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Phenotypic diversity of pedunculate oak (*Quercus robur* L.) for targeted genetic resource management

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Abstract: Pedunculate oak (*Quercus robur* L.) is a key forest tree species in Europe, valued for its ecological significance and economic importance. However, its genetic resources are increasingly threatened by climate change, habitat fragmentation, and anthropogenic pressures. This study assessed phenotypic variability among 11 natural populations from the Republic of Srpska (Bosnia and Herzegovina) and the Northern Serbia region (Autonomous Province of Vojvodina) to support conservation planning and sustainable genetic resource management. Ten leaf morphological traits were analyzed from ten trees per population. Variance components were estimated using two-way ANOVA, and multivariate differentiation was evaluated through principal component analysis, canonical discriminant analysis, and cluster analysis. All examined traits showed statistically significant variation both among and within populations. Size-related traits (blade length, leaf width, lobe width, sinus depth, and leaf area) defined the main axis of morphological differentiation, while venation traits and width of the principal lobe had the strongest discriminative power among populations. According to previous genetic studies, the populations of Kupinovo, Bački Monoštor, and Morović in Serbia have been recognized as priority units for conservation due to their high levels of genetic diversity and strong spatial representativeness within the species' natural range, and their importance is further supported and confirmed by the results of the present study. Building upon these findings, the results of the present study identify the Lukavica population as the primary candidate for conservation in the Republic of Srpska, with the Ugljevik population representing a secondary priority. Their clear morphological differentiation and distinctive ecological characteristics further highlight their significance for safeguarding both neutral and adaptive genetic diversity of pedunculate oak.

Keywords: pedunculate oak, phenotypic variability, leaf morphology, forest genetic resources, conservation.

1. Introduction

Pedunculate oak (*Quercus robur* L.) is a widely distributed and ecologically important deciduous tree species in Europe, playing a central role in forest ecosystems. It contributes significantly to

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biodiversity by providing habitat and food resources for numerous plant and animal species (Eaton et al. 2016). Moreover, it has substantial economic value, being utilized in timber production, landscape restoration, and silvicultural management. The species occurs across a wide range of climatic and edaphic conditions, which contributes to both its adaptability and phenotypic variability among populations (Eaton et al. 2016). Understanding the phenotypic diversity of *Q. robur* is essential for developing effective conservation strategies and sustainable management practices (Kremer et al. 2002).

Phenotypic variability, encompassing measurable morphological and physiological traits, reflects both genetic differences among populations and their adaptive responses to environmental conditions. In *Q. robur*, traits such as leaf size, shape, and petiole length, as well as growth-related parameters, exhibit considerable variation across populations (Rellstab et al. 2016). Assessing this variability is crucial for understanding the adaptive potential of populations and for identifying individuals or populations with desirable traits for conservation, breeding, and restoration programs. Moreover, phenotypic analyses provide insights into population structure, gene flow, and the effects of environmental gradients, forming a foundation for effective management of genetic resources (Fady et al. 2022).

Genetic resources of forest tree species represent a cornerstone for ecosystem resilience, adaptive capacity, and long-term sustainable productivity. *Q. robur* populations are threatened by a combination of drivers, including climate change, habitat fragmentation, and anthropogenic pressures, which can lead to erosion of genetic diversity and reduced adaptive potential (Fady et al. 2022). Conservation of genetic variation at both intra- and inter-population levels is therefore essential for safeguarding evolutionary potential and ecosystem functioning.

Targeted use of genetic resources, through selection of superior genotypes and ecotypes, enhances resistance to biotic and abiotic stressors, supports breeding programs, and informs reforestation strategies that are better adapted to future environmental conditions (Kesic et al. 2021). Integrating phenotypic variability into management frameworks allows for identification of populations with traits desirable for timber quality, growth performance, and stress tolerance (Climent et al. 2024). Consequently, systematic phenotypic characterization contributes not only to effective conservation planning but also to sustainable utilization of genetic resources in forestry practices, ensuring that adaptive diversity is maintained for generations to come.

Pedunculate oak populations in the Republic of Srpska (Bosnia and Herzegovina) and the Northern Serbia (Autonomous Province of Vojvodina) represent an important component of lowland and submontane forest ecosystems in the western Balkans (Banković et al. 2008; Ballian et al. 2010). These populations are located at the edge of the natural distribution range of *Q. robur*, making them particularly valuable for conservation and for maintaining the species' adaptive genetic potential (Kesić et al. 2021; Hodžić and Ballian, 2023). Populations at the distribution margin are often exposed to more extreme or variable environmental conditions, which can lead to the expression of unique phenotypic traits and potentially increased resilience to stress factors (Sáenz-Romero et al. 2017).

Bearing this in mind, the primary objective of this study is to assess the phenotypic variability of *Q. robur* populations from the Republic of Srpska and the Autonomous Province of Vojvodina, with the aim of informing conservation strategies and targeted genetic resource management. Specific objectives include quantifying morphological and growth-related traits across populations, identifying traits that contribute most to population differentiation, evaluating the adaptive potential of edge-of-range populations, and providing recommendations for the sustainable use of genetic resources. By addressing these objectives, this study contributes to evidence-based management strategies that maintain adaptive genetic diversity while supporting sustainable forestry practices.

2. Material and methods

2.1. Study area and site descriptions

The investigation of variability in the morphological traits of leaves of *Q. robur* was conducted across five natural populations within the Republic of Srpska, Bosnia and Herzegovina (Brčko, Ugljevik, Srbac, Prnjavor, and Lukavica) and six natural populations growing across Autonomous Province of Vojvodina, Serbia (Morović, Apatin, Bački Monoštor, Kupinovo, Klenak, and Odžaci) (Table 1; Figure 1).

Climatic data for the investigated sites were retrieved from the interactive platform ClimateCharts for the reference period 1985–2015 (Zepner et al. 2020).

Table 1. General characteristics of the investigated populations of *Quercus robur* L.

Locality	Latitude	Longitude	Elevation (m)	T (°C)	P (mm)
Vojvodina Province - Populations					
Morović	44°55'	19°07'	93	12.8	769
Apatin	45°37'	18°56'	88	11.5	625
Odžaci	45°28'	19°09'	85	12.2	657
Bački Monoštor	45°54'	18°53'	84	11.5	625
Kupinovo	44°44'	19°59'	83	12.9	711
Klenak	44°47'	19°45'	86	12.9	711
The Republic of Srpska - Populations					
Prnjavor	44°52'	17°44'	135	11.0	960
Srbac	45°02'	17°33'	126	11.5	865
Ugljevik	44°41'	19°04'	149	12.8	769
Brčko	44°49'	18°57'	106	11.6	866
Lukavica	43°48'	18°23'	580	8.2	1108

Legend: T – mean air temperature (°C); P – annual sum of precipitations (mm)

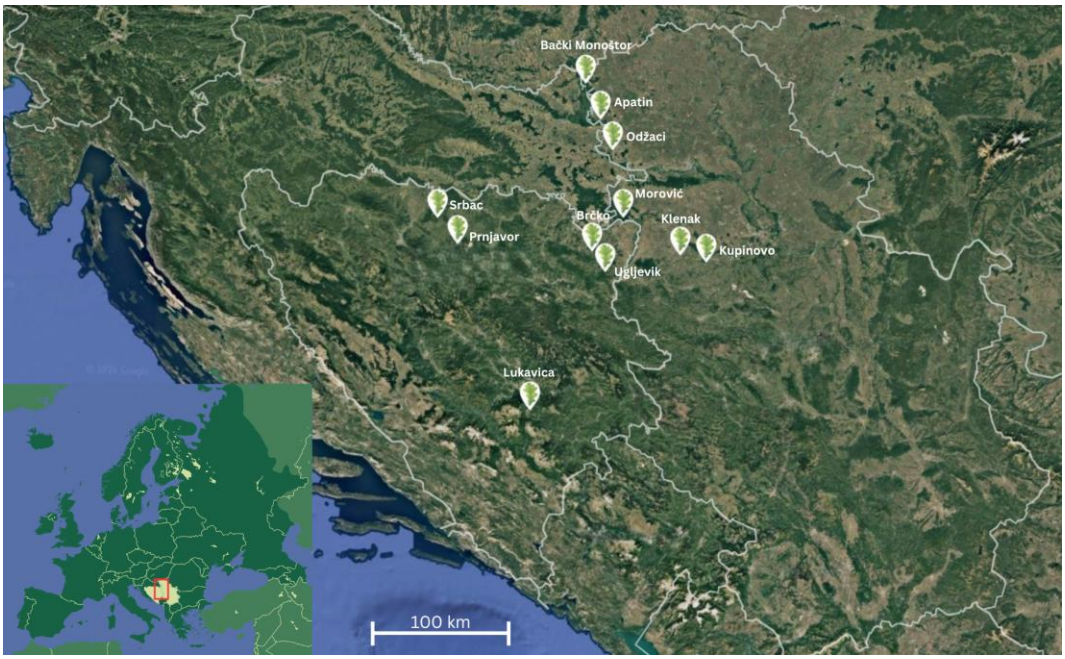

Figure 1. Spatial distribution of studied *Quercus robur* L. populations (Source: Google Earth, 2026).

2.2. Material collection and leaf trait measurement

For morphological analyses, leaves of *Q. robur* were sampled from ten trees per population. From each tree, twenty fully expanded, healthy, and undamaged leaves from the upper canopy were selected. To maximize sample uniformity, only sun-exposed leaves, oriented southwards and free from visible signs of disease or pathogen attack, were collected. The collected material was transported to the laboratory and processed into herbarium specimens. These specimens were subsequently scanned using a flatbed scanner (A3 2400S, Mustek System Inc.), and the resulting digital images were analyzed with tpsDig2 software. Leaf area (cm^2) was quantified using ImageJ.

The study assessed the following morphological traits: leaf area (LA) [cm^2], petiole length (PL) [cm], blade length (BL) [cm], maximum leaf width (LW) [cm], width of the principal lobe (WMLo) [cm], depth of the sinus of the principal lobe (SDMLo) [cm], number of lobes (LoN), width of the widest lobe (LoW) [cm], and the number of veins on the left (VNL) and right (VNR) sides of the leaf (Figure 2). This approach allowed for a detailed and standardized characterization of leaf morphology across populations.

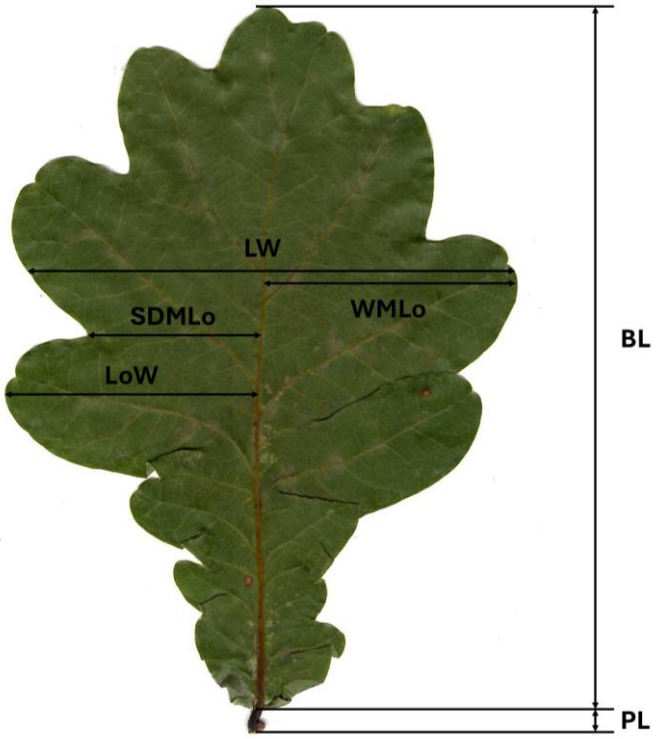

Figure 2. Measured phenotypic traits in *Quercus robur* L. populations.

2.3. Statistical analyses

The significance of different factors contributing to overall trait variation in *Q. robur* was assessed using appropriate statistical models. Morphological traits were analyzed with a two-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test to examine differences within and between populations and within-three variability. Expected components of variance were calculated for populations, and residual error, using a random-effects model, as both populations were considered random sources of variation.

Prior to analysis, normality of distributions and homogeneity of variances were evaluated using the Shapiro–Wilk and Levene's tests (Sánchez-Gómez et al. 2013), and variables deviating from normality were appropriately transformed. Data transformation was applied only to the traits describing the number of veins on the left (VNL) and right (VNR) sides of the leaf, as these variables

exhibited a continuous distribution. A square-root transformation was performed according to the following formula: $\sqrt{x+1}$.

To further explore differences among populations based on morphological traits, multivariate analyses were performed, including principal component analysis (PCA), canonical discriminant analysis (CDA), stepwise discriminant analysis, and hierarchical clustering. Hierarchical cluster analysis was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. Euclidean distance was used as the measure of similarity among the analyzed samples, as it is appropriate for continuous quantitative morphological data and provides a statistically robust basis for grouping patterns. Correlation analysis among the analyzed morphological traits was performed using Pearson's correlation coefficient. Pearson correlation was selected because the analyzed variables were continuous and approximately normally distributed after data transformation. All statistical analyses were conducted using STATISTICA, version 13 (TIBCO Software Inc. 2017).

3. Results

The study results revealed that all examined morphological leaf traits of pedunculate oak examined significant variation both among and within populations (Table 2). The highest coefficients of variation were recorded for the residual effect (CVR), reflecting differences among individual trees within trees.

Table 2. Results of the analysis of variance for the measured parameters of the studied *Quercus robur* L. populations.

Examined traits	MS_B	MS_W	MS_R	F_B-value	F_W-value	CV_B (%)	CV_W (%)	CV_R (%)
PL	3.89	0.72	0.05	5.37 **	15.87 **	20.92	30.79	35.66
BL	133.03	43.00	4.71	3.09 **	9.14 **	5.82	12.07	18.89
LW	119.91	21.50	2.45	5.58 **	8.78 **	10.18	14.23	22.78
WMLo	282.03	10.49	1.02	26.88 **	10.29 **	32.04	19.01	27.85
SDMLo	11.18	2.41	0.30	4.64 **	7.90 **	11.93	18.56	31.55
LoN	7.06	0.90	0.08	7.86 **	11.07 **	5.43	6.28	8.83
LoW	55.86	5.80	0.64	9.63 **	9.12 **	14.34	14.63	22.92
VNL	14.12	0.32	0.06	43.50 **	5.49 **	10.59	4.66	9.83
VNR	16.12	0.35	0.06	46.40 **	5.87 **	11.36	4.88	9.87
LA	11624.73	2299.46	188.5	5.06 **	12.20 **	15.62	23.61	31.15

Significance indicators for the F-test: *$p < 0.05$; **$p < 0.01$

Legend: MS_B – mean square between populations; MS_W – mean square between trees within populations; MS_R – mean square of the residual; F_B value – F-value for variation among populations; F_W value – F-value for variation among trees within populations; CV_B – coefficient of variation among populations (%); CV_W – coefficient of variation among trees within populations (%); CV_R – coefficient of residual variation (%).

Analysis of mean values for the examined traits revealed that the Ugljevik population displayed the highest averages for most leaf morphological characteristics, including blade length (BL), leaf width (LW), sinus depth of the principal lobe (SDMLo), number of lobes (LoN), width of the widest lobe (LoW), and leaf area (LA) (Table 3). The highest mean values for the number of veins on the left (VNL) and right (VNR) sides were observed in the Bački Monoštor population, while the Lukavica and Apatin populations exhibited the highest mean values for petiole length (PL) and width of the principal lobe (WMLo), respectively. The Lukavica population showed the lowest mean values for BL, LW, and WMLo, whereas the lowest mean numbers of veins on both sides of the leaf (VNL and VNR) were recorded in the Srbac population. The Morović population was characterized by the lowest mean values

of LoN and LA, while the Bački Monoštor and Apatin populations exhibited the lowest mean values of SDMLo and LoW.

Table 3. Tukey's HSD test for the mean values of the measured parameters in the studied *Quercus robur* L. populations. Mean values followed by the same letter are not significantly different ($p > 0.05$).

Population	PL	BL	LW	WMLo	SDMLo	LoN	LoW	VNL	VNR	LA
Ugljevik	0.60 ^{c}	12.88 ^{a}	8.76 ^{a}	3.94 ^{bc}	2.25 ^{a}	12.79 ^{a}	4.69 ^{a}	6.36 ^{de}	6.27 ^{d}	64.25 ^{a}
Prnjavor	0.58 ^{cd}	11.96 ^{bc}	7.11 ^{bcd}	3.04 ^{de}	1.90 ^{bc}	10.27 ^{de}	3.93 ^{b}	4.76 ^{f}	4.54 ^{e}	48.82 ^{b}
Srbac	0.56 ^{cde}	11.26 ^{cd}	6.72 ^{cde}	2.77 ^{ef}	1.66 ^{def}	10.17 ^{de}	3.79 ^{bc}	4.22 ^{h}	4.17 ^{f}	44.79 ^{bcd}
Brčko	0.53 ^{de}	11.17 ^{d}	6.43 ^{efg}	2.68 ^{f}	1.79 ^{cd}	11.86 ^{b}	3.55 ^{cd}	4.95 ^{fg}	4.86 ^{e}	44.13 ^{cd}
Lukavica	0.96 ^{a}	9.58 ^{e}	5.98 ^{g}	2.49 ^{f}	2.01 ^{b}	10.96 ^{c}	3.26 ^{e}	5.14 ^{f}	4.87 ^{e}	37.65 ^{ef}
Branjevina	0.67 ^{b}	12.36 ^{ab}	7.48 ^{b}	3.67 ^{c}	1.77 ^{cd}	10.14 ^{de}	3.62 ^{c}	7.70 ^{b}	7.67 ^{b}	45.79 ^{bc}
Bački Monostor	0.54 ^{cde}	11.51 ^{cd}	6.37 ^{efg}	3.19 ^{d}	1.48 ^{g}	10.65 ^{cd}	3.12 ^{e}	8.38 ^{a}	8.52 ^{a}	41.93 ^{cde}
Apatin	0.53 ^{de}	11.46 ^{cd}	6.20 ^{fg}	6.82 ^{a}	1.55 ^{fg}	10.49 ^{cde}	2.79 ^{f}	6.48 ^{d}	6.43 ^{d}	37.67 ^{ef}
Klenak	0.70 ^{b}	11.70 ^{bcd}	7.23 ^{bc}	4.04 ^{b}	1.74 ^{cde}	9.02 ^{f}	3.31 ^{de}	7.11 ^{c}	7.16 ^{c}	41.51 ^{cde}
Morovic	0.41 ^{f}	11.03 ^{d}	6.66 ^{def}	3.30 ^{d}	1.51 ^{fg}	8.41 ^{g}	3.05 ^{e}	5.99 ^{e}	6.10 ^{d}	35.67 ^{f}
Kupinovo	0.50 ^{e}	11.31 ^{cd}	6.56 ^{ef}	3.90 ^{bc}	1.57 ^{efg}	10.00 ^{e}	3.13 ^{e}	6.91 ^{c}	6.99 ^{c}	40.95 ^{de}

Legend: LA – leaf area [cm²]; PL – petiole length [cm]; BL – blade length [cm]; LW – maximum leaf width [cm]; WMLo – width of the principal lobe [cm]; SDMLo – sinus depth of the principal lobe [cm]; LoN – number of lobes; LoW – width of the widest lobe [cm]; VNL – number of veins on the left side; VNR – number of veins on the right side.

Differences among the studied populations contributed only weakly to moderately to the total variation, largely due to the high proportion of variation within trees and the residual effect (Figure 3). The greatest population-level contributions were observed for WMLo, VNL, and VNR. No single trait exhibited the highest contribution from within populations.

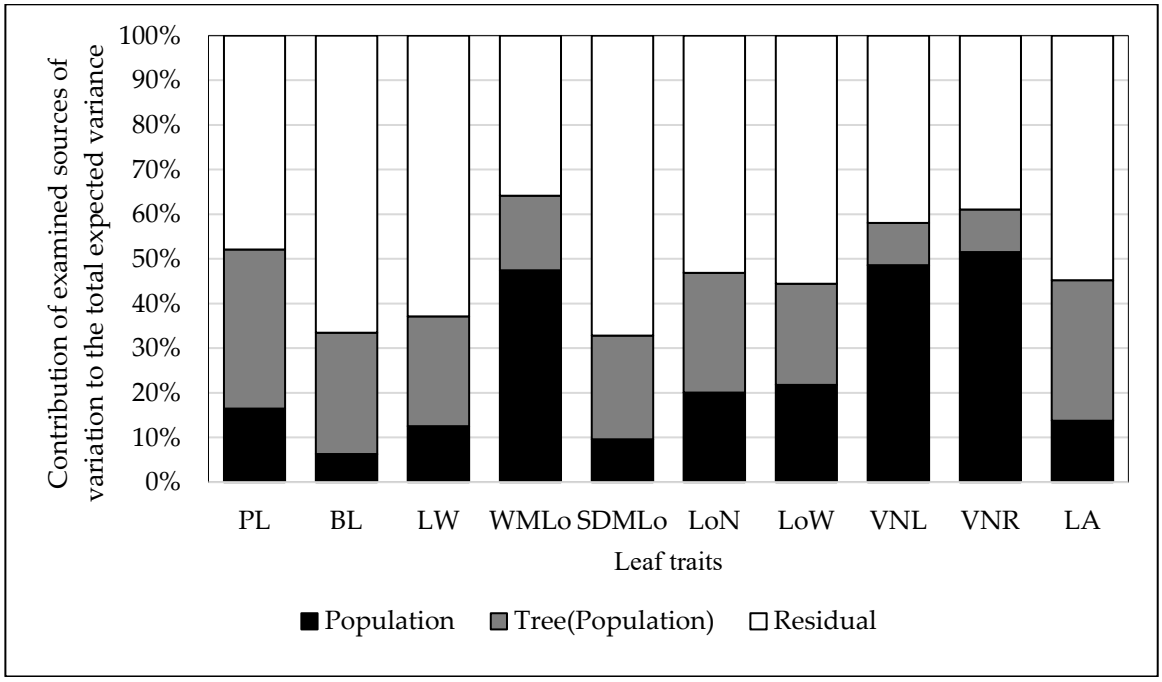

Figure 3. Contribution of the examined sources of variation to the total expected variance for the measured parameters of the studied *Quercus robur* L. populations.

A statistically significant correlation was observed between the LW trait, on the one hand, and BL, SDMLo, LoW, and LA, on the other (Table 4). SDMLo was also positively correlated with LoN, LoW, and LA. As expected, LA showed a positive correlation with LoN and LoW.

Table 4. Correlation coefficients for the measured parameters of the studied *Quercus robur* L. populations.

	BL	**LW**	**WMLo**	**SDMLo**	**LoN**	**LoW**	**VNL**	**VNR**	**LA**
PL	-0.398	-0.055	-0.241	0.549	0.216	0.096	-0.082	-0.138	-0.007
BL		0.831 **	0.295	0.219	0.215	0.583	0.356	0.359	0.734 *
LW			0.025	0.620 *	0.314	0.829 **	0.159	0.152	0.882 **
WMLo				-0.242	-0.059	-0.328	0.382	0.381	-0.093
SDMLo					0.654 *	0.801 **	-0.306	-0.352	0.740 **
LoN						0.585	-0.150	-0.185	0.672 *
LoW							-0.295	-0.311	0.944 **
VNL								0.997	-0.049
VNR									-0.067

The first three principal components, whose eigenvalues were greater than 1, explained 84.8% of the total variability among the studied populations. The highest loadings on the first principal component (PC1) were observed for the traits BL, LW, SDMLo, LoW, and LoN. PC2 was primarily defined by the parameters WMLo, VNL, and VNR, while PL showed the strongest correlation with PC3 (Table 5).

Table 5. Loadings between the measured parameters of *Quercus robur* L. populations and the first three principal components after their rotation using the Varimax method.

Examined traits	**RC1**	**RC2**	**RC3**
PL	-0.008	-0.067	**0.952**
BL	**0.776**	0.398	-0.433
LW	**0.924**	0.186	-0.091
WMLo	-0.054	**0.544**	-0.279
SDMLo	**0.744**	-0.281	0.549
LoN	**0.620**	-0.148	0.367
LoW	**0.938**	-0.301	0.072
VNL	-0.020	**0.980**	0.018
VNR	-0.038	**0.974**	-0.038
LA	**0.991**	-0.039	0.017
Explained variation	4.261	2.595	1.624
Proportion of the total variation	0.426	0.259	0.162

The analysis of the relationships among the studied populations (Figure 4) indicated a clear differentiation of the Ugljevik population along PC1. With respect to PC2, populations originating from Republika Srpska were generally positioned opposite to those from the Autonomous Province of Vojvodina, with the exception of the Morović and Ugljevik populations. The 3D PCA plot was generated using the mean values of all analyzed leaf samples within each population. Thus, each point shown in the PCA ordination represents the average trait values (population centroid) calculated for a given population, rather than individual leaf samples. This approach was used to summarize the

overall morphological characteristics of each population and to facilitate clearer visualization of interpopulation relationships.

The trait WMLo contributed most strongly to model discrimination, as indicated by the lowest Wilks' lambda values and a highly significant F-test ($p < 0.001$) (Table 6). The relationships among the studied pedunculate oak populations are presented based on the first three canonical variables (Figure 5). The ordination plot reveals the formation of two distinct groups: one comprising populations originating from the Autonomous Province of Vojvodina and the other including populations from Republika Srpska, with the exception of the Apatin population, which was differentiated along the second canonical axis.

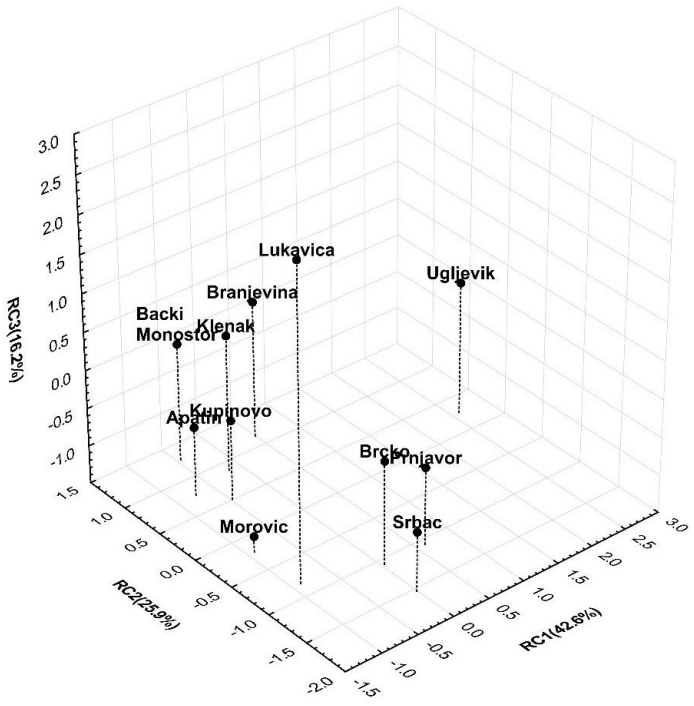

Figure 4. Ordination of the studied *Quercus robur* L. populations based on the first three principal components following Varimax rotation.

Table 6. Results of the assessment of the significance of the contribution of measured parameters to the discrimination of the studied *Quercus robur* L. populations.

Examined trait	Wilks' lambda	Partial Wilks' lambda	F-remove	p-value	Tolerance
PL	0.003	0.657	4.745	0.000	0.881
BL	0.003	0.747	3.083	0.002	0.251
LW	0.003	0.734	3.298	0.001	0.138
WMLo	0.010	0.220	32.205	0.000	0.552
SDMLo	0.003	0.875	1.305	0.240	0.490
LoN	0.004	0.556	7.263	0.000	0.568
LoW	0.003	0.762	2.847	0.004	0.260
VNL	0.003	0.868	1.380	0.202	0.126
VNR	0.003	0.849	1.619	0.114	0.129
LA	0.002	0.963	0.345	0.966	0.251

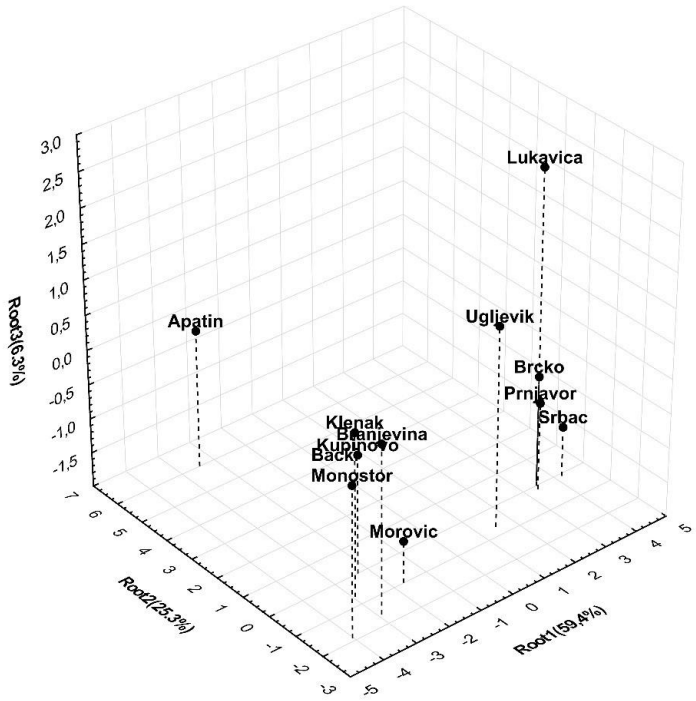

Figure 5. Relationship among the studied *Quercus robur* L. populations based on the first three canonical variables.

Discriminate loadings of the investigated traits across the first five canonical variables indicated that the highest loadings on the first canonical variable were observed for SDMLo, VNL, and VNR, while WMLo exhibited the highest loading on the second canonical variable. The trait PL showed the strongest loading on the third canonical variable (Table 7). Most morphological traits (BL, LW, LoN, LoW, and LA) exhibited their highest loadings on the third canonical variable.

Table 7. Discriminant loadings of the studied *Quercus robur* L. population traits according to the derived canonical variables.

Traits	Root1	Root2	Root3	Root4	Root5
PL	0.270	-0.082	**0.868**	0.346	0.146
BL	-0.266	-0.147	-0.257	**-0.791**	0.328
LW	0.011	-0.339	0.042	**-0.725**	0.591
WMLo	-0.521	**0.791**	-0.017	-0.255	0.188
SDMLo	**0.570**	-0.155	0.565	-0.394	0.375
LoN	0.446	0.086	0.441	**-0.677**	-0.320
LoW	0.524	-0.369	0.074	**-0.654**	0.357
VNL	**-0.926**	-0.170	0.222	-0.213	-0.112
VNR	**-0.940**	-0.179	0.163	-0.210	-0.112
LA	0.300	-0.258	0.116	**-0.846**	0.271
Explained variation	10.416	4.431	1.111	0.853	0.508
Proportion of the total variation	0.594	0.253	0.063	0.049	0.029

The discriminative ability of pedunculate oak populations was assessed for the analyzed traits using stepwise discriminant analysis. The model including all examined morphological traits achieved 67% correct classification (Table 8; Figure 6). The first parameter included was VNR, and the model with this trait alone achieved 36% correct classification. The model incorporating VNR, WMLo, LoW, LoN, PL, LW, and BL together reached 67% correct classification, which is the same percentage obtained with the model including all examined traits.

Table 8. Results of the stepwise discriminant analysis.

Step	Entering trait	F to enter	p-value	Wilks' lambda	F-value	p-value
1	VNR	46.534	0.000	0.177	46.534	0.000
2	WMLo	23.815	0.000	0.052	33.540	0.000
3	LoW	16.715	0.000	0.019	27.342	0.000
4	LoN	9.352	0.000	0.010	22.078	0.000
5	PL	5.823	0.000	0.006	18.185	0.000
6	LW	4.101	0.000	0.004	15.383	0.000
7	BL	3.574	0.000	0.003	13.446	0.000
8	VNL	1.661	0.102	0.003	11.625	0.000
9	SDMLo	1.313	0.235	0.002	10.227	0.000

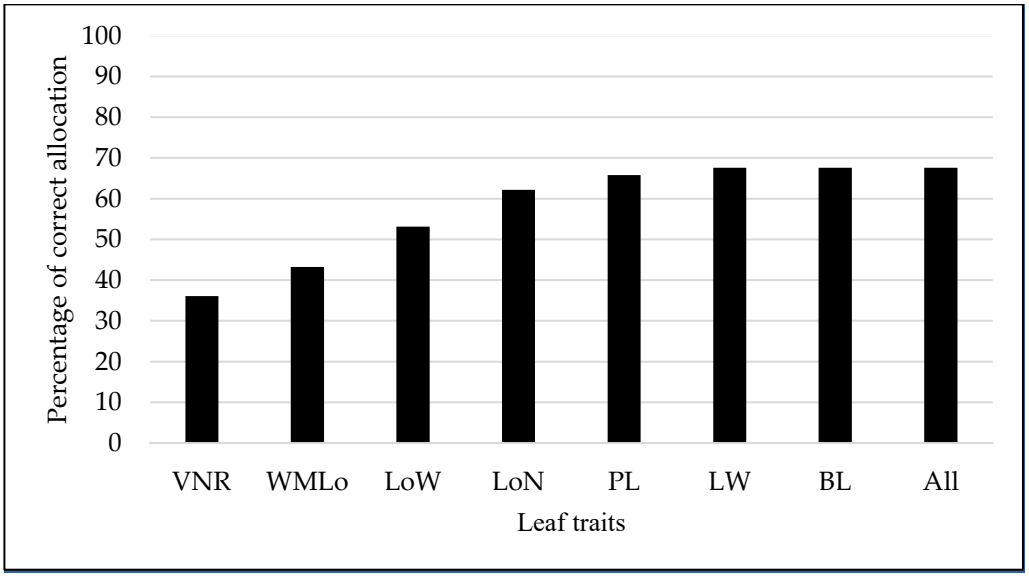

Figure 6. Percentage of correct classification for models generated based on stepwise discriminant analysis.

A dendrogram was constructed using hierarchical clustering based on the UPGMA method, and the grouping of the studied pedunculate oak populations was analyzed accordingly (Figure 6). The results indicated that the populations were clustered into four groups. The first and fourth clusters each consisted of a single population from Republika Srpska, namely Ugljevik and Lukavica. The second cluster comprised the remaining populations from Republika Srpska (Srbac, Brčko, and Prnjavor), while populations originating from the Autonomous Province of Vojvodina formed the third cluster.

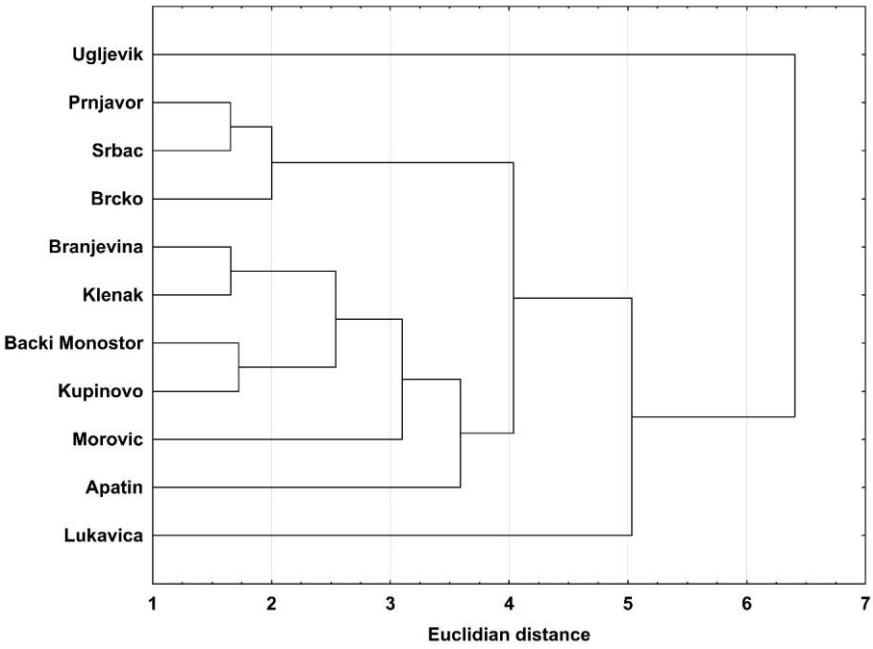

Figure 7. Dendrogram of the cluster analysis of the studied *Quercus robur* L. populations, aggregated using the UPGMA method based on standardized measured parameters.

4. Discussion

The results of the present study demonstrate pronounced morphological variability among and within populations of *Q. robur*, confirming that leaf traits in this species exhibit a complex pattern of differentiation. All examined traits showed statistically significant variation at multiple hierarchical levels, yet the largest proportion of variability was attributed to within-population and residual components. This pattern is consistent with the biological characteristics of long-lived, wind-pollinated, predominantly outcrossing forest tree species, which are generally characterized by high levels of intrapopulation genetic diversity and comparatively low interpopulation differentiation (Kremer et al. 2002).

The high coefficients of residual variation (CVR) observed for most traits indicate substantial individual level heterogeneity within trees. Such variability may reflect both additive genetic variation and phenotypic plasticity. In oaks, phenotypic plasticity of leaf morphology is well documented and often associated with environmental heterogeneity, including variation in light availability, water regime, and soil properties (Ramírez-Valiente et al. 2010; Ramírez-Valiente et al. 2011; Rolando et al. 2025). Given the broad ecological amplitude of *Q. robur*, which occupies diverse habitats ranging from alluvial plains to mesic lowland forests, plastic responses likely contribute significantly to the observed morphological diversity (Sáenz-Romero et al. 2011; Abadie et al. 2012; George et al. 2020).

Although population-level variance components were smaller than within-population components, multivariate analyses revealed clear geographic structuring. The differentiation between populations originating from Republic of Srpska and those from the Autonomous Province of Vojvodina suggests that regional ecological conditions may have influenced morphological divergence. Similar patterns of moderate but significant morphological and genetic differentiation across ecological gradients have been reported for *Q. robur* and related oak species throughout Europe (Kremer et al. 2002; Gómez Quijano et al. 2024). Environmental factors such as temperature regime, soil moisture availability, and flood dynamics are known to influence leaf size, lobation, and venation patterns, potentially driving adaptive divergence at regional scales (Wang et al. 2022).

The strong positive correlations among blade length (BL), leaf width (LW), width of the widest lobe (LoW), sinus depth (SDMLo), and leaf area (LA) reflect a high degree of morphological integration.

Leaf area emerged as a central integrative trait, strongly associated with linear dimensions and lobe development. Such coordinated variation is consistent with functional and developmental constraints in leaf architecture, where overall leaf expansion influences both lobe formation and venation density (Peng et al. 2022; Matos et al. 2024). The first principal component (PC1), accounting for the largest share of total variation, was primarily defined by these size-related traits, suggesting that overall leaf size and degree of lobation represent the dominant axis of morphological differentiation.

In contrast, the second principal component was largely associated with venation traits (VNL and VNR) and the width of the principal lobe (WMLo), indicating that venation pattern and specific aspects of lobe architecture constitute a partially independent morphological axis (Hegedüs et al., 2025). The near-perfect correlation between VNL and VNR confirms the strong bilateral symmetry typical of oak leaves and highlights the developmental stability of venation traits. Stepwise discriminant analysis identified VNR and WMLo as the most powerful discriminative variables, underscoring the taxonomic and ecological relevance of venation and lobe configuration in population differentiation (Tantray et al. 2017).

The results of the morphometric analysis of *Q. robur* leaves indicate a high level of variability in the analyzed morphological traits among individual trees as well as among populations. The highest proportion of variability was detected at the individual and within-population levels, suggesting a pronounced intraspecific diversity within populations. Similar findings were reported by Ballian et al (2010), who demonstrated that variability within populations of *Q. robur* in the Western Balkan region is often greater than variability among populations, indicating that populations may share a relatively similar genetic structure, while observed differences are largely influenced by local ecological conditions.

Since the studied populations are located at the edge of the species' distribution range, where environmental conditions are generally less favorable and often more variable compared to the central part of the range, the detected morphological differences may reflect both genetic differentiation and phenotypic plasticity in response to local environmental conditions. Edge populations are frequently exposed to ecological constraints such as increased temperature variability, periodic drought stress, and habitat fragmentation, which may contribute to morphological divergence among populations. Therefore, the observed variation in leaf morphology likely represents the combined influence of genetic background and environmental adaptation to marginal habitat conditions (Vakkari et al. 2020).

The moderate overall classification success (67%) suggests that while populations exhibit detectable morphological structuring, substantial overlap exists among them. This overlap may result from ongoing gene flow, which is known to be extensive in pedunculate oak due to effective long-distance pollen dispersal (Kremer et al. 2002), as well as from phenotypic plasticity that can reduce apparent differentiation. Notably, a substantial portion of variation was detected for most traits, suggesting extensive gene flow and a low degree of spatial genetic structuring. The clustering pattern, where certain populations formed distinct groups while others overlapped, likely reflects a balance between local adaptation and genetic connectivity (Rellstab et al. 2016).

Therefore, the observed morphological variability of *Q. robur* leaves can be interpreted as the result of complex interactions between genetic factors and environmental conditions. Such findings highlight the importance of integrating morphological and molecular approaches in future research to better understand the genetic structure, adaptive potential, and evolutionary processes within populations of this ecologically and economically important forest tree species.

4.1. Implications for the conservation of pedunculate oak genetic resources

The predominance of within-population variability has important implications for genetic resource management. Since most variation resides within populations rather than among them, conservation and breeding programs should prioritize the maintenance of large and genetically diverse stands to preserve adaptive potential. Sampling strategies for seed collection should include a high number of unrelated mother trees within each population to adequately capture existing diversity. The

detected regional structuring suggests that geographic origin should not be disregarded in reforestation and afforestation efforts. Even moderate morphological differentiation may reflect adaptive responses to local climatic and edaphic conditions. Therefore, the use of locally adapted or ecologically matched reproductive material may enhance plantation performance and resilience, particularly under changing climatic conditions.

Previous studies on the genetic diversity of *Q. robur* in the Republic of Serbia clearly indicated which populations require particular attention in terms of conserving the genetic resources of this species (Kesić et al. 2021). It should be recalled that, based on several indicators of genetic diversity, the aforementioned authors identified the Kupinovo (KU) population as a primary candidate for inclusion in the European Information System on Forest Genetic Resources (EUFGIS), as it exhibited the highest expected heterozygosity, allelic richness, and number of private alleles. The Bački Monoštor population was also highlighted, given that it is located within the Mura–Drava–Danube Biosphere Reserve. In this way, not only the genetic resources of individual populations be preserved, but a significant step would also be taken toward conserving spatial genetic diversity, as these populations (together with the Morović population, which is already registered in EUFGIS) cover the natural distribution range of pedunculate oak in Serbia, extending from the north toward the south of the country. Research conducted within the framework suggests that additional populations, particularly those originating from the Republic of Srpska, should also be considered for conservation (i.e., inclusion in the EUFGIS database). In this regard, special attention should be given to the Lukavica and Ugljevik populations, which were clearly differentiated from the other studied populations. Interestingly, these two populations were positioned on opposite sides of the dendrogram, reflecting contrasting trends across the majority of the analyzed leaf morphological traits. Furthermore, the Lukavica population is geographically isolated from the other studied populations and occurs at the site characterized by the highest altitude (580 m), the highest annual precipitation (1108 mm), and the lowest mean annual air temperature (8.2°C).

Based on the results obtained in this study, the Lukavica population is therefore proposed as the primary candidate for inclusion in the EUFGIS database of forest genetic conservation units, followed closely by the Ugljevik population. Their inclusion would contribute to the preservation of both neutral and adaptive spatial variability of pedunculate oak across the regions of the Autonomous Province of Vojvodina and the Republic of Srpska.

5. Conclusion

The results of this study demonstrate substantial phenotypic variability in leaf morphological traits among and within populations of *Q. robur* originating from the Republic of Srpska and the Autonomous Province of Vojvodina. All examined traits showed statistically significant differentiation; however, the largest proportion of total variation was attributed to within-population and residual components. This pattern is consistent with the biological characteristics of long-lived, outcrossing forest tree species and indicates high intrapopulation diversity and considerable gene flow. From a conservation perspective, the high level of within-population variability emphasizes the importance of maintaining large, genetically diverse stands to preserve adaptive potential. The confirmation of previously identified priority populations in Serbia (Kupinovo, Bački Monoštor, and Morović), together with the distinctiveness of Lukavica and Ugljevik, provides a strong basis for evidence-based conservation planning. In particular, Lukavica is proposed as a primary candidate for inclusion in conservation networks, followed by Ugljevik, in order to safeguard both neutral and adaptive genetic diversity of pedunculate oak across the western Balkan region.

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