

Intraspecific competition in ordinary plantings of northern white-cedar cultivars

In tall hedges of northern white-cedar and its columnar and pyramidal forms, after planting, each individual develops without experiencing any particular phytocoenotic influence on each other. However, when the crowns begin to touch each other with their lateral shoots, competition begins, which deepens over time, causing the plants to differentiate in height and diameter. Three experimental studies were carried out to determine the peculiarities of plant development under conditions of dense row planting.

As we can see in Table 1, most of the trees with small trunk diameters are in the 4–12-cm class. Most of them are stunted due to underutilisation of light resources. The struggle for light and its result is also evidenced by the removal of crowns by almost 2–3 m to the south.

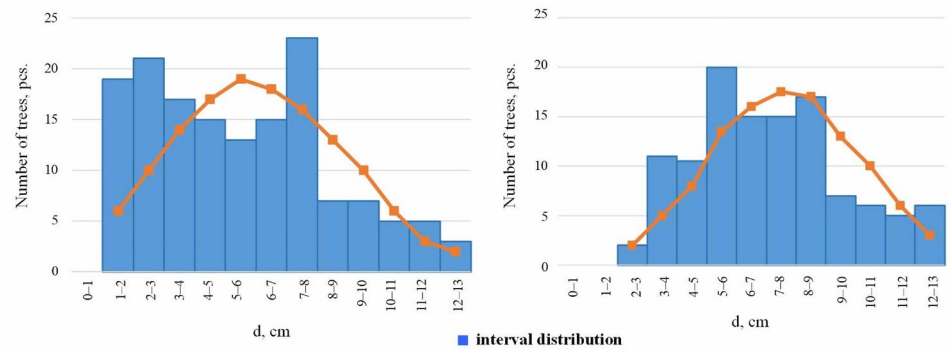
Table 1. Indicators of the distribution of trees by thickness.

Level of thickness, cm	Less than 4	4–8	8–12	12–16	16–20	20–24	24–28
Number of trees, pcs.	1	15	21	7	8	5	2

The distribution of trees with a diameter of less than 4–8 cm indicates the futility of their further growth and subsequent falling off. Their place in the green wall space will be taken by trees that are more developed (8–12; 12–16; 16–20; 20–24; 24–28).

Alley green wall of northern white-cedar “Fastigiata” along Yefremova Street

The differentiation of northern white-cedar “Fastigiata” individuals was studied in a 30-year-old row planting created along the perimeter of tennis courts along the street (next to the sidewalk) and in an alley planting inside the courts (Figure 1).



Diameter intervals. Quantitative distribution of individuals in a row planting of Th.o. “Fastigiata” along the sidewalk on Yefremova Street

Diameter intervals. Quantitative distribution of individuals ordinary alley planting of Th.o. “Fastigiata” in the middle of tennis courts

Figure 1. Distribution of Th.o. “Fastigiata” in a row planting according to the degree of thickness.

The row planting along the sidewalk on Yefremova Street is 56.4 m long and contains 106 individuals. The individuals are spaced 53.2 cm apart. In a row planting of cedar in the alley located directly on the courts (52.6 m long), there are 116 individuals. The distance between the plants is 45.3 cm. It should be noted that the distance between the trees during planting was the same in the first and second cases – 1.5 m high and equivalent in habit.

The decline and slowdown in the growth of northern white-cedars that have lagged behind in their development is a consequence of lateral crown closure, which has led to a decrease in light flux. Significant soil compaction and dehydration have also contributed to this (Table 2).

Table 2. Edaphic conditions and viability of Th.o. “Fastigiata”.

Location of the research object	Resistance to crushing (according to Kaczynski), kg/cm^2	Humidity of the soil, %	Vitality, score
Yefremova Street, along the sidewalk	32.7	38.5	1.92
Yefremova Street, alley planting in the middle of the tennis courts	19.3	50.4	2.19

The competition between the individual cedars led to their morphological differentiation, which can be seen in the distribution of trunk diameter intervals.

In the alley planting, which divides the territory of the tennis courts into half, there are much more individuals with diameters of 12–14, 10–12 and 8–10 cm than in the plantings along the sidewalk. The vitality of these plants is much higher here. It should also be noted that in the alley planting, where better soil care (loosening, watering) is provided, the distance between the trees is 6.9 cm less than in the planting along the sidewalk. The average diameter of the tree trunks in the alley planting is 1.67 cm larger than in the street planting, as evidenced by the interval distribution of the individuals.

In the process of development of individuals, which is accompanied by the struggle for light, there is a decrease in the crown towards a slowdown in the strength of the light flux (Table 3).

Table 3. Effects of the size of Th.o. “Fastigiata” on the light flux and growth dynamics.

Yefremova Street (sidewalk)			Sports courts (alley)		
Levels, cm	Tree height Amount of light, lux	Tree height Amount of light, lux	Levels, cm	Tree height Amount of light, lux	Tree height Amount of light, lux
7–8	8.2	36000	5–6	8.7	36700
2–3	7.5	32500	8–9	8.2	36100
1–2	7.7	32450	6–7	7.5	4200
3–4	6.9	12150	7,8	7.4	4200
4–5	6.5	11900	3–4	4.2	2600
6–7	6.9	11700	4–5	4.0	2500
5–6	6.4	10100	9–10	3.0	2100
6–7	6.9	11700	10–11	2.4	1800
8–9	2.1	2200	11–12	2.0	1700
9–10	2.1	2150	12–13	2.5	1650
10–11	2.0	2000	2–3	1.2	800
11–12	2.0	1950	–	–	–
12–13	1.1	1900	–	–	–

This pattern is reproduced in the green wall tiering diagram. The green wall in the alley planting, where the plantings were regularly tended and watered, has a high crown density and is highly decorative (Figure 2).

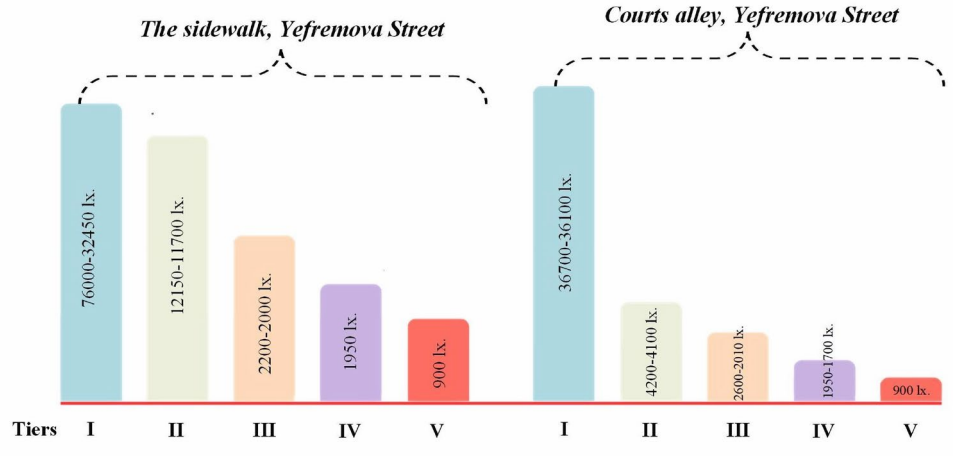


Figure 2. Tiering of green walls in alley planting.

As the quantitative distribution of individuals by thickness in the alley planting of the courts shows, there are no trees with a trunk thickness of 0–2 cm, and less than 10 plants with a thickness of 2–4 cm, while in the planting along the sidewalk, these thicknesses account for more than 40 individuals. This situation confirms to a certain extent the degree of tree mortality, namely, it decreases as habitat conditions deteriorate.

The alley of the arboretum of the botanical garden of the National Forestry University of Ukraine

Two ornamental forms of *Thuja occidentalis* columnar "Fastigiata" and spherical "Globosa" – were planted alternately 50 years ago: Th.o. "Fastigiata" – Th.o. "Globosa" – Th.o. "Fastigiata" (complex rhythm). To obtain such results, special studies are needed that would take into account the aspect of studying the growth and development of the studied cultivars in the context of different age periods, starting from the time of planting, the formation of the habitus of the crowns of the specimens, the influence of the soil type and the local microclimatic conditions. Since no similar scientific works have been found, there is no information on this period. Therefore, we use the results of our own research.

The distance between the columnar cedar trees was 3.0 m, with spherical cedar trees planted in the middle between them. In a row, 32 columnar cedar trees and 31 spherical cedar trees were planted. When planted, this complex rhythm

looked proportionate and large-scale in terms of the composition: spherical bushes with a crown diameter of 0.4 m were between the columnar trees about 1.0 m high. The distance from the bush to the edge of the tree crown was about 1.1 m. This planting rhythm was perceived harmonious and was optimal from an environmental point of view (fertile soil, especially good lighting). The overlap of the crowns of the studied cultivars of *Thuja occidentalis* L. is presented graphically (Figure 3). The studied areas are located in the Frankivsk district of Lviv, which covers the territory of the central and southern part of the city. The green wall of *Thuja occidentalis* "Fastigiata" cultivars on the square next to the Frankivsk District Administration consists of 58 trees, and the green wall of *Thuja occidentalis* "Fastigiata" along Yefremova Street consists of 106 trees, and along the perimeter of the tennis courts – 116 specimens.

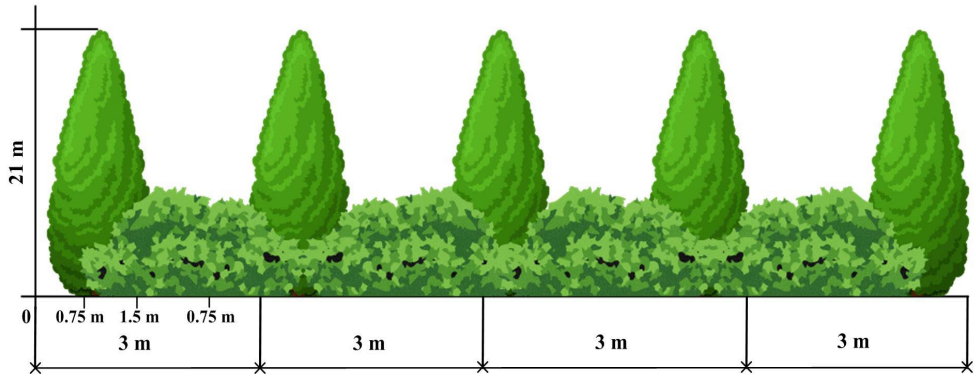


Figure 3. The arrangement of columnar and spherical cedars in a row planting.

After 50 years, the configuration of the row has changed due to the morphological differentiation of the individuals, which was the result of competition for light. First of all, the proportional ratio of the elements has changed: "column-ball", which was at the time of planting. The ratio of the height of the columnar cedar to the height of the spherical cedar (1.0 m: 0.4 m) was 25, today it is different (12.0 m: 4.0 m), i.e., 3. There is a clear disproportion, which reduces the level of aesthetic value of the alley.

Overgrown spherical cedar bushes create a certain negative effect: the width reaches up to 4.35 m, as can be clearly seen in Figure 4. However, the biggest problem is the removal of the crowns beyond the regular line of the row, which was formed by the columnar cedar. The diameter of the crown in the north-south direction indicates that the plants have not only taken over the space equal to the distance between the planting spaces (3.0 m), but have also begun to cover the crowns of the columnar cedar located on the left and right.

The overlap of the lower part of the columnar cedar cultivars leads to shading and, as a result, to the gradual drying out of the lower branches. Overlapping the crowns of columnar cedars, whose diameter at a height of 1.5 m ranges from 1.1 m to 1.6 m, causes the trunks to be exposed, reducing the aesthetic value of the alley. At the same time, the removal of the crowns of the spherical cedar beyond the designed row towards the openness of the alley space contributed to a negative phenomenon – gravity.

Influence of insolation intensity on the development of Th.o. “Columna”

Northern white-cedar and the vast majority of its ornamental forms are typical heliophytes, but they can tolerate slight shading, acting in this case as facultative heliophytes. The cause of the crown deformation was the concentration of significant masses of snow and glaciation due to the amplitude of daily temperatures in the study area.

However, as our research has shown, the lack of full lighting, which has been confirmed by many authors, negatively affects plant growth rates. The observations made at the experimental site on the growth of columnar cedar “Columna” in a regular planting (age 15 years) under different lighting conditions revealed a difference in growth. Plants that were located in an open space and under full insolation flow grew better (Figure 4) compared to those that were overlapped under an overhanging tent of tall (3.2 m) hazel bushes (*Corylus avellana* L.). This trend was also observed in terms of seasons.

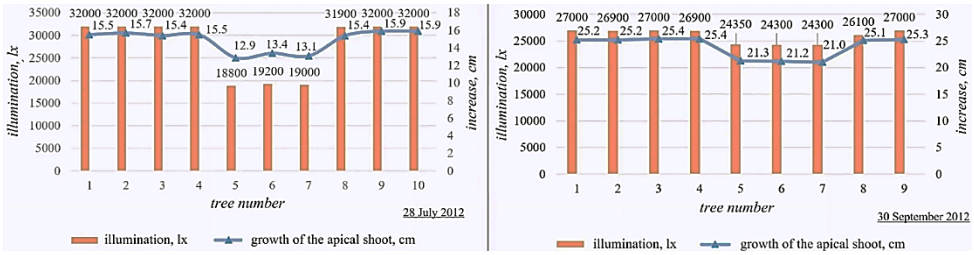


Figure 4. Effects of lighting on the growth of apical shoots of Th.o. “Columna”.

It should be noted that in the first half of the season the difference in the growth of illuminated and shaded individuals was 16%, and in the second half, i.e., at the end of the growing season, it was less – 11%, which indicates a lower growth rate of illuminated and shaded plants with lower radiation activity.

The introduction of cedars and their decorative forms, mainly columnar, pyramidal and oval-ovoid, into mixed plantings with the dominance of hardwoods confirmed the need to take into account the light requirements of cedars. The fact is

that cedar is a forest-forming species on the North Atlantic coast, i.e., a unifier tree, and therefore a light-loving species. Therefore, once under the shading canopy of crowns, the tree begins to slow down its growth, and then gradually dies with light at 5–12 thousand lux. Northern white-cedar species planted in the 50s of the last century in the park edges of Lviv, subsequently shaded by overhanging crowns of deciduous trees, slowed down their growth, their trunk deformed, turning towards the illuminated space, the coverage of the shoots was thinned out, and later, with even greater shading, the plants gradually died.

The role of lighting in the colouring of northern white-cedar colours

It is known that the pigment composition and its parameters characterise the state of the plant photosynthetic apparatus. Under optimal environmental conditions, the ratio of chlorophyll a+b/carotenoids is usually stable. According to many authors, this ratio is disturbed under stressful conditions, which negatively affects the activity of photosynthesis, and therefore not only the assimilative capacity of the plant, but also its decorative quality. This is especially true for those ornamental forms that differ in colour, in particular those that shade each other with their crowns.

For the study, two compositional groups were selected as follows: “Aureospicata” + “Globosa”; “Aureospicata” + “Aureovariegata”. In each of these variants, the pigment composition of the needles of individuals returned to the open space and those covered by the neighbouring crown was studied. It was found that the ratio of chlorophyll a+b/carotenoids in the needles of “Aureospicata” and “Globosa” depended on the level of crown exposure (Figure 5).

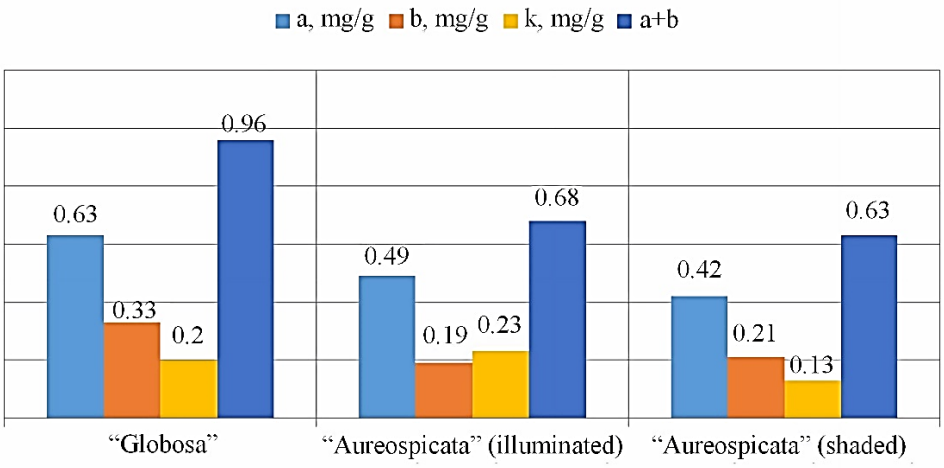


Figure 5. The effect of lighting on the pigment content and colour of needles.

As seen, the needles of the spherical cedar, which have a dark green colour, contain more chlorophylls a and b compared to the golden-tipped “Aureospicata”. As for carotenoids, their content in “Aureospicata” was slightly higher at 0.23 mg/g, which indicates that they are involved in the characteristic golden colour of this ornamental form.

The needles of thuja “Aureospicata”, which have been in contact with *Globosa* for three years, have slightly changed their pigment composition. In particular, the amount of pigments a and b changed, although their ratio remained almost the same. However, the amount of carotenoids increased by 43.5%. In our opinion, the pale green colour of the needles of the golden cedar, which are not exposed to sunlight, is due to a sharp decrease in the amount of carotenoids.

The discolouration of the needles of coloured decorative forms was also observed in the case of mutual shading of the sides of the crowns of “Aureospicata” and “Aureovarigiata”. The parameters of the pigment complex are shown in Figure 6. As we can see, similarly to the first case, the individuals of Th.o. “Aureospicata” and Th.o. “Aureovarigiata” showed a decrease in carotenoids: in “Aureospicata” – by 0.04 mg/g, and in “Aureovarigiata” – by 0.08 mg/g.

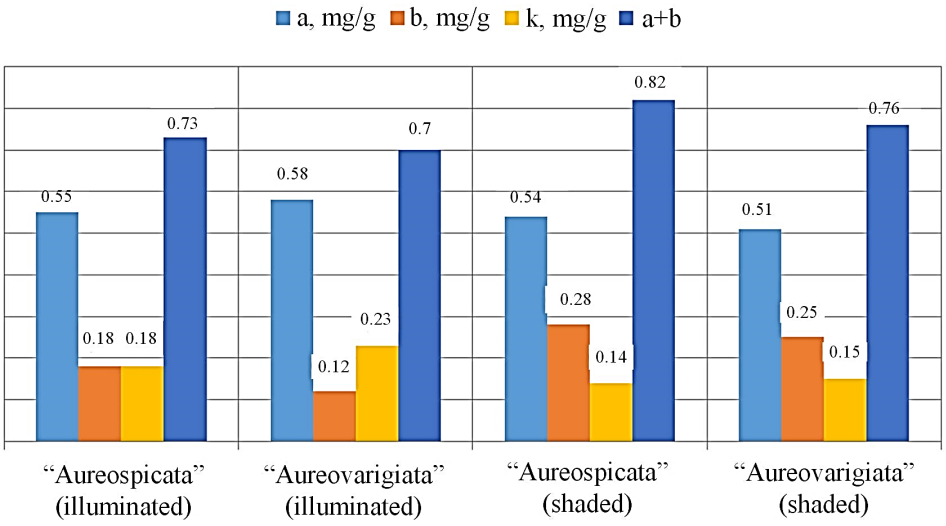


Figure 6. The effect of lighting on the pigment content and colour of the needles under mutual shading of “Aureospicata” and “Aureovarigiata” crowns.

A sharp decrease in insolation leads to a decrease in the colour inherent in these ornamental forms, and therefore such cultivars should be planted in isolation from each other.

Seasonal colour phases and the role of pigments in the colour of needles of northern white-cedar and its cultivars

Observations of seasonal changes in the colour of the needles of white-cedar and its cultivars have revealed certain dynamics, which indicates the possibility of using this factor in ensuring the decorative effect of a particular decorative form (Table 4). The study of the ratio of chlorophylls a and b to carotenoids in the needles by pigment separation was conducted in January.

Table 4. Seasonal colour changes of northern white-cedar and its cultivars.

No.	Name of the species, cultivar	months											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Columnar and pyramidal shapes													
1	*Th.o. occidentalis* L.	br.g.	br.g.	br.g.	br.g.	bl.g.	bl.g.	bl.g.	bl.g.	bl.g.	bl.g.	br.g.	br.g.
2	"Fastigiata"	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.
3	"Dougleasii Piramidalis"	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.
4	"Rosenthalii"	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.
5	"Wagneriana"	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.
6	"Wareana"	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.	bt.g.
Weeping forms													
1	"Filiformis"	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.
2	"Pendula"	br.g.	br.g.	br.g.	br.g.	g.	g.	g.	g.	g.	g.	g.	br.g.
3	"Pendula glauca"	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.	gr.g.
Spherical and cushion shapes													
1	"Globosa"	br.g.	br.g.	br.g.	br.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	br.g.	br.g.
2	"Hoveyi"	br.g.	br.g.	br.g.	br.g.	l.g.	l.g.	l.g.	l.g.	l.g.	l.g.	br.g.	br.g.
3	"Umbraculifera"	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.
4	"Woodwardii"	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.	d.g.
Juvenile forms													
1	"Ericoides"	b.g.	b.g.	b.g.	b.g.	g.	g.	g.	g.	g.	g.	b.g.	b.g.

Note: g. – green; bl. g. – brilliant green; d. g. – dark green; y. g. – yellow-green; gr. g. – grey-green; l. g. – l.g; l.g. – light green; bt. g. – bright green; br. g. – brownish-green; b. g. – brown-green.

The study of the changes, namely the browning of the studied needles, was recorded in winter, when the intensity of the insolation flux is very low.

Summarising the data of phenological observations in 2010–2011, it can be concluded that the main colour is green (Figures 7 and 8).

The green colour of northern white-cedar and its forms is dominant in summer, but in winter, some forms turn brown-green and brownish-green. The green colour, which prevails in summer, has 6 shades with a predominance of green and dark green (about 60%). In winter, green colours predominate in most

forms (70.7%), which is an important indicator of their high decorative value. This contrast in winter with the achromatic tones of deciduous trees and shrubs and urban development is advantageous from an aesthetic point of view.

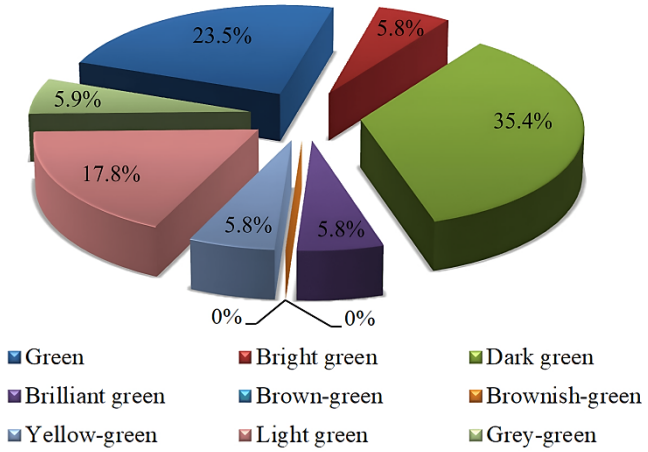


Figure 7. Distribution of summer colours and shades of northern white-cedar and its cultivars.

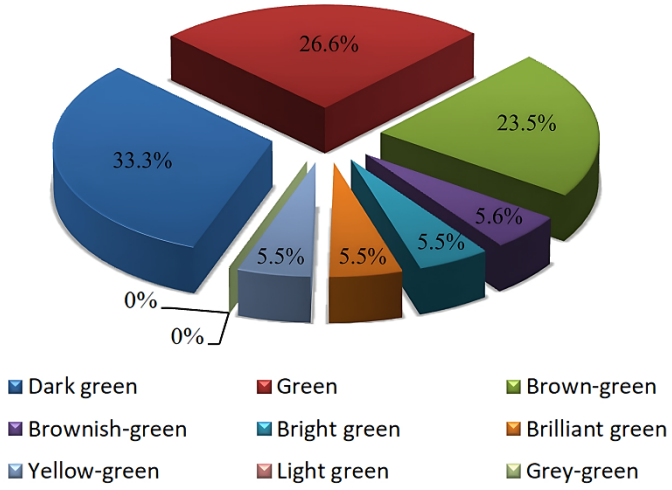


Figure 8. Distribution of the winter colours of northern white-cedar and its cultivars.

Some forms that change the colour of their needles in autumn and winter are of lesser aesthetic value. For example, a brown-green tone appears in 5.8% of forms, and a brownish-green tone in 3.5%. The juvenile form of northern white-cedar "Ericoides" has an original colour in winter – brown-green with a purple tint.

It is known that the colour of green plants is provided by plastid pigments, which are the most important components of the photosynthetic system. This method, used in this research, involves passing a pounded mass of pine needles dissolved in acetone through a sorbent (starch, talc). Due to the absorbing properties of starch and talc, the solution passed through the column was divided into layers, in which the chlorophylls a and b and the carotenoids were separated.

Experiment 1: Dark green needles taken from *Thuja occidentalis* "Fastigiata". The extract, made on the basis of acetone, had a dark green colour. The solution was passed through a column with a powdered mass of starch and talc: variant 1 (starch) chlorophyll a – 83.5%, chlorophyll b –16.5%; variant 2 (talc) chlorophyll a – 84.3%, chlorophyll b – 15.7%. As it can be seen, the chromatographic study in both cases revealed a close ratio of chlorophyll a to chlorophyll b. Chlorophyll a prevailed – 83.5% and 84.3%, respectively.

Experiment 2: Brownish needles taken from *Thuja occidentalis* "Globosa". The acetone-based extract had a greenish-brown colour. As in the previous case, the solution was passed through a powdered mass of starch and talc: variant 1 (starch) carotenoids – 9.5%, chlorophyll – 90.5%; variant 2 (talc) – carotenoids – 8.7%, chlorophyll – 91.3%. It is evident that the carotenoids accounted for less than 10% of the extract, but were capable of giving the solution a rich dark brown colour.

Changes in the summer green colour of *Thuja occidentalis* to autumn-winter, brownish-green and brown-green, occur due to an increase in the concentration of carotenoids in the cells, which, together with chlorophylls, are involved in the absorption of light by plants, taking part in the process of photosynthesis. Carotenoids easily form peroxides, in which oxygen molecules are attached at the double bond site and can then participate in the oxidation of various compounds. In general, carotenoids are involved in the processes of plant respiration and growth.

Conclusion

Seasonal measurements (summer – peak of vegetation, late autumn – end of vegetation) of the growth of apical shoots of Th.o. "Columna" trees in a row planting (simple rhythm: Col.-Col.-Col...) confirm the slowdown of growth of plants that were shaded by *Corylus avellana* L. While the growth of apical shoots in well-lit plants was 25 cm over the entire growing season, it was 13 cm in shaded plants. If the hazel bushes adjacent to the row are not removed, the shaded individuals will eventually disappear, which will lead to a violation of the rhythmic series.

The mutual shading of the lateral shoots of cedars with golden colour, "Aureospicata", leads to the replacement of the golden tone with a pale green one,

which leads to a decrease in the content of chlorophylls a from 0.49 mg/g to 42 mg/g, and carotenoids from 9.23 mg/g to 0.13 mg/g. In this regard, there should be a distance between the planting places of coloured forms that would ensure full lighting of the crown during the growth process in the future.

The dominance of green colours among the cultivars in the autumn-winter period gives great opportunities to use them in an achromatic architectural environment, especially in the autumn-winter period. Only a small part of cedars (9.3%), due to the accumulation of carotenoids in the autumn-winter period, acquires brownish-green and brown colours, and turns green colour in the spring-summer period. The carotenoids, which accounted for less than 10% of the studied extracts, give the solution a rich dark brown colour. The change of summer green colour to autumn-winter, brown-green and brown-green is due to the ratio of carotenoids to chlorophylls. In addition, the carotenoids are involved in the oxidation of compounds, affecting the colour of the needles.

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EKOLOŠKA I BIOLOŠKA ULOGA SVETLOSTI U RAZVOJU I SEZONSKOJ DINAMICI ČETINA ZAPADNE TUJE (*THUJA OCCIDENTALIS* L.) I NJENIH UKRASNIH FORMI U LAVOVU

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R e z i m e

Za ovo istraživanje smo odabrali urbane zelene prostore sa monotonim i kompleksnim ritmovima biljnih kompozicija gde smo ispitivali uticaj svetlosti na komepticiju izmedju individua, a u zavisnosti od prirode i toka svetlosti: od vrha ka dnu krošnje i ka otvorenom prostoru. U pogledu debljinskih razreda stabala kultivara zapadne tuje stubastog habitusa potvrdjena je diferencijacija, a napravljena je i prognoza daljeg razvoja biljaka koje zaostaju u rastu i koje bi trebalo pravovremeno ukloniti kako bi se očuvala ornamentalna kompozicija zelenih zidova. Istraživane su korelacije stepena osvetljenosti krošnje *Thuja occidenatlis* L. (Th.o.) "Fastigiata" u cilju pojašnjenja njene uloge u kompeticiji individua tokom leta i jeseni i njihovog rasta. Takođe, proučavani su sezonski uticaj svetlosti na promene boja četina kultivara izdvojenih prema koloritu i značaj hlorofila a i b, kao i karotenoida u tom procesu. Zlatno žute krošnje kultivara Th.o "Aureaspicata" i Th.o. "Aureovarigeata" u zaseni, usled smanjenja sadržaja karotenoida, su se transformisale u svetlo zelenu boju. Istovremeno, braon-zelena boja četina tokom zime je rezultat povećanja sadržaja karotenoida u ćelijama. Razumevanje mirovanja i dinamike boja dekorativnih formi zapadne tuje omogućava odabir koloritno najornamentalnijih kultivara za različite vrtne i parkovske kompozicije.

Ključne reči: zeleni zid, ritam sadnje, insolacija, spratnost, fotosinteza, hloroplasti, pigmenti, rast, untarvrsna kompeticija.

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OCENA KOEFICIJENATA NASLEDNOSTI ZA DUŽINU BREMENITOSTI I TELESNU MASU NA ROĐENJU U POPULACIJI HOLŠTAJN FRIZIJSKIH GOVEDA U REPUBLICI SRBIJI

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Rezime: Trajanje bremenitosti kod krava i telesna masa teladi na rođenju ima veliki uticaj na tok i težinu partusa kod mlečnih krava, te samim tim i vrlo značajan uticaj na ekonomičnost govedarske proizvodnje. Cilj istraživanja bio je da se utvrdi fenotipska varijabilnost i koeficijenti naslednosti navedenih osobina kao i mogućnost selekcije na dužinu bremenitosti i telesnu masu na rođenju u populaciji mlečnih goveda u Republici Srbiji. Istraživanjem je obuhvaćeno 1348 krava holštajn frizijske rase koje su se telile 4247 puta u periodu od 2007. do 2016. godine na jednoj farmi u okolini Beograda. Ispitivanje značajnosti faktora urađeno je u okviru procedure GLM statističkog paketa SAS. Komponente varijanse izračunate su primenom metodologije REML u okviru procedure VARCOMP statističkog paketa SAS (v. 9.4). Prosečno trajanje bremenitosti u ispitivanoj populaciji iznosilo je 278,5 dana, dok je prosečna telesna masa teladi na rođenju iznosila je 37,1kg. U istraživanju je utvrđen visoko statistički značajan uticaj laktacije po redu, godine i sezone teljenja kao i pola teleta na obe ispitivane osobine, sem u slučaju sezone teljenja na telesnu masu teladi, gde je sezona imala statistički značajan uticaj. Trajanje bremenitosti imalo je nisku naslednost od 0,018, dok je utvrđena vrednost koeficijenta naslednosti za telesnu masu na rođenju iznosila 0,355.

Ključne reči: koeficijent naslednosti, telesna masa na rođjenju, trajanje bremenitosti, holštajn rasa.

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Uvod

Bremenitost je period od začeća do partusa i predstavlja ključan period za razvoj sisara. Trajanje bremenitosti kao i događaji koji se odvijaju u toku nje imaju značajan uticaj na vitalnost, zdravlje i kasniju produktivnost teladi (Arnott et al., 2012). Telesna masa teladi na rođenju ima veliki uticaj na tok i težinu partusa kod mlečnih krava. Umerenu do jaku pozitivnu genetsku povezanost između telesne mase na rođenju sa teškim teljenjima, perinatalnim uginućima i dužinom bremenitosti utvrdio je veći broj autora u svojim istraživanjima (Johanson et al., 2011). Povećanu učestalost teških teljenja i mrtvorođenih teladi u populaciji holštajn frizijskih krava utvrdili su u svom istraživanju Meyer et al. (2001), što je za posledicu imalo gubitke u proizvodnji od 125,3 miliona dolara godišnje. Bicalho et al. (2008) ispitujući uticaj teških teljenja na produktivnost holštajn krava u SAD-u su utvrdili da 4,5% od svih teljenja čine teška teljenja koja se završavaju uginućem teleta (engl. *stillbirth*) i uzrokuju smanjenje dnevne proizvodnju mleka za 1,1kg.

Telesna masa teladi na rođenju je osobina na koju utiču kako genetski, tako i negenetski faktori. Određeni broj autora navodi mogućnost selekcije na nižu telesnu masu kod teladi, kako bi se smanjila učestalost pojave teških teljenja (Steinbock et al., 2003; Atashi et al., 2013). U većem broju istraživanja utvrđene su niske do srednje vrednosti koeficijenta naslednosti, kako direktnog tako i materinskog. Te vrednosti iznosile su od 0,08 do 0,26 u istraživanju sprovedenom od strane Johanson et al. (2011). Više vrednosti koeficijenta naslednosti za telesnu masu teladi na rođenju utvrdili su Yin i König (2018). Oni su utvrdili vrednost direktnog koeficijenta naslednosti koja je iznosila 0,47, dok je procenjeni materinski koeficijent naslednosti iznosio 0,19. Utvrđene vrednosti koeficijenta naslednosti za telesnu masu teladi na rođenju ukazuju da postoji mogućnost da se selekcijom postigne optimalna masa, koja će omogućiti laka teljenja sa jedne strane, a u isto vreme i dobru osnovu za adekvatne stope rasta nakon partusa. Kada je reč o uticaju negenetskih faktora na težinu teljenja i telesnu masu teleta na rođenju, najveći broj istraživanja je odredio godinu i sezonu teljenja, laktaciju i uzrast majke, dužinu bremenitosti i pol teleta kao najznačajnije faktore koji utiču na ispitivane osobine (Meyer et al., 2001; Johanson i Berger, 2003; Steinbock et al., 2003; Ettema i Santos, 2004).

Cilj istraživanja bio je da se utvrdi fenotipska varijabilnost i procene koeficijenti naslednosti za trajanje bremenitosti i telesnu masu na rođenju i mogućnost uključenja ovih osobina u selekcijske programe u populaciji mlečnih goveda, kako bi se skratilo trajanje bremenitosti i smanjila učestalost javljanja teških teljenja i mrtvorođene teladi kao direktnih posledica, tako i problema sa zdravljem i gubitaka u proizvodnji kod krava sa teškim teljenjem, kao sekundarnom posledicom.

Materijal i metode

Istraživanjem je obuhvaćeno 1348 krava holštajn frzijske rase koje su se telile 4247 puta u periodu od 2007. do 2016. godine na jednoj farmi u okolini Beograda. U pitanju je bila moderna farma, sa novoizgrađenim objektima za slobodno držanje krava, sa izmuzištem tipa rotolaktor gde su se krave muzle tri puta dnevno. Grla su pripadala holštajn frizijskoj rasi i prvobitno stado je bilo uveženo iz Kanade. Grla su hranjenja obrokom TMR prilagođenim fazi laktacije i nivou mlečnosti, ujednačenog sastava u toku cele godine.

Preuzeti set podataka je sadržao identifikacioni broj grla, paritet, datum osemenjavanja, datum teljenja, pol i telesnu masu teleta na rođenju, ocenu teljenja kao i oca teleta. Prvobitni set podataka je sadržao 5142 teljenja, ali su iz seta podataka isključeni zapisi koji nisu imali sve podatke ili ti podaci nisu bili logični. Deskriptivni statistički pokazatelji izračunati su primenom procedure PROC MEANS u okviru statističkog paketa SAS (SAS v9.4). Ispitivanje značajnosti faktora urađeno je u okviru procedure GLM statističkog paketa SAS.

Komponente varijanse izračunate su primenom metoda REML u okviru procedure VARCOMP statističkog paketa SAS (v. 9.4). Model koji je korišćen za izračunavanje komponenti varijanse imao je sledeći oblik:

$$Y_{ijklm}= \mu + G_i + S_j + L_k + P_l + s_m + e_{ijklm} \quad (1)$$

U kojem je:

Y_{ijklm} – fenotipska ispoljenost trajanja bremenitosti (TB/GL) i telesne mase na rođenju (TMNR/CBW),

μ – opšti prosek populacije;

G_i – fiksni uticaj i-te godine teljenja (i=2007.....2016);

S_j – fiksni uticaj j-te sezone teljenja (godina je podeljena na 4 sezone: I – zima: decembar, januar, februar; II – proleće: mart, april, maj; III – leto: jun, jul, avgust; IV – jesen: septembar, oktobar, novembar);

L_k – fiksni uticaj k-te laktacije;

P_l – fiksni uticaj pola teleta (I - muški pol, II - ženski pol);

s_m – slučajni efekat oca teleta;

e_{ijklm} – slučajna greška.

Koeficijent naslednosti je izračunat upotrebom sledeće formule:

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2}, \quad (2)$$

gde je:

h^2 – koeficijent naslednosti za ispitivane osobine, σ_s^2 – aditivna genetska varijansa, σ_e^2 – varijansa okoline.

Rezultati i diskusija

Prosečne vrednosti i pokazatelji varijabilnosti za telesnu masu na rođenju kod teladi i trajanje bremenitosti prikazani su u tabeli 1.

Tabela 1. Prosečne vrednosti i pokazatelji varijabilnosti za ispitivane osobine.
Table 1. Average values and variability of calf birth weight and gestation length.

Osobina/Trait	$\overline{X}$	SD	Var	min	max	Cv (%)
TMNR/CBW (kg)	37,1	5,1	25,85	20	65	13,7
TB/GL (dan/day)	278,5	5,19	26,92	266	298	1,86

TMNR/CBW – telesna masa na rođenju/calf birth weight, TB/GL – trajanje bremenitosti/gestation length.

Prosečno trajanje bremenitosti u ispitivanoj populaciji iznosilo je 278,5 dana, uz nisku varijabilnost, od svega 1,86%, što ukazuje da se više radi o biološkoj konstanti nego o klasičnoj osobini na koju je moguće u većoj meri uticati selekcijom. Prosečna telesna masa teladi na rođenju iznosila je 37,1kg. Slične vrednosti za dužinu trajanja bremenitosti utvrdili su u svom istraživanju Atashi i Asaadi (2019) u populaciji holštajn frizijskih krava u Iranu. Isti autori su utvrdili znatno višu telesnu masu na rođenju kod teladi holštajn frizijske rase u datoj populaciji. Kraće trajanje bremenitosti utvrdili su u svom istraživanju Kašná et al. (2020) u populaciji holštajn frizijskih goveda u Češkoj. Duže trajanje bremenitosti utvrdili su Norman et al. (2009) u populaciji holštajn krava u SAD-u, kao i Nienartowicz-Zdrojewska et al. (2018) u poljskoj populaciji holštajn frizijskih goveda. U istraživanju je utvrđeno da se sa porastom pariteta povećavalo i trajanje bremenitosti, tako da je najduža bremenitost bila u petoj laktaciji (grafikon 1). Na istom grafikonu se može videti da je duže trajanje bremenitosti utvrđeno kod krava koje su telile mušku telad. Veću telesnu masu na rođenju imala su telad oteljena u višim paritetima, kao i telad muškog pola (grafikon 2).

U tabeli 2 prikazana je statistička značajnost faktora uključenih u model.

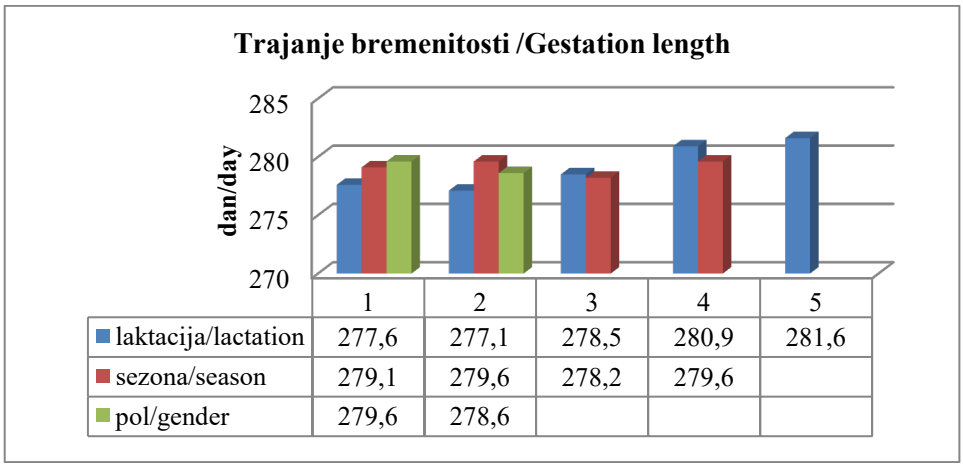
Tabela 2. Uticaj faktora na telesnu masu teladi na rođenju i dužinu bremenitosti.
Table 2. The influence of factors on calf birth weight and gestation length.

Osobina/Trait	Laktacija/ Lactation	Godina teljenja/ Year of calving	Sezona teljenja/ Season of calving	Pol teleta/ Gender of calf	R^2
TMNR/CBW (kg)	p<0,01	p<0,01	p<0,05	p<0,01	0,138
TB/GL (dan/day)	p<0,01	p<0,01	p<0,01	p<0,01	0,116

TMNR/CBW – telesna masa na rođenju/calf birthweight; TB/GL – trajanje bremenitosti/gestation length.

U istraživanju je utvrđena visoka statistička značajnost laktacije, godine i sezone teljenja kao i pola teleta na obe ispitivane osobine, sem u slučaju sezone teljenja na telesnu masu teladi, gde je sezona imala statistički značajan uticaj. Statistički značajan uticaj pola teleta, godine i sezone teljenja kao i pariteta na trajanje bremenitosti utvrdili su u svom istraživanju Kašná et al. (2020). Statistički značajan uticaj godine i meseca osemenjavanja na trajanje bremenitosti utvrdili su Norman et al. (2009). Visoko statistički značajan uticaj sezone teljenja na dužinu bremenitosti i telesnu masu na rođenju teladi utvrdili su u svom istraživanju Nienartowicz-Zdrojewska et al. (2018).

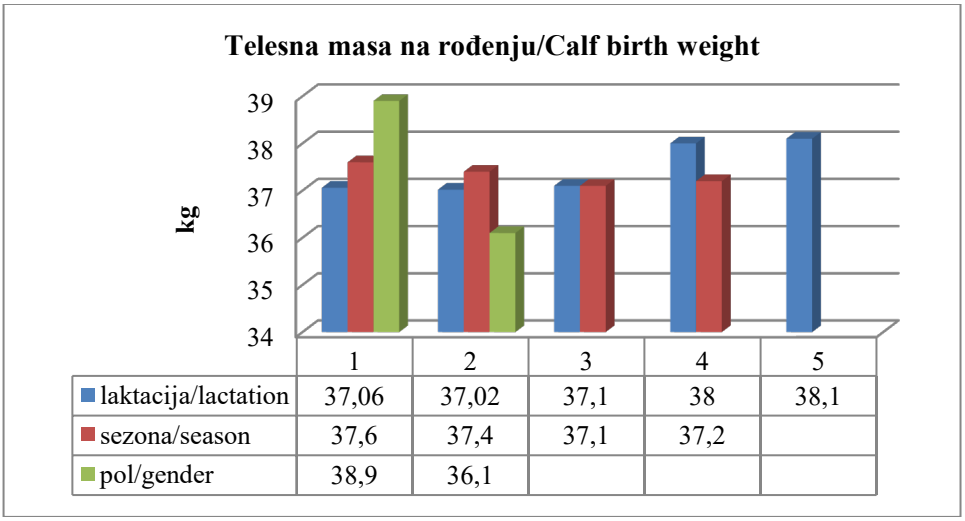
Trajanje bremenitosti u zavisnosti od nivoa uključenih faktora prikazani su na grafikonu 1.



	1	2	3	4	5
laktacija/lactation	277,6	277,1	278,5	280,9	281,6
sezona/season	279,1	279,6	278,2	279,6	
pol/gender	279,6	278,6			

Grafikon 1. Trajanje bremenitosti u zavisnosti od nivoa uključenih faktora.
Graph 1. Gestation length depending on the level of included factors.

Laktacija po redu imala je statistički visoko značajan uticaj na trajanje bremenitosti i telesnu masu teladi na rođenju. Najkraća bremenitost zabeležena je u u drugoj laktaciji dok je najduže trajanje bremenitosti utvrđeno u petoj laktaciji. Najveću telesnu masu imala su telad oteljena u petoj laktaciji, dok je najmanja telesna masa utvrđena u drugoj laktaciji (grafikon 2). Pol teleta je uticao da su krave koje su otelile mušku telad imale duže trajanje bremenitosti u odnosu na krave koje su telile žensku telad. Delimično kao posledica toga muška telad su imala veću telesnu masu u odnosu na žensku telad. Sezona teljenja je imala statistički značajan uticaj na telesnu masu na rođenju, dok je na trajanje bremenitosti imala visoko statistički značajan uticaj. Najveću telesnu masu su imala telad oteljena u toku zime, dok su najmanju telesnu masu imala telad oteljena u toku leta.



Grafikon 2. Telesna masa teladi na rođenju prema nivoima uključenih faktora.
Graph 2. Calf birth weight according to the levels of included factors.

Vrednosti varijansi, koeficijenata naslednosti i njihove greške utvrđene u istraživanju prikazane su u tabeli 3.

Tabela 3. Koeficijenti naslednosti za trajanje bremenitosti i telesnu masu na rođenju kod teladi.
Table 3. Coefficients of direct heritability of calf birth weight and gestation length.

Traits	σ_a^2	σ_e^2	h^2	SE_h^2
TMNR/CBW (kg)	0,16	35,17	0,018	0,003
TB/GL (dan/day)	2,087	21,192	0,355	0,04

TMNR/CBW – telesna masa na rođenju/calf birth weight; TB/GL – trajanje bremenitosti/gestation length.

Za osobinu trajanje bremenitosti procenjena je niska vrednost koeficijenta naslednosti i iznosila je 0,018. Značajno više vrednosti koeficijenta naslednosti u svom istraživanju utvrdili su Jamrozik et al. (2005) i Haile-Mariam i Pryce (2019). Posledica ovako velikih razlika jeste primenjena metodologija, kao i sam model koji su autori koristili. U ovom istraživanju utvrđena je srednja vrednost koeficijenata naslednosti za telesnu masu na rođenju i iznosila je 0,355. Blisku vrednost za naslednost mase na rođenju kod teladi utvrdili su Nienartowicz-Zdrojewska et al. (2018) u poljskoj populaciji holštajn frizijskih goveda. Nižu vrednost koeficijenta naslednosti za telesnu masu na rođenju utvrdili su Johanson et al. (2011), kao i Hansen et al. (2004) u populaciji holštajn goveda u Danskoj.

Zaključak

Genetska evaluacija trajanja bremenitosti pokazala je da je u pitanju osobina sa niskom naslednošću i da bi selekcijski odgovor bio neznatan ako ovu osobinu stavimo u fokus selekcijskog rada. Za razliku od nje, telesna masa na rođenju pokazala je znatno višu naslednost (h^2=0,355) i potencijal da se selekcijom, pre svega bikova koji će se koristiti za veštačko osemenjavanje, može uticati na postizanje optimalne telesne mase na rođenju, što bi uticalo na smanjenje pojave teških teljenja i postpartalnih komplikacija i kod krava i kod teladi, koje nastaju kao posledica teških teljenja. U narednom periodu neophodno je razmotriti uključivanje ove ekonomski važne osobine u odgajivačke programe za holštajn frizijsku rasu u Republici Srbiji, kako bi se smanjili gubici i troškovi koji nastaju kao posledica teških teljenja i povećala profitabilnost govedarske proizvodnje.

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ESTIMATION OF HERITABILITY COEFFICIENTS FOR GESTATION LENGTH AND CALF BIRTH WEIGHT IN THE POPULATION OF HOLSTEIN CATTLE IN THE REPUBLIC OF SERBIA

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A b s t r a c t

The gestation length in cows and the birth weight of calves significantly influence the course and difficulty of parturition in dairy cows, thereby having a considerable impact on the economic efficiency of cattle production. The aim of the study was to determine the phenotypic variability and heritability coefficients of these traits, as well as the potential for selection for these traits in the dairy cattle population in the Republic of Serbia. The research included 1,348 Holstein-Friesian cows, which calved 4,247 times between 2007 and 2016 on a farm near Belgrade. The significance of the factors was analyzed using the GLM procedure of the SAS statistical package. The variance components were calculated using the REML methodology within the VARCOMP procedure of the SAS statistical package (version 9.4). The average gestation length in the studied population was 278.5 days, while the average birth weight of the calves was 37.1 kg. The study showed a high statistical significance of parity, year, calving season, and calf sex for both traits, except for the effect of calving season on calf birth weight, where the season had a statistically significant effect. Gestation length showed a low heritability (0.018), whereas the heritability coefficient for birth weight was determined to be 0.355.

Key words: heritability, calf birth weight, gestation length, Holstein breed.

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CLASSIFICATION OF THE SOILS OF RIVER ISLAND MICRO-DEPRESSIONS (GREAT WAR ISLAND, SERBIA)

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Abstract: Great War Island (GWI) is a river island formed at the confluence of the Sava and Danube rivers, heavily exposed to groundwater and flooding and is therefore suitable as a case study for the investigation of hydromorphic soils. The aim of this study was to classify the soils in two different micro-depression on Great War Island according to the local (Škorić 1985) and international (WRB 2022 and USDA Soil Taxonomy 1999) systems, with particular attention to the soil-forming factors that influenced the classification of the soils. The results obtained could help to improve the existing local classification system or to create a new system in the future. The soil of the closed (less flooded) micro-depression is Eugley, Hipogley, Mineral, Calcareous (Škorić 1985) or Calcaric Oxygleyic Gleysol (Loamic, Humic) (WRB 2022). The soil of the micro-depression open to the Danube (more flooded) is Humogley, Calcareous, Weakly alkalized, Loamy (Škorić 1985) or Calcaric Oxygleyic Mollic Tidalic Gleysol (Loamic, Fluvi-Loaminovic) (WRB 2022). The both soils are Typic Endoaquolls (USDA Soil Taxonomy 1999). The high level and amplitude of the groundwater and the duration of the flood caused by the topography, as well as the texture of the alluvial sediments, are the main soil-forming factors that have influenced the classification of the soils. The local soil classification mostly corresponds to the two international soil classifications with regard to the influence of pedogenetic factors/characteristics. To increase its accuracy, quantitative thresholds for soil type and lower levels are required.

Key words: WRB, soil taxonomy, eugley, humogley, gleysols, typic endoaquolls.

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Introduction

Hydromorphic soils are formed when there is an excess of water, which can cause anaerobic conditions in the soil in whole or in part. Saturated and waterlogged soils occupy about 6% of the Earth's surface (2.1 million km^2) (Zhang et al., 2016). These hydromorphic soils are mainly distributed along coasts, lakes, shores, river deltas, river islands and their tributaries, and their properties have been studied worldwide (Bandyopadhyay et al., 2017). The formation of the hydromorphic soils is primarily the result of the interaction of topography and hydrological processes under specific geological and geomorphological conditions (Lin et al., 2007). Parent material and bedrock, groundwater table fluctuations, past and present river flow velocities, relief positions and anthropogenic factors are summarized by Yakovenko et al. (2023) as the most important factors for the formation of hydromorphic soils in river valleys.

Hydromorphic soils have long been recognized by morphological and chemical characteristics, but in the past different criteria were used for their classification, and the different soil classification systems were not satisfactorily interchangeable (Okusami, 1985). The World Reference Base (WRB) soil classification was introduced as an international soil correlation and communication system, which was approved and adopted from 1998 to 2022 (fourth edition) (IUSS Working Group WRB, 2022). The soils traditionally referred to as hydromorphic belong to soils that differ in Fe/Al chemistry and belong mainly to the WRB reference soil groups (RSGs) Gleysols (groundwater influenced, submerged or tidal), Planosols (stagnant water, abrupt textural difference) or Stagnosols (stagnant water, structural difference and/or moderate textural difference). The RSG of Fluvisols (stratified fluviatile, marine or lacustrine sediments) is categorized under soils with little or no profile differentiation. By using well-defined and quantified diagnostic horizons, properties and materials, the WRB has become one of the applied international soil classification systems. Hydromorphic soils, i.e., the wettest soils, are classified at the second level of the United States Department of Agriculture (USDA) Soil Taxonomy (Soil Survey Staff, 1999) as wet (Aqu) suborders within various soil orders or as subaqueous (Wass) suborders of the orders of Entisols and Histosols (Rabenhorst et al., 2017).

The soil classification system developed in the former Yugoslavia (Škorić et al., 1985) is still used in its original or modified version in Serbia (Đorđević and Radmanović, 2018) and other countries that emerged from Yugoslavia (Filipovski, 2001; Resulović et al., 2008; Husnjak, 2014; Repe, 2020). Hydromorphic soil is one of the four soil divisions (the highest level unit), which is subdivided into the classes Surface water gley, Undeveloped, Semigleys, Gleys and Histosols, each containing one or more soil types. In general, the classification units are qualitatively defined and have few quantitative boundaries.

At the turn of the 18th and 19th centuries, extensive river regulation was carried out in Europe and in the late 18th and during the 19th and 20th centuries in Serbia, in order to minimize flood damage (Prokof'eva et al., 2010; Đorđević and Radmanović, 2018). Drained floodplains have been converted into agricultural, forestry or urban areas (Prokof'eva et al., 2010; Łabaz and Kabala, 2016). The prevention of flooding and groundwater recharge significantly alter soil conditions and soil formation, leading to a change in soil morphology and other properties and their classification (Łabaz and Kabala, 2016; Kawalko et al., 2021). Great War Island is a river island (Figure 1) with little topographic variation, yet highly exposed to groundwater and flooding, making it a suitable case study for the study of hydromorphic soils. The aim of this study was to classify the soils in two diverse micro-depressions of the Great War Island, according to the local (Škorić et al., 1985) and international WRB 2022 (IUSS Working Group WRB, 2022) and the USDA Soil Taxonomy (Soil Survey Staff, 1999) systems, with particular reference to the pedogenetic factors that affect soil classification. The results obtained will contribute to increase the knowledge on the basis of which the existing local soil classification system could be improved or a new system created in the future.

Material and Methods

The GWI is located (Figure 1A) at the contact of the Carpatho-Balkan Massif and the Pannonian Plain and at the confluence of the Sava and Danube rivers. The rivers connect at an obtuse angle, their water slows down and leads to the deposition of transported material. These Holocene alluvial sediments are about 25 m thick. The process of sedimentation is still ongoing and is changing the shape and surface area of the island. As a result of the uneven accumulation effect of the flood waters, there are zones of micro-elevations (the highest 73.5 m a.s.l. on the upstream part of the coast), micro-depressions (~ 69.5 m a.s.l.) and generally flat areas (~ 72 m a.s.l. on average). The Great War Island currently covers an area of 2.11 km². The land is covered with natural forest or grass-herbaceous vegetation. Since 2005, the GWI has been declared an area of importance for the protection of the environment and cultural and historical heritage (JKP Zelenilo-Beograd, 2018). For the period 1991–2020 (RHSS, 2024), the average annual precipitation was 698.9 mm, with the lowest monthly value being 43.5 mm in February and the highest value being 95.6 mm in July. The average annual air temperature was 13.2°C, with the warmest months being July and August (average 23.8°C) and the coldest January (1.9°C).

The field investigation (creation and description of profiles) was carried out using the field guide required for soil classification according to WRB (IUSS Working Group WRB, 2022). The groundwater level was visually estimated in the soil profiles. The soil color was determined using the Munsell color charts. Disturbed soil samples were taken from genetic horizons and their layers (if

present). Laboratory analyses were carried out using standard methods: particle size distribution by the sieve and pipette method, preparation with Na-pyrophosphate, soil texture class according to IUSS Working Group WRB (2022), soil organic carbon by the Tiurin method, $CaCO_3$ by the volumetric Scheibler method and pH in distilled H_2O (soil/water = 1/2.5).

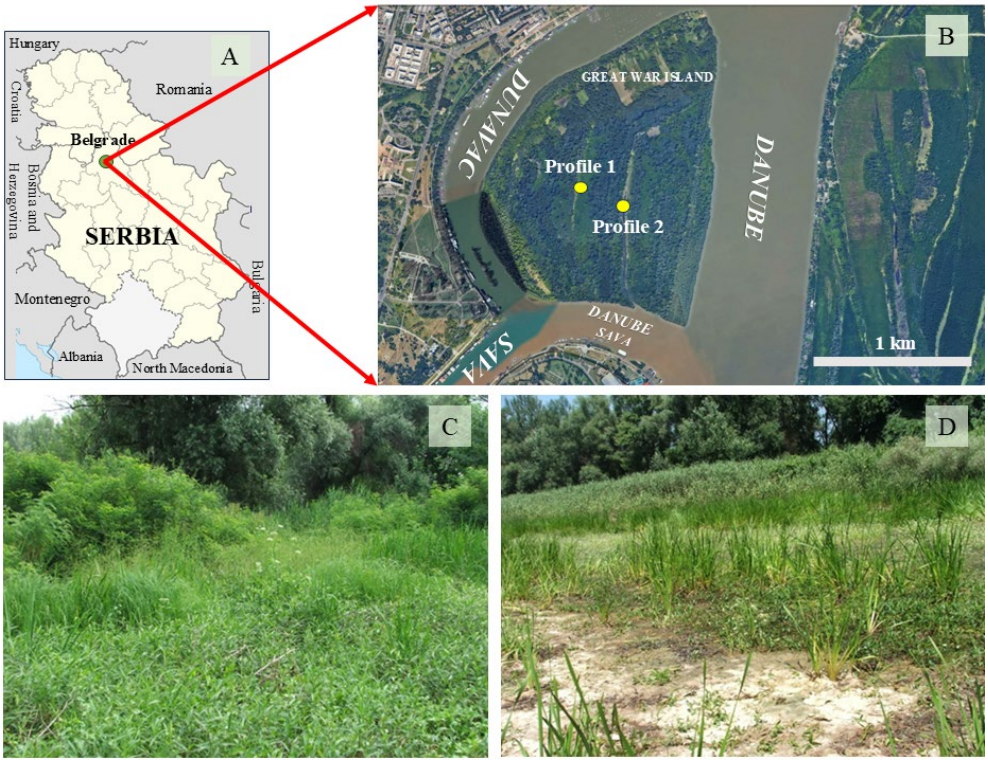

Figure 1. A – Geographical position of the study area; B – Great War Island, profiles 1 (44° 49' 47.60" N/20° 26' 4.44" E) on closed micro-depression (CMDS), and 2 (44° 49' 54.16" N/20° 26' 13.27" E) on open micro-depression (OMDS); C – vegetation of CMDS; D – vegetation of OMDS.

The soil profiles were created in two existing micro-depressions (Figure 1B), which differ in altitude, i.e., the presence of groundwater and floodwater, causing soil hydromorphism. Profile 1 was opened in a closed micro-depression (CMDS in the following text) in the central part of the GWI, where the groundwater level rises to the soil surface for most of the year. The micro-depression is occasionally flooded in wet years and when the water level of the Danube is high. The ground surface is flat. Profile 2 is located in the Veliki Galijaš canal micro-depression open to the Danube (OMDS in the following text). The Veliki Galijaš canal was at times

in history a canal and a lake, which was last reunited with the Danube in 2007. In addition to the high groundwater level (up to soil surface, except in the driest part of the year), the OMDS profile has been flooded and inundated in the past and is currently flooded every year. The ground surface slopes gently to the south-east towards the Danube. The parent material consists of fluvial and lacustrine sediments, especially sands and clayey sands in the closed micro-depression (CMDS) and sands and silts in the Veliki Galijaš lake/canal (OMDS) (Marković et al., 1984). The vegetation of the CMDS in the closed micro-depression consisted mainly of herbs and grasses and only rarely of aquatic (non-woody) plants (Figure 1C). Aquatic (non-woody) plants and bare soil surfaces formed by a layer of tin litter were present in the Veliki Galijaš canal, OMDS (Figure 1D).

The determination of the genetic horizons and the classification of the soils were carried out in parallel according to Škorić et al. (1985) (Škorić 1985 in the following text) and IUSS Working Group WRB (2022) (WRB 2022 in the following text). The soils were also classified according to the USDA Soil Taxonomy (Soil Survey Staff, 1999) (USDA Soil Taxonomy in the following text).

Results and Discussion

The soil properties required for classification are presented in Table 1. Figures 2A and 2B represent the CMDS and OMDS, respectively. The soils were wet from the surface, and the water table was stabilized in the CMDS and OMDS at depths of 90 and 75 cm, respectively.

Table 1. Morphological, physical and chemical properties of the soil studied.

Depth	Genetic horizons		Soil matrix color		Texture	SOC	$CaCO_3$	pH
cm	a[2]	b[2]	dry	moist	class	%	%	H_2O
CMDS[1]	[a]**Eugley**, Hipogley, Mineral, Calcareous; [b]Calcaric Oxygleyic **Gleysol** (Loamic, Humic); [c]Typic Endoaquolls							
0–20	A/Gso	Al1	2.5Y 4/2	2.5Y 3/2	clay loam	2.1	11.3	7.4
20–40	A/Gso	Al2	2.5Y 5/2	2.5Y 4/2	clay loam	1.3	13.6	7.6
40–60	Gso	Bl1	2.5Y 6/2	2.5Y 4/2	clay loam	1.0	14.5	7.8
60–80	Gso	Bl2	2.5Y 6/3	2.5Y 4/3	clay loam	1.1	11.5	7.7
80–90	Gso	Bl3	2.5Y 7/2	2.5Y 5/2	clay loam	1.0	18.1	7.7
OMDS[1]	[a]**Humogley**, Calcareous, Loamy; [b]Calcaric Oxygleyic Mollic Tidalic **Gleysol** (Loamic, Fluvi-Loaminovic); [c]Typic Endoaquolls							
0–20	Amo/Gso	Al	10YR 4/2	10YR 3/2	clay loam	2.4	14.6	7.8
20–35	Amo/Gso	2Alb1	10YR 3/2	10YR 2/2	clay loam	3.7	11.0	7.7
35–55	Amo/Gso	2Alb2	10YR 5/2	10YR 3/2	clay loam	2.0	11.9	7.9
55–75	Gso	3Bl	10YR 5/3	10YR 4/3	clay loam	1.0	15.4	8.0

[1]CDMS – closed micro-depression soil; OMDS – open micro-depression soil. [2]Horizons and classification according to: a – Škorić et al. (1985); b – IUSS Working Group WRB (2022); c – Soil Survey Staff, 1999.

The surface A horizon of the CMDS is divided into two layers (Table 1, Figure 2A), with the upper Al1 containing redoximorphic features. The lower layer (20–40 cm), which has more pronounced redoximorphic features, is referred to as Al2. The layer below A (40–90 cm) is designed as the Bl horizon and is divided into three layers. These layers are calcareous, contain about 1% SOC and have very pronounced redoximorphic features (reductimorphic features predominate over oxymorphic features at a soil depth of about 60 cm).

Figure 2. Genetic horizons according to Škorić et al. (1985) (left) and IUSS Working Group WRB (2022) (right) of the soil from: A – the closed micro-depression (CMDS) and B – the open micro-depression (OMDS).

The decrease in the humus content and the change in the frequency of oximorphic and reductimorphic features with soil depth led to a change in soil color. The color of the soil matrix was: dark gray-brown, gray-brown (A), light brownish gray, light yellowish brown, light gray (B), and moist: very dark gray-brown, dark gray-brown (A), olive brown, gray-brown (B). Although the

proportion of sand decreases with increasing depth and silt and clay increase, the texture class remains the same over the entire soil depth. The subangular blocky structure in the A horizon changes to an angular blocky structure in the B horizon. Only a few roots are present in the lower part of the soil profile.

According to Škorić 1985, CMDS with an A – humic genetic horizon of less than 50 cm depth and a genetic G – gley horizon with clearly differentiated subhorizons Gso and Gr (not registered up to a depth of 90 cm) belongs to division – Hydromorphic, class – Gleys, type – Eugley, subtype – Hipogley (process of gleysation by groundwater), variety – Mineral, form – Calcareous.

Figure 3. Redoximorphic features of the soil of: A – the closed micro-depression (CMDS) (Gso or Bl horizon, 40–60 cm depth) and B – the open micro-depression (OMDS) (Gso or Bl horizon, 55–75 cm depth). The soils are 20 cm high.

According to WRB 2022, CMDS shows gleyic properties and reducing conditions in some part of the soil material, calcareous and mineral material, SOC, and no diagnostic horizon. Gleyic properties in the upper 60 cm were confirmed with 50% oximorphic features found predominantly on the biopore walls and aggregate surfaces, fine to very coarse, non-cemented, reddish brown in color

(2.5YR 5/4), moist (Figures 2A and 3A). Reductimorphic features, light gray (2.5Y 7/1) in color, moist, were distributed throughout the layer and surrounded areas of oximorphic features. Reductimorphic features predominated over oximorphic features deeper than 60 cm from the soil surface and were gray in color (2.5Y 5/1 and 6/1). With gleyic features at the soil surface and reducing conditions in some parts of each sublayer, the CMDS meets criterion 1 of the RSG Gleysols. Since there is no layer with ≥ 95% reductimorphic features within 100 cm of the soil surface, the Oxigleyic principal qualifier fits with the CMDS. As the CMDS also contains over 11.3% primary carbonate, it meets the criteria for the Calcaric principal qualifier. The Loamic supplementary qualifier results from the texture class of clay loamy over the entire soil depth and Humic with a SOC content of ≥ 1.46% as a weighted average up to a depth of 50 cm from the soil surface. According to WRB 2022, the CMDS is therefore classified as Calcaric Oxygleyic Gleysol (Loamic, Humic).

A very thin (1 cm) litter layer has been formed on the soil surfaces of the OMDS (Figure 2B), under flood water and probably at high groundwater levels. The genetic A horizon is a first mineral layer, calcareous, 55 cm thick (Table 1). The A horizon is divided into three sub-layers, which differ in color, humus content and redoximorphic features. Below the Al horizon is a layer with a higher humus content and a darker color as well as more redoximorphic features. This is obviously a buried A horizon, which is divided into two sub-layers, 2Alb1 and 2Alb2. Sublayer 2Alb2 has a lower humus content and a lighter color. Below 55 cm, the humus content decreases, the redoximorphic features are strong, but the color remains dark, probably due to the hydrogenic conditions caused by the high water table during most of the year. This layer is referred to as the genetic 3Bl horizon. The color of the soil matrix changes with depth as follows: dry – dark gray-brown, very dark brown, gray-brown, brown, and moist – very dark gray-brown, very dark brown, very dark gray-brown, dark brown. The horizons differ in sand, silt and clay content (no trend is apparent), but the soil texture class remains the same over the entire soil depth. Soil aggregate structure was weak, fine to medium across the depth. Few (coarser in the upper layer) roots are present on the soil. Snails in deeper layers and earthworms in the surface layers (< 10 cm) were the observed soil fauna.

According to Škorić 1985, the OMDS with a genetic mollic humus horizon above 50 cm and a predominantly oximorphic gley horizon (Gso) up to the soil profile (75 cm) belongs to the division – Hydromorphic, class – Gleys, type – Humogley, subtype – Calcareous, form – Loamy.

According to WRB 2022, the OMDS has a mollic diagnostic horizon, gleyic properties and reducing conditions in some part of the soil materials, calcaric and mineral materials and SOC. The mollic horizon is characterized by a granular aggregate structure with an average aggregate size of 3 cm, ≥2%

SOC and a Munsell color value of ≤ 3 moist and 4 dry as well as a chroma value of ≤ 2 moist, a very high base saturation (pH ≥7.7) and a thickness of 55 cm. The gleyic properties are expressed from the soil surface to the bottom of the soil profile. Oximorphic features were present > 50% of the exposed area, predominantly on biopore, walls and aggregate surfaces, and were red in color (10R 5/8 and 4/8), moist (Figures 2B and 3B). Reductimorphic features, gray in color (10Y 4/1 and 5/1) moist, were distributed throughout the layer and surrounded areas with oximorphic features. The OMDS met criterion 2 of the RSG Gleysols: a mollic horizon 55 cm thick and gleyic properties and reducing conditions in some parts from the soil surface to the soil profile (75 cm). The principal qualifiers associated with the OMDS are: Tidalic, Mollic, Oxigleyic and Calcaric. The Tidalic qualifier is the result of tidal water from the Danube River (Veliki Galijaš canal) acting on the ground surface every year and causing the formation of a tin litter layer (Figure 2B). The Mollic qualifier requires a mollic diagnostic horizon. As in the OMDS, there is no layer with ≥ 95% reductimorphic features within 100 cm of the soil surface, so the Oxigleyic principal qualifier matched the OMDS. With a primary carbonate content of over 11%, the OMDS meets the criteria for the Calcaric principal qualifier. The clay loamy soil texture caused the Loamic supplementary qualifier. The surface layer (20 cm), which has a lower SOC content than the subsurface (20–40 cm), is obviously of fluvial origin, but does not fulfill the criteria for Fluvic material (>25 cm required) and the Fluvic principal qualifier. Therefore, the loamy clay fluvial surface layer already implies a Fluvi-Loaminovic supplementary qualifier. The subsurface layer (20–40 cm) of the A horizon in the OMDS, which has a higher SOC content than the surface layer, is referred to as buried, but does not fulfill the criterion of the Panpaic horizon, which has no lithic discontinuity at its upper boundary. Finally, the OMDS is classified as Calcaric Oxygleyic Mollic Tidalic Gleysol (Loamic, Fluvi-Loaminovic) according to WRB 2022.

According to USDA Soil Taxonomy, both soils exhibit a mollic epipedon and satisfactory base saturation at depth, meeting the criteria of the order Mollisols. With chroma 2 and pronounced redox concentrations in the lower part of the mollic epipedon, the soils belong to the suborder Aquolls. The great group Endoaquolls refers to soils with endosaturation. The groundwater level is located at or near the soil surface during wet periods (winter, spring and early summer in the study area) and is deeper during dry periods (late summer). The subgroup is Typic Endoaquolls. In summary, the two soils investigated are Mollisols, Aquolls, Endoaquolls, Typic Endoaquolls.

As is well known (Collins, 2005), the water supply in soil wetland depression comes from groundwater discharge (high groundwater table), precipitation, surface runoff and possibly spring water. It is obvious that the investigated soils belong to

the same RSG of Gleysols due to the dominant influence of groundwater on their formation and the resulting gleyic properties. The gleyic properties develop in layers that are saturated with groundwater for a period of time that allows reducing conditions to occur (several weeks) and in the overlying capillary fringes that are saturated long enough for the soil to become partially anaerobic. The gleyzation process leads to an underlying highly reduced layer and an overlying layer with oximorphic features on or adjacent to the surface of the soil aggregates (IUSS Working Group WRB, 2022). In the studied Gleysols, there are no strongly reduced layers with permanently wet conditions (with ≥ 95% reductimorphic features) at the bottom of the soil pits. The oximorphic features over the soil depth indicate oxidizing conditions, i.e., high groundwater level fluctuations. Consequently, the Oxygleyic principal qualifiers are characteristic of the both soils. Iron oxides/hydroxides were concentrated on the surfaces of the soil aggregates and the walls of larger pores (e.g., old root channels), which is typical for these Gleysols with a clay loamy texture according to the IUSS Working Group WRB (2022). In addition, the Calcaric principal qualifiers and the Loamic supplementary qualifier are characteristic of the stand.

In contrast to the CMDS, the OMDS, which is located in a canal normally subject to a high level of groundwater and flooding, is characterized by two more interconnected principal qualifiers, Mollic and Tidalic. In general, the Tidalic is more characteristic of the soils of the marine intertidal zones, which cover the largest area compared to the fluvial zones (Gröngröft et al., 2020). The A horizon of the OMDS, which has mollic attributes, is so deep because it contains the buried humus horizon above which the river flood has deposited the alluvial layer, also enriched with humus. The dark color of the two layers, the buried and the novic, is a consequence of the humification process, which partly takes place under saturated soil conditions. In addition, the aquatic plants produce more slowly decomposable litter compared to herbs and grasses, which leads to an increase in the humus content. The process of accumulation of already humified organic matter transported by the river water probably also took place.

Gleysols are widespread (more than 720 million ha worldwide) at all latitudes and climatic zones (perhumid to arid), on low elevations in landscapes with high groundwater tables, tidal areas, shallow lakes and seashores (IUSS Working Group WRB, 2015). Consequently, various principal and secondary qualifiers can be assigned to these WRB RSGs. For example, Rubinić et al. (2020) reported on Eutric Reductigleyic Stagnic Gleysols along the Sava and Drava rivers, where the stagnic properties, i.e., periodically stagnant surface water (precipitation), are caused by a heavy clay texture. In the Dnipro River valley in Ukraine (Yakovenko et al., 2023), most of the Gleysols are Fluvic and Calcic, which is mainly due to the relief and sediments variances formed by fluvial and eolian processes. Zhangurova et al. (2023) reported Reductaquic Gleysols in the mountain tundra of the Ray-Iz

massif in the polar Urals, while Łachacz and Nitkiewicz (2021) classified some soils formed from deposits of bottom lakes in north-eastern Poland as Eutric Gleysols. According to a national Romanian soil classification, the calcaric mollic subtype of Gleysols is found in a poorly drained lower part of a Danube river basin (Moraru et al., 2020).

According to Škorić 1985, the differences between the soils were manifested on the third level, the soil type, chiefly due to the thickness of the humus-accumulative horizons. One of several quantitative limits in Škorić 1985 is the thickness of the A horizon, less than 50 cm for Eugley and over 50 cm for Humogley. In addition, for Humogley, a mollic form of the A horizons was required, based on soil color and base saturation. Soil properties that are important for distinguishing soil types according to the Škorić 1985 system came to the fore at the second level of the WRB 2022 system, resulting in the Mollic principal qualifier for the same soil.

The amplitude of underground water fluctuation is another criterion at the soil type level. The authors are not aware of any data on the height of the GWI groundwater table. The long-term averages of the mean groundwater level on the surrounding left (Banat) and right (Ušće) coasts are 68 m a.s.l. (Tošović, 2002). Furthermore, the frequency and duration of floods have not been quantified. Therefore, as usual (Lin et al., 2005), soil morphology is used as a method to indicate drainage conditions and pedogenetic processes in the field. However, the relationships between water regimes and the resulting morphological properties are often complex, and interpretation can be more complicated than simplified schemes offer (Bouma, 1983). For example, the OMDS, which is wetted by groundwater and floodwater for a certain time of the year, could have an amfigley character (Škorić 1985), meaning a surface layer with stagnic properties, a bottom layer with gleyic properties and a less gleyic intermediate layer. A layer with clearly pronounced stagnic properties is not recognisable in either soil. Some lighter tubular features are observed, but these are not sufficient for WRB 2022 Stagnic principal or Uterquic supplementary qualifiers, or for Škorić 1985 Amfigley subtype of Eugley.

Determining the genetic horizons of the soil was one of the challenges of morphological observation. A layer with redoximorphic features as a result of excessive wetting with groundwater, traditionally referred to as the G horizon (Table 1, Figure 2), is still used in many national and regional soil classifications (Pollmann et al., 2018; Moraru et al., 2020; Łachacz and Nitkiewicz, 2021; Zhangurova et al., 2023). According to the international systems USDA Soil Taxonomy and WRB 2022, some master horizons (H, A, B or C) were changed by the gleyization process. The layers below the A horizons in the studied Gleysols are referred to as B horizons, which is due to the formation of a soil aggregate structure.

As the area has been declared a protected area, the direct impact of agriculture, forestry and urban development on the future development of the soils under investigation should be limited. The groundwater level and flooding, as crucial pedogenetic factors that depend on the water level and flow velocity of the Danube and Sava rivers, can be altered by anthropogenic and natural influences. The Đerdap dam already influences the water flows of the Danube and Sava rivers (JKP Zelenilo-Beograd, 2018), and future regulation could do the same. The predicted climate change, leading to rising temperatures, melting ice, rising sea levels and oceans, and a change in precipitation regimes, with global and local impacts (Đorđević et al., 2020; Trajković and Milanović, 2021), will certainly affect the water balance of the studied soils. The changes caused by waterlogging will affect plant composition and diversity, primary production, soil properties, i.e., nutrient status, humus accumulation (Collins, 2005), will certainly change the taxonomy of soils as well.

Conclusion

On the Great War Island, which was formed at the confluence of the Sava and Danube rivers, the soils from two micro-depressions were classified according to the local system of Škorić 1985 and the international systems WRB 2022 and USDA Soil Taxonomy 1999. The soil from the closed micro-depression is a Eugley, Hipogley, Mineral, Calcareous (Škorić 1985) or Calcaric Oxygleyic Gleysol (Loamic, Humic) (WRB 2022). The soil of the micro-depression open to the Danube is Humogley, Calcareous, Loamy (Škorić 1985) or Calcaric Oxygleyic Mollic Tidalic Gleysol (Loamic, Fluvi-Loaminovic) (WRB 2022). The both soils are Typic Endoaquolls (Soil Taxonomy 1999).

Groundwater (high level and amplitude), caused by topography, is the main soil-forming factor that has caused the gleyzation process and redoximorphic features that have influenced the classification of soils at the higher levels of the local (division, class, type) and WRB system (RSG and Oxygleyic principal qualifier) and the second level (suborder) of the USDA Soil Taxonomy in the both micro-depressions. In addition, prolonged flooding periods have led to principal (Mollic and Tidalic) and supplementary (Fluvi-Novic) qualifiers of the soils in the open micro-depression. Alluvial sediments as parent material have produced the Calcaric principal and the Loamic supplementary qualifiers for the two soils.

The local soil classification reflects most of the soil-forming factors and properties as the two international soil classifications. In order to increase accuracy, quantitative limits for the soil type and lower levels are required. The results obtained should contribute to the expansion of knowledge, on the basis of which the existing local soil classification system could be improved or a new one created.

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KLASIFIKACIJA ZEMLJIŠTA U MIKRODEPRESIJAMA REČNOG OSTRVA (VELIKO RATNO OSTRVO, SRBIJA)

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R e z i m e

Veliko ratno ostrvo, nastalo na ušću reke Save u Dunav, veoma je izloženo podzemnim i poplavnim vodama i stoga pogodno za istraživanje hidromorfinih zemljišta. Cilj ovog rada je detaljna klasifikacija zemljišta iz dve različite mikrodepresije na Velikom ratnom ostrvu, prema lokalnom klasifikacionom sistemu (Škorić 1985), Svetskoj referentnoj bazi za zemljišne resurse (engl. *World reference base for soil resources*, WRB 2022) i američkoj taksonomiji (engl. *USDA Soil Taxonomy* 1999), sa posebnim osvrtom na faktore pedogeneze koji su uticali na klasifikaciju. Zemljište zatvorene mikrodepresije je euglej, hipoglej, mineralni, karbonatni (Škorić 1985) ili kalkarični oksiglejični glejsol (loamični, humični) (engl. *Calcaric Oxygleyic Gleysol* [*Loamic, Humic*]) (WRB 2022). Zemljište mikrodepresije otvorene prema Dunavu je humoglej, karbonatni, ilovasti (Škorić 1985) ili kalkarični oksiglejični molični tidalični glejsol (loamični, fluvi-loaminovični) (engl. *Calcaric Oxygleyic Mollic Tidalic Gleysol* [*Loamic, Fluvi-Loaminovic*]) (WRB 2022). Oba zemljišta su tipični endoakvolsi (engl. *Typic Endoaquolls*) (Soil Taxonomy 1999). Pozemne vode (visok nivo i amplitude) uzrkovane topografijom, glavni su faktor formiranja zemljišta, što je dovelo do procesa oglejavanja i redoksimorfnih osobina (engl. *redoximorphic features*) koje su uticale na klasifikaciju zemljišta na višim nivoima lokalnog sistema (red, klasa i tip) kao i sistema Svetske referentne baze za zemljišne resurse (referentne grupe zemljišta [engl. *reference soil group* – RSG] i oksiglejni [engl. *Oxygleyic*] osnovni kvalifikator) kao i na drugom nivou (engl. *suborder*) američke taksonomije zemljišta u obe mikrodepresije. Pored toga, produženi periodi poplava doveli su do osnovnih (molični [engl. *Mollic*] i tidalični [engl. *Titalic*]) i dodatnih (fluvi-loaminovični [engl. *Fluvi-Loaminovic*]) kvalifikatora zemljišta u otvorenoj mikrodepresiji. Aluvijani sedimenti kao matični supstrati prouzrokovali su kalkarični (engl. *Calcaric*) osnovni i loamični (engl. *Loamic*) dopunski kvalifikator za oba zemljišta. Lokalna klasifikacija odražava većinu faktora pedogeneze/karakteristika kao i dve međunarodne klasifikacije. Da bi se povećala njena preciznost, neophodne su kvantitativne granice na nivou tipa i nižih klasifikacionih jedinica. Dobijeni rezultati treba da doprinesu sakupljanju znanja na osnovu kojih bi bilo moguće unaprediti postojeći lokalni sistem klasifikacije zemljišta ili formirati novi.

Ključne reči: svetska referentna baza za zemljišne resurse, američka taksonomija, euglej, humoglej, glejsoli, tipični endoakvolsi.

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ENHANCING THE STORAGE LIFE AND MARKETABILITY OF ORANGE CAPE GOOSEBERRY FRUIT: MELATONIN TREATMENT BOOSTS THE ENZYMATIC ANTIOXIDANT SYSTEM

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Abstract: The cape gooseberry fruit (*Physalis peruviana* L.) is a climacteric fruit that experiences significant color and texture changes during storage due to increased ethylene synthesis. While its storage life with its calyx is one month, it only lasts 4 to 5 days without it. Therefore, strategies to reduce postharvest losses and extend storage life are essential. In this study, entirely ripe orange cape gooseberry fruits with yellow calyces were harvested and transferred to the laboratory. After washing, the fruits were immersed in melatonin solutions at concentrations of 100, 200, and 300 µM for 5 minutes, with distilled water as a control. The fruits were stored at 10°C and 90 ± 5% relative humidity for 21 days and evaluated weekly. The results showed that all melatonin treatments significantly controlled weight loss and fruit softening. Melatonin-treated fruits also had a comparable taste index and performed better than the controls. Melatonin treatment improved the antioxidant enzymatic system, with fruits treated with 300 µM melatonin showing the highest activities of superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase enzymes, and the lowest hydrogen peroxide content, indicating reduced oxidative stress. Additionally, the lowest decay (17.4%) was observed in fruits treated with 300 µM melatonin, while the highest decay (43.83%) occurred in control fruits. Melatonin treatment proved to be effective in improving the quality and extending the shelf life of cape gooseberry fruits, acting as a valuable and environmentally friendly postharvest preservation method by delaying ripening, enhancing enzymatic antioxidant activity, and preserving taste index.

Key words: ascorbate peroxidase, cape gooseberry, free radicals, melatonin treatment, taste index, tissue firmness, weight loss.

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Introduction

Physalis peruviana L., commonly known as cape gooseberry, is a non-native plant with significant economic potential. Its fruit is classified as a superfruit due to its exceptional flavor and aroma, as well as its nutritional and medicinal properties (Fischer et al., 2011). Cape gooseberry grows at various altitudes and tolerates cold temperatures, but it is damaged by subzero temperatures and grows optimally at 18°C. The plant requires adequate sunlight, protection from strong winds, and moderate irrigation during its growth. It prefers well-drained, slightly acidic soils. The productive lifespan of the plant ranges from 9 to 11 months, and it reaches the harvest stage about nine months after seed germination (Puente et al., 2011).

Colombia is the world's largest producer of cape gooseberry, with an annual production of about 18,134 tons, of which approximately 80% is exported as fresh produce. There are also commercial farms in India, the United States, Portugal, France, Bulgaria, Brazil, and South Africa (dos Santos et al., 2023). In recent years, cape gooseberry cultivation has expanded in Iran, both in open fields and greenhouses, although precise statistics are not available.

While the yield of cape gooseberry in Colombia varies between 9 and 28 tons per hectare, yields in other regions are typically lower, usually between 2 and 6 tons per hectare (dos Santos et al., 2023). Additionally, countries other than Colombia often produce fruits with lower quality indices that cannot compete with Colombian produce. The primary challenge in expanding production in these regions is the lack of improved cultivars. In other words, few commercial varieties are currently available, most of which have been selected for Colombian conditions and may not be suitable or adaptable to other regions (dos Santos et al., 2023).

Cape gooseberry fruit is highly perishable and exhibits a climacteric ripening pattern characterized by increased ethylene synthesis and significant changes in color and texture during storage. The storage life of cape gooseberry fruit is about one month when kept with its calyx, but only 4 to 5 days without it (Oliveira et al., 2015).

These abovementioned challenges require the development of strategies to reduce waste and extend the storage life of fruits. Therefore, maximizing benefits for producers and exporters relies on genetic improvements of the plant and postharvest management strategies. These strategies aim to develop genotypes with higher yield and quality, along with enhanced postharvest quality and extended storage life.

Melatonin, a compound naturally found in plants, animals, and humans, shows significant potential in maintaining postharvest quality and extending the storage life of fruits and vegetables. The exogenous application of melatonin has been shown to slow down the decay rate, reduce weight loss and respiration rate, and preserve tissue firmness and quality indices (Feng et al., 2014). The exogenous

treatment of this compound enhances endogenous melatonin levels and activates antioxidant enzymes, thereby increasing antioxidant capacity, reducing oxidative stress, and delaying senescence (Gurjar et al., 2022). These findings demonstrate the potential of melatonin as a safe and effective postharvest treatment. Several studies have investigated the effects of melatonin on fruits during the postharvest period, reporting varying results across different species and cultivars, indicating the need for further research on various horticultural products (Wang et al., 2020; Ze et al., 2021).

The maturity index of cape gooseberry fruit, commonly used by most farmers and traders, is the visual determination of the maturity stage based on the color of the calyx, which correlates with the fruit color. Typically, a change in the calyx color from green to yellow, with fruits being yellow or orange, indicates the optimal harvest time for export and local markets, respectively. These changes are easily detectable and are, therefore, usually considered by farmers (Balaguera-López et al., 2016).

Given that the fruits are usually harvested at the orange stage for fresh consumption and local markets, the present study aimed to investigate the effects of exogenous melatonin application on preserving and enhancing the quality and storage life of orange-colored cape gooseberry fruits.

Material and Methods

Plant material

Entirely orange cape gooseberry fruits with completely yellow calyxes were harvested from a commercial greenhouse in Pasargad (Fars Province, Iran). The fruits were immediately transported to the laboratory using cardboard boxes. Suitable fruits were selected through visual inspection, focusing on uniformity in size and color and the absence of damage or contamination. The selected fruits were washed with deionized water and dried at room temperature.

Treatments

Initially, melatonin (Sigma-Aldrich, Madrid, Spain) was dissolved in ethanol and then diluted with distilled water to achieve the desired concentrations of 100, 200, and 300 μM. Immediately following washing and air drying of fruits, sixty fruits per treatment were immersed in the melatonin solutions for 5 minutes, with distilled water as a control. The treated fruits were air-dried at room temperature for 30 minutes and then packaged in 0.03-mm polyethylene fresh-keeping bags (10 × 5 × 20 cm^3) with a 3% perforation ratio. The fruits were stored at 10 ± 1°C and 90 ± 1% relative humidity (RH) for 21 days. The fruits were removed from storage weekly for evaluation during 21 days. Additionally, the parameters were assessed

for informational purposes and not for statistical comparison on the initial day of the experiment, before the start of the storage period.

Experimental design

The experimental design was a completely randomized design (CRD) using a factorial arrangement comprising 12 treatments with three replications per treatment (20 fruits per replicate). The experimental factors included immersing the fruits in four melatonin solutions (0 µM [distilled water as a control], 100 µM, 200 µM, and 300 µM) and sampling at three intervals on days 7, 14, and 21 of storage.

Fruit weight loss

The mass variation was measured using an FZ-300iWP precision scale (A&D Co.). The weight loss was calculated as the difference between the initial mass and the mass at each time point (Taghipour and Assar, 2022), expressed as a percentage of the initial mass using Equation (1) (Hayati et al., 2023a):

Weight loss (%) = [(initial weight of each sample (g) − final weight of each sample (g))/ initial weight of each sample (g)] × 100 [1]

Fruit decay rate

The decay rate was calculated as a percentage by examining the number of decayed fruits and using Equation (2) (Hayati et al., 2023b):

Decay rate (%) = (the number of decayed fruits/the total number of fruits) × 100 [2]

Fruit firmness

This parameter was measured using a hand-held penetrometer (I-OSK-10576). For each fruit, the firmness was measured at two opposite points of its equatorial area using a 3-mm probe. The results were expressed as mN (Hayati et al., 2023a).

Juice total soluble solids/titratable acidity (TSS/TA) ratio (taste index)

Juice TSS was measured with a hand-held refractometer (ATAGOB933475) and expressed as a percent. Titration was performed to determine titratable acidity (TA) using 0.1 N NaOH to reach a pH of 8.2. The results were expressed as a percent of citric acid. The TSS/TA ratio was calculated by dividing TSS by TA (Hayati et al., 2023a).

Juice hydrogen peroxide (H_2O_2) content

The hydrogen peroxide content was measured following the method outlined by Velikova et al. (2000). In this procedure, 500 mg of fruit tissue was homogenized in 5 mL of 0.1% (w/v) TCA in an ice bath. The homogenate was then centrifuged at 12,000×g for 15 minutes, and 0.5 mL of the resulting supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. The absorbance was recorded at 390 nm using a spectrophotometer. The H_2O_2 concentration was calculated using a standard curve and expressed as µmol H_2O_2 per gram of fresh weight (FW) of the fruit.

Juice superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activity

The antioxidant enzyme activities in the juice were determined according to the method described by Taghipour et al. (2021). Briefly, 1 g of tissue was homogenized in 4 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 2 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 10,000 × g for 10 minutes at 4 °C, and the supernatant was stored in vials at -80 °C for several days until the enzymatic assays were performed.

SOD activity was measured by observing the decrease in absorbance of the superoxide-nitro blue tetrazolium complex. The reaction mixture (1 mL) consisted of 50 mM potassium phosphate buffer (pH 7.8), 50 µL enzyme extract, 13 mM L-methionine, 0.1 mM EDTA, 75 µM nitro-blue tetrazolium (NBT), and 2 µM riboflavin, which was added last. The reaction was initiated by exposing the tubes to light from a 15 W fluorescent lamp for 15 minutes. The tubes were then covered with a black cloth. Control tubes without enzyme extract and blank tubes without light exposure were used as references. One unit of SOD activity was defined as the enzyme amount required to reduce the absorbance at 560 nm by 50% relative to the control, with the specific activity expressed as units per gram of fresh weight.

CAT activity was assessed by mixing 950 µL of reaction solution (50 mM phosphate buffer, pH 7.0, and 15 mM H_2O_2) with 50 µL enzyme extract. The decrease in absorbance at 240 nm was used to quantify CAT activity, with specific activity expressed as units per gram of fresh weight.

POD activity was measured by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation in the presence of H_2O_2. The reaction mixture (1 mL) included 50 mM potassium phosphate buffer (pH 7.0), 33 µL enzyme extract, and 13 mM guaiacol, with the reaction initiated by adding 5 mM H_2O_2. The specific activity was expressed as units per gram of fresh weight.

APX activity was determined by measuring the decrease in absorbance at 290 nm due to ascorbate oxidation. The reaction mixture (1 mL) consisted of 50 mM

potassium phosphate buffer (pH 7.0), 50 µL enzyme extract, 0.5 mM ascorbate, 0.15 mM H_2O_2, and 0.1 mM EDTA. The specific activity was expressed as units per gram of fresh weight.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) at a significance level of $P < 0.05$, and mean comparisons were carried out with the least significant difference (LSD) test. All statistical analyses were conducted using SAS software version 9.4.

Results and Discussion

The maturity stage of fruit at harvest is a critical factor influencing postharvest behavior and quality, closely tied to commercial demand and consumer preferences. Fruits harvested at immature or overripe stages are significantly more susceptible to physiological damage and generally exhibit lower quality than those picked at optimal maturity. Harvesting at the optimal stage ensures the desirable flavor while preventing excessive softening associated with overripening (Proebsting and Murphey, 1987). Immature fruits are more prone to physical damage and quality degradation due to internal water loss, resulting in a weaker flavor upon ripening. Conversely, overripe fruits rapidly become soft, tasteless, and mealy after harvest (Proebsting and Murphey, 1987). These conditions are also relevant to cape gooseberry fruits, leading to reduced quality, sensory attributes, nutritional value, and profitability within the production and supply systems.

Four maturity stages are defined for harvested cape gooseberry fruits: S1 (25% yellow and 75% green with a green calyx), S2 (50% yellow and 50% orange with a yellow-green calyx), S3 (100% orange with an entirely yellow calyx), and S4 (100% orange with a dry brown calyx) (Balaguera-López et al., 2016). Storage of cape gooseberry fruits at various maturity stages and without calyx at 18°C and 60% relative humidity for 15 days showed that fruits harvested at stage S1 exhibited the lowest postharvest quality, the highest weight loss, the least firmness, and inconsistent color development. Consequently, stage S1 was identified as an unsuitable maturity stage for harvesting. It was also recommended that cape gooseberry fruits should be harvested at maturity stages S2 and S3 for export and the local market supply, respectively, due to their more favorable postharvest behavior. In contrast, fruits at stage S4 should only be harvested for immediate consumption and are unsuitable for commercial purposes (Balaguera-López et al., 2016).

Based on the abovementioned findings, the authors decided to harvest and treat cape gooseberry fruits at stage S3 to enhance postharvest quality and storage

life to meet the requirements of the local market and fresh consumption, as previously mentioned in the introduction section.

Our results on the analysis of variance (Table 1) indicated a significant effect of all experimental factors and their interactions on all evaluated traits, except for the lack of the interaction effect on catalase enzyme activity. Moreover, mean comparisons, as shown in Figures 1, 2, and 3, demonstrated that postharvest melatonin treatment generally reduced fruit weight loss and decay, decreased hydrogen peroxide content, and maintained or improved the other indices, including tissue firmness, TSS/TA ratio, and antioxidant enzyme activities in the juice.

The results indicated that weight loss and decay rates in cape gooseberry fruits increased significantly over the storage period. However, this trend was slower in melatonin-treated fruits compared to the control (Figures 1a and 1b). After 21 days of storage, the weight loss in fruits treated with different concentrations of melatonin was similar and significantly lower than that of the control fruits (Figure 1a). At this point, the fruits treated with higher concentrations of melatonin exhibited the lowest decay rate, which was significantly lower than that of the fruits treated with 200 μM melatonin (Figure 1b).

At each sampling point, a significant decrease in fruit firmness was observed compared to the previous sampling. After seven days of storage, the treatments with the two higher concentrations of melatonin showed the highest fruit firmness. After 14 days, this was observed in the treatment with the highest concentration, and after 21 days, all three concentrations maintained the highest fruit firmness. Throughout the storage period, control fruits consistently exhibited lower firmness compared to melatonin-treated fruits (Figure 1c).

The taste index of both the control and treated fruits showed a significant increase by the end of the storage period. At each sampling point, there was no difference in the taste index among the treated fruits, and their index was lower than that of the control fruits (Figure 1d).

The changes in hydrogen peroxide levels in all experimental groups showed a significant increase. However, fruits treated with higher concentrations of melatonin had statistically lower levels of hydrogen peroxide compared to other groups at each sampling point (Figure 2a).

The activity of the SOD enzyme increased until the seventh day of storage and then decreased until the end of the storage period. A significant reduction in enzyme activity was observed at each sampling time compared to the previous sampling, with fruits treated with higher concentrations of melatonin showing higher enzyme activity than those in other groups at each sampling point (Figure 2b).

CAT enzyme activity increased with the increase in melatonin concentration (Figure 2c). On the other hand, enzyme activity levels were higher on day 7

compared to the start of the experiment, but a significant decrease was observed over time during the storage period (Figure 2d).

Regarding APX enzyme activity, as the storage period progressed, the difference in enzyme activity levels between fruits treated with 300 µM melatonin and other experimental groups became more pronounced. Between the 7th and 14th days of storage, the enzyme activity in the fruits treated with 100 µM melatonin showed a significant decrease, while no significant change was observed with 200 µM melatonin. From the 14th to the 21st days, enzyme activity remained unchanged with 100 µM, but significantly increased with 200 µM. In contrast, fruits treated with 300 µM melatonin consistently showed a significant increase in enzyme activity throughout the storage period, with levels always higher than those in the other groups (Figure 3a).

The activity of the POD enzyme in the control fruits decreased consistently, with a significant reduction observed at each sampling point compared to the previous one. Fruits treated with melatonin exhibited higher enzyme activity levels throughout storage compared to the start of the experiment. However, a significant decrease in enzyme activity was noted between the 7th and 14th days, followed by a significant increase in the final 7-day period of the experiment. At all sampling points, fruits treated with higher concentrations of melatonin showed higher enzyme activity levels compared to those treated with lower concentrations (Figure 3b).

Table 1. Analysis of variance of the effect of exogenous melatonin treatment on the physicochemical properties of *Physalis peruviana* L. fruit during storage.

Source of variation	df	Fruit weight loss	Fruit decay rate	Fruit firmness	Juice taste index	Juice hydrogen peroxide	Juice superoxide dismutase activity	Juice catalase activity	Juice ascorbate peroxidase activity	Juice peroxidase activity
Dip in melatonin	3	35.73**	624.95**	21630.75**	3.60**	46.15**	5.07**	7.42**	21.59**	10.98**
Time	2	87.36**	996.91**	71951.62**	6.44**	4.63**	2.33**	4.96**	17.07**	2.55**
Dip in melatonin × time	6	3.00**	48.97**	1908.02**	0.38**	6.82**	0.02*	0.01^{ns}	1.32**	0.27**
Error	24									
CV (%)		6.77	14.29	1.42	2.18	3.71	1.92	3.61	3.80	4.18

**, *, and ns: significantly different at 1%, 5% and no significant differences, respectively.

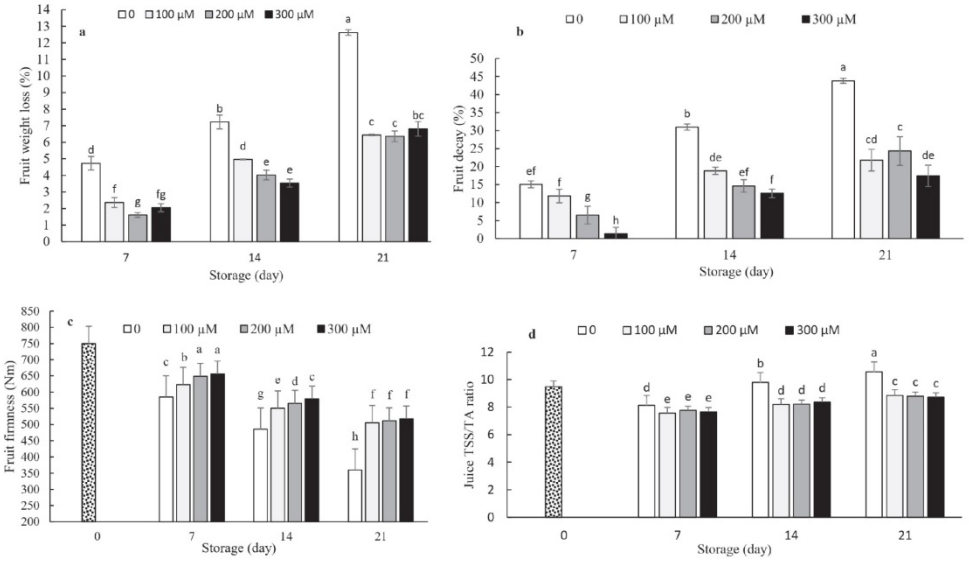

Figure 1. Effects of exogenous melatonin treatment on selected properties of *Physalis peruviana* L. fruits during 21 days of storage. For each property, similar letters indicate no significant difference between the experimental groups at the 5% level, based on the least significant difference (LSD) test.

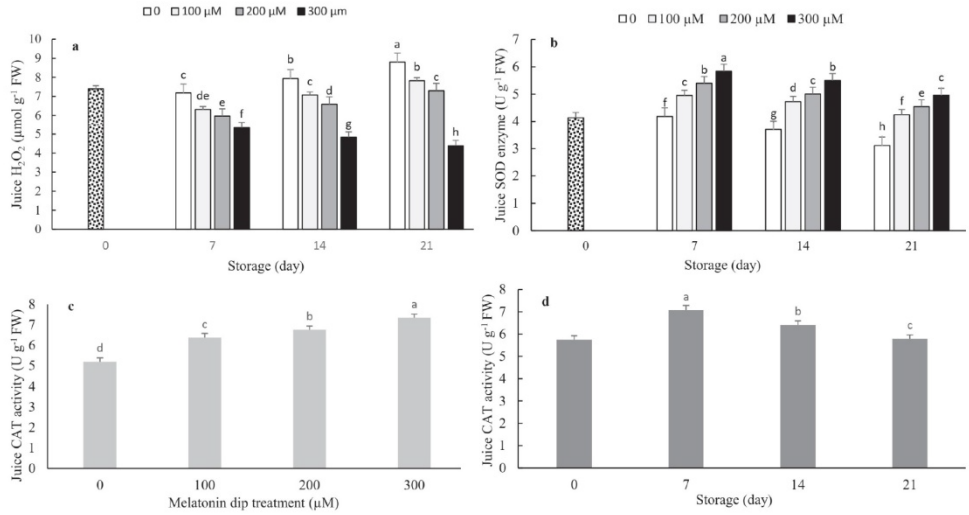

Figure 2. Effects of exogenous melatonin treatment on selected properties of *Physalis peruviana* L. fruits during 21 days of storage. For each property, similar letters indicate no significant difference between the experimental groups at the 5% level, based on the least significant difference (LSD) test.

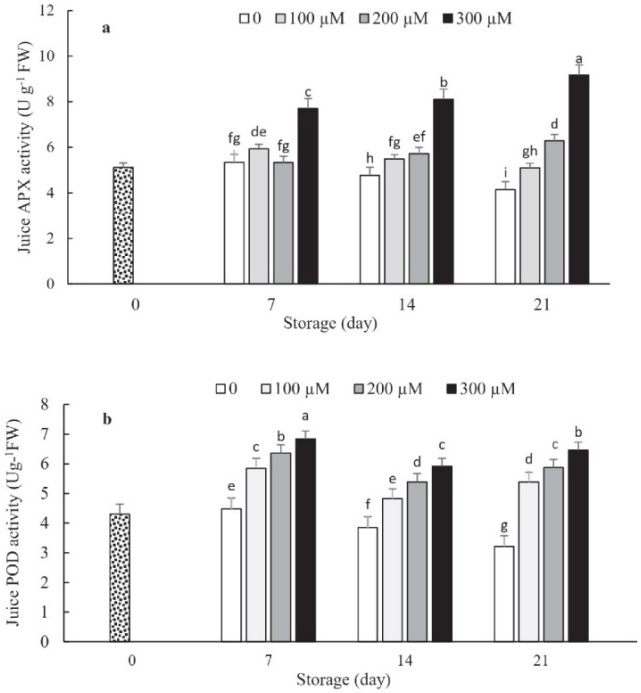

Figure 3. Effects of exogenous melatonin treatment on selected properties of *Physalis peruviana* L. fruits during 21 days of storage. For each property, similar letters indicate no significant difference between the experimental groups at the 5% level, based on the least significant difference (LSD) test.

Oxidative damage during postharvest storage of horticultural products such as fruits and the destructive effects of reactive oxygen species (ROS) on macromolecules such as nucleic acids, lipids, and proteins, ultimately leading to decreased quantity, quality, and storage life, is a well-known phenomenon (Zhang et al., 2018). Fruits have developed enzymatic antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), to combat ROS. These systems reduce ROS accumulation and lipid peroxidation, enhancing cellular integrity and prolonging postharvest storage life (Zhang et al., 2018).

Melatonin is an eco-friendly and safe compound naturally present in various fruits and vegetables, including tomatoes, bananas, oranges, strawberries, cherries, and apples (Feng et al., 2014). It acts as a primary signaling molecule during biotic and abiotic stresses, helping regulate many plant functions, and is recognized as a potent free radical scavenger (Arnao and Hernández-Ruiz, 2014; Tan et al., 1993).

Melatonin synthesis is mediated by several key enzymes, including serotonin N-acetyltransferase (SNAT) and hydroxyindole-O-methyltransferase (HIOMT).

Exogenous melatonin application has been demonstrated to upregulate the expression of genes encoding these enzymes, thereby enhancing the melatonin biosynthetic pathway and increasing endogenous melatonin levels (Kumari et al., 2023). This increase in melatonin aids in quality maintenance under postharvest stress conditions (Hu et al., 2017; Dubbels et al., 1995).

It has been reported that as the maturity stage progresses and the storage duration of cape gooseberry fruits increases, ethylene production, weight loss, and TSS content increase while tissue firmness and titratable acidity decrease (Balaguera-López et al., 2016). Maintaining membrane integrity, limiting lipid peroxidation, reducing malondialdehyde levels, and decreasing membrane leakage in horticultural products treated with melatonin are evidence of improved stress tolerance and postharvest quality preservation (Gao et al., 2016; Jimenez et al., 2002). Consequently, delayed fruit senescence, better retention of fruit firmness, and slower weight loss rates occur (Onik et al., 2021; Rastegar et al., 2020). As shown in previous studies on melatonin application, melatonin's ability to inhibit respiration and ethylene production, along with inhibiting polyphenol oxidase (PPO) activity, helps preserve quality indices such as firmness, TA, and TSS, and prevent browning and color changes in the peel and flesh (Kumar et al., 2014; Zhang et al., 2018). In this study, the stability of the TSS/TA ratio, as an indicator of fruit taste, demonstrates the efficacy of melatonin treatment in maintaining fruit marketability throughout the storage period.

Melatonin stimulates the expression of antioxidant-related genes and enhances ROS scavenging mechanisms, effectively limiting hydrogen peroxide production as a primary indicator of oxidative stress (Jannatizadeh et al., 2019; Saxena et al., 2016). A significant reduction in hydrogen peroxide content was observed in fruits treated with 300 μM melatonin while an increasing trend was seen in fruits treated with 200 μM. However, after 21 days of storage, the hydrogen peroxide levels in the treated fruits were lower than at the start of the experiment. This indicates that melatonin treatment effectively stimulates antioxidant system activity and controls hydrogen peroxide levels, which is consistent with the results of this study regarding antioxidant enzyme activity.

Similarly, in a study examining the effect of melatonin treatment on improving the storage life, quality, and antioxidant enzyme activity of papaya fruits, the effects of 1.5 mM exogenous melatonin were evaluated during 28 days of storage at 10 ± 2°C. The researchers reported that the treatment significantly delayed postharvest senescence, preserved higher titratable acidity levels, and reduced weight loss compared to control fruits (Wang et al., 2022). Moreover, the treatment not only enhanced the activity levels of superoxide dismutase, peroxidase, and catalase enzymes, but also significantly inhibited hydrogen peroxide accumulation. Additionally, the treated fruits showed a better taste index than the controls.

The protective mechanisms of melatonin against pathogens and pathogen-induced decay in horticultural products are multifaceted. These mechanisms include enhancing the antioxidant defense system by stimulating the activity of enzymatic antioxidants (such as SOD, CAT, POD) and increasing the levels of non-enzymatic antioxidants, strengthening hormonal signaling, and affecting levels of plant hormones such as abscisic acid, jasmonic acid, ethylene, and salicylic acid. They also involve inducing the phenylpropanoid pathway and stimulating activities that lead to synthesizing cell walls, lipids, and waxes in the fruit peel, serving as physical barriers against pathogen invasion (Fan et al., 2022). Similar findings have been reported for cherry fruits (Wang et al., 2019).

Conclusion

The authors believe that this study elucidates the key mechanisms contributing to the extended storage life of orange-colored cape gooseberry fruits and provides valuable insights into postharvest management. The results demonstrate that exogenous melatonin application, particularly at a concentration of 300 µM, serves as a promising postharvest strategy to enhance the commercial potential and marketability of these fruits. This is attributed to the positive effects of melatonin treatments in alleviating weight loss, decay, and softening, while better maintaining the taste index. Furthermore, the 300 µM melatonin treatment showed the most significant impact on enhancing the enzymatic antioxidant system, as evidenced by increased activities of superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase enzymes. This enhancement was accompanied by a substantial reduction in hydrogen peroxide content, indicating an effective mitigation of oxidative stress. However, further research is necessary to fully elucidate the underlying molecular mechanisms and evaluate the feasibility of melatonin treatments under commercial-scale storage conditions.

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PRODUŽAVANJE VEKA SKLADIŠTENJA I POBOLJŠANJE TRŽIŠNE VREDNOSTI PLODOVA NARANDŽASTE PERUANSKE JAGODE: TRETMAN MELATONINOM POJAČAVA ENZIMSKI ANTIOKSIDATIVNI SISTEM

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R e z i m e

Voćka peruanska jagoda (*Physalis peruviana* L.) daje klimakterične plodove koji tokom skladištenja prolaze kroz značajne promene boje i teksture usled povećane sinteze etilena. Dok mu je vek skladištenja sa čašicom jedan mesec, bez nje traje samo 4 do 5 dana. Stoga su strategije za smanjenje gubitaka nakon berbe i produženje veka skladištenja od suštinskog značaja. U ovoj studiji, potpuno zreli plodovi narandžaste peruanske jagode sa žutim čašicama ubrani su i prebačeni u laboratoriju. Nakon pranja, plodovi su potopljeni u rastvore melatonina u koncentracijama od 100, 200 i 300 µM tokom 5 minuta, dok je destilovana voda korišćena kao kontrola. Plodovi su skladišteni na temperaturi od 10°C i relativnoj vlažnosti od 90 ± 5% tokom 21 dana i ocenjivani svake nedelje. Rezultati su pokazali da su svi tretmani melatoninom značajno kontrolisali gubitak mase i omekšavanje plodova. Plodovi tretirani melatoninom imali su uporediv indeks ukusa i pokazali su bolje rezultate od kontrolnih uzoraka. Tretman melatoninom poboljšao je antioksidativni enzimski sistem, pri čemu su plodovi tretirani sa 300 µM melatonina pokazali najveću aktivnost enzima superoksid dismutaze, katalaze, askorbat peroksidaze i peroksidaze, kao i najniži sadržaj vodonik peroksida, što ukazuje na smanjen oksidativni stres. Pored toga, najniži procenat propadanja plodova (17,4%) zabeležen je kod plodova tretiranih sa 300 µM melatonina, dok je najviši procenat propadanja (43,83%) primećen kod kontrolnih plodova. Tretman melatoninom pokazao se kao efikasan u poboljšanju kvaliteta i produženju roka trajanja plodova peruanske jagode, delujući kao vredna i ekološki prihvatljiva postharvest tehnologija, usporavanjem sazrevanja, povećanjem enzimske antioksidativne aktivnosti i očuvanjem indeksa ukusa.

Ključne reči: askorbat peroksidaza, peruanska jagoda, slobodni radikali, tretman melatoninom, indeks ukusa, čvrstoća tkiva, gubitak mase.

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CONCEPTUAL FRAMEWORK FOR THE INTEGRATED SUSTAINABLE MANAGEMENT OF SOILS AND BOTTOM SEDIMENTS

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Abstract: This study explores a sustainable framework for bottom sediment management, utilizing both SWOT and PESTEL analyses to assess its strategic viability. Through structured evaluations, the study identifies key strengths, including the circular economy benefits of sediment reuse, job creation potential, and improvements in soil fertility. However, challenges such as contamination, regulatory compliance, and logistical costs are also highlighted as factors that need careful management. The PESTEL analysis reveals strong external support in the form of favorable environmental and regulatory conditions, while the SWOT analysis provides a positive strategic potential score, indicating a feasible path forward. Additionally, the study addresses the role of sediment management in post-war recovery, particularly relevant for areas in Ukraine, suggesting its application for landscape restoration and infrastructure reinforcement. By integrating both internal and external factors, this research offers a comprehensive model for sediment repurposing, supporting sustainable development goals in the areas of resource management, environmental protection, and agricultural productivity. The results emphasize the potential of applying sustainable sediment management practices in the agricultural sector to support sustainable development.

Key words: sustainable sediment management, agricultural waste reuse, environmental resource management, circular economy in agriculture, remediation techniques, bioremediation in agriculture, post-war environmental recovery, bottom sediment recycling.

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Introduction

A sustainable approach to managing sedimentary deposits is increasingly important due to the environmental and logistical challenges of their removal and disposal. Sedimentary deposits, formed through natural weathering processes, are transported by water, wind, and gravity, often accumulating far away from their original sources. These particles range from large boulders (>256 mm) to fine colloids (<1 μm) and typically consist of materials such as sand, silt, and clay. Routine dredging is crucial for maintaining the capacity of rivers, lakes, and urban water bodies, protecting against flooding, and ensuring uninterrupted navigation (Helms et al., 2012). In Europe alone, approximately 200 million cubic meters of sediment are extracted annually, primarily from coastal, riverine, and lacustrine environments, as well as from artificial and urban water bodies (Helms et al., 2012).

Sediment accumulation in reservoirs and dams reduces water storage capacity, obstructs flow, and can damage critical infrastructure, such as hydroelectric turbines, necessitating periodic removal (Guy, 1975). Effective sediment management involves assessing factors such as water depth, sediment cohesiveness, organic content, and pollution levels prior to dredging (Puccini et al., 2013; Bates et al., 2015). The extracted sediments are sorted and reused based on their mechanical and chemical properties. For instance, the sediments may be employed in construction, including riverbank reinforcement or beach restoration (De Vincenzo et al., 2019). However, urban sediments often contain anthropogenic contaminants, complicating their direct use in agriculture or construction without treatment (Stojiljkovic et al., 2019).

As natural “accumulators”, sedimentary deposits capture persistent and potentially hazardous compounds from various pollution sources (Perelo, 2010). Contaminants such as heavy metals and organic pollutants often necessitate specialized treatment or even classification as hazardous waste (Vervaeke et al., 2003). The European Union Regulation 850/2004 governs permissible sediment reuse, setting contamination thresholds and imposing stricter restrictions on industrial sites where hazardous substances may be concentrated (Frohne et al., 2015).

Interest in the sustainable reuse of sediments is growing, particularly in agriculture, as researchers explore their potential as soil nutrient sources. Matej-Łukowicz et al. (2021) analyzed urban water body sediments and found significant amounts of iron and sulfur, which enhance phosphorus availability for plants, despite the low content of nitrogen and organic carbon. This finding suggests that nitrogen- and carbon-enriched sediments could serve as alternatives to synthetic fertilizers, aligning with circular economy principles and reducing dependence on non-renewable phosphorus fertilizers (Sandor and Homburg, 2017; Christel et al., 2014).

Sediment reuse has proven to be promising in practice. For example, a project by Studds and Miller (2010) under British Waterways applied sediments for canal

bank reinforcement. After confirming the absence of hazardous substances, the project reduced disposal costs and highlighted the potential of sediment reuse in construction, promoting environmental and economic sustainability (Council Directive 67/548/EEC, 1967; Council Regulation (EC) No. 850/2004, 2004).

The diversity of sediment sources, compositions, and contamination levels necessitates a flexible yet regulated approach to sustainable management. While sediment reuse offers significant potential, particularly in agriculture and construction, proper treatment and thorough planning are essential to maximize benefits and ensure compliance with environmental standards.

This paper has three objectives. First, it explores a conceptual framework for the sustainable management of soils and bottom sediments, focusing on environmental safety and economic viability, while addressing their reuse potential in agriculture and construction. Second, the study identifies the critical processes, challenges, and opportunities associated with sediment remediation, utilizing methods such as SADT and IDEF0 modeling to develop an integrated management framework. Third, it evaluates the strategic potential of sediment reuse in post-conflict recovery, particularly within Ukraine, and its implications for broader sustainable development goals.

The study involved a comprehensive methodological approach designed to address the complex challenges of sediment management. Specifically, (i) the SADT and IDEF0 models were used to structure and optimize the sediment management processes, ensuring a clear hierarchy of operations from analysis to reuse; (ii) SWOT and PESTEL analyses were applied to identify the strengths, weaknesses, opportunities, and threats of sediment reuse, as well as to examine external factors influencing its feasibility; (iii) bioremediation techniques, including biostimulation and bioaugmentation, were analyzed to determine their suitability for purifying contaminated sediments; and (iv) economic, environmental, and legal factors were systematically evaluated to ensure alignment with international standards for sediment reuse. These methodologies collectively contribute to a robust framework for advancing sustainable resource management and supporting post-war recovery efforts.

Material and Methods

A comprehensive approach was utilized to design a sustainable management framework for sedimentary deposits, incorporating established SADT (Structured Analysis and Design Technique) (Ross, 1985) and IDEF0 (Integration Definition for Function Modeling) (Presley and Liles, 1995) modeling techniques to address the unique challenges of environmental sediment management. This methodology facilitated the development of a structured management framework aimed at achieving both environmental safety and economic feasibility in the reuse of sediment materials. The final graphic flowchart representing this framework was

created using MyMap.ai [https://www.mymap.ai], which helped to visually organize and clarify the sequential operations within the model.

Approaches and Methods

1. Core models (SADT and IDEF0)

- SADT and IDEF0 structured sediment management and remediation processes. SADT provided a hierarchical framework for defining key stages, including initial analysis, cleaning, processing, and reuse options. IDEF0 detailed functional blocks and interconnections between sediment cleaning, processing, and reuse tasks.
- These methodologies facilitated the identification of critical operations and ensured a systematic approach to the preparation and treatment of sedimentary deposits.

2. Adaptation of the sustainable management framework

- The framework builds on Renella's recommendations (Renella, 2021) for sediment reuse in agriculture and construction. It incorporates sequential steps for environmentally safe sediment treatment, ensuring compliance with agricultural quality standards.
- A multi-level cleaning approach, including physical fraction separation, bioremediation, and other methods, enhances material processing efficiency and reduces environmental impact.

3. System of analysis and evaluation

- Sediment management begins with analyzing physicochemical properties to classify materials for further processing or reuse. Verification against European pollution standards ensures regulatory compliance.
- SWOT and PESTEL analyses evaluate the feasibility and strategic implications of sustainable sediment management.
- PESTEL systematically scores political, economic, social, technological, environmental, and legal factors (Kucher et al., 2019) to align the recommendations with the regulatory and economic contexts.
- SWOT highlights internal strengths and weaknesses, as well as external opportunities and threats, offering a balanced perspective on strategic potential (Dankevych, 2018).

To assess strategic feasibility quantitatively, scores were assigned to each factor in the SWOT and PESTEL analyses. The scoring methodology, with impact levels categorized by score ranges, is presented in Table 1.

For the SWOT analysis, we applied a structured approach inspired by Dankevych (2018), which quantifies strategic potential as follows:

$$SP=(S+O)-(W+T), \text{ where:}$$

SP – strategic potential;

S – strengths;

O – opportunities;
W – weaknesses;
T – threats.

This formula, adapted from Dankevych (2018), provides an overall strategic viability score by balancing positive (S + O) and negative (W + T) factors. A positive result suggests that the strengths and opportunities outweigh the weaknesses and threats, indicating a favorable strategic environment. Conversely, a negative or low score signals a challenging scenario, requiring mitigation strategies.

Unlike SWOT, the PESTEL factors were analyzed independently, rather than summed into a single numerical score. Each category—political, economic, social, technological, environmental, and legal—was evaluated separately to ensure a contextual assessment of external influences on sustainable sediment management.

This approach allows for a structured, quantitative analysis while maintaining analytical depth and clarity in assessing strategic feasibility.

Table 1. Criteria for assigning relative importance levels in PESTEL and SWOT analyses.

Score range	Relative importance level	Description
8–10	High	Factors scoring within this range are highly influential and critical to the success or feasibility of sediment management. These factors require primary consideration.
5–7	Medium	Factors in this range have a moderate impact and provide valuable support to the strategy. They are important, but secondary to the high-impact factors.
1–4	Low	Factors scoring in this range have minimal influence and are less likely to affect the overall outcomes significantly. These factors can be deprioritized in strategic focus.

Source: authors' research, refined from previous studies (Dankevych, 2018).

Results and Discussion

Sedimentary deposits accumulating in natural water bodies play a crucial role in maintaining ecological balance and forming fertile soils. However, they may also contain significant concentrations of organic and inorganic contaminants.

Figure 1 illustrates the sustainable sediment management framework developed in this study, detailing the sequence of operations and the connections between stages of sediment analysis, treatment, and reusing/recycling. This framework provides a systematic approach to sediment management, ensuring that each phase – from initial evaluation to final application – meets environmental and regulatory standards while optimizing both ecological and economic outcomes.

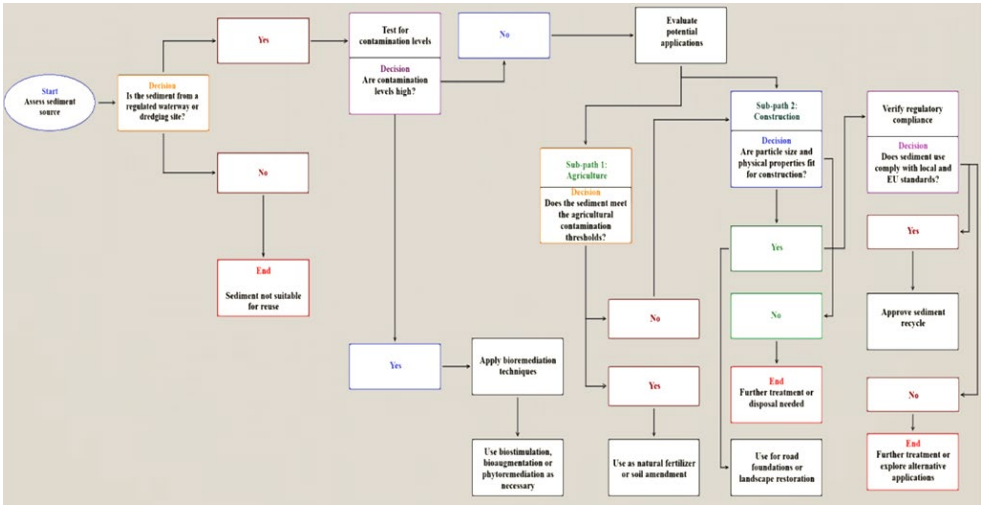

Figure 1. Decision-making flowchart for sustainable sediment recycling.
Source: Authors' adaptation, based on existing research (Renella, 2021).

The structured approach of the framework emphasizes the essential stages of sediment analysis and classification, identifying contaminants and selecting appropriate treatment methods according to sediment type and contamination levels. This approach supports a closed-loop system, enhancing resource efficiency and advancing sustainable development goals by promoting the circular use of sedimentary deposits.

For the safe and sustainable management of sedimentary deposits, it is crucial to adhere to environmental safety principles, employing both modern remediation techniques and regulatory restrictions that ensure the environmentally acceptable use of such materials.

The primary step in sustainable sediment management involves a comprehensive analysis of their physical and chemical properties. Particle size, organic matter content, and pollutant concentrations are critical factors influencing subsequent decisions regarding the use of these deposits. In the Czech Republic (Ministerstvo zemědělství & Ministerstvo životního prostředí, 2009), regulatory frameworks permit the application of sedimentary deposits to agricultural soils, provided that contaminant levels do not exceed established limits. Specifically, the concentrations of pollutants such as heavy metals must fall below the permissible thresholds set for soil conditioners and fertilizers.

If the analytical results indicate that the contaminant levels in the sediments are within acceptable ranges, these materials can be used for a variety of environmentally significant purposes. Experience shows that sedimentary deposits can be employed effectively for riverbank reinforcement, where they help stabilize

soils and prevent erosion. Additionally, these materials contribute to landscape restoration and can be used for beach reconstruction, especially in areas where the natural coastal topography requires rehabilitation.

In the context of quarry and abandoned mine reclamation, sedimentary deposits hold considerable potential. They not only aid in stabilizing reclaimed sites but also enhance the physicochemical properties of soils, improving their suitability for ecosystem restoration. In Ukraine's post-war landscape restoration efforts, sedimentary deposits could serve as a valuable resource, potentially useful for filling craters left by military activities.

The processing of sedimentary deposits begins with particle-size-based separation (UNI EN 13242, 2002). This approach divides the sediments into coarse and fine fractions, which is essential for targeted reuse. The primary aim of this step is to isolate materials with different physicochemical properties, each requiring specific treatment and processing methods.

The coarse fraction, containing larger particles, has broad applications in engineering and construction. For example, sand, gravel, and small stones from this fraction are well-suited for road construction, foundation strengthening, and other infrastructure projects. Additionally, the coarse fraction can be used to create artificial embankments or as an aggregate for concrete, reducing the reliance on natural resources such as sand and gravel, thus lowering extraction and transportation costs (Kucher et al., 2024), which may be especially valuable in the context of Ukraine’s post-war landscape reconstruction.

By facilitating the sustainable management and the targeted reuse of sedimentary deposits, these practices support both environmental goals and economic efficiency, offering a pathway to reduce environmental impact and conserve natural resources.

The fine fraction of sedimentary deposits, mainly composed of sand, silt, and clay particles, requires further purification after separation from the coarser components. Chemical and biological processes are considered some of the most effective cleaning methods, with bioremediation being recognized as the most environmentally sustainable option. Bioremediation minimizes chemical use and promotes natural pollutant degradation without harming the environment (Mattei et al., 2017). For this reason, bioremediation was chosen in our framework as the optimal method for purifying the fine fraction of sedimentary deposits. Table 2 presents a summary of the main bioremediation techniques and their characteristics.

Once purification is complete, the fine fraction can be reused as a substrate for growing ornamental plants or as a natural fertilizer. This approach is both environmentally sustainable and economically beneficial, reducing reliance on traditional substrates such as peat, a limited resource whose extraction damages ecosystems.

Table 2. Summary of bioremediation techniques for fine fraction sediment purification.

Technique	Key Characteristics	Advantages
Biostimulation	Adjusts conditions (nutrients, moisture, temperature) to promote natural microbial activity.	Facilitates organic contaminant degradation naturally.
Bioaugmentation	Introduces pollutant-degrading microorganisms.	Speeds up contaminant breakdown effectively.
Phytoremediation	Uses plants to extract/stabilize heavy metals and other pollutants.	Lowers contaminant levels while enhancing ecosystem sustainability.

Source: Authors' research.

The purified fine fraction of the sediments serves as an effective substitute for peat-based substrates traditionally used in ornamental horticulture. Peat is a non-renewable resource, and its extraction degrades peatlands—critical ecosystems for biodiversity conservation and carbon sequestration. Coconut substrates, another alternative, have a high environmental footprint due to the long-distance transportation from tropical regions.

Using treated sedimentary deposits offers an eco-friendly and resource-efficient alternative, supporting sustainable horticulture while reducing the environmental impact of traditional substrate materials. These purified sediments are agronomically effective, containing essential macro- and micronutrients necessary for plant growth. Additionally, the fine fraction of purified sediments can function as a natural fertilizer due to its high nutrient content, including nitrogen, phosphorus, potassium, and calcium, which enhance soil fertility (Baran et al., 2019). Replacing synthetic fertilizers with natural materials reduces the chemical burden on soils and water resources, thus contributing to sustainable agroecosystem management.

Integrating sedimentary deposits into agricultural soil promotes environmental sustainability and productivity. Studies show sediments improve soil structure and nutrient content, reduce reliance on synthetic fertilizers, and enhance ecological safety. Leonard et al. (2002) found that sediment application in deteriorated marsh soils improved elevation and plant biomass without disrupting ecological balances. Controlled sediment placement in agriculture could yield similar benefits, enhancing soil stability, supporting root development, and increasing water retention—especially in erosion-prone areas. These sediments supply essential nutrients such as nitrogen and phosphorus, enabling robust plant growth without chemical fertilizers, reversing degradation over time (Leonard et al., 2002).

Expanding on these findings, Spearman and Benson (2023) highlighted sediment recycling for habitat restoration, especially in coastal regions. They identified sediment as a valuable resource to offset environmental degradation by promoting soil health and preventing erosion. When applied to agricultural land,

sediments enrich the nutrient-rich topsoil, fostering sustainable crop growth. Additionally, sediment recycling reduces pressure on natural fertilizer resources, allowing local agriculture to rely less on imported or synthetic inputs. This practice aligns with broader conservation efforts by reducing waste directed to offshore disposal, supporting biodiversity, and mitigating pollution risks in water bodies through beneficial land use (Spearman and Benson, 2023).

Research by Szara-Bąk et al. (2023) further emphasizes the nutrient-rich composition of sediments and their agricultural potential, particularly when combined with organic waste. Sediment-based growing media enriched with biomass ash or coffee hulls improve soil quality and reduce potential metal contamination. These substrates exhibit beneficial properties, such as pH balancing, low toxicity, and enhanced soil sorption, critical for long-term agricultural productivity. When blended with organic amendments, sediment mixtures mitigate acidity, improve soil structure, and enhance nutrient density, supporting a circular approach to resource use. This integrated approach minimizes waste, reinforces the resilience of agricultural soils, and ensures high productivity over time (Szara-Bąk et al., 2023).

In summary, the use of sedimentary deposits in agriculture promotes environmental resilience, reduces waste, and supports soil fertility through organic nutrient recycling. These findings suggest that sediment application not only serves as a sustainable alternative to traditional fertilizers, but also bolsters ecosystem health and agricultural productivity, providing a viable solution to some of the pressing challenges in sustainable soil management.

In cases where sediment contaminants exceed the permissible levels, purification procedures are essential before reuse or further processing. To ensure environmental safety, bioremediation is typically the preferred approach, due to its environmental benefits and efficiency, as we have discussed previously. Following the purification process, the sediments must undergo additional analysis to confirm that the pollutant concentrations meet the established standards. If bioremediation successfully reduces the contaminant levels to acceptable thresholds, the sediments will then be redirected for reuse or recycling. Depending on the intended application – such as for riverbank stabilization, landscape restoration, or use as substrates or fertilizers in agriculture – the cleaned sediments are employed in ways that align with both environmental safety and the specific requirements of each project.

For instance, the thresholds for beach restoration may be more flexible than for substrates in ornamental horticulture or agricultural fertilizers. In all cases, regulatory compliance is essential to ensure safe and effective use. If processing is selected after purification, additional bioremediation may not be necessary. Thus, processed materials are also suitable for safe use in agriculture or engineering projects, supporting environmental and economic sustainability goals.

Table 3. SWOT analysis of sustainable sediment management strategies.

Indicators	Helpful to achieve the objective		Harmful to achieve the objective	
	Strengths	Score (1–10)	Weaknesses	Score (1–10)
Structural factors (inherent to sediment management practices)	Environmental sustainability: Sustainable sediment management supports a circular economy, repurposes sediments, reduces waste, and minimizes reliance on non-renewable resources	9	Contamination challenges: High levels of contaminants (heavy metals, organic pollutants) in sediments can complicate and increase the cost of treatment, making some sediments unsuitable for immediate reuse	9
	Economic benefits: Sediment management can create jobs, lower fertilizer costs, and promote the use of local materials, leading to economic advantages for regional communities	8	Regulatory compliance: Ensuring sediment meets regulatory standards for reuse, especially in agriculture, can be challenging and may require extensive testing and purification efforts	8
	Resource efficiency: Treated sediments can substitute for traditional substrates and fertilizers, conserving resources such as peat and reducing reliance on synthetic fertilizers	8	Technical complexity: Advanced purification methods, such as biostimulation, bioaugmentation, and phytoremediation, require specialized knowledge, infrastructure, and financial investment	7
	Improved soil fertility: Nutrient-rich sediments enhance soil structure, water retention, and fertility, which benefits agricultural productivity and reduces erosion risks	9	Logistical requirements: Collecting, transporting, and processing large volumes of sediments can be challenging, especially in regions with limited infrastructure	8
	Opportunities		Threats	
Contextual factors (influences from the broader environment)	Growing demand for sustainable agriculture: Increasing awareness of sustainable practices opens up opportunities for using treated sediments as natural fertilizers in place of synthetic alternatives	9	Environmental and health risks: Improperly managed sediments or inadequate treatment can lead to environmental contamination and health risks, especially if pollutants are not effectively removed	9
	Construction applications: Sediments have promising uses in construction (e.g., road foundations, landscape restoration, riverbank stabilization), presenting new markets for treated sediments	8	Market acceptance and awareness: Public perception and market readiness may be barriers, as stakeholders might view sediment-derived products with scepticism due to potential contamination concerns	7
	Climate change mitigation: Using sediments for natural fertilization and peat replacement helps reduce greenhouse gas emissions associated with synthetic fertilizers and peat extraction	8	Regulatory hurdles: Regulatory standards and permissible limits for contaminants may tighten, restricting the reuse options for sediments and increasing treatment requirements	8
	Post-war reconstruction: In post-conflict areas, such as Ukraine, sediment reuse offers cost-effective materials for landscape restoration, filling craters, and rebuilding infrastructure	7	Economic and infrastructural challenges: High costs of transporting and processing sediments, especially in regions with limited infrastructure, may reduce the economic viability of sediment management practices	8

Source: Authors' research.

Table 4. Scored PESTEL analysis for sustainable bottom sediment management.

Category	Factor	Score (1–10)	Description
Political	Regulatory standards	10	Strict EU and local regulations on allowable contaminant levels in reused sediments are critical. Compliance directly impacts the feasibility of using sediments, particularly in agriculture, where contamination thresholds are stringent
	Government support for sustainability	8	Political support for sustainable practices can facilitate funding and policy incentives, making sediment management more economically viable
	Post-conflict recovery policies	7	In regions such as Ukraine, government prioritization of post-war recovery may boost support for sediment use in landscape restoration, especially in areas with limited resources
Economic	Cost savings in agriculture	9	The substitution of treated sediments for synthetic fertilizers represents a significant cost-saving opportunity for farmers, enhancing agricultural profitability and sustainability
	Job creation	8	Employment opportunities created through sediment management contribute positively to local economies, fostering social acceptance and economic resilience
	Logistics and transportation costs	9	High transportation and handling costs are a major factor in the economic feasibility of sediment management projects, particularly in remote or rural regions
	Local resource utilization	8	By using locally sourced sediments, dependency on imported materials, such as peat, is reduced, lowering costs and supporting local industries
Social	Public perception and acceptance	7	Public support is essential, particularly for agricultural applications. Perceptions around safety and contamination must be managed through transparency and education
	Community engagement and employment	6	Local job creation through sediment management can enhance community support, creating positive social impacts and fostering regional development
	Health and safety	8	Ensuring that reused sediments are free from harmful contaminants is crucial for public health, especially in agriculture where there is direct exposure to treated soils
Technological	Bioremediation techniques	7	Advanced bioremediation methods are essential for meeting safety standards in sediment treatment. Innovation in these techniques can improve purification efficiency and reduce treatment costs
	Particle separation technologies	6	Technologies that categorize sediments into fractions for agriculture or construction increase flexibility and scalability in sediment reuse.
	Monitoring and testing	7	Rapid, accurate testing for contaminants ensures compliance and safety. Advances in monitoring technology can streamline treatment processes and support regulatory adherence
Environmental	Reduction of waste	9	Recycling sediments reduces waste that would otherwise accumulate in landfills or waterways, contributing to the circular economy and environmental sustainability
	Climate impact reduction	8	Using sediments as a substitute for synthetic fertilizers and peat substrates reduces greenhouse gas emissions, supporting climate action goals
	Soil and waterway health	10	Sustainable sediment management promotes waterway health by preventing sediment build up and enhancing soil structure and stability, which is crucial for long-term environmental health
	Contamination risks	9	Effective treatment is necessary to mitigate contamination risks, ensuring sediments do not introduce harmful substances into ecosystems
Legal	Compliance with environmental regulations	10	Legal compliance with EU environmental standards directly impacts the scope of sediment reuse. Strict regulations can limit applications, while flexibility could broaden reuse options
	Waste classification	8	Legal classifications of sediments as hazardous or non-hazardous dictate handling, treatment, and disposal requirements, affecting project feasibility
	Land use and agricultural policies	9	Regulations on land use and soil additives influence sediment reuse in agriculture. Compliance with these policies is essential for project approval and sustainability

Source: Authors' research.

The results of the SWOT analysis, which identifies key strengths, weaknesses, opportunities, and threats associated with sustainable sediment management, are summarized in Table 3. This analysis provides insights into the internal and external factors that influence the feasibility and effectiveness of sediment reuse strategies.

The PESTEL analysis, presented in Table 4, evaluates the external political, economic, social, technological, environmental, and legal factors influencing sustainable sediment management. Rather than aggregating these factors into a single total, each category is examined individually to ensure a precise assessment of external influences without diluting their impact.

Following the PESTEL assessment, the SWOT analysis was conducted to determine the strategic feasibility of sediment management. Using the quantitative approach described in the Methods section, the strategic potential score was calculated as follows:

SWOT analysis calculations

1. Sum of positive factors (strengths + opportunities)

Strengths: 9 + 8 + 8 + 9 = 34

Opportunities: 9 + 8 + 8 + 7 = 32

Total positive factors: 34 + 32 = 66

2. Sum of negative factors (weaknesses + threats)

Weaknesses: 9 + 8 + 7 + 8 = 32

Threats: 9 + 7 + 8 + 8 = 32

Total negative factors: 32 + 32 = 64

3. Strategic potential calculation

SP = (S + O) - (W + T)

SP = 66 - 64 = 2

A strategic potential score of 2 suggests that the strengths and opportunities slightly outweigh the weaknesses and threats (Dankevych, 2018). While this reflects a moderately favorable outlook, the narrow margin indicates that addressing risks and leveraging opportunities is crucial to ensure long-term viability.

Qualitative insights from the PESTEL analysis

Since the PESTEL factors are not summed into a single total, their individual impact is analyzed based on previous research by Dankevych (2018) and Renella (2021):

- Political (regulatory influence = moderate): Environmental policies and sediment reuse regulations provide clear guidelines, but bureaucratic delays can slow implementation.
- Economic (investment and cost feasibility = significant): Market-driven incentives encourage sediment reuse, but funding gaps and financial constraints remain obstacles.

- Social (community engagement = low-moderate): Public awareness is relatively low, indicating a need for greater advocacy and educational outreach.
- Technological (infrastructure and innovation = moderate): Advancements in sediment processing and reuse technologies present growth potential, though implementation challenges exist.
- Environmental (sustainability and risk mitigation = high): Regulatory pressures on contamination control and ecosystem health make environmental considerations a dominant factor.
- Legal (compliance and policy stability = strong): A well-defined regulatory framework supports sediment reuse, yet inconsistent enforcement may introduce uncertainty.

Strategic implications

The SWOT and PESTEL analyses suggest that sustainable sediment management exists in a moderately supportive yet complex environment. While the internal strengths and opportunities outweigh the risks, the external landscape requires proactive strategies to strengthen feasibility.

- To enhance strategic viability, future initiatives should focus on the following:

Regulatory adaptation – streamlining compliance to reduce bureaucratic delays.

- Financial incentives – expanding investment and funding opportunities.
- Technological innovation – improving infrastructure to enhance adoption.
- Community engagement – increasing awareness to drive public and industry support.

By addressing these key areas, sustainable sediment management can fully capitalize on its strategic potential while mitigating existing challenges.

Conclusion

To enhance strategic viability, future sediment management initiatives should prioritize several key areas. Regulatory adaptation is necessary to streamline compliance procedures and reduce bureaucratic delays that may hinder the implementation of sustainable sediment reuse. Expanding financial incentives and investment opportunities will be essential to support the economic feasibility of sediment processing and transportation, particularly in regions where infrastructure limitations increase costs.

Technological innovation must be further encouraged to improve sediment purification methods, particularly in the areas of bioremediation and contaminant monitoring, ensuring that treated sediments meet regulatory and environmental standards. Community engagement and public awareness campaigns should be strengthened to address safety and contamination concerns while increasing the acceptance of sediment-based products in agriculture and construction.

By addressing these challenges and capitalizing on the identified opportunities, sustainable sediment management can become a viable strategy for environmental conservation, economic efficiency, and post-war recovery initiatives. In particular, the potential for sediment reuse in Ukraine's reconstruction efforts highlights its role in restoring landscapes, stabilizing damaged infrastructure, and reducing the need for imported raw materials. A structured approach that integrates strategic planning, regulatory compliance, and technological advancement will be crucial in realizing the full benefits of sediment reuse.

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KONCEPTUALNI OKVIR ZA INTEGRISANO ODRŽIVO UPRAVLJANJE ZEMLJIŠTEM I DONJIM SEDIMENTIMA

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R e z i m e

Ova studija istražuje održivi okvir za upravljanje donjim sedimentima, koristeći *SWOT* i *PESTEL* analize za procenu njegove strateške izvodljivosti. Kroz strukturirane evaluacije, studija identifikuje ključne prednosti, uključujući koristi cirkularne ekonomije kroz ponovnu upotrebu sedimenata, potencijal za otvaranje novih radnih mesta i poboljšanje plodnosti zemljišta. Međutim, istaknuti su takođe i izazovi, poput kontaminacije, usklađenosti sa regulativama i logističkih troškova kao faktori koji zahtevaju pažljivo upravljanje. *PESTEL* analiza otkriva snažnu eksternu podršku u vidu povoljnih ekoloških i regulatornih uslova, dok *SWOT* analiza pokazuje pozitivan strateški potencijal, ukazujući na izvodljiv put napred. Pored toga, studija se bavi ulogom upravljanja sedimentima u posleratnom oporavku, što je posebno relevantno za područja u Ukrajini, sugerišući njegovu primenu u obnovi pejzaža i jačanju infrastrukture. Integracijom internih i eksternih faktora, ovo istraživanje nudi sveobuhvatan model za prenamenu sedimenata, podržavajući ciljeve održivog razvoja u oblastima upravljanja resursima, zaštite životne sredine i poljoprivredne produktivnosti. Rezultati naglašavaju potencijal primene održivih praksi upravljanja sedimentima u poljoprivrednom sektoru kako bi se podržao održivi razvoj.

Ključne reči: održivo upravljanje sedimentima, ponovna upotreba poljoprivrednog otpada, upravljanje ekološkim resursima, cirkularna ekonomija u poljoprivredi, tehnike remedijacije, bioremedijacija u poljoprivredi, posleratni ekološki oporavak, reciklaža donjih sedimenata.

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Key words are terms or phrases which describe best the content of the article for the needs of indexing and browsing purposes. The number of key words should be 3 to 10. They should appear below the abstract. The title of key words should be bolded and indented by pressing the tab key. The colon should be used after the title, and then the list of key words in lower-case letters should be given with the full stop at the end. Key words should be provided in Serbian and English after abstract on both languages.

Introduction
The introduction should contain all the relevant information on past researches according to the stated problem and what can be achieved by further research. Reviewing the references, the author and the year should be provided, and the mentioned author should be cited in References. The title of the introduction should be centred and bolded, written in lower-case letters, below which using one line spacing, the text of the introduction should follow, justified. Each new paragraph should be indented pressing the tab key. These rules should be applied to all parts of the paper.

Material and Methods
The material and methods should be clearly outlined explaining all applied procedures in the paper. Generally known methods should be presented briefly, and a detailed explanation should be given if there is a deviation from previously published procedures. Papers, which have an experimental character, should provide the way of statistical data processing. This part, as well as the part Results and Discussion, if needed, may comprise certain subparts, too.

Results and Discussion
In the part Results and Discussion data obtained on the basis of observation and conducted experiments should be interpreted. In the comment of the results, references should be quoted at the end of the paper, providing the comparison between the obtained results and previous knowledge of the certain area.

Conclusion
All relevant items achieved in the researched area should be mentioned in the conclusion. Listing of all results with repetition of numbers previously specified in Results and Discussion should be avoided. Conclusion should not contain references.

Acknowledgements
Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

References

The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon, France.

Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

Tables
Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

Illustrations
All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae
All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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All future associates are asked to prepare the paper according to the given instructions in order to facilitate the work of the Editorial Board. Unless the paper is prepared according to the given instructions it will not be accepted for the prospective publishing.

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Za obradu teksta treba koristiti program MS-Word. Rukopise treba slati u jednom od sledećih formata .doc, .docx, koristiti font Times New Roman, veličina 12, jednostruki prored, margine 2,5 cm. Strane ne treba numerisati.
Originalan naučni rad – Rad koji sadrži prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
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Obavezna poglavlja svakog originalnog naučnog rada i prethodnog saopštenja su sledeća: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, materijal i metode, rezultati i diskusija, zaključak, zahvalnica, literatura i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Pregledni rad mora da sadrži: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, analizu-diskusiju određene teme, zaključak, literaturu i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Ako su radovi na engleskom jeziku, prednost se daje britanskoj varijanti ovog jezika.

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Naslov rada treba što vernije da opiše sadržaj rada i da ima što manje reči. U interesu je autora da se u naslovu koriste reči prikladne za indeksiranje i pretraživanje. Naslov se piše velikim slovima i centrirano. Ako je rad prethodno bio izložen na nekom skupu u vidu usmenog saopštenja, pod istim ili sličnim naslovom, podatak o tome treba navesti pri dnu prve stranice, posle podataka autora za kontakt.

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Navodi se puno ime, srednje slovo i prezime svih autora, u originalnom obliku. Imena se pišu ispod naslova, malim slovima, centrirano i boldovano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu, iza prezimena, označiti ustanovu u kojoj radi svaki autor. Autor za kontakt označava se zvezdicom u superskriptu, iza prezimena, komandom „insert footnote“, a njegova e-mail adresa navodi se ispod crte pri dnu prve stranice članka.

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Sažetak
Sažetak je kratak informativni prikaz sadržaja članka koji čitaocu omogućava da brzo i tačno odredi njegovu relevantnost. U interesu je autora da sažetak sadrži termine koji se koriste za indeksiranje i pretraživanje. Sažetak ne sme da sadrži reference. Sastavni delovi sažetka su cilj istraživanja, metode, rezultati i zaključak. Sažetak treba da ima od 200 do 250 reči. Reč „Sažetak“ piše se boldovano i uvlači jednim tabulatorom, nakon čega slede dve tačke, a zatim tekst sažetka.

Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

Rezultati i diskusija

U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

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Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

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Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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