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Original scientific paper

MORPHOLOGICAL VARIABILITY OF *QUERCUS ROBUR* L. LEAF IN SERBIA

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This paper presents the results of a study dealing with leaf morphological variability of *Quercus robur* L. 148 trees were sampled from 5 population across Serbia and 17 morphological traits were assessed. Interpopulation variability was confirmed by the results of the analysis of variance (ANOVA). A statistically significant ($p < 0.05$) effect of population was obtained for most of the studied morphological characters. Intrapopulation variability was confirmed by statistically significant tree effects for all of the studied leaf characters (all $p < 0.05$). The results of the multivariate analysis of variance (MANOVA) confirmed a significant population and tree share in the total phenotypic variability (all $p < 0.05$). By applying the canonical discriminant analysis (CDA), the first discriminant function accounted for 63% of the variability between populations and the second accounted for 20% of the population variability. The leaf area (AREA), specific leaf area (SLA) and surface area to perimeter ratio (ARPE) had the greatest effect on population differentiation (CDA). It is assumed that different environmental conditions affect population differentiation and that high intrapopulation variability is due to intraspecific variability.

Keywords: leaf morphology, pedunculate oak, population, variability

INTRODUCTION

Quercus robur L. belongs to the largest genus *Quercus*, of the family Fagaceae. The undefined taxonomic status of some oak species is due to their wide range of distribution, absence of phenological barriers and occurrence of intra- and inter-specific hybridization and introgression (BRUSCHI *et al*., 2000; BORAZAN and BABAC 2003; BORATYNSKI *et al*., 2008). Pedunculate oak is the most common oak species in Europe. The Balkan Peninsula is an area with high diversity of oak species and as such it is valuable in the study of their evolution (BROSHTILOV, 2006).

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In recent decades, there has been an evident decline of pedunculate oak forests in Europe, Asia, the Balkan Peninsula, as well as in Serbia. This has been caused by a number of biotic and abiotic factors, such as climate change, site conditions, over-exploitation, or insufficient and inadequate regeneration of pedunculate oak forests (YAKOVLEV and KLEINSCHMIT, 2002; BOBINAC, 2003; THOMAS *et al*., 2003; BALBOA-MURIAS *et al*., 2006; HELAMA *et al*., 2009).

Pedunculate oak is one of the most valued forest species in Serbia (BOBINAC, 2011). It is one of 10 oak species native to the territory of Serbia, where it usually occurs in the valleys of major rivers (Danube, Sava, and Morava). The most valuable pedunculate oak forests in Serbia grow - along the river Sava in the area of Srem, where there are individual specimens of pedunculate oak aged hundreds of years ("Smogva" and "Vratičina" localities in Bosutska forest were set aside as nature reserves) (BOBINAC, 2007).

Individual variability in leaf morphology is induced by the interactions between the genetic structure and the effects of the environment. At the same time, it makes the potential of a species to adapt to changing environmental conditions (CASTRO-DIEZ *et al*., 2000; BAYRAMZADEH *et al*., 2012).

The study of leaf morphology from the aspect of genetic differentiation provides useful information on population and intrapopulation variability and can be the basis for the determination of species and lower categories as well as intraspecific or interspecific hybrids. The similarity between individuals of the same or different populations or between distant and separate populations can point to their historical connections and common descent. Morphological determination is a good basis for further research studies of this kind and it is often combined with chemotaxonomic, cytological and molecular analyses (KELLEHER *et al*., 2005; FRANJIĆ *et al*., 2006; PRIDA *et al*., 2006; JORDAO *et al*., 2007).

The aim of this study was assess inter- and intrapopulation variability of the pedunculate oak leaf morphology in Serbia.

MATERIALS AND METHODS

Study populations

The research was conducted in 5 populations in the territory of Serbia: in the central part (Belgrade surroundings: Ada Ciganlija (AC) and Bojčin forest (BS), in the northwest - Sombor (SO), in the north - Subotica (SU), and in the northeast of the country - Vršac (VR) (Figure 1). These populations are mixed-structured, uneven-aged, of coppice and seed origin, 60 - 130 years old.

Population Ada Ciganlija is at N 44˚48', E 20˚24', altitude 70-76 m, ass. *Fraxino angustifoliae - Quercetum roboris* Jov. and Tom. 1979., soil type *fluvisol calcaric*, mean annual temperature 11.8°C and precipitation 654 mm; population Bojčin forest is at N 44˚43', E 20˚10', altitude 77-78 m, ass. *Carpino - quercetum roboris* Rauš 1969., soil type *planosol dystric* mean annual temperature 11.8°C and precipitation 691 mm; population Sombor is at N 45˚46', E 18˚56', altitude 83-86 m, ass. *Carpino betuli-Quercetum roboris* Anić 1959., soil type gleysol calcaric, mean annual temperature 10.6°C and precipitation 583 mm; population Subotica is at N 46˚07', E 17˚18', altitude 137 m, ass. *Quercetum roboris* Jov. and Tom. 1979., soil type *arenosol calcaric*, mean annual temperature 10.9°C and precipitation 555 mm; and population Vršac is at N 45˚07', E 21˚25', altitude 76-80 m, ass. *Carpino–Fraxino-Quercetum roboris* Miš. et Broz 1962., soil type gleysol mollic, mean annual temperature 11.5°C and precipitation 659 mm.

Soil types were determined in soil profiles opened at each study population, and soil samples for laboratory analyses were taken at fixed depths - ICP Forests – methods (Examination laboratory of Institute of Forestry, Belgrade, Department of soil and plant material) (BATOS *et al*., 2010).

Weather information are from Republic Hydrometeorogical Service of Serbia.

Figure 1. Location of analysed populations of *Quercus robur* L. in Serbia: in the central part Ada Ciganlija (AC), and Bojčin forest (BS); in the northwest - Sombor (SO); in the north - Subotica (SU); and in the northeast of the country - Vršac (VR)

Leaf morphological assessement

The morphological study of the pedunculate oak leaf included 17 characters (10 primary - measured and 7 secondary - derived), in a sample of 10 leaves per tree, on 148 trees in 5 populations, which makes a total of 1480 leaves (28 – 31 trees per population). Leaves were collected in the same period of the year (in September), from spring short fertile shoots on the branches that grew on approximately the same height (3-4 m), on the outer parts of the crown with four different aspects (KREMER *et al*., 2002). A sample of 10 fresh leaves from each tree was dried and preserved. The images were recorded on a scanner (Hewlett Packard Jet 3400 C) at the resolution of 200 dpi and saved in JPG format. The obtained images were further processed in Image Tool in order to obtain data for the given morphological traits (Figure 2).

Primary leaf parameters:

- leaf area with petiole (AREA) (cm^2),
- lamina perimeter with petiole (PERI) (cm),
- length of petiole PL (cm),
- length of lamina (LL) (cm),
- maximum width of lamina (MWL) (cm),
- width of the main lobe (LW) (cm),

- height of maximum width (length of lamina from the base to the widest part) (HMW) (cm),
- depth of sinus of the main lobe (SW) (cm),
- number of lobes (NL), and
- dry leaf mass (MASS) (g).

Secondary leaf parameters:

- total length of lamina (TL = PL + LL) (cm),
- rattio from length of petiole to total lamina length (PR = 100*PL/TL),
- lobe depth ratio (LDR = 100*(LW – SW)/LW),
- leaf surface area to perimeter ratio (ARPE = 100*AREA/PERI),
- lamina shape or obversity (OB = 100*MWL/LL),
- leaf mass per area (SMASS = 100*MASS/AREA) (gcm^{-2}), and
- specific leaf area (SLA = AREA/MASS) (cm^2g^{-1}).

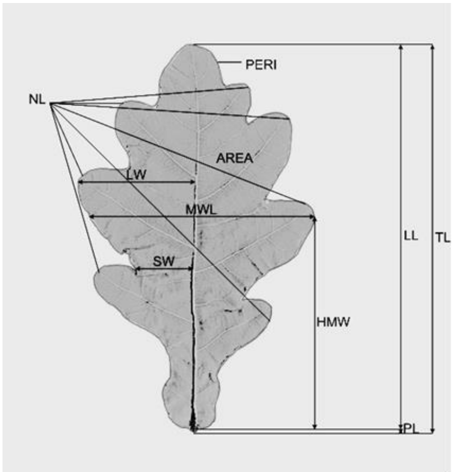

Figure 2. Analysis of primary characters of *Quercus robur* L. leaf

Statistical analysis

The statistical analysis were performed using the appropriate procedures of the SAS version 9.1.3 (SAS Institute, Cary, NC 2011) and Statistica 10 (StatSoft, Inc.)

The most important data on population and individual variability are described by the results of descriptive statistics.

Pearson`s correlation coefficients, which describe the degree and significance of correlations between pairs of leaf morphological traits, were obtained using the PROC CORR procedure of the SAS software package.

Statistical significance of the differences in the mean values of the studied traits between populations is shown in the results of Scheffe's test. Statistical significance of different sources of variation, with the population as a fixed and the tree as a random factor, was determined by using the analysis of variance (ANOVA, MANOVA) (PROC GLM - SAS software package). The analysis used logarithmically transformed data, except for SW, HMW and OB. They were square rooted in order to achieve normal distribution of data, which is the basis for the application of the analysis of variance.

Multivariate relationships between the studied morphological characters were assessed using the Canonical Discriminant Analysis (CDA) of the data from all five populations. This analysis used only the characteristics that showed statistical significance as determined by the results of ANOVA.

RESULTS

According to the results of the leaf morphological assessement, the variability of most of the studied characters was very high, both between the sampled leaves within individual trees and between trees in a population or among populations.

The obtained mean trait values for the most important morphological leaf traits, of *Quercus robur* L. trees from 5 population of Serbia, in the whole sample were LL = 11.74 cm, MWL = 7.20 cm, PL = 0.53 cm, NL = 11.68, and OB = 61.48%.

According to the results of descriptive statistics and the calculated coefficient of variation (results not shown) the character with the highest variability is PL (CV = 21.92% - 32.96%), followed by PR (CV = 21.75% - 30.75%), AREA (CV = 17.46% - 24.93%) and MASS (CV = 19.99% - 28.17%). The lamina length (CV = 9.73% - 11.12%), MWL (CV = 9.64% - 13.41%) and NL (CV = 9.89% - 12.61%) are among the characters with lower variability. The lamina shape or obversity (CV% = 6.44 – 9.36%) is the least variable character.

The leaf area had a strong positive correlation with MWL (r = 0.913), and with PERI, LL, LW, MASS, and TL. The length of lamina had a strong positive correlation with TL (r = 0.995) and with MWL, LW, HMW, and MASS (all $p < 0.05$) (Table 1).

Table 1. The Pearson's correlation coefficients *among the quantitative leaf variables of Quercus robur L. The bold and underline values present a strong statistical significant correlation with r values under 0.7 (all p < 0.05)*

	PERI	PL	LL	MWL	LW	SW	HMW	NL	MASS	TL	PR	LDR	ARPE	OB	SMASS	SLA
AREA	**0.831**	0.210	**0.895**	**0.913**	**0.876**	0.457	**0.666**	0.191	**0.835**	**0.894**	-0.164	0.063	**0.790**	0.210	0.087	-0.075
PERI		0.213	**0.826**	**0.828**	**0.820**	0.154	**0.624**	0.337	**0.683**	**0.828**	-0.136	0.328	0.339	0.178	0.056	-0.051
PL			0.193	0.196	0.185	0.070	0.174	-0.109	0.263	0.288	**0.899**	0.041	0.130	0.053	0.178	-0.158
LL				**0.770**	**0.748**	0.352	**0.784**	0.263	**0.719**	**0.995**	-0.229	0.098	**0.644**	-0.141	0.032	-0.027
MWL					**0.936**	0.375	**0.570**	0.179	**0.761**	**0.771**	-0.128	0.179	**0.664**	**0.514**	0.083	-0.072
LW						0.301	**0.528**	0.180	**0.731**	**0.749**	-0.131	0.290	**0.609**	0.445	0.078	-0.065
SW							0.420	0.064	0.379	0.350	-0.074	**-0.801**	**0.619**	0.107	0.034	-0.022
HMW								0.265	**0.548**	**0.783**	-0.161	-0.109	0.481	-0.165	0.044	-0.036
NL									0.078	0.246	-0.217	0.044	-0.041	-0.074	-0.139	0.151
MASS										**0.728**	-0.046	0.052	**0.673**	0.209	**0.587**	**-0.525**
TL											-0.134	0.099	**0.641**	-0.132	0.049	-0.042
PR												-0.007	-0.141	0.117	0.158	-0.140
LDR													-0.256	0.152	0.016	-0.023
ARPE														0.167	0.100	-0.088
OB															0.087	-0.081
SMASS																**-0.908**

marked correlation coefficient are statistically significant ($p < 0.0001$); bold and underline r > 0.70, bold r (0.50 - 0.70)

The population of Ada Ciganlija has trees with the highest AREA, PERI, LL and MWL, which is the least elongated in this population. The population of Bojčin forest has trees with the smallest AREA, LL and MASS. Trees of Vršac population have the longest petiole, while the trees of Subotica population have the shortest one. Although the petiole length is at the individual level a very variable character, at the population level, the difference is not highly significant. Regarding the leaf shape, the leaves of Ada Ciganlija and Sombor populations show the greatest differences, the former being the least elongated and the latter the most elongated and the least lobed. Trees of Subotica population have the most distinctly lobed leaf with the highest number of lobes (Figure 3).

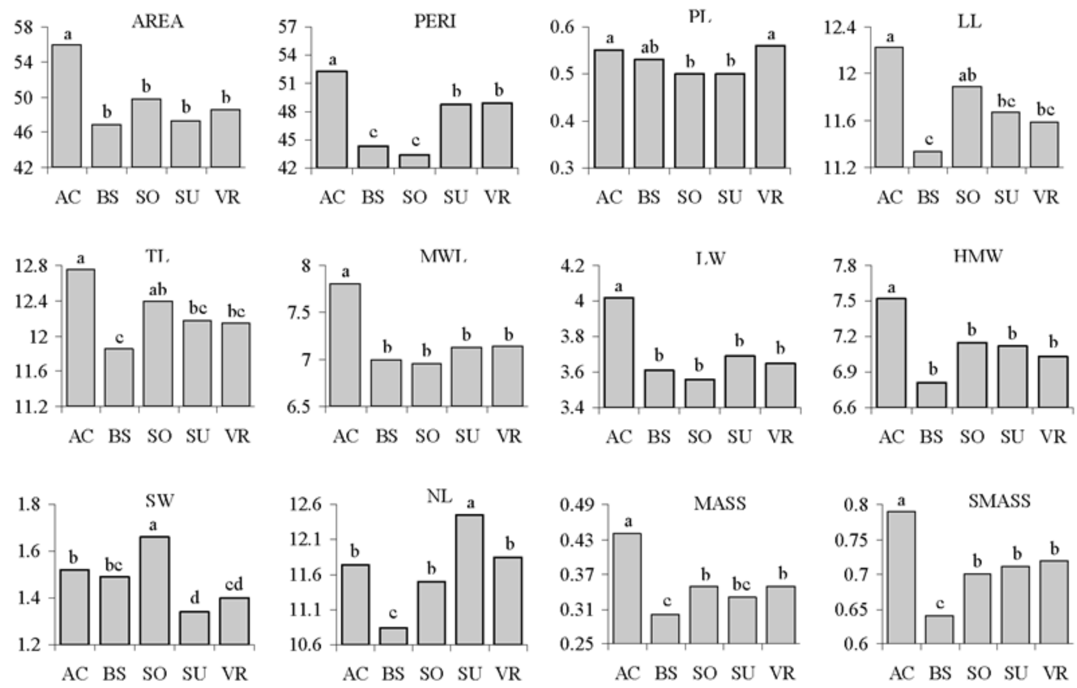

Figure 3. Variability of morphometric characters of Quercus robur L. leaves. The populations with different letters differ significantly ($P < 0.05$) according to a Scheffé-test

The results of Scheffe's comparison test revealed that the mean values of the studied traits showed among-population differences in PERI, LL, TL, MASS, SMASS, SLA. The populations of Ada Ciganlija and Bojčin forest are two extreme groups while the populations of Sombor, Subotica and Vršac form a homogeneous group between these two extreme variants. The population of Vršac is distinguished from other populations with regard to the PL, PR, ARPE (Figure 3).

The share of phenotypic variability, influenced by environmental differences (population effect) is statistically significant for all leaf traits, except for PL, LL, TL and PR. A statistical effect of the tree was obtained for all studied traits (Table 2). The results of multivariate analysis of variance (MANOVA) confirmed a significant population and tree share in the total phenotypic variation (Table 3).

Table 2. ANOVA results for the mean primary characters of Quercus robur L. leaves

Source	df	F value									
		Analyzed primary characters of leaf									
		AREA	PERI	PL	LL	MWL	LW	HMW	SW	NL	MASS
Population	4	4.26**	10.30****	0.89ns	2.37ns	4.99***	5.39***	3.04*	7.12****	5.93***	9.77****
Trees	143	5.28****	6.29****	7.99****	5.35****	5.72****	4.79****	3.49****	3.21****	7.76****	6.59****

Table 2 Continue

Source	df	F value						
		Analyzed secondary characters of leaf						
		TL	PR	LDR	ARPE	OB	SMASS	SLA
Population	4	2.23ns	1.32ns	9.31****	8.36****	4.49**	6.26****	6.23****
Trees	143	5.59****	6.79****	3.12****	7.92****	8.06****	14.90****	14.94****

ns = not significantly; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

Table 3. MANOVA results for 17 characters of Quercus robur L. leaves

Source	df	den df	F-Value	P-Value
Population	76	5178.3	20.18	<.0001
Trees	2717	24891	4.03	<.0001

Having determined significant differences between the populations, we studied which character had the greatest impact on their differentiation. The obtained discriminant axes (CDA1 and CDA2) showed a significant correlation with their own values (eigenvalue) (Table 4). The first two discriminant functions account for 83% of the variability (CDA1 for 63% and CDA2 for 20%) (Table 5). Leaf area had the highest value of the absolute standardized coefficient (Table 5) for the first discriminant axis. According to the first canonical discriminant axis (CDA 1), which describes the 63% of the whole variability between populations, we obtained the trend of differentiating the trees from the population of Ada Ciganlija (with the highest mean value of AREA in compared with Bojčin forest populations (Figure 4).

Table 4. χ^2 test for characters of Quercus robur L. leaves

Roots Remove	Eigen-value	Canonicl R	Wilks' Lambda	Chi-Sqr.	df	p-level
0	0.3592	0.5141	0.6018	681.3363	36	0.0000
1	0.1158	0.3221	0.8181	269.3996	24	0.0000
2	0.0768	0.2671	0.9128	122.3114	14	0.0000
3	0.0172	0.1301	0.9830	22.9374	6	0.0008

Table 5. Standardized coefficients for multivariate analysis on log-transformation *or* square-root transformation *on the morphological traits values of Quercus robur L. leaves*

Variable	Root1	Root2	Root3	Root4
SLA	0.5249	-0.6750	0.1152	0.0453
NL	-0.3173	0.2700	-0.7950	0.2244
ARPE	1.4027	0.9232	-0.5151	1.0521
AREA	-2.5344	-0.6267	2.4052	-3.2211
OB	-0.9717	-0.4263	1.2981	-0.1666
MWL	1.4166	0.3139	-2.3410	0.8877
HMW	-0.3247	-0.1753	0.3933	0.5362
LW	0.3837	0.1529	0.1026	1.7576
SW	0.1568	0.1353	0.0759	-0.4393
Eigenval	0.3592	0.1158	0.0768	0.0172
Cum.Prop	0.6311	0.8346	0.9697	1.0000

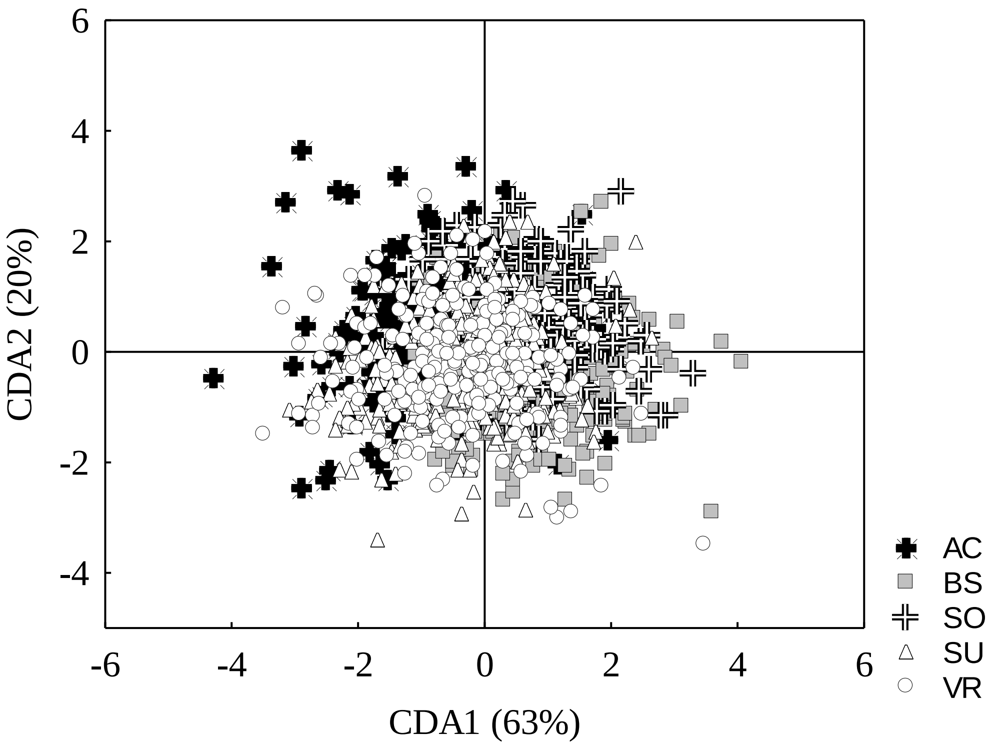

Figure 4. Canonical discriminant analysis scattergram of trees data from five populations of *Quercus robur* L. in Serbia, each canonical coordinate (canonical discriminant function) is based on the nine analyzed morphological leaf traits

DISCUSSION

The obtained values of the morphological traits of the pedunculate oak leaf from 5 population across Serbia are more or less different from the published data on pedunculate oak in the neighboring and other countries in Europe (PONTON *et al*. 2004; SAINTAGNE *et al*. 2004; BROSCHTILOV 2006; Figure 4. Canonical discriminant analysis scattergram of trees data from five populations of Quercus robur L. in Serbia, each canonical coordinate (canonical discriminant function) is based on the nine analised morphological leaf traits BAŠIĆ *et al*., 2007; BORATYNSKI *et al*., 2008; BALLIAN *et al*., 2010; BATOS, 2012; ENESCU *et al*., 2010). The difference is also clear in relation to data on pedunculate oak in Serbia (BALLIAN *et al*., 2010) and other European countries displayed in Table 6. A comparative review reveals that the pedunculate oak leaf in 5 population in Serbia is somewhat larger and with a greater number of lobes compared to the pedunculate oak leaf in Croatia and Bosnia and Herzegovina. It also has a longer petiole and a more elongated shape than the leaf in Croatia, while it has a smaller petiole and a less elongated shape than the leaf in Bosnia and Herzegovina. Like the results from Serbia, the results of the research studies of the pedunculate oak in Croatia and Bosnia and Herzegovina show that the intrapopulation variability is higher than the population variability, which is common in woody species (ŠKVORC *et al*., 2005; BAŠIĆ *et al*., 2007). The results indicate a significant intraspecific variability of the pedunculate oak leaf morphology.

Most traits do not vary independently of each other, and particular combinations of morphological and physiological traits are more correlated than it would be expected randomly (KUDOH *et al*., 2001). The correlation analysis carried out within the study confirmed the expected correlation between some of the studied characters (lamina length/width of lamina), previously stated by BAŠIĆ *et al*. (2007). The correlation between the characters that usually aren't in direct logical correlation was also confirmed (e.g. the correlation of the lamina length with the maximum width of lamina, and the width of the main lobe, which had also been stated in the relevant literature (KREMER *et al*., 2002; ENESCU *et al*., 2010).

Variability in leaf size and characters that are derived as their ratios (LDR) reveals the degree of transpiration and thus the adaptability of individuals to environmental conditions (Camarero and Gil-Pelegrin 2003). In comparison to other study populations, the population of Subotica is the most arid. Therefore, the most deeply incised leaf, which is typical of this population, may be the result of its adaptation to dry climate conditions.

As in our study, BALLIAN *et al*. (2010) and BAŠIĆ *et al*. (2007) in their studies of pedunculate oak in the Western Balkans and Bosnia and Herzegovina found that, besides the incision of leaf blade and the incision of a leaf to a central nerve, the petiole length is the most variable trait of character. According to BAŠIĆ *et al*. (2007), populations showed the highest differences in lamina length, length of lamina from the base to the widest part and maximum width of lamina, the differences are somewhat smaller for incised of lamina base, petiole length and lamina shape or obversity while there is no difference between the populations only for the length of sinus from the midrib. The study of pedunculate oak in Croatia also show that the petiole length is a very variable character, but the authors state that this is due to environmental differences between the study populations (ŠKVORC *et al*. 2005). BRUSCHI *et al*. (2002) found that the petiole length is a character significant for the discrimination of sessile oak populations in Italy, while the depth of sinus has significance in the tree differentiation.

Table 6. Comparative data morphological leaf characteristics of Quercus robur L. in Europe

Refferences	Analyzed properties															
	AREA (cm^2)	PERI (cm)	PL (cm)	LL (cm)	TL (cm)	MWL (cm)	LW (cm)	HMW (cm)	SW (cm)	NL (broj)	MASS (g)	SMASS (g cm^{-2})	PR (%)	LDR (%)	ARPE (%)	OB (%)
Ballian et al. 2010 Bosnia and Hercegovina			0.39	8.22	8.62	8.62		4.92	0.99							60.0
Ballian et al. 2010 Croatia			0.49	9.96		10.45		6.02	1.31							61.0
Ballian et al. 2010 Serbia			0.52	10.74		11.26		6.60	1.30							60.0
Ballian et al. 2010 Montenegro			0.35	9.78		10.14		5.95	1.05							61.0
Bašić et al. 2007 Bosnia and Hercegovina			0.65	9.9	10.55	6.02		5.97	7.13							62.75
Batos 2012 Serbia	49.67	47.53	0.53	11.74	12.23	7.20	3.71	7.13	1.48	11.68	0.35	0.73	4.32	59.15	103.06	61.48
Boratynski 2008 Poland	46.3	41.95	0.95	11.3	12.25	6.6				5.50			7.73		109.0	58.65
Broshtilov 2006 Bulgaria			0.31	8.88	9.19					14.6						
Enescu et al. 2010 Romania			0.64	11.6				6.83								
Ponton et al. 2004 France	28.0		0.5	9.2	9.7					10.6		0.65				
Saintagne et al. 2004 France			0.44	7.75	8.19	4.44	2.45		1.34	8.7			5.4	44.5		57.3

CONCLUSION

The results suggest that all of the studied leaf characters showed highly significant statistical differences between the trees and most of the characters showed differences that are significant for population differentiation. Both the populations and the pedunculate oak trees within them are isolated from other oak species in the surroundings, and the trees within a population live and grow in similar environmental conditions, thus the resulting variability within the population can be considered as intraspecific. The study populations are geographically distant and they develop in different site conditions, pointing to the assumption about the effects of the environment on leaf morphology. There are obvious differences between the soil composition, the mean annual temperature and annual precipitation in the analyzed populations. The combination of these factors certainly have an impact on leaf morphology. The trees whose values are extremely deviant from the obtained average values were entered into a separate register. In this respect, further research should comprise the study of the genotypes whose characters have extreme values as well as the study of microsite conditions and determine whose influence is more significant.

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MORFOLOŠKA VARIJABILNOST LISTA *QUERCUS ROBUR* L. U SRBIJI

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Izvod

Lužnjak je u Srbiji jedna od najrasprostranjenijih i najcenjenijih šumskih vrsta. Morfološka analiza lista sa aspekta genetičke izdiferenciranosti pruža korisne informacije o populacionoj i unutarpopulacionoj varijabilnosti i može da bude osnov u determinaciji vrsta i hibrida. Cilj rada je bio definisanje inter i intrapopulacione varijabilnosti morfologije lista lužnjaka na području Srbije. Analizirano je 17 morfoloških karaktera lista sa 148 stabala iz 5 populacija. Inter populaciona varijabilnost je potvrđena rezultatima analize varijanse (ANOVA) za većinu analiziranih karaktera. Unutar populaciona varijabilnost je potvrđena statistički značajnim efektom stabla za sve analizirane karaktere lista (all $p < 0.05$). Rezultatima multivarijantne analize (MANOVA) potvrđen je značajan udeo populacije i stabla u ukupnoj fenotipskoj varijabilnosti (all $p < 0.05$). Primenom kanonijske doscriminantne analize (CDA) dobijeno je da su razdvajanju populacija najviše doprineli površina lista (AREA), specifična površina (SLA) i odnos površine i obima lista (ARPE). Pretpostavka je da različiti ekološki uslovi utiču na odvajanje populacija a da je velika unutarpopulaciona varijabilnost posledica unutarvrsne varijabilnosti

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