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VARIABILITY OF BLACK POPLAR (*Populus nigra* L.) LEAF MORPHOLOGY IN VOJVODINA, SERBIA

MORFOLOŠKA VARIJABILNOST LISTOVA CRNE TOPOLE (*Populus nigra* L.) NA PODRUČJU VOJVODINE, SRBIJA

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Summary

Morphological study of intra and interpopulation variability of black poplar leaves was carried on four natural populations located in the basin of the major rivers at the area of Vojvodina, Serbia. Research was conducted on the basis of nine leaf morphometric parameters, with descriptive and multivariate statistical analysis. Results show that within and between studied populations exists considerable variability, with the variability much more pronounced within than between populations. Given that the environmental conditions of investigated locations are uniform, it is assumed that the variability is consequences of the specific gene pool of these populations.

KEY WORDS: Black poplar, Vojvodina, leaf morphology, interpopulation and intrapopulation variability

INTRODUCTION
UVOD

Area of Vojvodina, Serbia, is one of the regions with lowest forest cover in Europe, less than 6.50% of its total area. Forests of Vojvodina are unevenly distributed and mainly located in the narrow and wider areas along rivers, representing smaller and larger forest complexes. Since the last few decades floodplains are increasingly under human's control, representation of flooded ecosystems in total area of Vojvodina is only 28%. Forest and other wooded land of riparian area, which are the products of mutual relations between biological and ecological characteristics of native poplars and willows and river alluvial action, have been tight between the embankment and the river. These natural habitats of native riparian species are disappearing and formation of new alluvial sediments and new pioneer stands has been significantly reduced. Within these riparian ecosystems black poplar (*Populus nigra* L.) as one of important pioneer tree species (Pospiškova et al. 2004) has been adapted to the specific conditions of floodplain.

Until now, smaller and larger areas of a poplar-willow forest are preserved in groups and as single trees. In the river valleys, where the river is still wild, black poplar is better preserved than on the rivers which are more regulated. Today, black poplar participate with only 15,87% in total area of native poplars, which is 8,45% of all poplars area (other 91,54% belongs to hybrid poplars) (Radosavljević 2009). Given that black poplar suffers from tremendous habitat losses, it has been considered as endangered tree species of riparian ecosystem.

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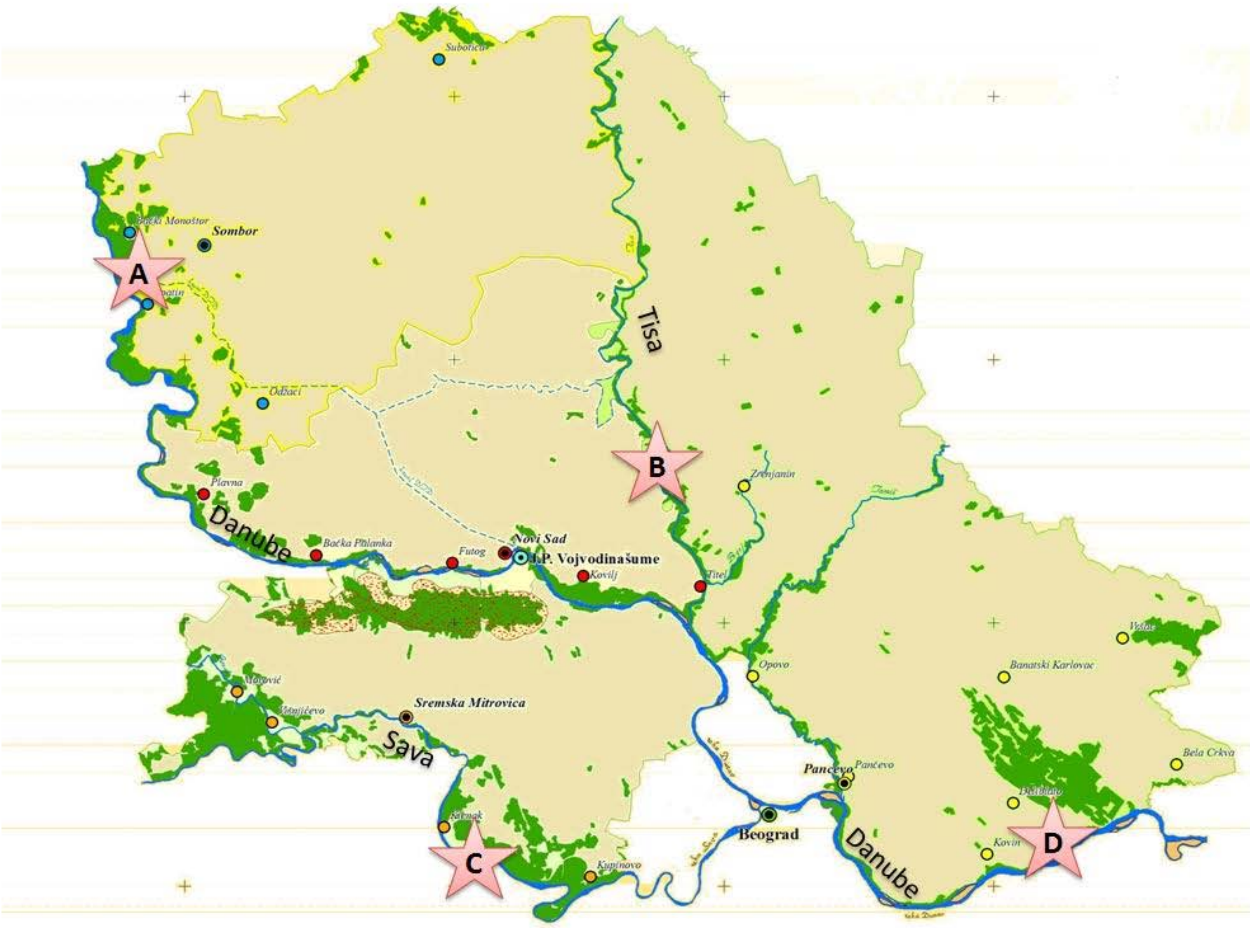

Figure 1. Studied Black poplar populations at the area of Vojvodina (Northern Serbia) (A – upper Danube population, B – Tisa population, C – Sava population, D – lower Danube population)

Slika 1. Istraživane populacije Crne topole na području Vojvodine (Sever Srbije) (A – populacija Gornje Podunavlje, B – populacija Tisa, C – populacija Sava, D – populacija donje Podunavlje)

Lefevre et al. (1998) lists three key factors for disappearance of natural populations of black poplar from reduced floodplain ecosystems. The first factor is the control of river systems which leads into the question possibility of riparian species natural regeneration and encourages the replacement of existing populations with hardwood populations; all this brings into the question the survival of the remaining gene pool of the species. Another important endangering factor is over-exploitation of autochthonous poplar in the last century and the mass introduction of superior hybrid Euro-American poplars and American black poplar clones (Romanić 2000), in order to meet the needs of the human population for fast-growing species. As the last given factor is introgression of cultivated poplars as a potential threat to natural populations of poplars. Besides hybrid poplars, which are threat for gene pool and sustainability of native poplar population, different poplar varieties present all over the Europe and wider could threaten the survival of black poplar natural population (Šijačić-Nikolić et al. 2012).

Assessment of black poplar variability along many rivers across Europe, using morphological and genetic markers, have been performed by a number of researchers (Samardžić 1996; Cottrell et al. 1997; Krstinić et al. 1997; Arens et al. 1998, Romanić 2000; Kajba et al. 2002; Alba et al. 2002; Van Dam et al. 2002; Gebhardt et al. 2002; Vanden Broeck et al. 2004; Pospiškova et al. 2004; Bruce et al. 2010; Čortan et al. 2013, 2014; Maksimović et al. 2014). Existence of variability is a key factor in the process of species adapting to environmental changes. The higher variability exists there are higher chances for species long-term survival.

In order to develop strategies for conservation as well as for reforestation, it is necessary to estimate the amount and distribution of diversity in existing natural populations (Flush et al. 2002). Prescribing appropriate conservation measures will neutralize losses and preserve the remaining gene pool, which is currently threatened by extinction.

Foregoing points to the need for variability research which have been exploring a number of black poplar (*Populus nigra* L.) leaf morphological parameters in four populations along Vojvodina area. Results of black poplar leaf morphologic variability have been shown in this paper.

MATERIAL AND METHODS
MATERIJAL I METODE

The examination of black poplar degree of leaf morphometric parameters variability within and between populations was performed on selected test trees in four populations at their natural habitat, in the area of Vojvodina (Figure 1). Populations are located in the basin of the major rivers in Vojvodina: two populations are located in the basin of Danube river (the upper course -population A and the lower course of Danube – population D), one is placed in the basin of the Tisa River – population B and the last one in the basin of the Sava River – population C (figure 1). These four sites have similar site characteristics; they are placed on flat ground right next to the riverside, which are under the periodical flooding (up to 65 days per year). The geological substrate consists of clayey sands with quartz and other silicate and it has sandy structure. The soil is alluvial on alluvial deposit, medium-deep (41–80 cm), loosely, fresh, skeletal (51–70% of the structure). Litter is medium represented, suggesting on favorable process of humification.

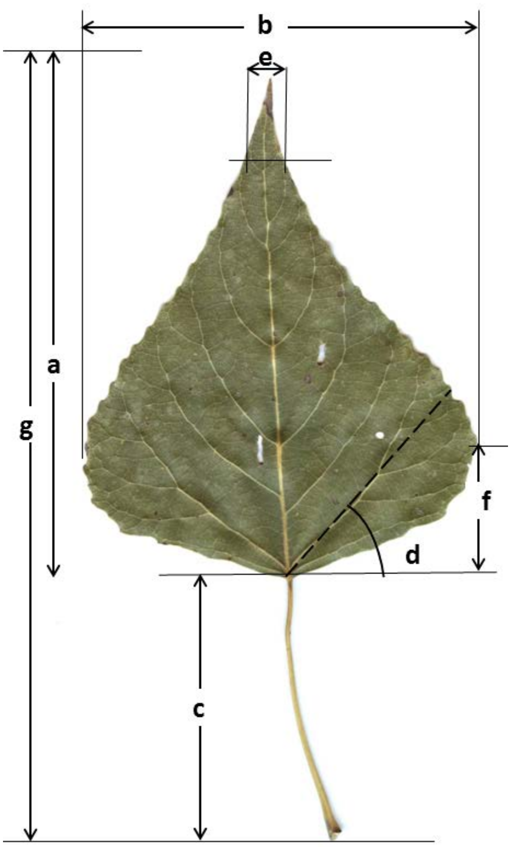

Figure 2 Analyzed morphometric leaf parameters
Slika 2. Analizirana mofometrijska svojstva lista

Within each of these studied populations 10 adult test trees were randomly selected. Leaves were randomly sampled from test trees during the growing season, when the leaves are fully developed (August-November). For the purpose of consistency, samples were collected from mature trees with approximately the same height (4–6 m) and the same part of the crown (outer part of crown, leaves of light, south side). In order to provide equivalent material required for comparison, samples were taken from the middle part of long shoot twig, because of the less pronounced polymorphism in relation to the leaves from basal and apical part of long shoot (Tucović 1965).

Analysis were carried out on a sample of 100 dried, healthy, undamaged leaves from each test tree, which is in total 4000 leaves from 40 test trees. On each leaf 9 morphometric parameters were analyzed, with a total of 36000 measurements. The measurements were performed with an accuracy of 1mm.

The analysis included the following morphometric parameters: a – length of leaf, b – width of leaf, c – petiole length, d – angle between the first vein and horizontal line, e – width of leaf at 1 cm from the top, f – distance between the base of leaf and the widest part of leaf, g – length of the whole leaf (leaf and stalk), h – number of veins on the left side of the leaf and i – number of veins on the right side of the leaf.

Obtained morphometric data were statistically analyzed using the software package Statistica 6.0. Results of measurements were statistically analyzed with descriptive statistic: mean value (x), minimum and maximum value (min – max), variation range (R), standard deviation (SD), relative standard deviation (RSD). The one-way ANOVA was used to test differences between mean values of measured leaf parameters. Mean values were separated using Tukey's HSD test, with significance levels of $p<0,05$ and $p<0,01$. The dendrogram of cluster analysis (Nearest Neighbor Method, Euclidean distance) was used to illustrate a hierarchical clustering of studied populations.

RESULTS
REZULTATI

Results of descriptive statistics are represented in Table 1. The largest mean values of leaf length (**a**=93,43 mm), leaf width (**b**=73,39 mm), petiole length (**c**=54,71 mm), distance between the base of leaves and the widest part of the

Table 1. Descriptive statistic for the measured morphological traits of leave for all analyzed Black poplar populations
Tabela 1. Deskriptivna statistika za mjerena morfometrijska svojstva listova za sve analizirane populacije Crne topole

Descriptive parameters Deskriptivni pokazatelji	Population Populacija	a(mm)	b(mm) **	c(mm)	d(°) **	e(mm)	f(mm) *	g(mm)	h **	i **
$\bar{X}$	A	89,18	$72,96^{bc}$	51,33	$38,72^{a}$	5,24	$21,77^{a}$	140,52	$5,07^{a}$	$5,32^{a}$
	B	93,43	$73,39^{c}$	54,71	$45,55^{bc}$	5,15	$24,89^{b}$	148,44	$5,78^{bc}$	$5,77^{ab}$
	C	86,64	$66,39^{a}$	51,49	$42,36^{ab}$	5,67	$23,03^{ab}$	137,80	$6,07^{c}$	$6,19^{b}$
	D	91,00	$68,71^{ab}$	50,19	$49,12^{c}$	5,25	$24,43^{b}$	141,19	$5,58^{b}$	$5,56^{a}$
	mean	90,06	70,36	52,00	43,94	5,33	23,54	141,91	5,63	5,71
min-max	A	74 98	70 76	41 63	33 46	4,0 9,4	19 26	118 161	4,0 5,6	4,5 6,0
	B	82 108	67 78	47 68	39 51	3,9 6,6	21 28	135 158	5,9 6,5	5,0 6,5
	C	77 103	62 76	36 69	37 47	3,3 7,5	19 26	116 170	5,5 6,8	5,7 6,6
	D	78 102	61 75	40 57	43 54	3,7 6,6	22 29	125 155	5,1 6,0	5,1 6,0
Sd	A	6.6505	2.0719	6.6584	4.4525	1.5498	1.8341	11.7430	0.5433	0.4554
	B	7.8168	3.3272	6.1018	3.9295	1.0692	2.6728	7.1297	0.5432	0.5399
	C	7.9787	4.6871	8.5544	3.7100	1.1547	2.0363	15.2070	0.3492	0.3294
	D	7.7785	3.9382	4.6863	3.4262	0.7826	2.3162	10.4321	0.3812	0.3609
	mean	7,556125	3,5061	6,500225	3,87955	1,139075	2,21485	11,12795	0,454225	0,4214
RSD(%)	A	7.46	2.84	12.97	11.50	29.56	8.42	8.36	10.71	8.56
	B	8.37	4.53	11.15	8.63	20.75	10.74	4.80	9.40	9.35
	C	9.21	7.06	16.62	8.76	20.37	8.84	11.04	5.76	5.32
	D	8.55	5.73	9.34	6.98	14.91	9.48	7.39	6.83	6.49
	mean	8.40	5.04	12.52	8.96	21.40	9.37	7.90	8.17	7.43

Legend: mean value (x), minimum and maximum value (min – max), variation range (R), standard deviation (SD), relative standard deviation (RSD), letters in superscript represent results of Tukey HSD test for significance level $p \le 0,01$** and 0,05*.

leaf (**f**=24,89 mm) were in population B (Table 1). Population D has the largest angle between the first vein and horizontal line (**d**=49,12°), population C has the largest width of leaf 1 cm from the top (**e**=5,67 mm). According to the average number of nerves on the left and on the right side of a leaf (**h**=6,07 and **i**=6,19) population C stands out, while in other populations the average number of nerves from both sides of a leaf is 5.

The parameter **e** has the highest variability (21,4%), unlike the parameter **b** (5,04%) which has the lowest variability. Relative standard deviation of other parameters is in the range of 7–13%. In total the highest variability is shown in population A (11,15%) and the smallest in population D (8,41%).

Statistically significant differences are recorded between test trees within each population, but there were no statistically significant differences between populations for most of measured parameters (Table 2). Parameters **b**, **d**, **h** and **i** are significant for p-value <0,01 while parameter **f** for p-value <0,05.

Furthermore Tukey's HSD test (table 1), that has been performed between populations, has determined which particular population is different in specific parameters, grouping populations with similar characteristics in same homogenous group. According to values of parameters **b**, **d** and **h** Tukey' test group populations into three different homogenous groups, showing the highest differentiation between studied populations, while according to values of parameters **f** and **i** populations were grouped just into two different homogenous groups. The populations A is the one that mostly separates in different homogenous group from other populations.

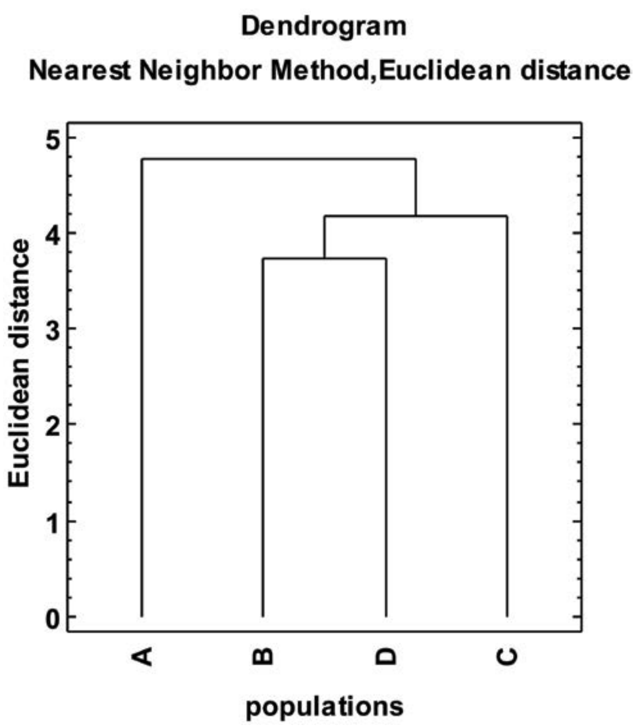

Figure 3. Cluster analysis diagram based on Euclidean distance
Slika 3. Diagram klaster analize baziran na Euklidskim udaljenostima

According to the dendrogram cluster analysis (Figure 3) of analyzed morphometric parameters it is clear that morphometric parameters are most similar between populations B and D. These populations are grouped on the lowest distance, while population C is very close to them. These three populations form a cluster, followed by population A which is on the highest distance.

DISCUSSION
RASPRAVA

Values of leaf parameters in this study are comparable with values given by Fitschen (2002) and Roloff (2006), where the leaf length is 5–12 cm, leaf width 4–10 cm and petiole length 2–6 cm. If we compare the obtained results with the results given by Tucović (1965) who reported leaf length in the range 89,28 – 94,80 mm, width 98,73 – 116,19 mm and petiole length 47,79 – 55,29 mm, it could be seen that leaf length and petiole length of studied populations are in this range, while leaf width is smaller from this range.

Krstinić et al. (1997), Romanić (2000), Kajba et al. (2002), Bruce et al. (2010) did research of black poplar leaf variability along the Drava and Sava River in Croatia, Drava and Mura River in Slovenia, compared to their data, it is evident that the morphometric parameters of the black poplar leaf in its natural habitat in Vojvodina area are much higher. But we must take into consideration that reasons for these differences, beside the obvious genetic and environmental factors, could be that for mentioned researches leaf samples were taken only from short shoot.

Observed characteristics are not equally under the genetic control. In general length and width of leaf are characteristics that are considered to be the most plastic one which are partially under genetic control (Hovanden and Vanden Schoor 2004). Given that the parameters that had a lowest variation (**b**, **a** and **g**) are considered as one under strong environmental influence (van Dam 2002, Krstinić *et al.* 1997), this indicates existence of similar environmental conditions between populations. The one that have highest variations (**e**, **c**, **f** and **d)** are considered to be under strong genetic control and less by the environmental factors (Kajba and Romanić 2002; Romanić 2000; Krstinić et al. 1997), which indicate a possible use of these parameters for estimation of intrapopulation and interpopulation variability, as well as to research the introgression of genes of other poplar species in local population of the European black poplar (Kajba and Romanić 2002).

Table 2. Results of the analysis of variance (ANOVA) for leaf morphological traits within and between studied populations
Tabela 2. Rezultati analize varijance (ANOVA) za morfometrijska svojstva lista unutar i između populacija

Population *Populacija*	Within populations / *Unutar populacija*								Between populations *Između populacija*	
	A		B		C		D			
Leaf parameter	F-ratio	P-Value	F-ratio	P-Value	F-ratio	P-Value	F-ratio	P-Value	F-ratio	P-Value
a	88,63	0,0000	152,11	0,0000	139,27	0,0000	177,24	0,0000	1,43	0,2496
b	14,46	0,0000	54,14	0,0000	107,24	0,0000	87,59	0,0000	8,68	0,0002
c	78,40	0,0000	58,42	0,0000	169,37	0,0000	54,34	0,0000	0,85	0,4738
d	83,78	0,0000	88,20	0,0000	77,54	0,0000	61,03	0,0000	12,98	0,0000
e	112,49	0,0000	97,79	0,0000	110,95	0,0000	54,33	0,0000	0,39	0,7628
f	52,96	0,0000	72,03	0,0000	55,97	0,0000	63,13	0,0000	4,00	0,0148
g	109,16	0,0000	44,05	0,0000	162,60	0,0000	131,18	0,0000	1,56	0,2156
h	49,59	0,0000	84,59	0,0000	18,95	0,0000	69,60	0,0000	8,18	0,0003
i	31,21	0,0000	99,11	0,0000	15,99	0,0000	61,04	0,0000	7,37	0,0006

Higher differentiation within populations than between populations, is consistent to previous results for other woody species (Ballian et al. 2005; Bašić et al., 2007; Ballian et al. 2010; Bruce et al. 2010; Ballian et al. 2014; Poljak et al. 2014), that could be considered as a general rule when it comes to forest tree species. This pattern of variability indicates the existence of gene flow between the populations so that each population has similar combination of genotypes (Poljak et al. 2014).

In general morphological research should be carried out successfully several years, in different conditions, in order to extract parameters that are highly environmentally influenced from those that are under genetic control. To clearly understand the relationship between and within populations, and to understand the variability within and between populations it would be necessary to do further research of numerous factors that affect the variability of the species, such as genetic, climatic, phytocoenological, pedological and other research which were not the subject of this paper.

CONCLUSIONS
ZAKLJUČCI

Analysis of black poplar morphometric leaf parameters was carried out to determine the morphological variability of leaf parameters on interpopulation and intrapopulation levels. The results indicate that there is considerable variability at both mentioned levels of research. Interpopulation and intrapopulation variability of leaf morphometric parameters is consequence of both genetic and environmental factors. Higher variability was perceived within populations than between populations and with relations to equal environmental conditions of researched populations, it could be concluded that the variability is not induced by environmental factors (climate and soil), but by specific genotype of studied populations.

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Sažetak

Crna topola (*Populus nigra* L.) predstavlja jednu od bitnih pionirskih drvenastih vrsta (Pospiškova et al. 2004) koja je prilagođena specifičnim uvijetima poplavnog područja. Budući da unatrag nekoliko desetljeća čovek intenzivno kontroliše plavna područja, utvrđeno je da prirodna staništa autohtonih ritskih vrsta polako nestaju. Uzevši u obzir nestanak ovih ekosustava, prekomjernu eksploataciju autohtonih topola, introdukciju superiornih hibrida topola i mogućnost introgresija gena kultiviranih topola, crna topola se smatra ugroženom vrstom. Kako bi se mogle primijeniti odgovarajuće metode konzervacije, potrebno je utvrditi postojanje varijabilnosti unutar preostalih prirodnih populacija (Flush et al. 2002). Varijabilnost postojećih prirodnih populacija crne topole na području Vojvodine je u ovom istraživanju ispitana pomoću niza morfoloških svojstava lista.

Istraživanja unutarpopulacijske i međupopulacijske morfološke varijabilnosti listova crne topole (*Populus nigra* L.) rađeno je na razini četiri prirodne populacije koje se nalaze u dolinama najvećih rijeka Vojvodine (Dunav, Tisa, Sava – Slika 1). Skupljanje uzoraka obavljeno je metodom slučajnog odabira u tijeku vegetacijskog perioda kada su listovi potpuno razvijeni. Prikupljeni su listovi srednjeg djela grančice dugorasta koje Tucović (1965) ističe kao najvažnije za karakteriziranje pojedinih sistematskih kategorija. Na herbariziranom materijalu analizirano je devet morfometrijskih svojstava (slika 2).

Standardna deskriptivna statistika (prosječna vrijednost, min/max vrijednost, raspon varijacije, standardna devijacija, relativna standardna devijacija), analiza varijance (one way ANOVA), post hoc Tukey HSD test i klaster analiza (metoda najbližeg susjeda, Euklidska udaljenost) su provedeni kako bi se utvrdile razlike na unutarpopulacijskom i međupopulacijskoj razini.

Rezultati analize varijance (tabela 2.) ukazuju na postojanje statistički značajnih razlika između individua u okviru populacija za sva ispitivana morfometrijska svojstva ($p<0{,}000$). Dok su rezultati analize varijance provedeni radi utvrđivanja značajnosti razlika između populacija pokazali da za svojstva **b**, **d**, **f**, **h** i **i** postoji statistički značajna razlika između populacija. Tukey testom i klaster analizom utvrđeno je da se populacija A najviše ističe, potom slijedi populacija C, dok su populacije B i D najsličnije. Rezultati analiza pokazuju izraženu varijabilnost kada su u pitanju parametri **c**, **e**, **f** i **d** za koje se smatra da su pod izrazitom genetskom kontrolom, dok parametri **b**, **a** i **g** koji se odlikuju velikom plastičnošću pokazuju manju varijabilnost, što ukazuje na slične stanišne uvjete istraživanih populacija.

Dobijene statističke analize ukazale su da na unutarpopulacijskoj i međupopulacijskoj razini postoji značajna varijabilnost, pri čemu je varijabilnost unutar populacija dosta izraženija od varijabilnosti između populacija. Imajući u vidu da su stanišni uvijeti istraživanih populacija ujednačeni i na osnovi utvrđenih statističkih značajnosti može se zaključiti da su njihove razlike zanemarive, možemo zaključiti da je unutarpopulacijska različitost uzrokovana izrazitom heterogenošću analiziranih genotipova ovih populacija.

KLJUČNE RIJEČI: Crna topola, Vojvodina, morfologija lista, unutarpopulacijska i međupopulacijska varijabilnost