BOSQUE 38(2): 337-345, 2017 DOI: 10.4067/S0717-92002017000200011

Leaf stomatal traits variation within and among black poplar native populations in Serbia

Variación de las características de los estomas de las hojas dentro y entre álamos negros de poblaciones autóctonas en Serbia

Dijana Čortan [a]*, Dragica Vilotić [b], Mirjana Šijačić-Nikolić [b], Danijela Miljković [c]

*Corresponding author: [a] University of Novi Sad, Faculty of Education, Department of Natural Science and management in education, Podgorička 4, Sombor, Serbia, dijanacortan@yahoo.com, dijana.cortan@per.uns.ac.rs

[b] University of Belgrade, Faculty of Forestry, Department of seedling, nursery and reforestation, Kneza Višeslava 1, Belgrade, Serbia.

[c] University of Belgrade, Institute for Biological Research "Siniša Stanković", Department of Evolutionary Biology, Bulevar Despota Stefana 142, Belgrade, Serbia.

SUMMARY

Populus nigra as a keystone riparian pioneer tree species is one of the rarest and most endangered species in Europe due to the loss of its natural habitats. Genetic diversity existence is a key factor in survival of one species, and stomata as genetically controlled trait could be used for differentiation studies. With the aim of proving stomatal phenotypic variation of the four native populations of *Populus nigra* located on the banks of three biggest river valleys (Dunabe, Tisa and Sava) in the region of Vojvodina in northern Serbia, we examined various leaf stomatal traits (stomatal length and width, pore length and width, stomatal density, shape coefficient and stomatal and pore area). We tested the differences of stomatal traits among populations, interindividual variability - differences among trees, the intraindividual variability, the differences between sun-exposed and shaded leaves, among leaves nested in exposition and the differences in adaxial and abaxial leaf surface. Based on mixed model ANOVA results, interpopulation variability, as statistically significant differences, observed only for stomatal pore length and shape, while all examined traits showed interindividual variability. On the intraindividual level the results showed differences for stomatal traits, except for stomatal width, stomatal shape coefficient and stomatal density regarding leaf exposure. For better understanding of how morphological and stomatal characteristics vary in black poplar populations, further studies should be necessary involving controlled environmental conditions with the aim of examining phenotypic plasticity to changing climate conditions.

Key words: *Populus nigra*, population differentiation, riparian forests, stomatal characteristics.

RESUMEN

Populus nigra es una especie importantes entre las pioneras de los ríos en Europa, debido a la pérdida de su hábitat natural. La existencia de diversidad genética es un factor clave en la supervivencia de una especie y los estomas, como rasgo genéticamente controlado, es usado para estudios de diferenciación. Con el objetivo de comprobar la variación fenotípica de los estomas de cuatro poblaciones autóctonas de *Populus nigra* en tres valles del río más grandes del norte de Serbia (Danubio, Tisa y Sava), región de Vojvodina, fueron analizados los estomas de las hojas (longitud y ancho de estomas, longitud y ancho de poros (ostíolos), densidad estomática, coeficiente de forma y área de estomas y poros). Se analizaron las diferencias de las características de los estomas entre poblaciones, la variabilidad interindividual, la variabilidad intraindividual, las diferencias entre hojas expuestas al sol y sombreadas, entre hojas anidadas en exposición y las diferencias de superficie adaxial y abaxial de las hojas. Según los resultados del modelo mixto ANDEVA, la variabilidad entre poblaciones, estadísticamente significativa, fue observada únicamente en la longitud y la forma de los poros estomáticos, mientras que todas las características analizadas mostraron variabilidad interindividual. En el nivel intraindividual, fueron observadas diferencias en las características de los estomas, excepto en su ancho, el coeficiente de forma y la densidad estomática respecto a la exposición de las hojas. Para comprender mejor cómo varían las características morfológicas y estomáticas en *Populus nigra*, es necesario que investigaciones futuras incluyan condiciones medioambientales controladas, para analizar la plasticidad fenotípica para el cambio de las condiciones climáticas.

Palabras clave: *Populus nigra*, diferenciación de población, bosques de ribera, características estomáticas.

INTRODUCTION

Stomata are the apertures found on the surface of leaves, flanked by guard cells, which regulate the gas exchange between the internal plant tissue and the atmosphere, especially water vapor and CO_2 (Zhang *et al.* 2012,), and the environment, allowing the plant to optimize and balance the photosynthetic performance with water availability and usage (Chaerle *et al*. 2005). The gas exchange regulation is achieved not only through the actual opening and closing of the stomatal pore, but by either increasing or reducing the stomatal conductance (Casson and Gray 2008), as well as by the number and size of stomata on leaf surfaces (Zhang *et al*. 2012).

Stomatal characteristics are highly dependent on the genetic background of the plants as well as on growth conditions or leaf ontogeny (Al Afas *et al*. 2006, Ruso *et al*. 2014). Stomatal density has shown significant variation within individuals, cultivars or ecotypes of a single species, as well as within community (Jones 1992). Within *Populus* genus, a wide inter-specific as well as inter-clonal variation in stomatal density, dimension and stomatal index has already been observed (Ceulemans *et al*. 1995, Ferris *et al*. 2002, Pearce *et al*. 2006). Stomatal traits could also be used as criteria for clonal discrimination within *Populus* genus (Ceulemans *et al.* 1995), which has six taxonomic sections (Eckenwalder 1996). The issue of sectional affiliation remains, pertaining to the relationship between the section *Aigeiros* and *Tacamahaca*, and status of *Populus nigra* L. (Eckenwalder 1996) which has a genetic affinity towards the species of section *Tacamahaca* (Hamzeh and Dayanandan 2004). Some species have been reported as possessing generally high heritability in their stomatal characteristics (Orlović *et al*. 1998), while others have been reported as being more sensitive to environmental factors. Many researchers have so far reported that stomatal density may vary depending on environmental factors such as atmospheric CO_2 concentration (Woodward *et al*. 2002), drought (Dunlap and Stettler 2001, Hetherington and Woodward 2003) or light (Batos *et al*. 2010). Even though they are showing a plastic physiological response to the environmental factors, they are also under genetic control and could go through evolutionary changes if selection differs among environments (Gailing *et al*. 2008).

Populus nigra as a keystone riparian pioneer tree species is one of the rarest and most endangered species in Europe due to the loss of its natural habitats. Considering that genetic diversity existence is a key factor in survival of one species, stomata as genetically controlled trait are used for differentiation studies. Trees display a remarkable phenotypic variation of stomatal leaf traits in growth habits as well. Hence, the main hypothesis of this research is that differences, based on stomatal characteristics of leaves, exist among examined *P. nigra* native populations considering that they are located in the valley of three big rivers (Dunabe, Tisa and Sava) in the region of Vojvodina in northern Serbia. With the aim of proving the default hypothesis, we test (i) the differences of anatomical traits among populations (interpopulation variability), (ii) the interindividual variability differences among trees in populations, (iii) the differences between sun-exposed and shaded leaves of every tree in population, the differences among leaves; (iv) the differences of adaxial and abaxial leaf surface. The last three goals present the intraindividual variability.

METHODS

Study species and area. *Populus nigra* is one of the most endangered riparian tree species in Europe due to the loss of its natural habitats. It occurs naturally in Vojvodina, which is considered as the least afforested area in Europe (7.1 %, Banković *et al.* 2009), where native poplars are represented in a very low percentage of 1.9 % within total forest area. In the total native poplars area *P. nigra* participates with only 15.87 % (Radosavljević 2009) is considered a rare species in the studied area as well. This species is a typical pioneer tree species of the riparian forest ecosystem; it is heavily dependent on the hydrologic conditions of the riverside environment for its regeneration. It has been used as a parent pool in breeding programs in many parts of the world.

The studied populations are situated in four localities in northern Serbia, in the Vojvodina region. These native populations belong to the basins of the three biggest rivers of this area, Danube, Sava and Tisa, as in Čortan *et al*. (2015). The selected locations (figure 1) have uniform environmental conditions; they are next to river banks as part of the willow and black poplar community on alluvial plane with regular flooding, on the flat grounds and without significant exposure, with altitude range between 62-87 m a.s.l. Annual mean air temperature is about 11 ^{o}C, while average sum of precipitation is about 615.7 mm per population (table 1).

Plant material and foliar analyses. In the present study, 10 adult trees from each of the four sites were used for the analyses. Leaf sampling was conducted in September of the same year (2013) around noon, on a sunny day without wind. From the south/south west side of the crown, at the height between 3-6 m; several short shoots were taken from the sunny and shade part of the crown (Batos *et al*. 2010). The sample consisted of ten leaves per one tree, five leaves from sunny and five from shade crown positions. Stomatal impressions from both leaf surfaces (adaxial and abaxial) were taken between the second and third leaf vein near the central vein of the leaf (Orlović *et al*. 1998), using colorless nail polish and adhesive cellophane tape (Ceulemans *et al*. 1995). In total 800 impressions (4 populations x 10 trees x 10 leaves x 2 leaf surfaces) were fixed to glass slides and examined under a light microscope.

Phenotypic variation of the four native populations of *P. nigra* were examined with various genetically controlled leaf stomatal traits (stomatal length and width, pore length and width, stomatal density, shape coefficient and stomatal and pore area) as described in Batos *et al.* (2010) and Balasooriya *et al*. (2009). Stomatal density (SD) was determined at 100x magnification under microscope CETI®MAGNUM-T/Trinocular Microscope, UK with an attached camera Si3000® (UK). The stomata were counted at five randomly selected microscopic fields at each impression (0.33 mm^2) using the "tpsDIG2" software (Rohlf 2001) and afterward calculated as the number of stomata per unit area (1 mm^2). Considering that *P. nigra* has amphistomatous leaves, we did analyses separately on the adaxial (SDd) and the abaxial (SDb) leaf surfaces, in total 4,000 microscopic fields were analyzed for stomatal density (4 populations x 10 trees x 10 leaves x 2 leaf surfaces x 5 microscopic fields) (figure 2). The ratio adaxial/abaxial stomatal density was calculated as well.

Stomatal size was determined at the magnification of 400× under ZEISS light microscope AxioVision Release 4.8.1. The image analyzing system Motic 2000 software was used to measure stomatal size, stomatal length (SL) and width (SW) and pore length (PL) and width (PW), separately on both leaf surfaces (adaxial – d and abaxial – b). Fifteen randomly selected stomata per one impression were measured (5 stomata per each of tree microscopic field), which is in total 12,000 analyzed stomata (4 populations x 10 trees x 10 leaves x 2 leaf surfaces x 3 microscopic fields x 5 stomata).

The following parameters were calculated from the directly measured parameters (Balasooriya *et al*. 2009):

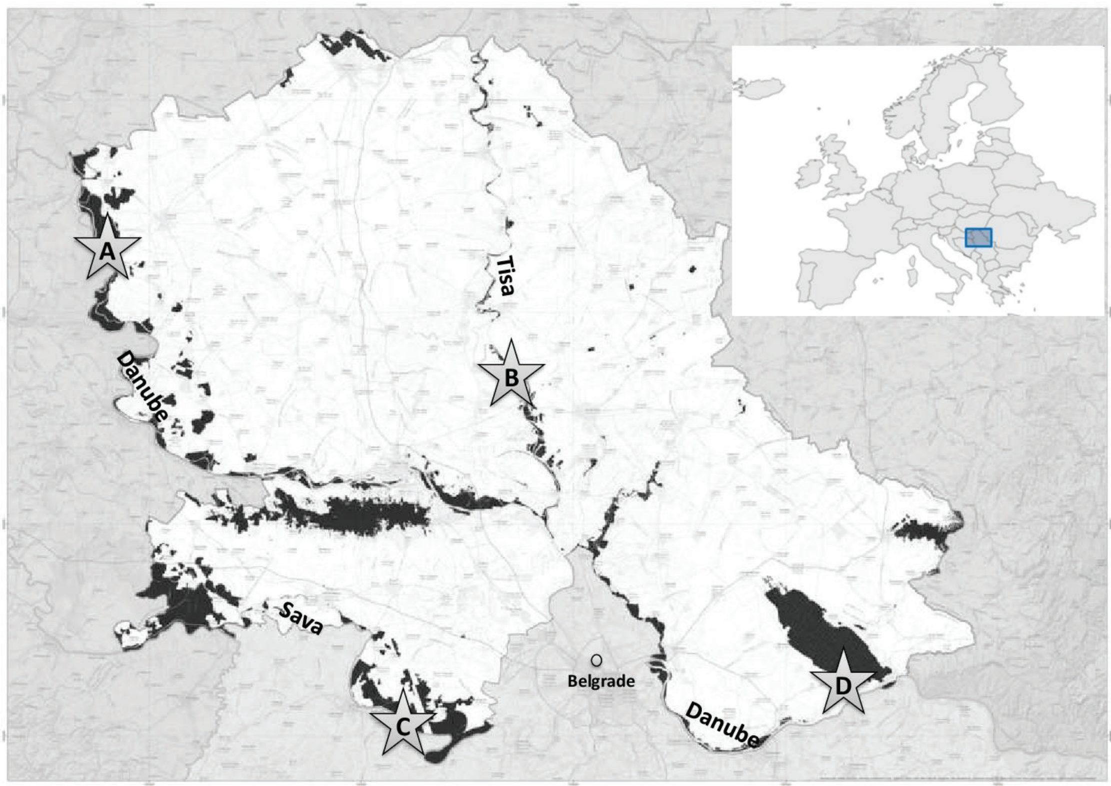

Figure 1. Forest coverage map of Vojvodina region, northern Serbia, with selected study sites: A) upper Danube, B) Tisa, C) Sava and D) lower Danube population (modified map by B. Tubić).

Mapa de cobertura de bosques de la región de Voivodina, norte de Serbia, con los sitios de estudio seleccionados: población de A) Danubio superior, B) Tisa, C) Sava, D) Danubio inferior (modificado por B. Tubić).

Table 1. Basic characteristics of four *Populus nigra* populations used in study.

Características básicas de cuatro poblaciones de *Populus nigra* utilizadas en el estudio.

Population	Location – River basin	Coordinates		Altitude range (m)	Mean annual temperature (°C)	Annual precipitations (mm)
		x	y			
A	Upper Danube	7,338,178	5,064,085	82 – 87	11.2	612.4
B	Tisa	7,446,577	5,008,043	72 – 80	10.9	583.2
C	Sava	7,413,348	4,951,019	76 – 78	11.3	614.2
D	Lower Danube	7,510,888	4,955,118	66 – 82	11.3	653.0

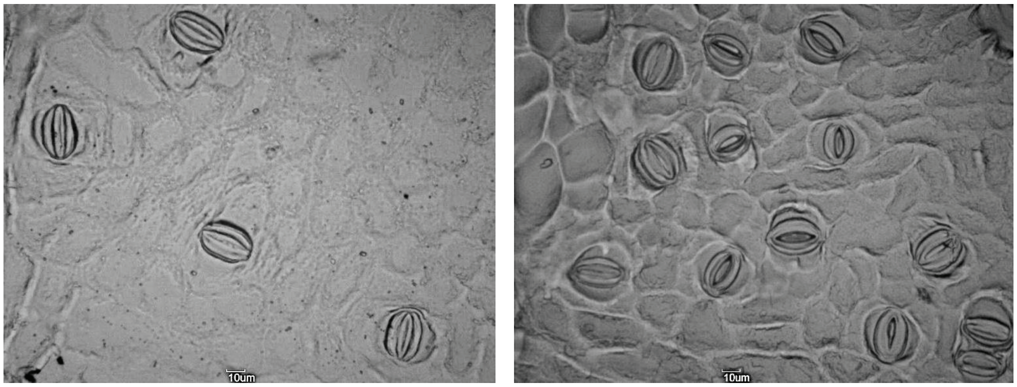

Figure 2. Stomata on adaxial (A) and abaxial (B) leaf surface, magnification 400×, ZEISS light microscope AxioVision Release 4.8.1.

Estomas en la superficie adaxial (A) y abaxial (B) de la hoja, magnificación 400×, microscopio ZEISS Light AxioVision Release 4.8.1.

– Stomatal area: $SA = (SL \times SW \times \pi)/4$ [μm^2] [1]

– Pore area (with widely opened stomatal pore):

$$PA = (PL \times PW \times \pi)/4 \ [\mu m^2] \quad [2]$$

– Stomatal shape coefficient:

$$SSC = 100 \times (SW / SL) \ [\%]; \quad [3]$$

– Pore shape coefficient:

$$PSC = 100 \times (PW / PL) \ [\%]. \quad [4]$$

Data analyses. Statistical analyses were conducted using procedure of the SAS statistical package (SAS Institute, Inc. 2011) for each of the analyzed characteristics. In order to satisfy normal distribution, values of stomatal characteristics were subjected to log-transformation. Statistical significance of different source levels of phenotypic variations was estimated with mixed model analysis of variance. The following sources of phenotypic variation were evaluated through the ANOVA model: population (variation among populations), tree (inter-individual variations within populations), exposure (variation depending of sunny or shaded leaf position in the crown for each tree of present populations), leaf (leaf intra-individual variation for each exposure on the sampled trees in present populations), surface (variation within each leaf surface - adaxial and abaxial, for each leaf exposure on the sampled trees in present populations). We employed a nested model of ANOVA where hierarchical low level subgroups nested in a hierarchical higher level and in a group variable. In the applied nested model of ANOVA tree nested in population - Tree (P), exposure nested in tree and population - Exposure (P T), leaf nested in exposure, tree and population (P T E), surface (leaf surface) nested in leaf, exposure, tree and population - Surface (P T E L). Descriptive statistic was provided for all analyzed stomatal characteristics, carried out separately for each locality, leaf exposure (sunny and shaded) and leaf surface (adaxial and abaxial).

RESULTS

Results of mixed model of ANOVA (table 2) showed statistically significant differences ($P < 0.05$) among populations just for pore length (PL, $P = 0.0154$) and stomatal pore shape coefficient (PSC, $P = 0.0012$), where population B was the one with longer and elongated pores while population A had oval shaped pores with short opening (figure 3). For other traits there is no difference among present populations. Statistical differences exist on individual level, between trees and leaves and between abaxial and adaxial sides of leaves ($P < 0.001$). Leaf position within tree, depending on light exposure (sunny or shaded), showed significant differences for stomatal (SL, $P = 0.0018$) and pore length (PL, $P = 0.0014$), for stomatal (SA, $P = 0.0034$) and pore area (PA, $P = 0.0050$), pore width (PW, $P = 0.0202$) and shape coefficient (PSC, $P = 0.0161$) (table 2). Stomatal and pore area had higher surface on sunny exposures, stomatal and pore length were higher on sunny exposure as well (figure 3).

Stomatal density (SD) did not show statistically significant differences between sun exposed and shaded leaves (table 2), but differences between adaxial and abaxial surface were more than evident (figure 3). Adaxial surface had density in range of 43.64 – 48.01 per mm^2, while abaxial surface of the leaf had a range of 154.22 – 171.79 per mm^2 (figure 3). The ratio of adaxial/abaxial stomatal densities showed the average value of about 0.29 (from 0.18 up to 0.44 per tree, results not shown).

DISCUSSION

Stomata are considered as one of the key regulation factors of the relation between plant and environmental factors (Bayramzadeh 2011). Stomatal traits are characteristics of species, although certain variations can present the micro-environmental conditions and global ecosystem changes (Batos *et al*. 2010). According to our analysis, the

Table 2. Results of mixed-model analyses of variance performed on nine traits of *Populus nigra* genotypes originating from four natural populations in Vojvodina. F-values with the star (*) are statistically significant for *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns – not significant.

Resultados del análisis de varianza de tipo modelo mixto realizado en nueve características del genotipo *Populus nigra* que proviene de cuatro poblaciones autóctonas en Voivodina. Los acrónimos de las características son definidos en Métodos. Valores F con asterisco (*) son estadísticamente significativos: *$P < 0,05$; **$P < 0,01$; ***$P < 0,001$; ns – no significativos.

Source of variation	Stomatal length			Stomatal width			Stomatal shape coefficient		
	df	MS	F	df	MS	F	df	MS	F
Population (P)	3	4.28	1.79 ns	3	0.01	0.13 ns	3	0.11	1.98 ns
Tree (T) (P)	36	2.39	8.31***	36	0.04	13.91***	36	0.06	7.93***
Exposure (E) (P T)	40	0.54	1.87**	40	0.00	1.36 ns	40	0.01	1.03 ns
Leaf (L)(P T E)	320	0.29	6.14***	320	0.00	4.33***	320	0.01	3.28***
Surface (P T E L)	400	0.27	5.83***	400	0.00	4.69***	400	0.01	3.56***
Error	11,200	0.05	-	11,200	0.00	-	11,200	0.00	-
Source of variation	**Pore length**			**Pore width**			**Pore shape coefficient**		
	df	MS	F	df	MS	F	df	MS	F
Population (P)	3	0.83	3.94*	3	1.13	0.58 ns	3	44.69	6.51**
Tree (T) (P)	36	0.21	6.89***	36	1.96	7.60***	36	6.87	8.68***
Exposure (E) (P T)	40	0.06	1.90**	40	0.40	1.56*	40	1.26	1.59*
Leaf (L)(P T E)	320	0.03	4.72***	320	0.26	3.17***	320	0.79	2.47***
Surface (P T E L)	400	0.03	5.29***	400	0.29	3.51***	400	0.94	2.93***
Error	11,200	0.01	-	11,200	0.81	-	11,200	0.32	-
Source of variation	**Stomatal area**			**Pore area**			**Stomatal density**		
	df	MS	F	df	MS	F	df	MS	F
Population (P)	3	0.01	0.5 ns	3	0.91	0.51 ns	3	0.59	0.21 ns
Tree (T) (P)	36	0.02	11.99***	36	1.78	6.84***	36	2.80	8.12***
Exposure (E) (P T)	40	0.00	1.79**	40	0.45	1.74**	40	0.40	1.17 ns
Leaf (L)(P T E)	320	0.00	5.56***	320	0.26	4.42***	320	0.35	10.97***
Surface (P T E L)	400	0.00	5.67***	400	0.28	4.80***	400	10.77	342.19***
Error	11,200	0.00	-	11,200	0.06	-	3,200	0.03	-

only trait that contribute to population differentiation are pore length (PL) and shape (PSC). Length is considered to be under strong genetic control and less under environmental influence (Zhang *et al*. 2012). However, stomatal density (SD), which is considered as a genetically determined quantitative trait (Gailing *et al*. 2008), did not show significant differences (P = 0.8884) in this study. Traits that characterized width are influenced by environmental conditions at the moment when samples were collected. Since width characteristics, as highly plastic trait, are not that prominent among populations, it could be confirmed that these populations have similar environmental characteristics as shown in table 2, while differences in length could be connected to micro-environmental conditions and to genetic variability as well. High influence of micro-environmental conditions might be confirmed by high inter-individual variability and by significant differences among single leaves for all analyzed traits as well.

The differences between sunny and shaded exposition leaves, as two micro-climatic sites within crown, present plant adaptation aiming at the best possible exploitation of available light (Batos *et al*. 2010). In contrast with the inner part of the crown, leaves from the outer part of the crown are more exposed to stressful environment such as higher solar irradiance, larger temperature variation, lower air humidity, various pests. Stomatal traits variation in relation to the leaf exposure (sun and shade exposure)

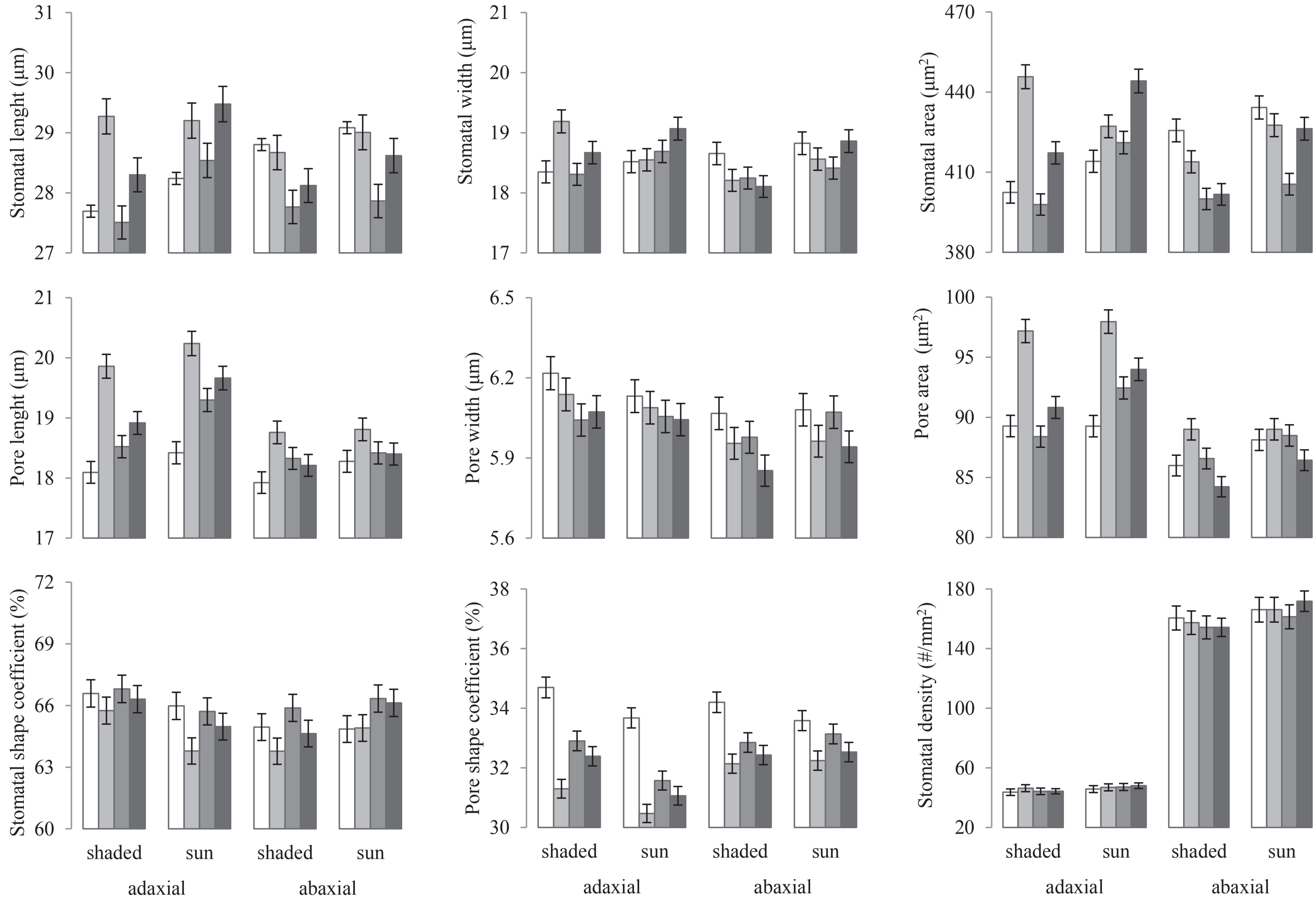

Figure 3. Mean values of nine analyzed traits of *Populus nigra* genotypes originating from four natural populations in Vojvodina. Values are separated by leaf exposure to light (shaded and sunny leaf positions), by leaf surface (adaxial and abaxial surface) and by populations (upper Danube: white bars, Tisa: light grey bars, Sava: dark grey bars, lower Danube: black bars).

Valores medios de nueve características analizadas del genotipo *Populus nigra* que proviene de cuatro poblaciones autóctonas en Voivodina. Los valores están separadas por la exposición a la luz de la hoja (sombreadas y posiciones soleadas de hojas), por la superficie de la hoja (superficie adaxial y abaxial) y por las poblaciones (Danubio superior: barras blancas, Tisa: barras grises claras, Sava: barras gris oscuro, Danubio inferior: barras negras).

in the tree crown showed significant differences for most of analyzed traits (table 2). Numerous investigations show high sensitivity of stomatal traits to light exposure, where in most cases stomatal density decreases by reducing light intensity, meaning that sun exposed leaves have higher stomatal density than the density presented by shaded leaves (Batos *et al*. 2010, Stojnić *et al*. 2015), but there are several studies that say otherwise (Bruschi *et al*. 2003). Leaf position did not influence significantly on stomatal density in our study, while stomatal and pore area from sun exposition had higher values than those presented by shaded leaves (sun SA = 425.01 μm² and PA = 90.71 μm²; shade SA = 413.07 μm² and PA = 88.93 μm², in average). Furthermore, stomatal and pore length also showed significant differences between different expositions, showing longer stomata on sun exposed leaves.

Stomatal patterning in the leaf epidermis is species dependent, and in most cases stomatal density is higher on the abaxial leaf surface (Casson and Gray 2008). However, the poplars are known as *amphistomatous* species, but there are some exceptions such as *Populus trichocarpa* Torr. *et* A.Gray ex Hook. and *Populus balsamifera* Lyall which belong to the section *Tacamahaca* which are the *hypostomatous* type, while *Populus maximowiczii* Henry*,* which belongs to the same section, is the *amphistomatous* type (Pearce *et al*. 2006, Al Afas *et al*. 2007, Dillen *et al*. 2008). *Populus nigra*, as a part of section *Aigeiros*, is an *amphistomatous* species (Ceulemans *et al*. 1995, Al Afas *et al*. 2006, 2007), which was confirmed in our research, where the abaxial stomatal density (in average 161.46 per mm^2) was considerably higher than the adaxial stomatal density (in average 45.79 per mm^2) for all individuals and for all canopy positions (figure 3). Our results are consistent with the results in Orlović (1992) who studied different poplar clones in a nursery. Those results showed that the adaxial density was between 69 (*P. nigra* clones) and 164 (*Populus deltoides* W.Bartram ex Marshall clones) stomata per mm^2, while the abaxial density was between 184 (*P. nigra* clones) and 208 (*P. deltoides* clones) stomata per mm^2. Significant differences among studied clones in Orlović (1992) were explained by different clonal reactions to environmental changes, *i.e*. low phenotypic stability and high adaptability of poplar clones. In general *P. deltoides* and some Euroamerican poplar clones have a high stomatal density on both leaf surfaces, while *P. nigra* showed the lowest density values (Orlović *et al*. 1999). This stomatal distribution pattern is present in most cases, where stomatal density is considerably larger on the abaxial leaf surface, it may help prevent water loss since abaxial surface is less exposed to heating (Casson and Gray 2008). Besides the density ANOVA analysis also showed significant differences between other stomatal characteristics of adaxial and abaxial leaf surface.

The ratio of adaxial/abaxial stomatal densities is considered as a significant characteristic in poplar taxonomy differentiation (Ceulemans *et al*. 1995, Orlović *et al*. 1999) and it showed the average value of about 0.29 within present *P. nigra* populations. Previous research showed the highest stomatal ratio present in *amphistomatous* poplar species within *Aigeiros* section, where *P. deltoides* had 0.8-0.9, followed by Euroamerican poplars with 0.6-0.7 and the lowest values were presented by *P. nigra* 0.3-0.4 (Orlović 1992, Orlović *et al*. 1999, Al Afas *et al*. 2006, Dillen *et al*. 2008), while few poplars from *Tacamahaca* section as *hipostomatous* species showed low ratio (Al Afas *et al*. 2006, 2007). Therefore, variations in stomatal characteristics among different poplar genotypes seem to be species specific (Al Afas *et al*. 2006). Poplar hybrids resulting from crosses between the *Tacamahaca* × *Aigeiros* sections and within the same section showed median values of stomatal density, length and ratio of adaxial/abaxial densities, when compared to parental species (Al Afas *et al*. 2007). Significant stomatal differences among different poplar clones infer that these stomatal characteristics are genetically controlled to a high degree (Orlović *et al*. 2002), which confirmed that there are no hybrids within sampled *P. nigra* genotypes in our study.

Numerous investigations conducted in populations or provenance trials of various tree species characteristics have shown the same results: that the differences among individuals at the intra-population level were statistically significant and most often considerably larger than differences among populations (Batos *et al*. 2010, Poljak *et al*. 2014, Čortan *et al.* 2015). The determined variability patterns, where variability is much more pronounced within rather than among populations, could indicate the existence of the gene flow among the populations so that each population has a similar combination of genotypes (Poljak *et al.* 2014); nonetheless thorough molecular analyses are need.

CONCLUSIONS

Our results showed that environmental conditions of studied populations did not influence on phenotypic differences of stomatal characteristics among populations, which did not confirm our hypotheses for most leaf stomatal characteristics that have been examined (except for stomatal pore length and shape). However, inter- and intra-individual variability was confirmed with gain results, which show us that these characteristics are more under micro environmental influence.

Hence, we could conclude that the variation in *Populus nigra* might have evolved due to inter-individual variability and environmentally induced morphological and stomatal leaf traits specific variation. Since there are no specific patterns of stomatal traits variations among and within populations, further studies involving controlled environmental conditions should be necessary for better understanding of how morphological and stomatal characteristics vary in black poplar populations across the studied area and wider area and their possible phenotypic plasticity to changing climate conditions.

CLARIFICATION

Authors Dijana Čortan and Danijela Miljković contributed equally to this work.

REFERENCES

Al Afas N, N Marron, R Ceulemans. 2006. Clonal variation in stomatal characteristics related to biomass production of 12 poplar (*Populus*) clones in a short rotation coppice culture. *Environmental and Experimental Botany* 58(1): 279-286. DOI: 10.1016/j.envexpbot.2005.09.003

Al Afas N, N Marron, R Ceulemans. 2007. Variability in *Populus* leaf anatomy and morphology in relation to canopy position, biomass production and varietal taxon. *Annals of Forest Science* 64(5): 521-532. DOI: 10.1051/forest:2007029

Balasooriya BLWK, R Samson, F Mbikwa, UWA Vitharana, P Boeckx, M van Meirvenne. 2009. Biomonitoring of urban habitat quality by anatomical and chemical leaf characteristics. *Environmental and Experimental Botany* 65(2): 386–394. DOI: 10.1016/j.envexpbot.2008.11.009

Banković S, M Medarević, D Pantić, N Petrović, B Šljukić and S Obradović. 2009. The growing stock of the Republic of Serbia-state and problems. *Bulletin of Faculty Forestry – University of Belgrade* 100: 7-29.

Batos B, D Vilotić, S Orlović, D Miljković. 2010. Inter and intrapopulation variation of leaf stomatal traits of *Quercus robur* L. in Northern Serbia. *Archives of Biological Sciences* 62(4): 1125-1136. DOI:10.2298/ABS1004125B

Bayramzadeh V. 2011. Stomatal characteristics of Fagus orientalis Lipsky in geograpgically separated locations in the Caspian forest of Northern Iran. *Research Journal* of *Environmental Sciences* 5(11): 836 – 840. DOI: 10.3923/rjes.2011.836.840

Bruschi P, P Grossoni, F Bussotti. 2003. Within- and among-tree variation in leaf morphology of *Quercus* (Matt.) Liebl. natural populations. *Trees-Structure and Function* 17(2): 164-172. DOI: 10.1007/s00468-002-0218-y

Casson S, JE Gray. 2008. Influence of environmental factors on stomatal development. *New Phytologist* 178(1): 9–23. DOI: 10.1111/j.1469-8137.2007.02351.

Ceulemans R, L Van Praet, XN Jiang. 1995. Effects of CO2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). *New Phytologist* 131(1): 99–107. DOI: 10.1111/j.1469-8137.1995.tb03059.x

Chaerle L, N Saibo, D van der Straeten. 2005. Tuning the pores: towards engineering plants for improved water use efficiency. *Trends in Biotechnology* 23(6): 308–315. DOI: 10.1016/j.tibtech.2005.04.005

Čortan D, B Tubić, M Šijačić-Nikolić, D Borota. 2015. Variability of Black poplar (*Populus nigra* L.) leaf morphology in Vojvodina, Serbia. *Šumarski list* 139(5-6): 245-252.

Dillen S, N Marron, B Koch, R Ceulemans. 2008. Genetic variations of stomatal traits and carbon isotope discrimination in two hybrid poplar families (*Populus deltoides* 'S9-2' x *P.nigra* 'Ghoy' and *P.deltoides* 'S9-2'x *P.trichocarpa* 'V24'). *Annals of Botany* 102(3): 399–407. DOI: 10.1093/aob/mcn107

Dunlap JM, RF Stettler. 2001. Variation in leaf epidermal and stomatal traits of *Populus trichocarpa* from two transects across the Washington Cascades. *Canadian Journal of Botany* 79(5): 528–536. DOI: 10.1139/b01-029

Eckenwalder JE. 1996. Systematics and evolution of *Populus*: Biology of Populus. *In* Stettler RF, HD Bradshaw, PE Heilman Jr., TM Hinckley eds. Biology of Populus and its Implications for Management and Conservation. Ottawa. Canada NRC Research Press. p. 7-32.

Ferris R, L Long, SM Bunn, KM Robinson, HD Bradshaw, A Rae, G Taylor. 2002. Leaf stomatal and epidermal cell development: identification of putative quantitative traits loci in relation to elevated carbon dioxide concentration in poplar. *Tree Physiology* 22(9): 633-640. DOI: 10.1093/treephys/22.9.633.

Gailing O, R Langenfeld-Heyser, A Polle, R Finkeldey. 2008. Quantitative trait loci affecting stomatal density and growth in a *Quercus robur* progeny: implications for the adaptation to changing environments. *Global Change Biology* 14 (8): 1934-1946.

Hamzeh M, Dayanandan, S. 2004. Phylogeny of *Populus* (Salicaceae) based on nucleotide sequences of chloroplast trnT–trnF region and nuclear rDNA. *American Journal of Botany* 91(9): 1398-1408.

Hetherington A, FJ Woodward. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424 (6951): 901-908. DOI:10.1038/nature01843

Jones H. 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology: second edition. New York, USA. University Press. 323 p.

Orlović S. 1992. Proučavanje morfologije i varijabilnosti stoma topola. Magistarski rad. Beograd, Serbia. Šumarski fakultet Univerziteta u Beogradu. 118 p.

Orlović S, V Guzina, B Krstić, Lj Merkulov. 1998. Genetic variability in anatomical, physiological and growth characteristics of hybrid poplar (*Populus x euramericana* Dode (Guinier)) and eastern cottonwood (*Populus deltoides* Bartr.) clones. *Silvae Genetica* 47(4): 183-190.

Orlović S, S Pajević, B Krstić. 1999. Anatomical and physiological parameters in selection of poplars (*Populus* sp.). *In* Proceedings for Natural sciences. Serbia and Montenegro. p. 27-39.

Orlović S, S Pajević, B Krstić. 2002. Selection of black poplars for water use efficiency. *In* Proceedings for Natural sciences. Serbia and Montenegro. p. 45-51.

Pearce D, S Millard, D Bray, S Rood. 2006. Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree Physiology* 26(2): 211 – 218.

Poljak I, M Idzojtić, I Sapić, J Vukelić, M Zebec. 2014. Population variability of grey (*Alnus incana* L.) and black alder (*A. glutinosa* L.) in the Mura and Drava region according to the leaf morphology. *Šumarski list* 1-2: 7-16.

Radosavljević N. 2009. The general management plan of forest and green lands in Autonomous Province of Vojvodina (APV), official document of Provenance government.

Rohlf FJ 2001. TPSDig2: a program for landmark development and analysis. Available in http://life.bio.sunysb.edu/morph/soft-dataacq.html

Ruso G, P De Angelis, J Mickle, MR Barone Lumaga. 2014. Stomata morphological traits in two different genotypes of *Populus nigra* L. *iForest – Biogeo Sciences and Forestry* 8: 547-551.

SAS Institute, INC. 2011. SAS/STAT Users Guide, Version 9.1.3. Cary, NC: SAS Institute, Inc.

Stojnić S, S Orlović, B Trudić, U Živković, G von Wuelisch, D Miljković. 2015. Phenotypic plasticity of European beech (Fagus sylvatica L.) stomatal features under water deficit assessed in provenance trail. *Dendrobiology* 73: 163-173. DOI: 10.12657/denbio.073.017
Woodward FI, JA Lake, WP Quick. 2002. Stomatal development and CO2: ecological consequences. *New Phytologist* 153(3): 477-484. DOI: 10.1046/j.0028-646X.2001.00338.x
Zhang L, H Niu, S Wang, X Zhu, C Lou, Y Li, X Zhao. 2012. Gene or environment? Species-specific control of stomatal density and length. *Ecology* and *Evolution* 2(5): 1065-1070. DOI: 10.1002/ece3.233

Recibido: 23.01.17
Aceptado: 04.04.17