

MORFOMETRIJSKA I MORFOLOŠKA ANALIZA LISTA CRNE TOPOLE (*Populus nigra* L.) U PLAVNIM I NEPLAVNIM PODRUČJIMA SLIVA DUNAVA

MORPHOMETRIC AND MORPHOLOGICAL ANALYSIS OF *Populus nigra* L. LEAVES IN FLOODED REGIONS

Danijela MILJKOVIĆ¹, Dijana ČORTAN²

SUMMARY

Human activity induced global changes in nature, particularly the flooding regime, which is a stress factor affecting wetland ecosystems. Knowledge of plant strategies of wetland vegetation across a range of flooding gradients is therefore very important. Natural flooding events are increasing as a consequence of constant climate changes. This research was focused on the area of Special Nature Reserve “Gornje Podunavlje” which represents a complex of peculiar marshland, originating from former vast inundated parts of the Danube basin. We have selected samples located on both sides of the embankment in the defended and in the flooded area. The main aim is the assessment of *Populus nigra* L. riparian tree leaf morphological traits variability (centroid size, shape, developmental instability, specific leaf area and petiole length) between two habitats (flooded and not flooded). The geometric morphometry methods were applied to provide visual representation of differences in the leaf shape. We employed leaf composite index indices of fluctuating asymmetry as a measure of developmental instability. A statistically significant impact of flooding was obtained only for the leaf geometric size and shape, so these traits could be an indicator of flooding as suboptimal environmental conditions.

KEY WORDS: riparian tree species, *Populus nigra* L., leaf developmental instability, leaf shape, flooding tolerant, Danube basin.

INTRODUCTION UVOD

The management of river flows has altered flooding patterns and reduced their frequency and duration in many European floodplains (Hughes et al., 2000). Riparian tree species are heavily dependent on the floods, both for providing new sites for their natural regeneration from seed and as well as for recharging water table levels in the rooting zone. These species may tolerate weeks and even months of flooding,

however *Populus nigra* L. cannot withstand flooding longer than 60 days (Herpka, 1963). A decrease in the number of floods has led to loss of the natural corridor that facilitates gene flow for many riparian species (Storme et al., 2004), with early successional riparian tree species being particularly adversely affected (Hughes et al., 2000). River damming and water diversion, by reducing the opportunities for regeneration of these pioneer species, contributed to the collapse of riparian pioneer populations along many river valleys, which threatens the stability of this vulnerable

¹ Dr. Danijela Miljković, Senior Research Associate, Institute for Biological Research “Siniša Stanković” - National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blvd. 142, Belgrade, Serbia, e-mail: danijela.miljkovic@ibiss.bg.ac.rs

² Dr. Dijana Čortan, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, CZ 165 21 Prague, Czech Republic, e-mail: cortan@fzp.czu.cz

ecosystem. Global warming induced by climate change increases risks of floods in Central and Western Europe (Alfieri et al., 2018). The biodiversity of flooding tolerant plants along river systems is very important in riparian ecosystems. The management of water resources and climate changes may have severe effects on species distributions and interactions. It also alters natural flow regimes and flooding patterns in the river system, reducing frequency, extent and duration of many European floodplains (Tonkin et al., 2018). The ecosystems of the Danube River Basin (Europe's second largest river basin) are under pressure due to pollution from agriculture, industry and cities (<https://www.icpdr.org/main/danube-basin/river-basin>).

In the process of evolution, the leaves as plants organs are sensitive to environmental changes and may exhibit phenotypic plasticity in their physiology, morphology and development as response to abiotic and biotic stress, which has been revealed in many studies (Xu et al., 2009; Miljković et al., 2018; Miljković et al., 2019). The fluctuating asymmetry of bilaterally symmetrical organs, as one of the aspects of morphological variability, is used as an assessment indicator for developmental instability in natural suboptimal environmental conditions. Random deviation from phenotypic symmetry is an indicator of the development instability, depending on the type of stressor and the length of its effect (Hagen et al., 2008; Miljković et al., 2018; Miljković et al., 2019). The flooding dramatically reduced leaf size and shape (Zhuang et al., 2011; Rood et al., 2003) and also it impacted the developmental stability process in the seasonal or perennial flooding areas. In the last decade the geometric morphometric analysis were employed instead of traditional morphometric analyses in numerous studies (Klingenberg, 2016). The geometric morphometry as a type of quantitative morphometric analysis allows statistical and visual defining of differences in the analyzed leaf shapes in complex of environmental surroundings (Rohlf and Corti 2000; Bookstein et al., 2003).

Vojvodina is a province in northern Serbia and it is well known for numerous protected wetlands such as swamps and marshes (Basarin et al., 2014). This research focused on the area of Special Nature Reserve "Gornje Podunavlje" which represents complex of peculiar marshland, originating from former vast inundated parts of the Danube basin. It covers an alluvial plain area of 19.648 ha on the left bank of the upper Danube Basin in Vojvodina, from the 1367th to 1433rd km of its course. The research area is located in the middle part of the Danube basin, which extends from the Gate of Devin (Bratislava) to the Iron Gate (between Serbia and Romania). This reserve covers aquatic and semi aquatic vegetation, wet meadows and riparian forests, without significant exposure at 82 - 87 m a.s.l. Forest vegetation is primarily dependent on floodwater or groundwater impacts related to the Danube water level (Bobinac et al., 2010).

As a result of work on the embankment and drainage systems, dating back to 1890's (Bobinac et al., 2010), the hydrological regime has changed considerably, considering the fact that a large quantity of water remained in a narrow area next to the riverbed and stagnated for a long time. This directed changes in the distribution and composition of riparian ecosystem, where fragmentation processes were dominated by ash, oak and elm forests, and willow and poplar forests started to dominate, while the situation was opposite on other side of embankment.

European black poplar (*Populus nigra* L.), an ecologically and economically significant species, is a key pioneer tree species colonizing alluvial sediments along large rivers in temperate climate zones of Europe, northern Africa and western Asia (Dickmann and Kuzovkina, 2008; DeWoody et al., 2015; Čortan and Tubić, 2017). The dynamics of *P. nigra* populations and the different phases of colonization are directly related to the dynamics of the rivers (details in Herpka 1986). In the last century, modifications of European river systems imposed significant threats to large riparian ecosystems. As a prominent early successional species on European floodplains, the absence of *P. nigra* populations indicates reduced geomorphological activity following river control (Hughes et al., 2000).

Considering the fact that the morphological traits of *P. nigra* have high phenotypic plasticity which allows adaptation and resistance to the fluctuating and prolonged floods (Čortan et al., 2019), the main aims of this exploratory study address the questions: i) Does flooding effect on leaf morphology? and ii) If it does, which of leaf morphological traits (centroid size, shape, developmental instability, specific leaf area or petiole length) could be considered an indicator of suboptimal environmental conditions for riparian tree *Populus nigra* L.

MATERIAL AND METHODS

MATERIJALI I METODE

2.1. Study locations

For this research we have selected samples located on both sides of embankment (Fig. 1), in defended area (localities A1, B1, C1 and E1) and in the flooded area (localities A2, C2, D2 and E2). Ten adult trees (over 30 years old) from each locality were used for the analysis; in total 80 trees. Ten full developed and undamaged leaves were sampled from each tree in July 2016, from the south/south west side of the crown, at the height between 3-6 m (about 800 leaves were analysed). The water level was measured and analysed at two hydrologic stations (Bezdan and Apatin) in the period of 2013 to 2016, covering all selected localities. The highest water level was recorded in 2013, and the lowest in 2015. The maximum water level in this period is recorded in May and June (Fig. 2).

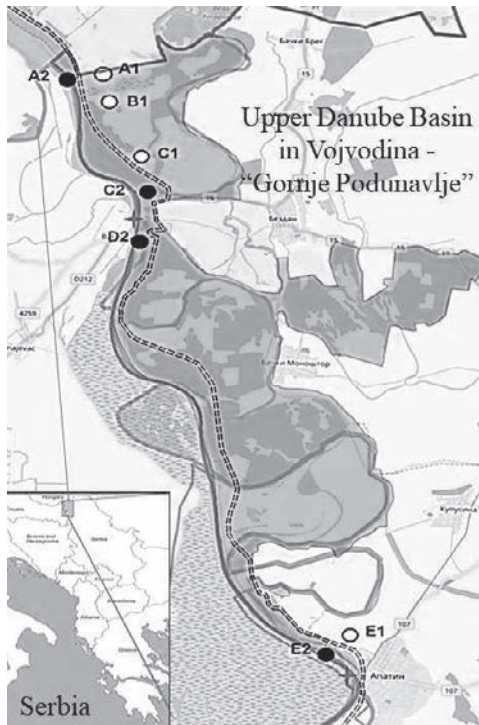


Figure 1. Selected localities in upper Danube Basin in Serbia – “Gornje podunavlje” - defended area not flooded (localities A1, B1, C1 and E1 – empty circles) and flooded area (localities A2, C2, D2 and E2 – fill circles) (modified by B.Tubić 2017).

Slika 1. Odabrani lokaliteti u slivu Gornjeg Podunavlja u Srbiji - “Gornje podunavlje” - zaštićeno područje koje u zoni plavljenja (lokaliteti A1, B1, C1 i E1 - prazni krugovi) i poplavno područje (lokaliteti A2, C2, D2 i E2 - punjenja krugovi) (modificirano od B.Tubić 2017).

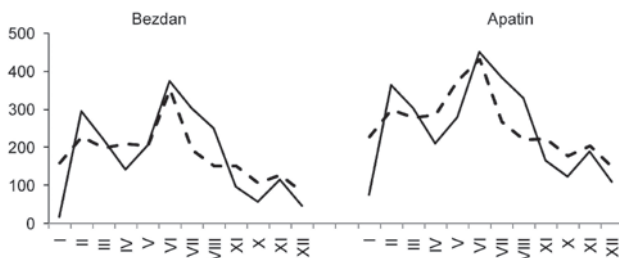


Figure 2. Mean water level (m) per month measured in two hydrological stations, Bezdan and Apatin, for 2016 (full lines) and mean values for period of 2013-2016. (dashed lines).

Slika 2. Srednja razina vode (m) mjesečno izmjerena u dvije hidrološke postaje, Bezdan i Apatin, za 2016. godinu (pune linije) i srednje vrijednosti za razdoblje 2013-2016. (Isprekidane linije).

2.2. Morphometric analysis and leaf traits analysis

The collected leaves were scanned (CANON LIDE 110) in 200 ppi resolution (Fig. 3). The digital images were used in the IMP (Integrated morphometrics package) software package (Sheets et al., 2004). In the MakeFan6 program a set of median landmarks (LM) (1 and 2) was labeled, according to which „comb“ with eight lines were constructed (Fig. 3, dashed thin lines across the leaf). According to the „comb“ with parallel axis a uniform series of semilandmarks pairs

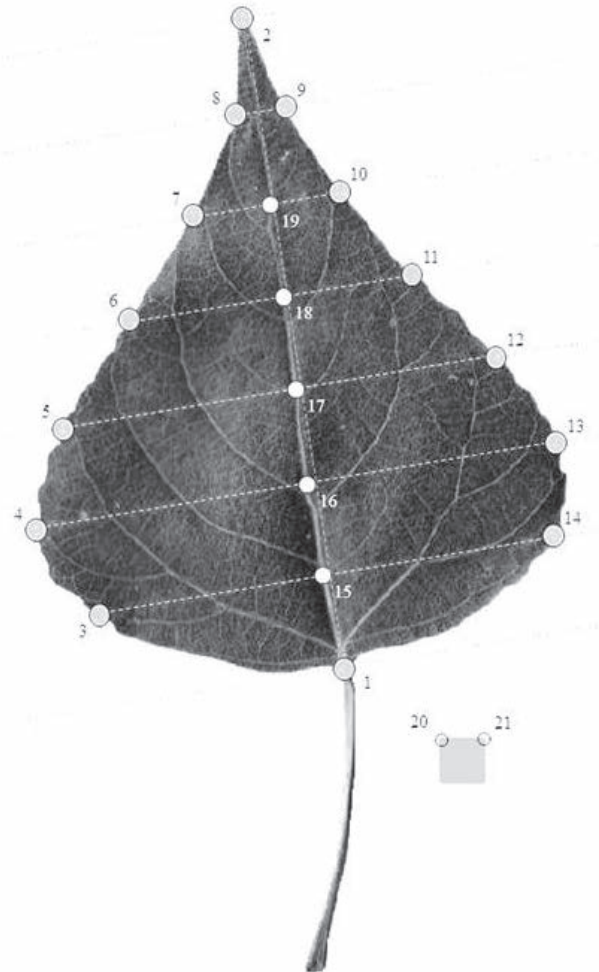


Figure 3. The image of European black poplar leaf (*Populus nigra* L.) with eight parallel line of comb fan two landmarks (1 and 2) and twelve semi-landmarks (3-8 and 9-14) which define the Curvature and shape of the leaf. The 21 and 22 marks used for calibration. The five marks along the leaf nerve, on the place of intersection with the parallel axes, (15-19 white dots) used for the distance from homologs marks on leaf curvature for differences between leaf and right side of leaf and calculate composite index of fluctuating asymmetry FA_{LEAF} .

Slika 3. Slika lista europske crne topole (*Populus nigra* L.) osam linija osa “ceslja” sa dva orijentira (1 i 2), dvanaest polu-oznaka (3-8 i 9-14) koje definiraju zakrivljenost i oblik list. Za kalibraciju se koriste oznake 21 i 22. Pet oznaka duž glavne ose lista, na mjestu raskrižja s paralelnim osima, (15-19 bijelih točkica) koje se koriste za udaljenost od oznaka homologa na zakrivljenosti lista za razlike između listova i desne strane lista i izračunavaju kompozitni indeks fluktuacije asimetrija FA_{LEAF} .

(SLM) (3-8 left leaf curvature, 9-14 right leaf curvature and 15 to 19 (across leaf midvein) for measuring the distance of homologous points of the leaf margin and the midvein were used. The marks 21 and 22 in gray square were used as the scale factor (mm/pixel) for every digital images (Fig. 3).

Software for the two-dimensional geometric morphometric analyses (tpsDig2) was used to mark the points where axes cross the lamina edge and midvein. The Tmorphgen6 procedure was used to obtain leaf lamina dimensions, the distance between marks on left and right leaf curvature and across the leaf midvein (left lamina side 3-8, right lamina side 9-14), and petiole length. The obtained data for lamina

distance between the midvein and the left and right curvatures were used for leaf composite index calculation:

$$FA_{LEAF} = \sum(\ln R_i - \ln L_i) / n \quad (1)$$

with i = as a distance between the midvein and the left and right curvatures for every axis, $n = 6$. The leaf composite index FA_{LEAF} presents the size-scale indicator of developmental instability of a whole leaf.

The observed Procrustes coordinates (MorphoJ software package, procedure Covariance Matrix Generate and Principal Components Analysis application) enabled analysis of leaf shape differences between the analyzed localities. The graphically visualized leaf shape variation were present with wireframe diagrams. The leaf centroid size (CS) were obtained too (Klingenberg, 2011).

We employed a Procrustes variance analyses (Klingenberg, 2016) to estimate statistically significant differences between habitats (fixed effect), trees (nested in habitat; random effect) and leaves (nested in habitat and trees; random effect) as source variations for centroid size and shape as a dependent variable (Palmer, 1994; Klingenberg and McIntyre, 1998; Klingenberg, 2003; Savriama and Klingenberg, 2011). The graphical patterns of leaf shape variation were constructed employing the Principal Component Analysis (PCA). The Canonical Analysis Variant (CVA) was used to create a visual display of shape differences between populations. The digital images were used in Image tools program to determine the surface area of each leaf.

Each leaf was dried at 70°C degrees 48 h, then the weight of the dry leaf mass was measured (balance Chyo JL-200).

The obtained surface area and mass leaf values were used to assess the specific leaf area (SLA) the ratio of the leaf area to the dry mass (cm²/mg).

2.3. Statistical analysis

The differences among habitats in centroid size, leaf shape, developmental stability (FA_{LEAF}), SLA and petiole length were tested with ANOVA, in SAS statistical package (SAS Institute, Inc. 2011). The habitat used in the model was set as a fixed factor (evaluation of environmental variability), while trees nested within habitat (evaluation of intra-population variability) and leaves nested in trees and populations (evaluation of intra-individual variability) were set as random factors.

RESULTS REZULTATI

The obtained results of the applied Procrustes ANOVA model showed that the geometric size of the leaf (centroid size) and shape of leaf differ between habitats (a significant impact of the habitat) ($P < 0.05$) (Table 1).

The results obtained for morphological traits (SLA and petiole length) did not differ statistically significantly between habitats (all $P > 0.05$). Intraindividual variability of the values was statistically significant among the individual trees ($P < 0.05$), which indicates an existence of genetic variability for the analysed traits (Table 2). The composite index of fluctuating asymmetry FA_{LEAF} was not statistically significant among habitats, indicating that the conditions in the flooding compared to the non-flooding habitat did not lead

Table 1. Results of Procrustes ANOVA computed for centroid size (A) and shape (B) for whole sample.

Tablica 1. Rezultati Procrustes ANOVA izračunati za centroid veličine (A) i oblik (B) za cijeli uzorak.

Source of variation	df	Centroid size		df	Shape	
		MS ($\times 10^4$)	F		MS ($\times 10^4$)	F
Habitat	7	17.51	90.60****	84	75.66	15.25****
Tree	16	0.33	1.71*	192	29.45	5.94****
Leaves	216	0.19	0.39	2592	4.96	4.57****
Error rep	240	0.49		5760	0.05	

Table 2. Results of three-way ANOVAs exploring the effects of habitat, populations and tree for specific leaf area (SLA), petiole length and composite index of fluctuating asymmetry (FA_{LEAF}) in leaves of *Populus nigra* plants from two habitats (flooding/ nonflooding).

Tablica 2. Rezultati three-way ANOVA analize utjecaja staništa, populacija i stabla na specifičnu površinu lista (SLA), dužine peteljčica i kompozitnog indeksa fluktuirajuće asimetrije (FA_{LEAF}) u listovima biljaka *Populus nigra* iz dva staništa (plavna / neplavna).

Source of variation	df	SLA (cm ² /mg)		Petiole length (mm)		FA_{LEAF}	
		MS	F	MS	F	MS (10^{-3})	F
Habitat (H)	1	93.16	1.61	0.20	0.15	0.52	2.25
Population (P)	6	40.78	0.71	7.23	12.49****	0.67	2.88**
Tree (H P)	72	57.75	15.02****	3.83	6.41****	0.23	1.17
Error	0	3.85		0.36		0.20	

P < 0.01; **P < 0.0001

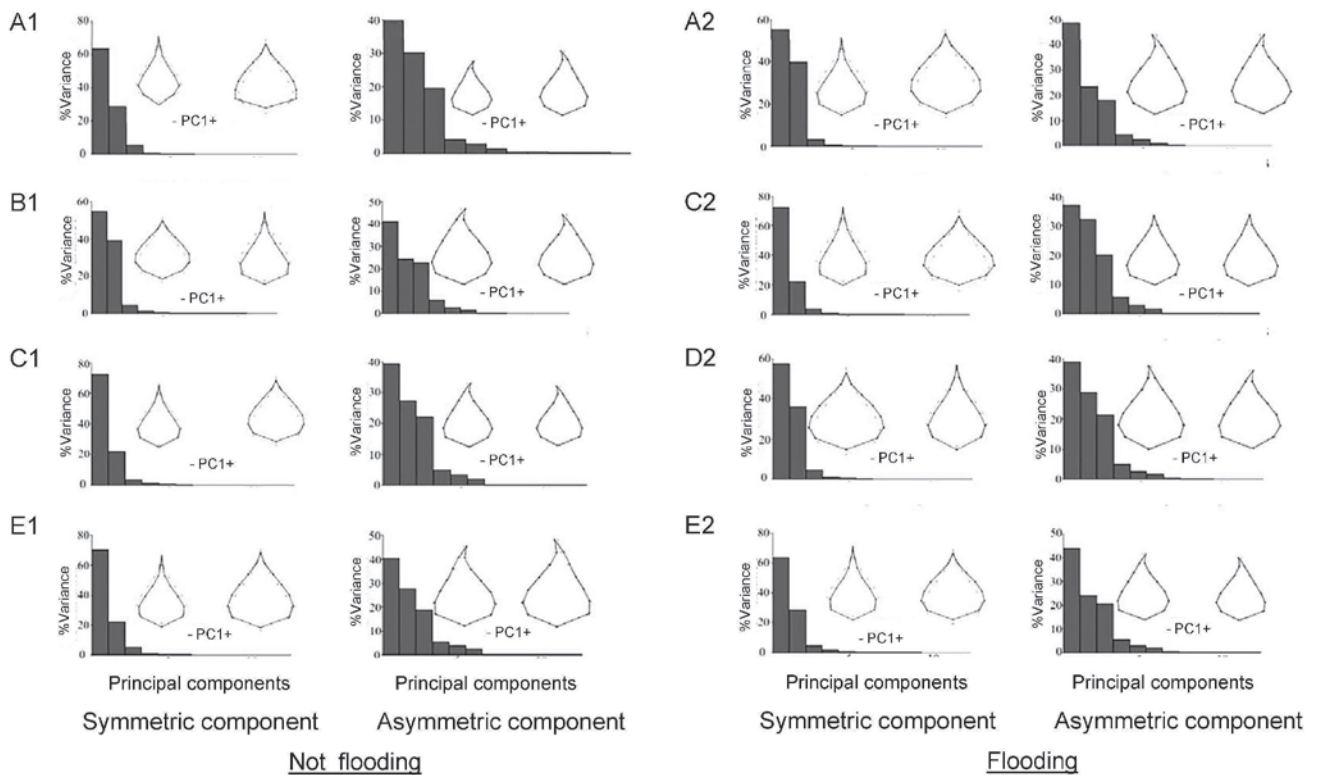


Figure 4. Patterns of shape variation (warped outline graphs) visualized through a principal component (PC) as the symmetric and asymmetric components for PC1 negative and positive scores, with portion of shape variation from principal component analysis, for every of population separately.

Slika 4. Obrazac varijacija oblika (iskrivljeni grafički prikazi) vizualizirani kroz glavnu komponentu (PC) kao simetrične i asimetrične komponente za PC1 negativne i pozitivne rezultate, s dijelom varijacije oblika od analize glavnih komponenti, za svaku populaciju zasebno.

to development instability, although the main value was slightly higher in the flooded habitat (Table 2; Fig.5).

The variation among individual leaves (symmetric components) showed a higher value (between 60 and 70%) relative to the variation of the position of the landmarks on both sides of a leaf (asymmetric component) (about 40%) for both habitats (Fig. 4).

In the visual presentation (scatterplots) of discriminant analysis of leaf shape derived from Canonical Variance Analyzes (CV), the first canonical variates (CV1) indicated 39.67% variance while the second (CV2) showed 23.47% variance (Fig. 5).

DISCUSSION RASPRAVA

Plant ecology and evolutionary biology studies focus on exploring the rules of plants organs development, growth and morphology diversity outputs, with temporal and spatial organization, which are under control of genetic structures closely correlated with habitat ecological characteristics (Lin et al., 2016, DeWoody et al., 2015).

In this study we used the plants present in a given study areas (flooded and non-flooded) as a passive model of bi-omonitoring (Nali and Lorenzini, 2007). Anthropogenic

activities induced fast climate changes. Flooding is a common natural disasters which increases impacts on ecosystem as a whole (Herzog and Pedersen, 2014). The tree survival, growth change in flooding areas, and the degree of damage depends on the ontogenetic stage of the tree characteristics (active growth or dormant), tolerance and the flood itself (time of the year and duration). Flood-stressed trees exhibit a range of symptoms including chlorosis, defoliation, reduced leaf size and shoot growth (Baughman, 2010).

The significant differences between habitats (flooding and not flooding) were observed in terms of the leaf centroid size (geometric size) and shape (Table 1). The changes in leaf size and especially in the shape are considered to have functional significance related to different natural conditions (Zhuang et al., 2011). Rood et al. (2003) stated that flooding dramatically reduced leaf size and slightly decreased some foliar gas exchange characteristic in poplar due to oxygen deficiency in the roots. The size, shape and biomechanical properties of its root and its specific aerial structures are well adapted to cope with these stressed inducing conditions. However, the poplar species are known its tolerance of fluctuating hydrological conditions, but *P. nigra* cannot stand flooding which last longer than 60 days (Herpka, 1963). The physiology, morphology and biomechanics of

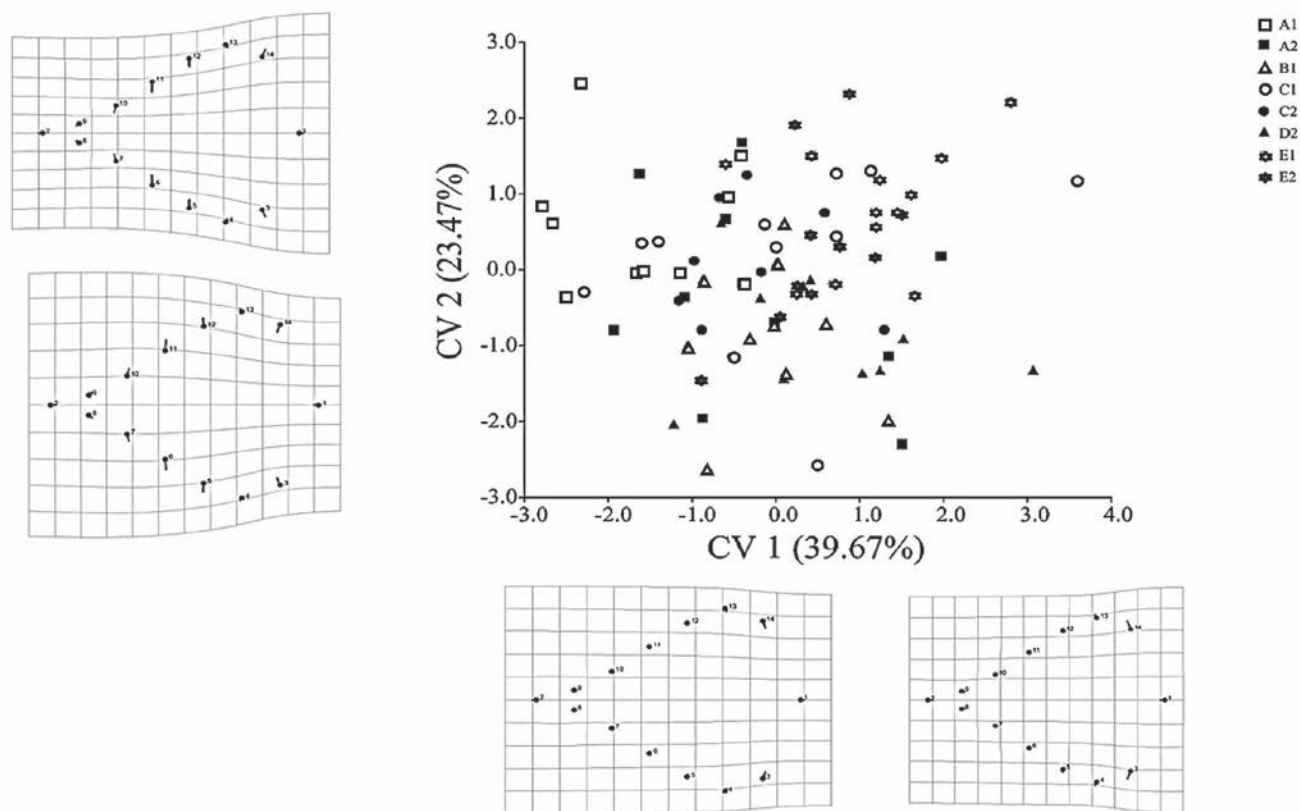


Figure 5. Discriminant analyses of leaf shape (means per tree) derived from Canonical Variance Analyses (CVA). Scatterplot of the first two canonical variate (CV1 and CV2), percentages indicate the amount of variance explained by the axis (39.67% vs. 23.67%; respectively).

Slika 5. Diskriminantne analize oblika lista (srednje vrijednosti po stablu) izvedene iz kanonične analize varijance (CVA). Dijagram prve dvije kanonične ose varijacije (CV1 i CV2), postoci ukazuju na veličinu varijance za svaku od osa (39,67% nasuprot 23,67%, respektivno).

P. nigra are adapted to resist hydraulic forces and prolonged submersions (Šiler et al., 2015).

The geometric morphometric leaf shape analyses provide valuable information about variance components obtained through the asymmetric component (such as random deviations from the bilateral asymmetry in the heterogeneous environment) and heritability of the leaf shape (symmetric component) (Albarran-Lara et al., 2010; Viscosi and Fortini, 2011; Miljković et al., 2019). According to the results for both habitats the percentage of shape variability was higher for the symmetric component, which implies that the genetic factor has more contribution in the variability of the leaf shape. The large number of gene activities and auxin have determined leaf shape in environmental conditions in which leaf mature (Drost et al., 2015).

In this study intraindividual variation was observed for the shape (statistically significant effect of leaves, Table 1). However, in other study the clonal variability (genetical variability) of petiole length and FA_{LEAF} index were observed, while for SLA there was no variability (Table 2) (Huber et al., 2008; Al Afas et al., 2005; Al Afas et al., 2007; Čortan and Tubić, 2017).

The observed results within the present research confirmed no significant differentiation between different habitats (flo-

oding/not flooding) (Table 2) for specific leaf area, petiole length and FA_{LEAF} (Table 2). Even though population „E“ is differentiated the most, with highest values, there are no significant differences between populations in terms of SLA and petiole length, while mild significant differences were observed for the composite index FA_{LEAF} (0.031 vs. 0.029; flooding vs. nonflooded) (Table 2). It is obvious that analyzed leaf traits showed great tolerance to flooding compared to the not flooding areas within the research area. In other studies the specific leaf area (SLA) decreased less by flooding than leaf area (Bacanamwo and Purcell, 1999; Dias-Filho and Carvalho, 2000; Herrera et al., 2009), which indicates that decreased leaf expansion was not associated with carbohydrate accumulation in leaves (Bacanamwo and Purcell, 1999). The dry leaf mass was lower in the flooding area but not significantly statistically different than in the non-flooding area (2.4 g vs. 2.6 g; respectively). The flooding causes significant decrease in leaf dry mass production (Dias-Filho, 2002; Caetano and Dias-Filho, 2008; de Oliveira and Joly 2010), and dramatically reduce leaf size and shape (Zhuang et al., 2011; Rood et al., 2003). The difference between and within species with regards to tolerance and response to flooding regime were confirmed. As flooded and drained conditions exerted different selection pressures on trait expression, the optimal values for constructive and

plastic traits depended on the frequency and duration of flooding (Huber et al., 2008).

On contrary, in more dry and warm conditions leaves had smaller blades and smaller petiole size and hairiness. The significant differences in leaf blade and petiole size were observed between the typical and hairy type of European black poplar which were in accordance with climate difference in respective habitats of continental riparian forests and Submediterranean type of climate (Kajba et al., 2004, 2015; Ballian, 2017).

We examine the variability of a specific leaf area, petiole length, and developmental stability (composite leaf index of fluctuating asymmetry FA_{LEAF}) with the aim of assessing which of them could be considered the key element of suboptimal environmental conditions for riparian tree *Populus nigra* L.. The statistically significant impact of flooding was obtained only for leaf geometric size and shape, so these traits could be recognized as indicators of flooding suboptimal environment. For all analysed leaf traits genetic variability were confirmed with statistical significant effect of tree variation (all $P > 0.05$), except for the developmental instability (FA_{LEAF}) (Table 1, Table 2). According to the study by Herrera et al. (2009) trees photosynthetic acclimation to flooding in the Mapire River could not be explained by leaf anatomy traits.

Plants have adaptive survival strategies in the period of flooding which depend of the seasonal or perennial flooding in certain areas. For example, plants reduce leaf expansion and new leaf production because the root systems are losing the capacity to absorb water and nutrients in an oxygen-poor environment (de Oliveira and Joly, 2010). The leaf shape and structure are defined mainly during a brief period of primary morphogenesis based on the possible role of the reaction-diffusion system and can be altered by the allometric expansion (Xu et al., 2009). A large variation in the values of the leaf traits exists within one individual species (Xu et al., 2009). Morphological adaptation can improve tissue aeration during flooding. However, flooding negatively affects the ultrastructure of leaves, especially photosynthetic organs, decreasing photosynthetic capacity (Du et al., 2010) and stomatal conductance (Rood et al., 2010). Negative effects on plant growth and total biomass accumulation in many species with different tolerance to flooding were also reported (Du et al., 2008; Rood et al., 2010).

The human activity-induced global changes in environment, especially changes in the flooding regime, represent the most important stress factor potentially affecting wetland ecosystems. The flooding regulation systems significantly altered the natural regeneration capacity of black poplar, and favoured the succession of poplar stands by hardwood forests (Ballian, 2017). The knowledge of plant

strategies of wetland vegetation across a range of flooding and not flooding gradients is therefore very important (Lou et al., 2016), and it is the base for future large-scale studies. The study showed the results of development instability and genotypic variability of the *P. nigra* leaf morphological traits in natural populations which depend on the changes in the habitat conditions, primarily from the water regime, which have been altered significantly in the last century. The results have confirmed that *P. nigra* is highly tolerant of long floodings as well of the changing water regime, so it could be used in a restoration programme aimed at the recovery of areas that are naturally subject to longer periods of flooding. In this way, we obtained the base for the conservation and the use of the available gene pool of these species, as well as the guidelines for breeding programs in riparian ecosystems. Preservation of genetic resources, or genetic variability of natural populations, is the basis for improvement of the existing state of black poplar forests, as well as precondition for further improvement of vitality and conservation of biodiversity. The assessment of endangered species may also give guidelines for a certain degree of protection of the investigated threatened areas.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia Grants No. 173025 title “Evolution in heterogeneous environments: mechanisms of adaptation, biomonitoring and conservation of biodiversity”. Many thanks to Jelena Mladjenović (English Professor) for manuscript language revision and Dr Bojan Tubić for producing map.

AUTHORS' CONTRIBUTIONS

DM conceived the idea and designed the study, performed morphometric and statistical analyses. DČ conducted fieldwork and performed laboratory measures. DM and DČ wrote and prepare the final version of the manuscript.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest in relation to this article.

REFERENCES

- Al Afas, N., Pellis, A., Niinemets, Ü., Ceulemans, R., 2005. Growth and production of a short rotation coppice culture of poplar. II. Clonal and year-to-year differences in leaf and petiole characteristics and stand leaf area index. *Biomass and Bioenergy*. 28(6), 536–547.
- Al Afas, N.A., Marron, N., Ceulemans, R., 2007. Variability in *Populus* leaf anatomy and morphology in relation to canopy

- position, biomass production, and varietal taxon. *Ann. Forest Sci.* 64(5), 521–532.
- Albarrán-Lara, A.L., Mendoza-Cuenca, L., Valencia-Avalos, S., González-Rodríguez, A., Oyama, K. 2010. Leaf fluctuating asymmetry increases with hybridization and introgression between *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae) through an altitudinal gradient in Mexico. *Int. J. Plant Sci.* 171(3), 310–322.
 - Alfieri, L., Dottori, F., Betts, R., Salamon, P., Feyen, L., 2018. Multi-model projections of river flood risk in Europe under global warming. *Climate* 6(1), 6.
 - Bacanamwo, M., Purcell, L.C., 1999. Soybean root morphological and anatomical traits associated with acclimation to flooding. *Crop Sci.* 39(1), 143–149.
 - Ballian D. 2017. Varijabilnost crne topole (*Populus nigra* L.) i neno očuvanje u Bosni i Hercegovini. Znanstvena monografija, Šumarski fakultet u Sarajevu - Silva Slovenica, 1- 210.
 - Basarin, B., Kržič, A., Lazić, L., Lukić, T., Đorđević, J., Janičijević Petrović, B., Čopić, S., Matić, D., Hrnjak, I., Matzarakis, A., 2014. Evaluation of bioclimate conditions in two special nature reserves in Vojvodina (northern Serbia). *Carpath. J. Earth Env.* 9(4), 93–108.
 - Baughman, M., 2010. Flooding effects on trees. Information part of the Trees and Woodlands program of the University of Minnesota. Information collected on a website: <http://www.extension.umn.edu/environment/trees-woodlands/flooding-effects-on-trees>.
 - Bobinac, M., Andrašev, S., Šijačić-Nikolić, M., 2010. Elements of growth and structure of narrow-leaved ash (*Fraxinus angustifolia* Vahl) annual seedlings in the nursery on fluvisol. *Period. Biol.* 112(3), 341–351.
 - Bookstein, F.L., Gunz, P., Mitteroecker, P., Prossinger, H., Schaefer, K., Seidler, H., 2003. Cranial integration in Homo: singular warps analysis of the midsagittal plane in ontogeny and evolution. *J. Hum. Evol.* 44(2), 167–187.
 - Caetano, L.P.D.S., Dias-Filho, M.B., 2008. Responses of six *Brachiaria* spp. accessions to root zone flooding. *Rev. Bras. Zoot.* 37(5), 795–801.
 - Čortan, D., Nonić, M., Šijačić-Nikolić, M. 2019. Phenotypic Plasticity of European Beech from International Provenance Trial in Serbia. In *Forests of Southeast Europe Under a Changing Climate* (pp. 333-351). Springer, Cham.
 - Čortan, D., Tubić, B., 2017. Viability and genetic diversity of *Populus nigra* population from riparian forest in SNR Gornje Podunavlje. *Dendrobiology* 78, 157–167.
 - de Oliveira, V.C., Joly, C.A., 2010. Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses. *Trees* 24(1), 185–193.
 - DeWoody, J., Trewin, H., Taylor, G., 2015. Genetic and morphological differentiation in *Populus nigra* L.: isolation by colonization or isolation by adaptation? *Mol. Ecol.* 24(11), 2641–2655.
 - Dias-Filho, M.B., Carvalho, C.J.R.D., 2000. Physiological and morphological responses of *Brachiaria* spp. to flooding. *Pesqui. Agropec. Bras.* 35(10), 1959–1966.
 - Dias-Filho, M.B., 2002. Tolerance to flooding in five *Brachiaria brizantha* accessions. *Pesqui. Agropec. Bras.* 37(4), 439–447.
 - Dickmann, D.I., Kuzovkina, J., 2008. Poplars and willows in the world. Chapter 2. Poplars and willows of the world, with emphasis on silviculturally important species. International Poplar Commission Thematic Papers. FAO.
 - Drost, D.R., Puranik, S., Novaes, E., Novaes, C.R., Dervinis, C., Gailing, O., Kirst, M., 2015. Genetical genomics of *Populus* leaf shape variation. *BMC Plant Biol.* 15(1), 166.
 - Du, K.B., Shen, B.X., Xu, L., Tu, B.K., 2008. Estimation of genetic variances in flood tolerance of poplar and selection of resistant F1 generations. *Agroforestry Syst.* 74, 243–257.
 - Du, K.B., Xu, L., Tu, B.K., Shen, B.X., 2010. Influences of soil flooding on ultrastructure and photosynthetic capacity of leaves of one-year old seedlings of two poplar clones. *Sci. Silve Sin.* 46, 58–64.
 - Huber, H., Jacobs, E. and Visser, E.J., 2008. Variation in flooding-induced morphological traits in natural populations of white clover (*Trifolium repens*) and their effects on plant performance during soil flooding. *Annals of Botany*, 103(2), pp.377-386.
 - Hagen, S.B., Ims, R.A., Yoccoz, N.G., Sørlibråten, O., 2008. Fluctuating asymmetry as an indicator of elevation stress and distribution limits in mountain birch (*Betula pubescens*). *Plant Ecol.* 195(2), 157–163.
 - Herpka, I., 1963. Postanak i razvoj prirodnih vrba u Podunavlju i donjoj Posavini. *Topola* 36/37, 18–27.
 - Herpka, I., 1986. A survey of development and possibilities of growing: natural forests of poplars and willows. *Poplars and Willows in Yugoslavia*, Poplar Research Institute, Novi Sad, 21–36.
 - Herrera, A., Escala, M., Rengifo, E., 2009. Leaf anatomy changes related to physiological adaptations to flooding in Amazonian tree species. *Braz. J. Plant Physiol.* 21, 301–308.
 - Herzog, M., Pedersen, O., 2014. Partial versus complete submergence: snorkelling aids root aeration in *Rumex palustris* but not in *R. acetosa*. *Plant Cell Environ.* 37(10), 2381–2390.
 - Hughes, F.M., Barsoum, N., Richards, K.S., Winfield, M., Hayes, A., 2000. The response of male and female black poplar (*Populus nigra* L. subspecies *betulifolia* (Pursh) W. Wettst.) cuttings to different water table depths and sediment types: implications for flow management and river corridor biodiversity. *Hydrol. Process.* 14(16-17), 3075–3098.
 - Kajba, D., Ballian, D., Idžojtić, M., Bogdan, S. 2004. The differences among hairy and typical European black poplars and the possible role of the hairy type in relation to climatic changes. *Forest. Ecol. Manag.* 197(1-3), 279-284.
 - Kajba, D., Ballian, D., Idžojtić, M., Poljak, I. 2015. Leaf morphology variation of *Populus nigra* L. in natural populations along the rivers in Croatia and Bosnia and Herzegovina. *SEEFOR*, 6(1), 39-51.
 - Klingenberg, C.P., 2003. A developmental perspective on developmental instability: theory, models and mechanisms. *Developmental instability: causes and consequences*, 14–34. Oxford University Press: New York, NY, USA.
 - Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11, 353–357.
 - Klingenberg, C.P., 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. *Dev. Genes Evol.* 226, 13–137.
 - Klingenberg, C.P., and McIntyre, G.S., 1998. Geometric morphometrics of developmental instability: analyzing patterns of

- fluctuating asymmetry with Procrustes methods. *Evolution* 52(5), 1363–1375.
- Lin, S., Zhang, L., Reddy, G.V.P., Hui, C., Gielis, J., Ding, Y., Shi, P., 2016. A geometrical model for testing bilateral symmetry of bamboo leaf with a simplified Gielis equation. *Ecol. Evol.* 6(19), 6798–6806.
 - Lou, Y., Pan, Y., Gao, C., Jiang, M., Lu, X., Xu, Y.J., 2016. Response of plant height, species richness and aboveground biomass to flooding gradient along vegetation zones in floodplain wetlands, Northeast China. *PLoS one*, 11(4), e0153972.
 - Miljković, D., Selaković, S., Vujić, V., Stanisavljević, N., Radović, S., Cvetković, D., 2018. Patterns of herbivore damage, developmental stability, morphological and biochemical traits in female and male *Mercurialis perennis* in contrasting light habitats. *Alpine Bot.* 28 (2), 193–206.
 - Miljković, D., Stefanović, M., Orlović, S., Stanković Nedić, M., Kesić, L., Stojnić, S., 2019. Wild cherry (*Prunus avium* (L.) L.) leaf shape and size variations in natural populations at different elevations.
 - Nali, C., Lorenzini, G., 2007. Air quality survey carried out by schoolchildren: An innovative tool for urban planning. *Environ Monit. Assess.* 131(1–3), 201–210.
 - Palmer, A.R., 1994. Fluctuating asymmetry analyses: a primer. In *Developmental instability: its origins and evolutionary implications* (pp. 335–364). Springer, Dordrecht.
 - Rohlf, F.J., Corti, M., 2000. The use of two-block partial least-squares to study covariation in shape. *Syst. Biol.* 49, 740–753.
 - Rood, S.B., Braatne, J.H., Hughes, F.M., 2003. Ecophysiology of riparian cottonwoods: stream flow dependency, water relations and restoration. *Tree Physiol.* 23(16), 1113–1124.
 - Rood, S.B., Nielsen, J.L., Shenton, L., Gill, K.M., Letts, M.G., 2010. Effects of flooding on leaf development, transpiration, and photosynthesis in narrow leaf cotton wood, a willow-like poplar. *Photosynth. Res.* 104, 31–39.
 - Savriama, Y., Klingenberg, C.P., 2011. Beyond bilateral symmetry: geometric morphometric methods for any type of symmetry. *BMC Evol. Biol.* 11(1), 280.
 - Sheets, H.D., Zelditch, M., Swiderski, D., 2004. Morphometrics software: IMP-Integrated morphometrics package. <http://www3.canisius.edu/~sheets/morphsoft.html>. 2013-05-16.
 - Šiler, B., Škorić, M., Mišić, D., 2015. General consideration of the European black poplar biology, significance and conservation prospects. In: *Variability of European Black Poplar (Populus nigra L.) in the Danube Basin*, Publisher: Public Enterprise “Vojvodinašume”. Novi Sad.
 - Storme, V., Broeck, A.V., Ivens, B., Halfmaerten, D., Van Slycken, J., Castiglione, S., Lefèvre, F., 2004. Ex-situ conservation of Black poplar in Europe: genetic diversity in nine gene bank collections and their value for nature development. *Theor. Appl. Genet.* 108(6), 969–981.
 - Tonkin, J.D., Merritt, D.M., Olden, J.D., Reynolds, L.V., Lytle, D.A., 2018. Flow regime alteration degrades ecological networks in riparian ecosystems. *Nat. Ecol. Evol.* 2(1), 86–93.
 - Viscosi, V., Cardini, A., 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLoS one*, 6(10), e25630.
 - Xu, F., Guo, W., Xu, W., Wei, Y., Wang, R., 2009. Leaf morphology correlates with water and light availability: what consequences for simple and compound leaves? *Prog. Nat. Sci.* 19(12), 1789–1798.
 - Zhuang, Li., YaNing, C., WeiHong, Li., Zhongke, W., 2011. Anatomical and morphological characteristics of *Populus euphratica* in the lower reaches of Tarim River under extreme drought environment. *J. Arid Land.* 3(4), 261–267.

SAŽETAK

Ljudska aktivnost izazvala je globalne promjene u prirodi, posebice režim poplava, kao faktor stresa koji potencijalno utječe na močvarne ekosustave. Stoga je vrlo važno poznavanje strategija biljnog razvoja močvarne vegetacije na nizu poplavnih gradijenta. Učestale poplave su posljedica stalnih klimatskih promjena. Ovo istraživanje bilo je usmjereno na područje Specijalnog rezervata prirode “Gornje Podunavlje” koje predstavlja kompleks osebujnog močvarnog područja, koje potječe iz nekadašnjih prostranih poplavnih dijelova dunavskog bazena. Odabrali smo uzorke koji se nalaze s obje strane nasipa u branjenom području i u poplavljenom području. Glavni je cilj procena varijabilnosti morfologije lista *Populus nigra* L. (priobalna stabla) između dva staništa (plavljeno i ne plavljeno). Metode geometrijske morfometrije primijenjene su kako bi se vizualno razlikovale oblike lista, dok smo kao mjeru razvojne nestabilnosti koristili kombinirane indeks listove fluktuirajuće asimetrije. Statistički značajan utjecaj poplava dobiven je samo za geometrijsku veličinu i oblik lista, te su ta svojstva prepoznata kao pokazatelji suboptimalnog sredinskog okruženja. Stoga je vrlo važno poznavanje strategija biljnog razvoja močvarne vegetacije na nizu poplavnih gradijenta.

KLJUČNE RIJEČI: obalne vrste drveća, *Populus nigra* L., nestabilnost razvoja lista, oblik lista, tolerantna na poplave, Podunavlja.