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VARIABILITY AND DISCRIMINATION POWER OF ANATOMICAL PARAMETERS IN JUGLANS REGIA HALF-SIB LINES ORIGINATING FROM NATURAL STANDS IN ĐERDAP GORGE, SERBIA IN NURSERY CONDITIONS

VARIJABILNOST I DISKRIMINACIJSKA MOĆ ANATOMSKIH PARAMETARA KOD LINIJA POLUSRODNIKA JUGLANS REGIA PODRIJETLOM IZ PRIRODNIH SASTOJINA U ĐERDAPSKOJ KLISURI, SRBIJA U RASADNIČKIM UVJETIMA

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SUMMARY

This paper presents analysis of anatomical parameters of leaves in *Juglans regia* half-sib lines from natural stands in Đerdap Gorge in Serbia. Thirty-five half-sib lines were examined, designated according to the number of their mother trees from R1-R35. Twenty measured and derived anatomical parameters were examined, describing stomatal and leaf blade cross-section traits. According to Hierarchical analysis of variance, variation of the majority of characters describing stomatal properties were significantly affected by effect of half-sib lines. The effect of variation between plants within half-sib lines was significant for all examined stomatal traits. However, in cross-section characters, the effect of differences between half-sib lines was significant only for thickness of spongy mesophyll (SL). The discrimination model with seven selected traits achieved 60.5% of correct allocation, while the correct allocation of the model with all 20 examined traits was 81.6%. According to the results of stepwise discriminant analysis, the most powerful trait in discrimination of these half-sib lines were stomatal aperture width/ stomatal aperture length ratio (Wb/La) and stomatal aperture length/ stomata guard cell length ratio (La/LA). Presented results strongly support implementation of stomatal and cross-section leaf traits in further studies that would deal with description of variability of *Juglans regia.*

KEY WORDS: Persian walnut, multivariate analysis, conservation

INTRODUCTION
UVOD

Persian walnut (*Juglans regia* L.) is noble broadleaved wind-pollinated monoecious and dichogamies long-lived tree species, mostly praised for edible fruits and hard, high-quality wood that is useful for versatile applications. It is also used in urban areas due to decorative crown and leaves, vigorous growth and resilience to toxic gases, smoke, and

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dust (SOROKOPUDOV et al. 2015; IANOVICI et al. 2017). Persian walnut is meso- and thermophilic species, susceptible to late and early frosts. Its edible fruits are important for many animal species which is its additional contribution to the idea of „green corridors“ and preservation and improvement of biodiversity. It is also basis for realization of secondary forest products, agroforestry, and ecosystem services (DE RIGO et al. 2016).

Study of variability is important step in preservation of a species, and basis for further selection, conservation and improvement of germplasm. Variability of numerous traits has been examined in Persian walnut. Usual morphological parameters used in variability studies of *Juglans sp.* are pomological and leaf morphological traits (MALVOLTI and FINESCHI, 1994; SHARMA and SHARMA, 2001; AKCA and SEN, 2001; JAFARI at al. 2006; ROSS-DAVIS et al. 2008; EBRAHIMI et al. 2009; EBRAHIMI et al. 2011; NOROUZI et al., 2013; ATTAR et al. 2014), but also wood quality and tolerance to biotic and abiotic agents, phenological traits (FADY et al. 2003; ARZANI et al. 2008), and leaf physiological traits (BOTU et al. 2001; IANOVICI et al. 2017). Numerous variability studies in Walnut used leaf anatomical traits. Most of them are related to stomatal traits (MURADOĞLU and GÜNDOĞDU, 2011) but also to cross-section characteristics (SKOROKOPUDOV et al., 2015). Leaf anatomical traits are of interest, as they are considered to be related to the drought tolerance. In their study on six species of genus *Juglans*, SKOROKOPUDOV et al. (2015) found *Juglans regia* to have most of xeromorphic features as highly developed palisade layer, thick leaf, elongated palisade cells, moderate partition of palisade layer, low stomatal density, and elongated stomatal guard cells. The importance of anatomical parameters is reflected in structure of the leaves and thicknesses of different layers of tissue, which play a significant role in various processes important for plant development. The main role of epidermis is to reduce the release of water from plant, palisade tissue is responsible for the process of photosynthesis and the main role of spongy tissue is to perform aeration (CUTLER et al., 2007).

The areal of this Persian walnut spans from Balkan peninsula, over Caucasus and Asia minor and further to Central Asia and northern parts of India. However, this species is disseminated and grown in much larger area that includes temperate regions of Europe, North and South America, Asia, Australia, and New Zealand (BERNARD et al. 2018). In Europe, this species is relatively neglected and endangered, so there are numerous projects considering it breeding and conservation (MALVOLTI and FINESCHI, 1994; FERNÁNDEZ-LÓPEZ et al. 2001; CEROVIĆ et al. 2010; IANOVICI et al. 2017). In Serbia, Persian walnut is present in autochthonous stands in its eastern parts, as western part of its global natural areal. In Đerdap gorge, through which flows river Danube, it is recognized as autochthonous species in numerous relict phytocoenoses of poly and oligodominant type (OSTOJIĆ et al., 2019). The aim of this study was to examine variability as well as discrimination power of anatomical traits in *Juglans regia* half-sib lines originating from autochthonous stands in Đerdap gorge, Serbia.

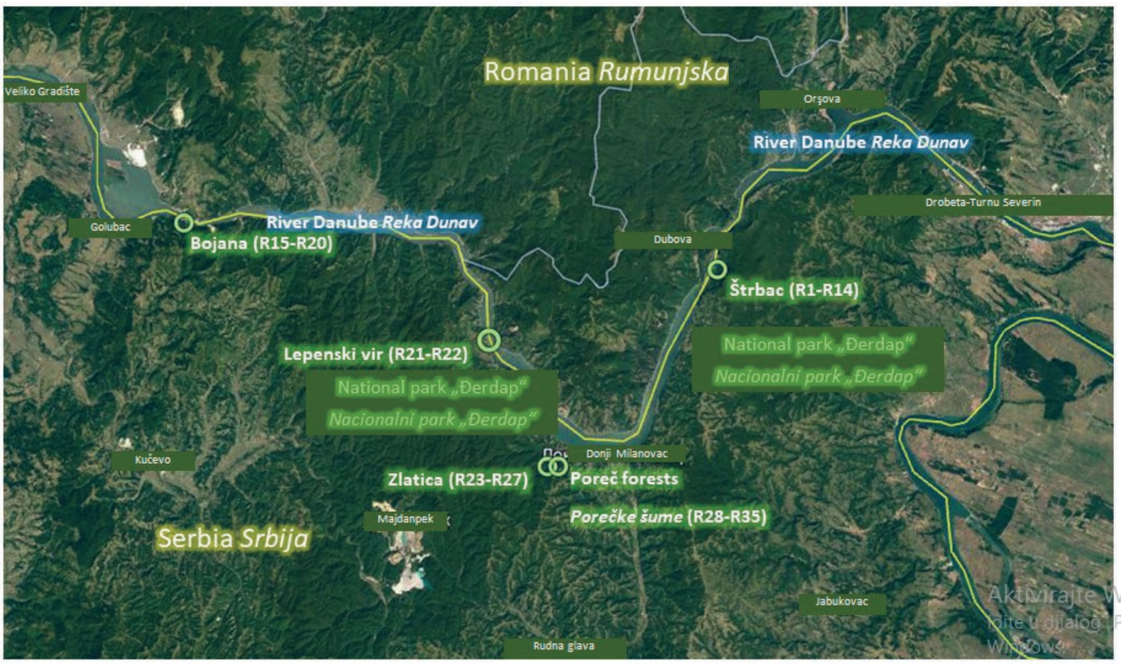

Figure 1. Location of mother trees of Persian walnut (*Juglans regia* L.) in Đerdap gorge, from which the examined half-sib lines were produced (according to geographic coordinates presented by KOVAČ et al.,2021)

Slika 1. Lokacija majčinskih stabala domaćeg oraha (Juglans regia L.) u Đerdapskoj klisuri od kojih su proizvedene ispitivane linije polusrodnika (prema geografskim kordinatama prezentiranim od KOVAČ et al.,2021)

MATERIAL AND METHODS
MATERIJAL I METODE

Plant material and examined parameters – *Biljni materijal i ispitivani parametri*

Examined half-sib lines originate from National park "Đerdap" (total area 63.608,02 ha) in the Eastern part of Serbia. For every mother tree the geographic coordinates were taken and presented by KOVAČ et al. (2021) (Fig. 1). Thirty-five half-sib lines (with 5-10 individuals per line), designated according to the number of their parent trees from R1-R35, were produced in nursery of Experimental estate "Kaćka šuma" (44°17'38.8"N 22°53'13.3"E) in the vicinity of Novi Sad, Serbia, managed by Institute of lowland forestry and environment of University of Novi Sad.

Half-sib lines were grown as seedlings in the open field in nursery conditions until the September 2018 when leaf anatomical parameters were measured on the dominant fully developed leaf from each plant of all half-sib lines in their third growing season, in the timescale between 9:00 AM and 11:00 AM, on a sunny and windless day. For stomatal parameters describing number and size of stomata, one imprint per plant was taken by fingerprint method or 'collodion' method. Imprints were made on the abaxial leaf surface of the top leaflet, between the 3rd and 4th leaflet vain, from a fully formed leaf of light, which were located at upper 1/3 of crown. There were five views per imprint with five stoma measured per view. For every stomatal parameter, average values for plant entered further statistical analysis. For cross-section analysis, the top leaflet from the largest fully developed leaf per plant which was cut-off and rinsed in 70% ethanol until used for preparation of cross-sections. Five cross sections were measured per plant and average values for plant of cross section parameters entered further statistical analysis.

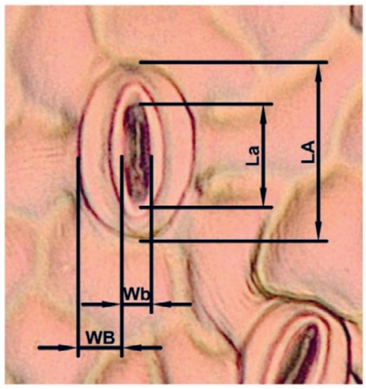

Figure 2. Examined measured stomatal traits on leaf of Juglans regia – LA - stomatal guard cell length, WB - stomatal guard cell, La – stomatal aperture length, Wb – stomatal aperture width

Slika 2: Mjereni parametri puči na listu Juglans regia - LA – dužina stanice zatvaračice, WB – širina stanice zatvaračice, La – dužina otvora puči, Wb – širina otvora puči

Following stomatal parameters were measured: stomatal density per mm^2 (SD), stomata guard cell length (LA [μm]) and width (WB [μm]), and stomatal aperture length (La [μm]) and width (Wb [μm]) were determined from leaf prints following the protocol described by STOJNIĆ et al. (2015) (Fig. 2). For measurements of stomata imprints the Olimpus SC50 light microscope was used. Stomatal density (SD) was determined by counting at five randomly chosen fields of view CellSens Standard software was used for measurements of size of stomata guard cells and stomata aperture were on five stomata per five randomly chosen fields of view. Beside mentioned, following stomatal characters were derived:

WB/LA, Wb/La, La/LA, and Wb/WB.

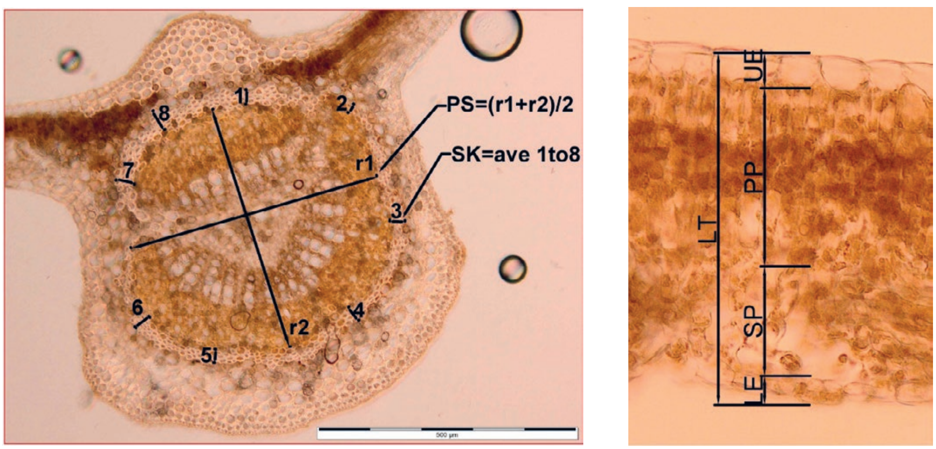

Figure 3. Examined measured leaf anatomical traits of Juglans regia * - a) leaflet midrib cross-section traits: PS – midrib vascular bundle diameter (average from r1 and r2), SK - midrib sclerenchyma thickness (average from measurements from 1 to 8); b) lamina cross-section traits: UE- adaxial epidermis thickness, PP - palisade mesophyll thickness, SP - spongy mesophyll thickness, LE - abaxial epidermis thickness, and LT- total lamina thickness

Slika 3. Ispitivani anatomski parametri lista Juglans regia: a) svojstva poprečnog presjeka glavnog nerva letka: PS – promjer sprovodnog snopića glavnog nerva (sredina od r1 i r2), SK – debljina sklerenhima glavnog nerva (sredina merenja od 1 do 8); b) svojstva poprečnog presjeka lisne plojke letka: UE – debljina adaksijalnog epidermisa, PP – debljina palisadnog tkiva, SP – debljina spužvastog tkiva, LE – debljina abaksijalnog epidermisa, i LT- ukupna debljina lisne plojke.

For cross-section traits measurements, samples were prepared on Slee mev device, and measured with same Olimpus SC50 light microscope and CellSens Standard software. Then, following leaflet cross-section characters were measured:

PS – diameter of midrib vascular bundle [μm] (average from two measurements), SK – thickness of midrib sclerenchyma [μm] (average from eight measurements), UE - adaxial epidermis thickness [μm], PP – palisade mesophyll thickness [μm], SP – spongy mesophyll thickness [μm], LE – abaxial epidermis thickness [μm], and LT- total lamina thickness [μm]. Also, following cross-section characters were derived: PP/LT, SP/LT, SP/PP, and (UE+LE/LT).

Statistical analysis – *Statistička analiza*

The variability of examined characters was analyzed by two-way hierarchical analysis of variance, with half-sib lines and plants within lines as controlled sources of variation according to following model:

$$X_{ijm} = \mu + a_i + b_{j(i)} + \varepsilon_{m(ij)},$$

Where X_{ijm} stands for measured value, μ for average value, a_i for effect of j_{th} half-sib line, $b_{j(i)}$ for effect of j_{th} plant within j_{th} half-sib line, and $\varepsilon_{m(ij)}$ for effect of residual variation. Based on these results the expected variances were calculated and their contribution to the total variation, as well as analog coefficients of variation. The stomatal density (SD) was transformed by square root transformation in order to meet normal distribution of frequencies, which is required for tests of parametric statistics (BARTLETT, 1936). Canonical discriminant analysis (CDA) and forward stepwise discriminant analysis were used for the study of relationship between examined parameters and their discrimination power. Hierarchical cluster analysis with UPGMA (unweighted pair group method with arithmetic mean) agglomeration method was used for the analysis of agglomeration of half-sib lines. Statistical methods were performed by STATISTICA for Windows version 13 (TIBCO SOFTWARE INC., 2020).

RESULTS AND DISCUSSION
REZULTATI I RASPRAVA

Variability of anatomical parameters – *Varijabilnost anatomskih parametara*

According to F-test of Hierarchical analysis of variance, most of characters describing stomatal properties were significantly affected by half-sib lines, except for LA and WB/LA. However, in cross-section characters, the effect of half-sib lines was significant only for thickness of spongy layer (SL) and (UE+LE)/LT ratio. All examined traits were under significant effect of variation between plants within half-sib lines (Table 1).

The highest coefficients of variation between half-sib lines among stomatal traits achieved Wb/La and among cross-section traits SL ($CV_{between}$ > 10%), except for SD, relatively high coefficients of variation within half-sib lines with CV_{within} > 10% were found only in cross-section traits, and the highest were for PS and PL with CV_{within} > 20%.

The further assessment of variability of examined anatomical traits was based on the contribution of examined sources of variation to the total expected variance. These results provided information about importance of differences between half-sib lines but also between plants within half-sib lines, allowing additional way of comparison of the importance of variability between and within half-sib lines (Figure 2). According to these results, the contribution of variation between half-sib lines considerably varies between examined traits. Generally, contribution of half-sib lines was higher in stomatal than in cross-section traits, with the highest contribution in Wb/La and La/LA of more than 45% of total variation. The highest contribution of half-sib lines in cross-section traits achieved SL and (UE+LE)/LT of more than 15%. The highest contribution of plants within half-sib lines among stomatal traits was found in SD (67.2%) and among cross-section traits in PS, PL, and (UE+LE)/LT (more than 70%). Except for SD, stomatal traits were generally more influenced by differences between lines than cross-section traits, while the contribution of variation of plants within half-sib lines in cross-section traits was considerably higher than in stomatal characters. This also suggest risk of decrease of variability of stomatal parameters if case of drop in size of *Juglans regia* population of Đerdap gorge, which could be of importance in its conservation. Significant differences in stomatal density, and length and width of guardian cells among eleven genotypes of *Juglans regia* has been studied by MURADOĞLU and GÜNDOĞDU (2011), who also cited similar findings of study of ÇAĞLAR et al. published in 2004, stressing the significance of stomata frequency and size for plant genetics and ecology. According to SKOROKOPUDOV et al. (2015), *Juglans regia* have the most xeromorphic leaf anatomical features among six species they examined. They found ranges of variability for thickness of leaf (86,3- 151,2 μm, palisade mesophyll (50,8-83,6 μm) and spongy mesophyll (28,9- 63,9 μm) for the group of several species within genus *Juglans*. Group of half-sib lines examined in our study achieved similar or even wider range of variability for the same leaf anatomical characters, suggesting the existence of considerable variability at the level of examined group of half-sib lines.

Considerable contribution of variance within half-sib lines that was found to be particularly high in cross-section traits,

Table 1. Results of analysis of variance for leaf anatomical parameters of examined half-sib lines of *Juglans regia* – F-test, coefficients of variation, variation range

Tablica 1. Rezultati analize varijance za anatomske parametre lista linija polusrodnika Juglans regia – F-test, koeficijent varijacije, interval varijacije

Character[a)] *Svojstvo*	F_B[b) c)]	F_W	CV_B(%)	CV_W(%)	CV_R(%)	Variation range *Interval varijacije*
LA	1.307[ns]	3.708**	1.588	4.438	6.030	27.115-31.860
WB	3,555**	3,032**	4,587	4,262	6,686	6,357-8,456
La	3.871**	5.362**	6.915	6.677	7.148	15.277-20.872
Wb	3.824**	2.789**	7.849	6.785	11.343	3.746-5.668
WB/LA	2,866**	2,496**	3,571	3,677	6,710	0,218-0,278
Wb/La	8.385**	2.458**	11.537	5.928	10.983	0.183-0.344
La/LA	7.519**	4.275**	5.704	3.546	4.385	0.541-0.684
Wb/WB	5,454**	2,713**	11,038	7,538	12,880	0,459-0,804
SD	1.805*	22.585**	5.661	11.185	5.383	38.052-82.569
PS	1.486[ns]	252.984**	8.684	22.366	3.147	432.29-755.26
SK	0.950[ns]	2.159**	0.000	17.394	36.093	19.638-35.035
UE	0.813[ns]	3.569**	0.000	11.839	16.464	11.862-15.64
PL	1.409[ns]	20.973**	9.054	24.708	12.322	52.448-110.80
SL	1.985**	7.311**	10.978	18.390	16.316	35.742-76.053
LE	1.056[ns]	1.819**	1.696	8.619	21.230	8.692-11.94
LT	1.543[ns]	27.754**	7.949	18.950	8.166	113.39-202.51
PL/LT	1.246[ns]	4.632**	2.570	8.226	9.620	0.413-0.561
SL/LT	1.221[ns]	2.358**	2.420	6.985	13.360	0.292-0.385
SL/PL	1.133[ns]	2.876**	3.789	15.004	24.413	0.543-0.961
(UE+LE)/LT	1.878*	10.601**	9.481	17.217	12.388	0.118-0.211

a) Abbreviations for characters: LA – stomatal guard cell length [µm]; WB – stomatal guard cell width [µm]; La – stomatal aperture length [µm]; Wb – stomatal aperture width [µm]; WB/LA – WB/LA ratio; Wb/La – Wb/La ratio; La/LA – La/LA ratio; Wb/WB – Wb/WB ratio; SD – stomatal density [mm^{-2}]; PS – diameter of midrib vascular bundle [µm]; SK – midrib sclerenchyma thickness [µm]; UE – adaxial epidermis thickness [µm]; PL – palisade mesophyll thickness [µm]; SL – spongy mesophyll thickness [µm]; LE – abaxial epidermis thickness [µm]; LT – total lamina thickness [µm]; PL/LT, SL/LT, SL/PL, and (UE+LE/LT);

b) Labels in subscript for F-value (F) and coefficient of variation (CV): B – between half-sib lines, W – plants within half-sib lines, R – residual;

c) Labels concerning significance of F-test: * – $p < 0.05$; ** – $p < 0.01$.

a) Kratice za svojstva: LA – dužina stanica zatvaračica [µm]; WB – širina stanica zatvaračica [µm]; La – dužina otvora puči [µm]; Wb – širina otvora puči [µm]; WB/LA – odnos WB/LA; Wb/La – odnos Wb/La; La/LA – odnos La/LA; Wb/WB – odnos Wb/WB; SD – gustoća puči [mm^{-2}]; PS – promjer provodnog snopa glavnog nerva [µm]; SK – debljina sklerenhima glavnog nerva [µm]; UE – debljina adaksijalnog epidermisa [µm]; PL – debljina palisadnog mezofila [µm]; SL – debljina spužvastog mezofila [µm]; LE – debljina abaksijalnog epidermisa [µm]; LT – ukupna debljina lisne plojke [µm]; PL/LT, SL/LT, SL/PL, i (UE+LE/LT);

b) Oznake u indeksu F vrednosti (F) i koeficijenata varijacije (CV): B – između linija polusrodnika, W – biljke unutar linija polusrodnika, R – rezidual;

*c) Oznake u vezi sa značajnošću F-testa: * – $p< 0.05$; ** – $p< 0.01$.*

could be explained by effective pollen dispersal that is characteristic of wind-pollinated forest tree species. It indicates extensive gene-flow which further high genetic diversity between individuals of the same population and low spatial genetic structure (KRAMER, 2012; POLJAK et al., 2014; JUMP et al., 2012). Walnut has heavy pollen, which, according to FERNÁNDEZ-LÓPEZ et al. (2001), could be considered to spread just over 100 m from pollinating tree, but ZHOU et al. (2021) suggested that that distance can be much longer (even around 1000 m). Low contribution of variation within lines is of particular importance in design of conservation and restoration strategies, since it suggests low heterozygosity of parent trees and in this way high dependance of the conservation of variability on the number of preserved trees. Among nine examined stomatal traits in 14 populations of *Fagus sylvatica* from six European countries (VASTAG et al. 2019), all of examined traits except potential conductance index (PCI) and stomatal aperture length (L_a) had relatively low contribution of differences between half-sib lines, but considerable contribution of plants within half-sib lines. Also, KOVACEVIC et al., (2014) in study of variation of leaf morphometric parameters of twelve populations of *Populus nigra* in Danube basin, found that in most examined traits contribution of variation between trees within population dominated contribution of variation between populations. Strong contribution of variance of half-sib lines within populations related to contribution of variation between population for

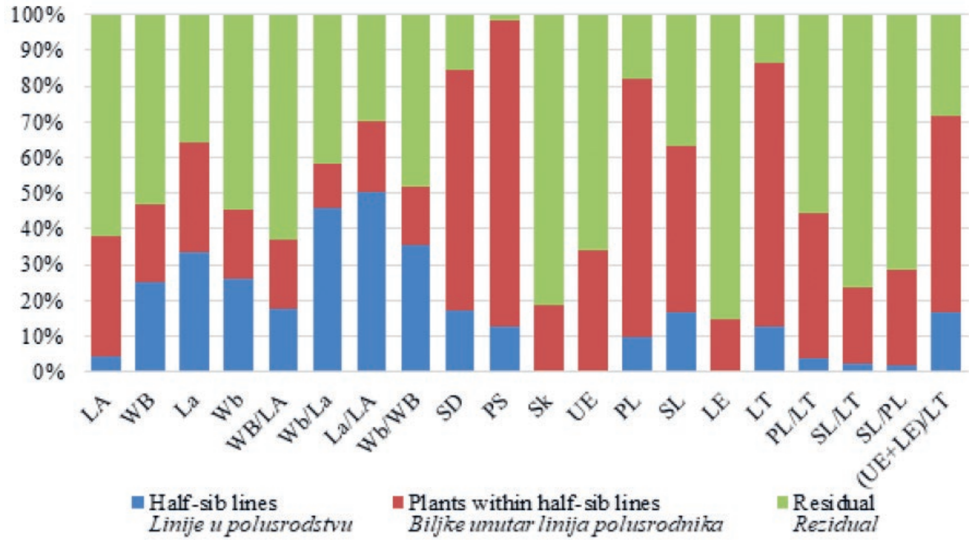

Figure 4. Contribution of expected variances of examined sources of variation to the total expected variance in half-sib lines of Juglans regia
Slika 4. Doprinos očekivanih varijanti ispitivanih izvora varijacije u ukupnoj očekivanoj varijanti linija polusrodnika Juglans regia

percentage of leaves damaged by *Myzus cerasi* in half-sib lines of six wild cheery populations in Bosnia and Hercegovina, was reported by POLJAKOVIĆ-PAJNIK et al. (2019).

Discrimination power of leaf anatomical parameters – *Snaga diskriminacije anatomskih parametara lista*

Canonical discriminative analysis allowed the evaluation of discrimination ability of examined traits. In our study, six successive canonical variables had eigenvalues higher than 1 (Table 2), but only for the first two of them χ^2-tests of canonical coefficient were significant (data not shown). The first canonical variable dominated, with 49,6% of contribution to the total variation. The highest loadings with this canonical variable had Wb/La and La/LA (-0.509 and 0.420, respectively), suggesting the dominant role of these traits in discrimination of examined half-sib lines. Traits Wb/La and La/LA were among traits with the highest contribution of half-sib lines to the total variation, with relatively low contribution of plants within lines.

Among rare studies that used canonical discriminant analysis in *Juglans regia*, MALVOLTI and FINESCHI, (1994), used this method to analyze relationship of leaf and fruit morphometric parameters in 21 population of *Juglans regia* in one region in Italy according to standardized canonical coefficients. In their study of *Fagus silvatica* populations that of anatomical traits included size characters of guard cells and stomatal aperture, as well as stomatal frequency, VAŠTAG et al. (2019) found strong relation of size traits with the first canonical variable, while SD had strong loading with the second canonical variable. DANIČIĆ et al. (2018) and MIKIĆ et al. (2012), used canonical discriminant analysis in the analysis of relationship between fruit traits in populations of *Castanea sativa* in Bosnia and Herzegovina and relationship between seven populations of wild cheery (*Prunus avium*) in Serbia, based on eight leaf morphological characters, respectively. KOVAČEVIĆ et al. (2014) used canonical discriminant analysis for differentiation of *Populus nigra* natural populations in Danube basin based on leaf morphometric traits, while ŠARAC et al., (2014) used same method in discrimination of *Pinus nigra* natural populations in Serbia based on terpene composition.

For further evaluation of discrimination power of anatomical traits, the forward stepwise discriminant analysis was used. By this statistical method, the discrimination models were formed subsequently by the addition of trait that maximizes the discrimination power of the model. Seven characters have been selected in following order: Wb/La, La/LA, Wb/WG, Wb, LA, SD and SP (Table 5). They had their highest loadings with the first (Wb/La and La/LA), second (Wb/WG), fourth (Wb, LA and SD) and fifth (SP) canonical variable. Since canonical variables are not correlated with each other, it is assumed that those parameters what have their highest loadings with the same canonical variable are more correlated between each other than with parameters that have their highest loadings with some other canonical variable. The fact that five last selected traits were in weak correlation with Wb/La and Wb/WB, are that they had their contribution of half sib lines to the total variance much smaller than that of the majority of other examined traits from the second RC group, suggest the ability of stepwise discriminant analysis to cope with multicollinearity. The increment of correct allocation of models calculated by forward stepwise analysis was relatively uniform, and almost linear. The discrimination model with seven selected traits achieved 60.5% of correct allocation, while

Table 2. Discriminative loadings between measured and the first six canonical variables (roots) based on leaf anatomical traits of examined half-sib lines of Persian walnut (Juglans regia).

Tablica 2. Diskriminatorna opterećenja između izmjerenih i prvih šest kanonskih varijabli (korijena) na osnovu anatomskih svojstava lista ispitanih linija polusrodnika domaćeg oraha (Juglans regia).

Original character *Originalna svojstva*	**Canonical variable** *Kanonička varijabla**)					
	Root1	Root2	Root3	Root4	Root5	Root6
LA	0,070	-0,103	0,177	-0,257	-0,168	0,016
WB	0,233	-0,178	0,447	-0,145	0,068	-0,406
La	0,296	-0,012	-0,079	-0,365	-0,351	0,176
Wb	-0,278	0,269	-0,054	-0,412	-0,316	0,000
WG/LA	0,171	-0,072	0,338	0,114	0,212	-0,413
Wb/La	-0,509	0,384	0,042	-0,116	0,001	-0,196
La/LA	0,420	0,098	-0,341	-0,339	-0,398	0,302
Wb/WG	-0,329	0,352	-0,327	-0,318	-0,294	0,191
SD	-0,111	0,155	-0,277	-0,337	-0,056	-0,210
PS	0,013	0,235	-0,215	0,098	-0,269	-0,096
Sk	0,070	0,056	-0,088	0,150	-0,032	0,025
El	0,029	0,071	-0,058	0,042	0,175	-0,205
PP	-0,032	0,142	-0,241	-0,113	-0,307	-0,373
SP	-0,019	0,258	-0,078	0,027	-0,552	-0,417
En	0,033	0,109	0,144	-0,088	0,037	0,077
LT	-0,024	0,195	-0,183	-0,066	-0,387	-0,403
PP/LT	-0,044	-0,033	-0,279	-0,199	-0,083	-0,197
SP/LT	0,028	0,131	0,205	0,194	-0,364	-0,050
SP/PP	0,043	0,102	0,234	0,208	-0,110	0,089
(El+En)/LT	0,039	-0,096	0,215	0,099	0,548	0,374
Eigenvalue *Karakteristični korijen*	12,566	2,717	2,047	1,720	1,148	1,101
Contribution to the total variance *Doprinos ukupnoj varijansi*	0,496	0,107	0,081	0,068	0,045	0,043

*) Bolded values represent the highest loading of original trait for the first six canonical variables

**) Pojačane vrijednosti predstavljaju najviše opterećenje originalnog svojstva za prvih šest kanoničkih varijabli*

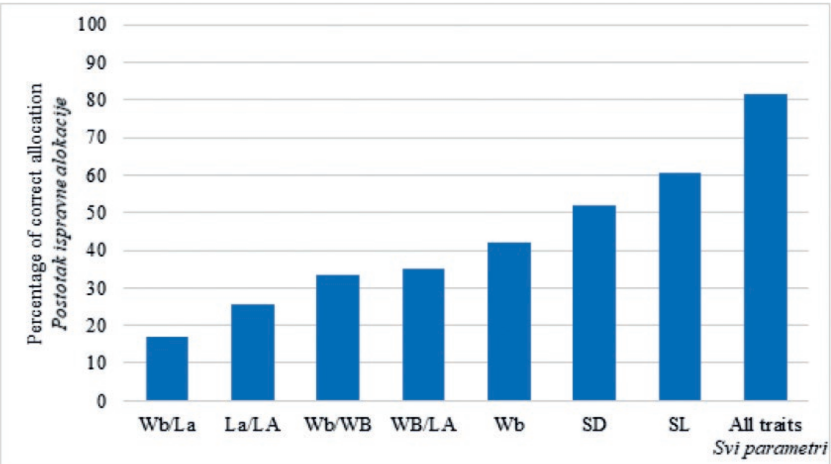

Figure 5. Percentage of correct allocation for models based on leaf anatomical traits and formed by forward stepwise discrimination analysis for examined half-sib lines of Juglans regia.

Slika 5. Postotak ispravne alokacije za modele zasnovane na anatomskim parameterima lista i formiranim stupnjevitom diskriminacijskom analizom sa uključivanjem parametara za linije polusrodnika Juglans regia.

the correct allocation of the model with all 20 examined traits was 81.6%. These results stress the significance of selected traits in discrimination of examined half-sib lines, but they are not sufficient to precisely discriminate all examined lines. For more precise discrimination it would be necessary to include some other traits (Figure 5).

Agglomeration of Persian walnut half-sib lines – *Grupiranje linija polusrodnika domaćeg oraha*

According to hierarchical cluster analysis with UPGMA agglomeration method, studied half-sib lines are grouped in five clusters (Figure 6). According to Scree-test, the linkage distance at which the clusters were defined was found to be at 6. Most of half-sib lines belong to one of two main clusters: the first and second cluster. Most of the first cluster consists of half-sib lines from sites „Štrbac" (81-332 m.a.s.l.) and "Zlatica" (166-172 m.a.s.l.), except for R-20, originating from site "Bojana" (228 m.a.s.l.) and R-21 originating from site "Lepenski vir" (87 m.a.s.l.). The second cluster consists mostly of lines originating from sites "Poreč forests" (236-286 m.a.s.l.) and „Bojana" (133-212 m.a.s.l.) except lines R-4 and R-13, originating from site „Štrbac" (265 and 94 m.a.s.l., respectively) and R-22 (92 m.a.s.l.) originating from site "Lepenski vir". The third cluster consists of two half sib lines that originates from site "Štrbac": R1 and R-2 (139 and 208 m.a.s.l., respectively), while lines R-33 (262 m.a.s.l., "Porečke šume") and R-17 (183 m.a.s.l., "Bojana") forms fourth and fifth cluster, respectively. The main difference between site "Štrbac" and other sites is that its bed rock is silicate, while the bed rock of other sites is calciferous. Also, mother trees on site „Zlatica" were close to river-bed of creek „Zlatica", and that differed this site from other sites on calciferous bed rock. According to the results of forward stepwise discriminant analysis, the most powerful trait in discrimination of these clusters was Wb/WB (data not shown).

Presented results strongly support implementation of stomatal and cross-section leaf traits in the further studies that would deal with description of variability in *Juglans regia*. Significant attention should be paid to derived leaf anatomical parameters that are rarely used in such studies. Most of examined traits the contribution of plants within half-sib lines to the total expected variance is considerable, but stomatal parameters presented higher contribution of half-sib lines to the total expected variance and higher discrimination power then cross-section parameters. Obtained results suggest that there is considerable variability of *Juglans regia* in National park „Đerdap", supporting further studies and preservation of *Juglans regia* autochthonous natural population in National park „Đerdap".

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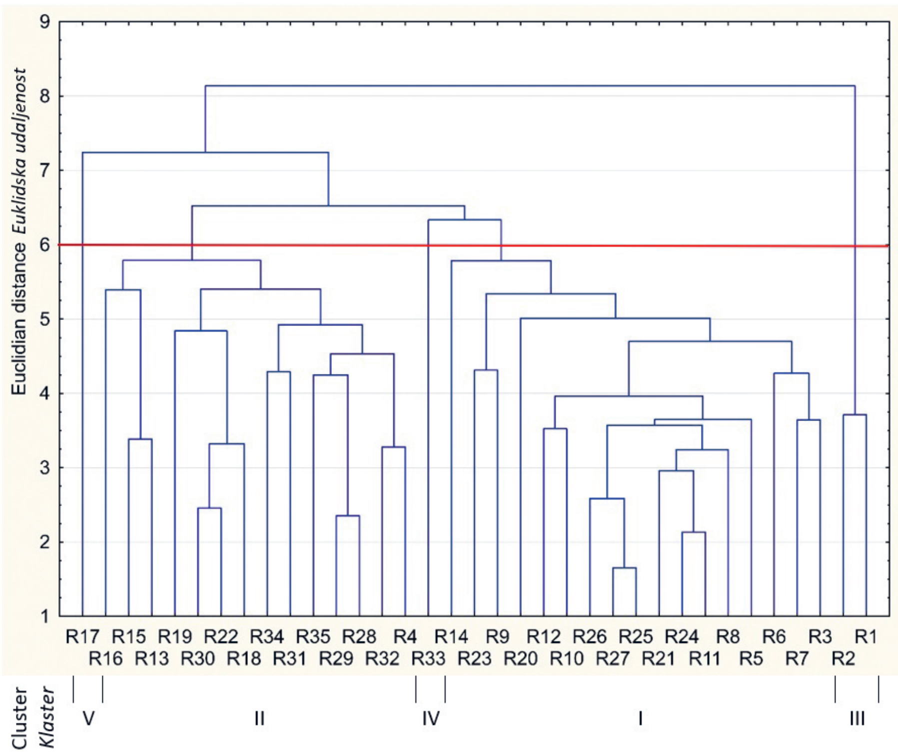

Figure 6. Dendrogram of cluster analysis of examined *Juglans regia* half-sib lines agglomerated by UPGMA method based on normalized leaf anatomical traits.

Slika 6. Dendrogram analize grupiranja ispitivanih linija polusrodnika Juglans regia grupiranih UPGMA metodom na osnovi normaliziranih anatomskih parametara lista

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SAŽETAK

Domaći orah (*Juglans regia* L.) jednodoma je dugovječna vrsta značajna ponajprije zbog svojih jestivih plodova i tvrdog visokokvalitetnog drveta, ali i zbog široke primjene u ozelenjavanju urbanih područja, kao i u agrošumarsrtvu i uslugama ekosustava. Proučavanje varijabilnosti je vrlo značajno za očuvanje vrste i temelj za daljnju selekciju, očuvanje i poboljšanje germplazme. U radu su prikazani rezultati analize anatomskih parametara listova u linijama polusrodnika *Juglans regia* koji potječu iz prirodnih sastojina u Đerdapskoj klisuri u Srbiji. Domaći orah smatra se autohtonom vrstom na ovome području, i zastupljen je u mnogim fitocenozama poli i oligodominantnog tipa. Na pet lokaliteta odabrano je 35 majčinskih stabala, a iz njihovog sjemena su tijekom tri vegetacijske sezone u rasadničkim uvjetima uzgajane linije polusrodnika, označene prema brojevima majčinskih stabala od R1-R35. Ispitano je dvadeset izmjerenih i izvedenih parametara koji opisuju svojstva puči i poprečnog presjeka letka. Prema analizi varijacije poduzorka, varijacija većine osobina koje opisuju svojstva puči značajno je ovisila o učinku linija polusrodinika. Učinak varijacije biljaka unutar linija polusrodnika bio je značajan za sva ispitivana svojstva puči. Međutim, uz svojstva poprečnog presjeka plojke, učinak varijacije biljaka unutar linija polusrodnika bio je značajan samo za debljinu spužvastog tkiva (SL). Diskriminantni model sa sedam selektiranih svojstava ostvario je 60,5 % ispravne alokacije, dok je model sa svih 20 ispitanih svojstava postigao 81,6 % ispravne alokacije biljaka u odgovarajuće linije polusrodnika. Prema rezultatima kanoničke i stupnjevite diskriminantne analize, svojstva s najjačom snagom diskriminacije bili su omjer širine aperture puči/duljinu aperture puči (Wb/La) i omjera duljine aperture puči/duljine stanice zatvaračice (La/LA). Klasterska analiza je pokazala da se linije polusrodnika grupiraju u 5 klastera, među kojima dominiraju prvi (koji potječu uglavnom od majčinskih stabala na lokalitetima Štrbac i Zlatica) i drugi (koji potječu uglavnom od majčinskih stabala na lokalitetima Porečke šume i Bojana). Prikazani rezultati snažno podupiru primjenu parametara puči i poprečnog presjeka plojke u budućim studijama varijabilnosti *Juglans regia*.

KLJUČNE RIJEČI: varijabilnost, anatomski parametri,polusrodnici, Juglans regia