UDC 575. 630
https://doi.org/10.2298/GENSR2203411K
Original scientific article

VARIABILITY OF SOME TRAITS OF WALNUTS (*Juglans regia* L.) IN THE TEST OF HALF SIB, ORIGINATING IN THE ĐERDAP FOREST

Milica KOVAČ[1*], Branislav KOVAČEVIĆ[2], Saša ORLOVIĆ[1,2]

[1] Faculty of Agriculture, University of Novi Sad, Serbia
[2] Institute of lowland forestry and environment, University of Novi Sad, Serbia

Kovač M., B. Kovačević, S. Orlović (2022). *Variability of some traits of walnuts (Juglans regia L.) in the test of half sib, originating in the Đerdap forest*- Genetika, Vol 54, No.3, 1411-1428.
As part of efforts for protection and restoration of variability of *Juglans regia* in Đerdap gorge, Serbia, where it grows as autochthonous relict species, the variability of ten measured and nine derived leaf morphometric parameters of half-sib progenies Persian walnut originating from Đerdap gorge was studied in this work. According to the contribution to total expected variance, parameters that were most effected by differences between half-sib progenies are leaf width (LW), side leaflet length (lL) and top leaflet length (20-30%). Using loadings with the first four rotated principal components, describing 90.1% of total variance, all parameters were grouped in four groups, where parameters LW, lL and lt were in the first group, suggesting multicollinearity between them. These parameters had also relatively high loadings with the first canonical variable from canonical discriminant analysis. Only one of them (lt) was selected by forward stepwise discriminant analysis, where model with four selected leaf parameters achieved 26.5% of correct allocation, while model with all studied parameters achieved 62.4% of correct allocation. Half-sib progenies agglomerated in three clusters, where two small clusters originate from trees that were close to sheltered valleys of small tributaries to river Danube, opposite to mother trees of the first cluster progenies, that were exposed to dominant winds.
Key words: Genetic diversification, gene transfer; *Hedera*, Sequence-related enhanced polymorphism Persian walnut, conservation, multivariate analysis, variability

INTRODUCTION

Walnut (*Juglans regia* L.) is noble broadleaved tree species with trees up to 30 m high, with broad, moderately branched crown. It is wind-pollinated monoecious and dichogamies long-

Corresponding author: Milica Kovač, University of Novi Sad,Faculty of Agriculture,Dositeja Obradovića Sq. 8,21000 Novi Sad, Serbia, E-mail: mici_kovac@hotmail.rs, milica.kovac@polj.uns.ac.rs

lived species, with usual lifespan of 100-200 years (SOROKOPUDOV *et al*., 2015). Walnut is 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 dust (IANOVICI *et al*., 2017). It is meso- and thermophilic species, susceptible to late and early frosts. Walnut is found in all types of substrates, mainly in fresh, fairly rich, and loose soils, avoiding gypseous, compact, or dry ones. The optimum soil pH ranges from 6.5 to 7.5. In preferred climatic conditions, altitude is not an important limiting factor, and it can be found from low elevations up to 2500 m a.s.l. (Himalayas, Morocco) (LESLIE and MCGRANAHAN, 1988). In Serbia walnut trees are most abundant between 100 and 400 m elevation, although individual trees can be found at elevations of up to 1,000 m in central Serbia and up to 1,200 m in southern Serbia (CEROVIĆ *et al*., 2010).

Origins of genus *Juglans* can be traced to the Middle Eocene in North America, while the center of Persian walnut domestication is considered to be in Central Asia. Nowadays, this species is disseminated and grown in temperate regions of Europe, North and South America, Asia, Australia, and New Zealand (BERNARD *et al*., 2018). As a wild species, the areal of this species spans from Balkan peninsula, over Caucasus and Asia minor and further to Central Asia and northern parts of India. In Europe, due to shortage and demand for its quality timber, this relatively neglected species is endangered in numbers and variability (FERNÁNDEZ-LÓPEZ *et al*., 2001). Its presence in autochthonous stands in Serbia is mostly found in its eastern parts. The most notable stands with Walnut in Serbia are those in Đerdap gorge through which flows river Danube. In the Đerdap gorge it forms significant forest association such as *Fagetum submontanus mixtum juglandetosum*, *Fagetum submontanum juglandetosum*, *Fago colurnetum mixtum juglandetosum*, *Celto juglandetosum*, at protected areas like “Veliki i Mali Štrbac”, “Bojana”, “Lepenski vir”, “Boljetinka” etc. “Ciganski potok” has a great floristic importance because we still find remanants of relict forest associations of beech and walnut (*Fagetum submontanum juglandetosum*) and walnut (*Parietario juglandetum*). There it is recognized as autochthonous species in numerous relict phytocoenoses of poly and oligodominant type (LAKUŠIĆ, 2001; MEDAREVIĆ, 2001). 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. It is basis for further selection, conservation and improvement of germplasm. Variability of numerous traits has been examined in Persian walnut. Phenological traits can show ability of species to adapt on environment conditions, based on that, it is decided whether to direct the selection on growth and wood quality, or to direct the selection exclusively on the quality of the fruit. (LITTVAY, 2011) Usual morphological parameters used in variability studies of *Juglans* sp. are pomological traits (SHARMA and SHARMA, 2001; AKCA and SEN, 2001; EBRAHIMI *et al*., 2011; ROSS-DAVIS *et al*., 2008; EBRAHIMI *et al*., 2009; NOROUZI *et al*., 2013), but also wood quality and tolerance to biotic and abiotic agents, phenological traits (FADY *et al*., 2003; ARZANI *et al*., 2008; LITTVAY, 2011), and leaf physiological traits (BOTU *et al*., 2001; IANOVICI *et al*., 2017). Also, numerous variability studies in Walnut used leaf morphological traits (MALVOLTI and FINESCHI, 1994; JAFARI *et al.*, 2006; ATTAR *et al*., 2014).

The aim of this study was to examine variability of *Juglans regia* half-sib progenies originating from autochthonous stands in Đerdap gorge, Serbia.

MATERIAL AND METHODS

Plant material

The seed from adult Walnut trees was taken in second half of September 2015 in National park "Đerdap" in the Eastern part of Serbia. Seeds were collected on five sites: one of them, site "Štrbac" (trees from R1-R14; 44°35'56.80"N 22°16'23.20"E), is on silicate base rock, and four of them: sites "Bojana" (trees from R15-R20; 44°39'10.40"N 21°42'22.30"E), "Lepenski vir" (trees from R21-R22; 44°33'12,18''N 22°01'39,69''E), "Zlatica" (trees from R23-R27; 44°27'26.36"N 22°05'03.60"E), "Poreč forests" (tress from R28-R35; 44°27'25.34"N 22°05'44.37"E) are situated on calcic base rock (KOVAČ et al. 2021). From each of thirty-five selected trees, up to 20 seeds were collected, labeled, and packed in bags. Then, they were stored in cool chamber at 4±2°C. In April 2016 the seeds were seeded to produce half-sib progenies 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 progenies, designated according to the number of their parent trees from R1-R35, were grown as seedlings until the May 2019 when following leaf morphometric parameters were measured and derived for five fully developed leaves per plant:

a) measured parameters: LL – leaf length, LW- leaf width, Nl – number of leaflets, lL – side leaflet length, lW – side leaflet width, LP – petiole length, LR – length of petiole from base up to the first basal leaflet, lV- number of side leaflet side veins, l-1 – width at 1 cm from the top of leaflet, lt – apical leaflet length (Figure 1);
b) derived parameters: LW/LL ratio, lW/lL ratio, LR/LP ratio, l-1/lW ratio, LW/LR ratio, lt/LL – apical leaflet length/length of the leaf ratio, lt/LR – apical leaflet length/length of petiole from base up to the first basal ratio, lt/LP - top leaflet length/length of petiole from base up to the first basal leaflet ratio, lt/LW - apical leaflet length/leaf width.

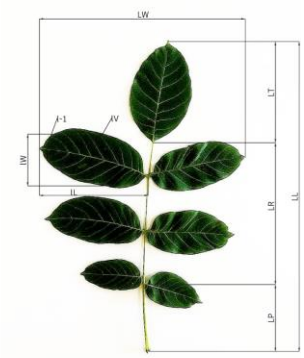

Figure 1. Examined measured leaf morphometric traits of *Juglans regia*[*)]

[*)] Labels of measured leaf morphometric traits: LL – leaf length, LW- leaf width, Nl – number of leaflets, lL – side leaflet length, lW – side leaflet width, LP – petiole length, LR – length of petiole from base up to the first basal leaflet, lV- number of side leaflet side veins, l-1 – width at 1 cm from the top of leaflet, lt – apical leaflet length

Statistical analysis

Five leaves per plant were measured for all survived plants within the 35 half-sib progenies. The number of leaflets (Nl) and number of leaflet side veins (lV) were transformed by square root transformation in order to meet normal distribution of frequencies of count data, which is required for tests of parametric statistics (BARTLETT, 1936).

The variability of examined group of half-sib progenies and relationship between them were described by Hierarchical analysis of variance. Based on these results, Tukey's HSD (honestly significant difference) test, contribution of expected variances to the total variance and coefficient of variance for examined sources of variation, and analog coefficients of variation were calculated. The relationship between examined parameters was described by Pearson's coefficient of variation at the level of half-sib progenies, as well as by loadings of principal components and results of canonical discriminant analysis (CDA). The matrix of coefficients of correlation was used as entering data for principal component analysis (PCA). The agglomeration of half-sib progenies was analyzed by hierarchical cluster analysis with UPGMA (unweighted pair group method with arithmetic mean) agglomeration method. All these statistical methods were performed by STATISTICA for Windows version 13 (TIBCO SOFTWARE INC, 2020).

RESULTS AND DISCUSSION

According to the results of analysis of variance, variation of all examined characters was significantly affected by differences between plants within half-sib progenies, and the most of characters differed significantly between half-sib progenies. Only lW, LP, l-1, lW/lL, and l-1/lW did not significantly differed between half-sib progenies (Table 1).

The highest coefficients of variation between half-sib progenies achieved lt and lt/LP (CV_B> 10%), while the highest coefficients of variation within progenies achieved LP, l-1, lW/lL, and l-1/lW with CV_W higher than 12%.

Beside F-test and coefficients of variation, analysis of variance provided results of contribution of examined sources of variation to the total expected variance of leaf morphometric traits. These were used as an additional information on the importance of the traits in discrimination of examined half-sib progenies and importance of variation between plants within half-sib progenies in relation to residual variation (Figure 2).

Walnut is anemophilous species, but its pollen is rather heavy, and it is considered that it spread over 100 m from pollinating tree (FERNÁNDEZ-LÓPEZ *et al*., 2001). However, it is evident that the contribution of variation of plants within half-sib progenies much higher than of variation between half-sib progenies, suggesting considerable gene-flow between trees on examined sites, probably due to rather frequent strong winds in the gorge that influence pollen dispersal and seed spread by birds. In study of variation of leaf morphometric parameters of twelve populations of *Populus nigra* in Danube basin, KOVAČEVIĆ *et al*. (2014) found that only number of leaf side veins on left and right blade side out of fourteen measured and derived leaf morphometric parameters were under considerably higher influence of differences between populations, than between trees within populations. Also, among nine examined stomatal traits in fourteen *Fagus sylvatica* half-sib progenies from 14 populations of six European countries (VASTAG *et al*., 2019), only potential conductance index (PCI) and stomatal aperture length (L_a)

had relatively high contribution of differences between half-sib progenies, that was similar to the contribution of plants within progenies. Strong contribution of variance of half-sib progenies within populations related to contribution of variation between population for percentage of leaves damaged by *Myzus cerasi* in half-sib progenies of six wild cheery populations in Bosnia and Hercegovina, was reported by POLJAKOVIĆ-PAJNIK *et al*. (2019).

Table 1. Results of the analysis of variance for examined leaf morphometric parameters of examined walnut (Juglans regia) half-sib progenies

Leaf morphometric traits[1)]	F-test						Coefficient of variation (%)[3)]		
	Between half-sib progenies			Plants within half-sib progenies					
	F-value[2)]	p-value		F-value	p-value		CV_B(%)	CV_W(%)	CV_R(%)
LL	2.343	0.001	**	5.958	0.000	**	7.379	10.505	10.532
LW	3.256	0.000	**	4.542	0.000	**	9.523	10.131	12.018
Nl	1.786	0.017	*	3.156	0.000	**	1.761	2.971	4.517
lL	3.244	0.000	**	4.444	0.000	**	9.690	10.305	12.396
lW	1.457	0.086		1.751	0.000	**	6.203	10.894	28.068
LP	1.445	0.091		3.727	0.000	**	5.945	13.802	18.660
LR	2.149	0.003	**	4.202	0.000	**	7.939	11.697	14.592
lV	1.748	0.021	*	3.134	0.000	**	3.006	5.191	7.932
l-1	1.171	0.278		5.996	0.000	**	4.396	17.570	17.549
lt	3.502	0.000	**	3.539	0.000	**	11.206	10.861	15.214
LW/LL	2.163	0.002	**	2.566	0.000	**	4.356	5.715	10.193
lW/lL	0.482	0.990		1.352	0.030	*	0.000	12.096	45.531
LR/LP	2.703	0.000	**	1.858	0.000	**	9.208	8.692	20.941
l-1/lW	1.521	0.064		4.525	0.000	**	9.487	20.992	24.956
LW/LR	1.808	0.016	*	2.207	0.000	**	4.632	6.905	14.031
LR/LL	1.854	0.012	*	2.033	0.000	**	2.850	3.985	8.751
lt/LL	2.055	0.004	**	1.801	0.000	**	4.592	5.411	13.487
lt/LR	1.688	0.029	*	1.845	0.000	**	5.764	8.525	20.706
lt/LP	2.725	0.000	**	1.794	0.000	**	10.936	10.041	25.161
lt/LW	1.865	0.012	*	1.974	0.000	**	3.739	5.114	11.568

[1)] Labels of leaf morphological traits: LL – leaf length, LW- leaf width, Nl – number of leaflets, lL – side leaflet length, lW – side leaflet width, LP – petiole length, LR – length of petiole from base up to the first basal leaflet, lV- number of side leaflet side veins, l-1 – width at 1 cm from the top of leaflet, lt – apical leaflet length, LW/LL ratio, lW/lL ratio, LR/LP ratio, l-1/lW ratio, LW/LR ratio, lt/LL – apical leaflet length/length of the leaf ratio, lt/LR – apical leaflet length/length of petiole from base up to the first basal ratio, lt/LP - top leaflet length/length of petiole from base up to the first basal leaflet ratio, lt/LW - apical leaflet length/leaf width;

[2)] Labels concerning significance of F-test: * - $p< 0.05$; ** - $p< 0.01$;

[3)] Labels for coefficients of variation: CV_B –coefficient of variation between half-sib progenies (%), CV_W– coefficient of plants within half-sib progenies (%), CV_R– coefficient of residual variation (%)

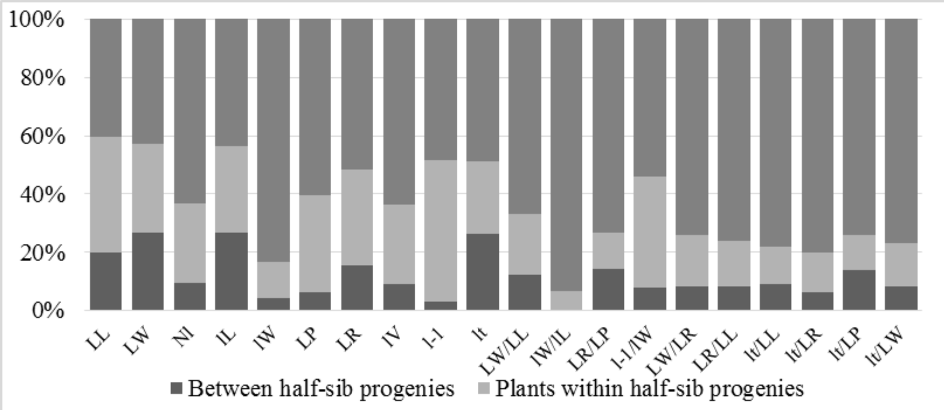

Figure 2. Contribution of examined sources of variation to the total expected variance for leaf morphometric traits*) of examined Persian walnut (*Juglans regia*) half-sib progenies

*) – Labels of leaf morphometric traits as explained in Table 1

Thus, it seems that traits that are more effected by differences between progenies and populations than by differences between plants within them are relatively rare. In our study, the highest contribution of half-sib progenies to the total variation achieved LW, lL and lt (20-30%). These results also show that in most traits contribution of variation within half-sib progenies dominated over contribution of variation between progenies, except for LW, lL, lt, LR/LP and lt/LW. Parameters LL and l-1 achieved the highest contribution of variation within progenies (>40%), while LL, LW, lL l-1, and lt had the highest contribution of summary effect of both controlled sources of variation (>50% of total expected variance). According to the results of JAFARI *et al*. (2006) for 32 leaf morphometric traits in Iranian populations of *Juglans regia,* the most distinctive and reliable leaf morphological characteristics were: length of the biggest leaflet, petiole length, leaflet marginal form, arctangent of average leaflet length to average leaflet width, and arctangent of half leaf width to petiole length. Their results suggest relatively high variability between nine populations they examined.

According to Pearson's coefficients of correlation there is close relationship between most of measured traits, except for Nl, LP and l-1. These three parameters were mostly in weak correlation with measured parameters as well as with each other. Also, high correlation between LL, LW, lL, and lt suggest that these traits that were under the highest effect of controlled sources of variation carry similar information about differences between half-sib progenies. On the other side, trait l-1, whose variation was also under considerable effect of controlled sources of variation was not in correlation with other measured traits, suggesting that this parameter gives additional information for discrimination between half-sib progenies. Moderate or strong correlations were found between most of derived traits, except for lW/lL, LR/LP, l-1/lW, and lt/LR. Among derived traits, significant correlation with most of measured traits achieved LR/LP and l-1/lW, while among measured traits LW and lL are significantly correlated with most of derived traits.

Table 2. Pearson's correlation coefficients based on half-sib progenies' mean values for leaf morphometric traits of examined Walnut (Juglans regia) half-sib progenies.

Leaf morphometric traits*)	LL	LW	Nl	lL	lW	LP	LR	lV	l-1	lt	LW/LL	lW/lL	LR/LP	l-1/lW	LW/LR	LR/LL	lt/LL	lt/LR	lt/LP	lt/LW
LL	1.00																			
LW	0.87 **	1.00																		
Nl	0.21	0.25	1.00																	
lL	0.88 **	1.00 **	0.26	1.00																
lW	0.89 **	0.90 **	0.22	0.89 **	1.00															
LP	0.54 **	0.19	-0.26	0.21	0.31	1.00														
LR	0.93 **	0.81 **	0.51 **	0.81 **	0.81 **	0.39 *	1.00													
lV	0.54 **	0.72 **	0.34 *	0.71 **	0.61 **	-0.06	0.55 **	1.00												
l-1	-0.23	-0.16	-0.15	-0.19	0.01	-0.30	-0.23	-0.01	1.00											
lt	0.88 **	0.92 **	0.04	0.91 **	0.87 **	0.24	0.72 **	0.61 **	-0.09	1.00										
LW/LL	0.00	0.48 **	0.14	0.47 **	0.24	-0.54 **	0.00	0.51 **	0.05	0.27	1.00									
lW/lL	-0.15	-0.37 *	-0.12	-0.38 *	0.06	0.10	-0.16	-0.27	0.40 *	-0.20	-0.51 **	1.00								
LR/LP	0.40 *	0.61 **	0.68 **	0.59 **	0.51 **	-0.50 **	0.60 **	0.56 **	0.07	0.48 **	0.50 **	-0.23	1.00							
l-1/lW	-0.70 **	-0.68 **	-0.26	-0.70 **	-0.57 **	-0.43 **	-0.67 **	-0.37 *	0.79 **	-0.57 **	-0.16	0.40 *	-0.25	1.00						
LW/LR	-0.06	0.32	-0.44 **	0.31	0.15	-0.28	-0.29	0.27	0.10	0.32	0.77 **	-0.35 *	0.00	-0.05	1.00					
LR/LL	0.06	0.07	0.85 **	0.07	0.03	-0.27	0.43 **	0.17	-0.09	-0.19	0.05	-0.10	0.62 **	-0.12	-0.60 **	1.00				
lt/LL	0.35 *	0.58 **	-0.25	0.56 **	0.51 **	-0.26	0.13	0.41 *	0.15	0.75 **	0.51 **	-0.15	0.37 *	-0.15	0.72 **	-0.49 **	1.00			
lt/LR	0.20	0.35 *	-0.58 **	0.34 *	0.32	-0.05	-0.12	0.18	0.14	0.59 **	0.31	-0.05	-0.05	-0.05	0.77 **	-0.80 **	0.90 **	1.00		
lt/LP	0.49 **	0.75 **	0.19	0.73 **	0.64 **	-0.40 *	0.43 **	0.61 **	0.14	0.79 **	0.61 **	-0.22	0.77 **	-0.24	0.48 **	-0.01	0.86 **	0.58 **	1.00	
lt/LW	0.38 *	0.22	-0.45 **	0.21	0.36 *	0.20	0.12	0.04	0.15	0.59 **	-0.28	0.30	-0.06	-0.01	0.16	-0.62 **	0.67 **	0.75 **	0.41 *	1.00

*) – Labels of leaf morphometric traits as explained in Table 1.; Labels below correlations coefficients concerning significance of t-test: * - $p < 0.05$; ** - $p < 0.01$

The relationship among examined leaf morphometric traits is further analyzed by principal component analysis (PCA). Considering Keiser's role, four first principal components that had eigenvalues higher than 1 had been selected for further analysis (data not shown). Their cumulative contribution to the total variance was 90,1%. The distribution of total variance between rotated principal component was not uniform. First rotated principal component dominated, with contribution to the total variation of 41.7% (Table 3).

These four principal components were rotated by Varimax method, by that variance of loadings within the four selected principal components were maximized. Since the principal components are orthogonal against each other (there is no correlation between them), it is supposed that traits that are in high correlation with the same principal components are highly correlated and in low correlation with traits that are in high correlation with another principal components. Based on this assumption, parameters had been grouped according to the rotated principal component with which they had the highest loadings. So, based on loadings with those four rotated principal components, examined traits were divided into four groups (Table 3).

This approach aloud further evaluation of significance of examined parameters in discrimination between half-sib progenies, since the traits that have high loadings with the first principal component are expected to contribute to the variability between half-sib progenies the most. Indeed, within the first group, which contains almost half of examined parameters, most of traits designated to have high contribution of half-sib progenies or plants within half-sib progenies to the total variance are included. However, high correlation between them suggests that they describe similar information on variability among progenies. Among them were not Nl, LP and l-1, as it has been already presented by Pearson's coefficients of correlation. In second group are: Nl, LR/LL, lt/LL, lt/LR, and lt/LW. The contributions of controlled sources of variation (half-sib progenies and plants within progenies) to the total variation of these traits were not as high as for the traits of the first group. However, considering weak correlations between traits from these two groups, the significance of this group is in additional information about differences between half-sib progenies they bring. The same stands for traits of third (LP, LW/LL and LW/LR) and fourth group (l-1, lW/lL, and l-1/lW) (Table 3).

In their study of variability of *Populus nigra* populations in Danube basin, KOVAČEVIĆ *et al*. (2014), divided 14 examined leaf morphometric traits in seven groups by the same method. in the first group were also measured parameters: length and width of the leaf blade, length of leaf petiole and length of the whole leaf. In the study of variability of leaf morphometric parameters of nine populations of wild pear (*Pyrus pyraster*) in Serbia (MIKIĆ *et al*., 2008), eight measured and derived leaf morphometric parameters were divided by the same method in two groups.

Table 3. Loadings between leaf morphometric parameters of examined half-sib progenies of Persian walnut (Juglans regia) and the first four principal components derived from them and rotated by Varimax method.*

Leaf morphometric traits	Rotated principal components			
	RC1	RC2	RC3	RC4
LL	0.948	0.028	-0.230	-0.184
LW	0.925	0.050	0.253	-0.231
Nl	0.303	-0.859	0.156	0.000
lL	0.920	0.046	0.238	-0.258
lW	0.946	0.063	-0.007	0.041
LP	0.274	0.218	-0.795	-0.383
LR	0.898	-0.346	-0.189	-0.148
lV	0.664	-0.114	0.391	-0.072
l-1	-0.097	0.085	0.161	0.826
lt	0.936	0.312	0.113	-0.041
LW/LL	0.182	0.032	0.921	-0.195
lW/lL	-0.093	0.046	-0.490	0.683
LR/LP	0.613	-0.496	0.528	0.199
l-1/lW	-0.618	0.053	0.090	0.713
LW/LR	0.054	0.651	0.695	-0.166
LR/LL	0.111	-0.979	0.075	0.010
lt/LL	0.529	0.612	0.517	0.190
lt/LR	0.297	0.882	0.307	0.123
lt/LP	0.709	0.151	0.613	0.224
lt/LW	0.420	0.686	-0.228	0.388
Explained variation	7.654	4.312	3.708	2.345
Proportion of the total variation	0.383	0.216	0.185	0.117

* - The underlined values represent the highest loading of examined trait for four selected and rotated principal components

To further analyze discrimination power of examined traits the canonical discrimination analysis and stepwise discrimination analysis were performed.

In canonical discriminative analysis five successive canonical variables had eigenvalues higher than 1 but only the first two of them χ^2-tests of canonical coefficient were significant. The first two canonical variables described 47,5% of total variation and the first five described 73.4% of total variation (Table 4).

Table 4. Canonical analysis statistics for successive canonical variables based on leaf morphometric traits of examined half-sib progenies of Persian walnut (Juglans regia)

Roots removed	Eigenvalue	Canonical coefficient	Wilks' lambda	χ^2-test	df	p-level
0	4.921	0.912	0.000	896.943	646	0.000
1	3.821	0.890	0.000	738.654	594	0.000
2	1.993	0.816	0.001	598.665	544	0.052
3	1.555	0.780	0.004	501.109	496	0.427
4	1.223	0.742	0.009	417.617	450	0.861
5	0.914	0.691	0.020	346.516	406	0.985
6	0.835	0.675	0.039	288.729	364	0.999
7	0.680	0.636	0.072	234.714	324	1.000
8	0.556	0.598	0.120	188.516	286	1.000
9	0.482	0.570	0.187	149.166	250	1.000
10	0.442	0.554	0.277	114.180	216	1.000
11	0.263	0.457	0.400	81.587	184	1.000
12	0.179	0.390	0.505	60.784	154	1.000
13	0.165	0.376	0.596	46.119	126	1.000
14	0.137	0.347	0.694	32.545	100	1.000
15	0.100	0.302	0.789	21.137	76	1.000
16	0.070	0.256	0.867	12.653	54	1.000
17	0.039	0.193	0.928	6.631	34	1.000
18	0.037	0.189	0.964	3.236	16	1.000

According to discriminative loadings with the first two canonical variables, only three traits could be notified: LW and lL that had moderate loadings with the first and lt with moderate loading with the first and second canonical variable (Table 5). So, in this case, the three traits that had the highest contribution of half-sib progenies to the total expected variance had also highest loadings with some of the first two canonical variables. All of them belong to the same (first) PCA group, which suggest multicollinearity among them.

MALVOLTI and FINESCHI (1994) used canonical discriminant analysis in analysis of significance of relationship leaf and fruit morphometric parameters in 21 population of *Juglans regia* in one region in Italy according to standardized canonical coefficients. They found that apical leaflet width, leaf area and number of left leaflets had the greatest standardized canonical coefficients with the first canonical variable. Other morphological leaf traits had their highest standardized canonical coefficients with the next two canonical variables, where petiole length dominated in second and apical leaflet length in third canonical variable. Considering some analog traits that were studied in our work, such as number of leaflets which belongs to the first PCA group, petiole length which belongs to the third PCA group and apical leaflet length which belongs to the first PCA group, it could be said that results of MALVOLTI and FINESCHI (1994) additionally stress the importance of lack of multicollinearity between traits included in discrimination of studied group of genotypes. DANIČIĆ *et al*. (2018), used canonical discriminant analysis in the analysis of relationship between fruit traits as well as between six examined populations of *Castanea sativa* in Bosnia and Herzegovina. In the first groups were represented both measured and derived parameters. Also, MIKIĆ *et al.* (2012), used canonical discriminant analysis in description of relationship between seven populations of wild cheery (*Prunus avium*) in Serbia, based on eight leaf morphological characters.

Table 5. Discriminative loadings between measured and the first five canonical variables (roots) based on leaf morphometric traits of examined half-sib progenies of Persian walnut (Juglans regia).

Leaf morphometric traits[1)]	Root1[2)]	Root2	Root3	Root4	Root5
LL	-0,264	-0,195	-0,180	0,007	-0,242
LW	<u>-0,352</u>	-0,283	0,120	0,193	-0,193
Nl	-0,065	0,005	-0,012	<u>0,368</u>	0,278
lL	<u>-0,346</u>	-0,282	0,114	0,182	-0,179
lW	-0,226	-0,129	-0,033	0,006	<u>-0,337</u>
LP	-0,042	0,005	-0,205	-0,237	-0,197
LR	-0,247	-0,137	-0,186	0,194	-0,115
lV	-0,187	-0,091	0,199	0,011	0,260
l-1	-0,022	0,006	0,132	-0,141	-0,215
lt	<u>-0,356</u>	<u>-0,367</u>	-0,036	-0,027	-0,327
LW/LL	-0,098	-0,098	0,461	0,293	0,084
lW/lL	0,006	0,059	-0,079	-0,123	-0,138
LR/LP	-0,204	-0,151	0,121	<u>0,540</u>	0,089
L-1/lW	0,122	0,092	0,107	-0,199	0,050
LW/LR	-0,028	-0,140	<u>0,396</u>	-0,055	-0,061
LR/LL	-0,054	0,090	-0,056	<u>0,469</u>	0,199
lt/LL	-0,123	-0,286	0,210	-0,073	-0,182
lt/LR	-0,040	-0,218	0,155	-0,256	-0,225
lt/LP	-0,254	-0,302	0,241	0,181	-0,055
lt/LW	-0,049	-0,170	-0,212	<u>-0,354</u>	-0,315
Explained variation	4.921	3.821	1,993	1,555	1,223
Proportion of the total variation	0.267	0.208	0,108	0,084	0,066

[a)] - The underlined values represent the highest loading whose absolute value is higher than 0.33 of particular trait with the first five canonical variables (total contribution to total variation 0.734)

Forward stepwise discriminant analysis, as a method where the discrimination model is improved by subsequent addition of the trait that maximizes the discrimination power of the model, provided final insight in the discrimination power of examined leaf morphological traits.

By this analysis, four traits were selected: lt and LR/LP from the first RC group, LW/LL from the third and l-1/lW from the fourth RC group (Table 6). So, by this method, there are lt and LR/LP that had relatively high contribution of half-sib progenies to the total variation, but also LW/LL and l-1/lW that has not as high contribution of half-sib progenies to the total variation but they are not in correlation with the first two traits. This suggests that they contribute new information about differences between half-sib progenies that is not described by lt and LR/LP.

Table 6. Statistics for forward stepwise discrimination analysis based on leaf morphometric traits of examined half-sib progenies of Persian walnut (Juglans regia).

	Wilk's lambda	F to enter	p-value*)	
lt	0.467	3,624	0,000	**
LR/LP	0.543	2,376	0,001	**
LW/LL	0.535	1,813	0,016	**
l-1/lW	0.530	1,781	0,019	**

*) Labels concerning significance of F-test: * p< 0.05; **p< 0.01

According to percentage of correct allocation, the model with these four selected traits achieved only 26.5%, while the model with all traits achieved 62.4% of correct allocation of plants in original half-sib line (Figure 3). These data suggest that examined leaf morphometric traits are not sufficient for discrimination of studied half-sib progenies and that additional markers should be used in further population studies in *Juglans regia*. This is probably due to the fact that dominant portion of variability is explained by differences between plants within half-sib progenies.

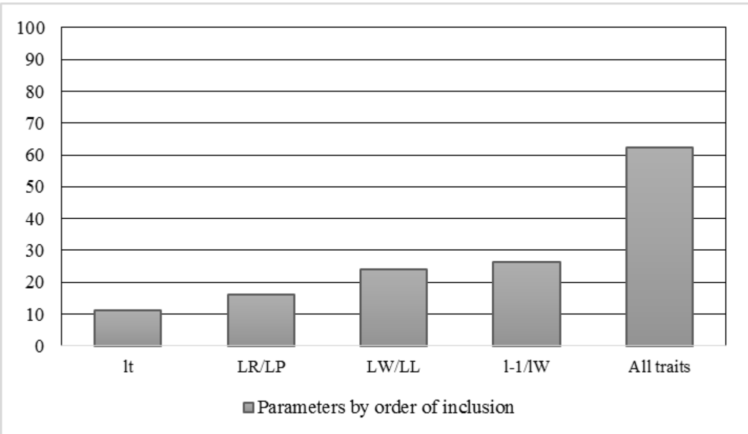

Figure 3. Percentage of correct allocation for models formed by forward stepwise discrimination analysis for examined half-sib progenies of Persian walnut (*Juglans regia*).

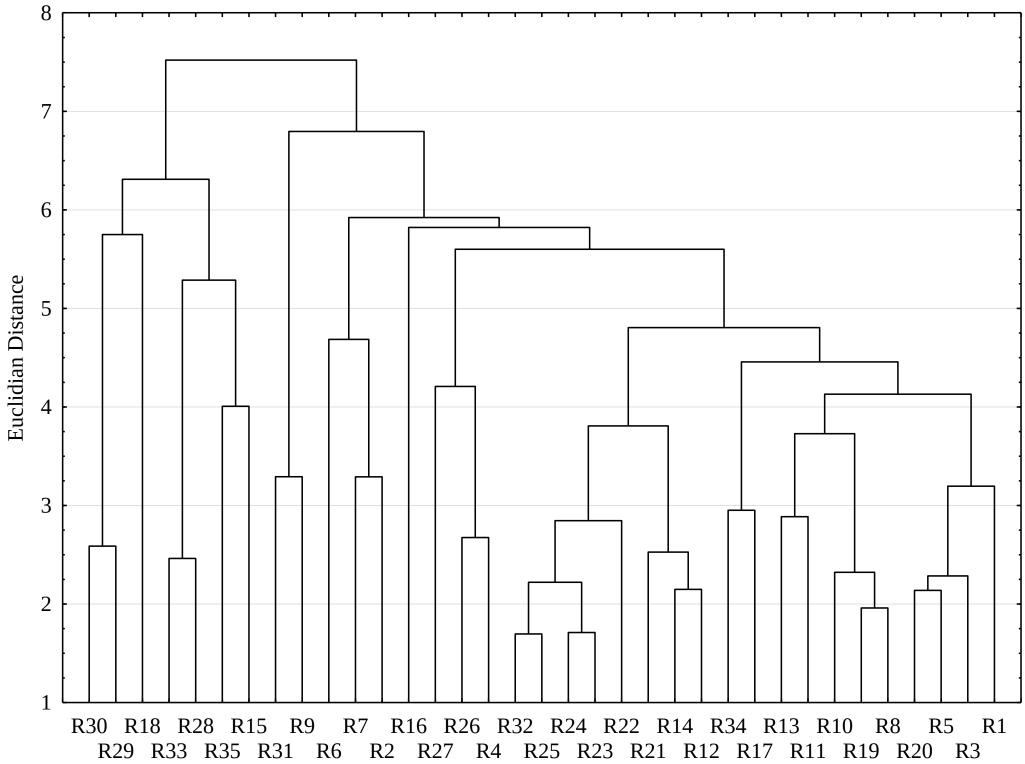

Figure 4. Dendrogram of cluster analysis of examined *Juglans regia* half-sib progenies agglomerated by UPGMA method based on normalized leaf morphometric traits.

Such results are in accordance with results of KOVAČEVIĆ (2014), who found that model with all thirteen leaf morphometric parameters studied in populations of European black poplar in basin of Danube achieved around 60% of correct allocation, but model with four parameters achieved around 52%. In work of VASTAG *et al.* (2019) on variability of stomatal parameters of fourteen European beach provenances, all discrimination model with all examined traits achieved only around 40% of correct allocation, and the same achieved model with five selected parameters.

According to hierarchical cluster analysis with UPGMA agglomeration method, studied half-sib progenies are grouped in three clusters (Figure 4). According to Scree-test, the linkage distance at which the clusters were defined was found to be at 6.5 (Figure 5). Most of half-sib progenies belong to the first (main) cluster. The second cluster include several half-sib families from site "Zlatica" (R28, R29, R30, R33 and R35), as well as two that originates from relatively low altitude of site "Štrbac" (R15) and site "Bojana" (R18). The second cluster includes another half-sib family from site "Zlatica" (R31) and one whose parent tree was on relatively high altitude of site "Štrbac" but in the riverbed of Alibeg's creek (R9). So, these results suggest that half-sib progenies from site “Zlatica” mostly clustered apart from the main group, except for half-sib progenies R26 and R27 were included.

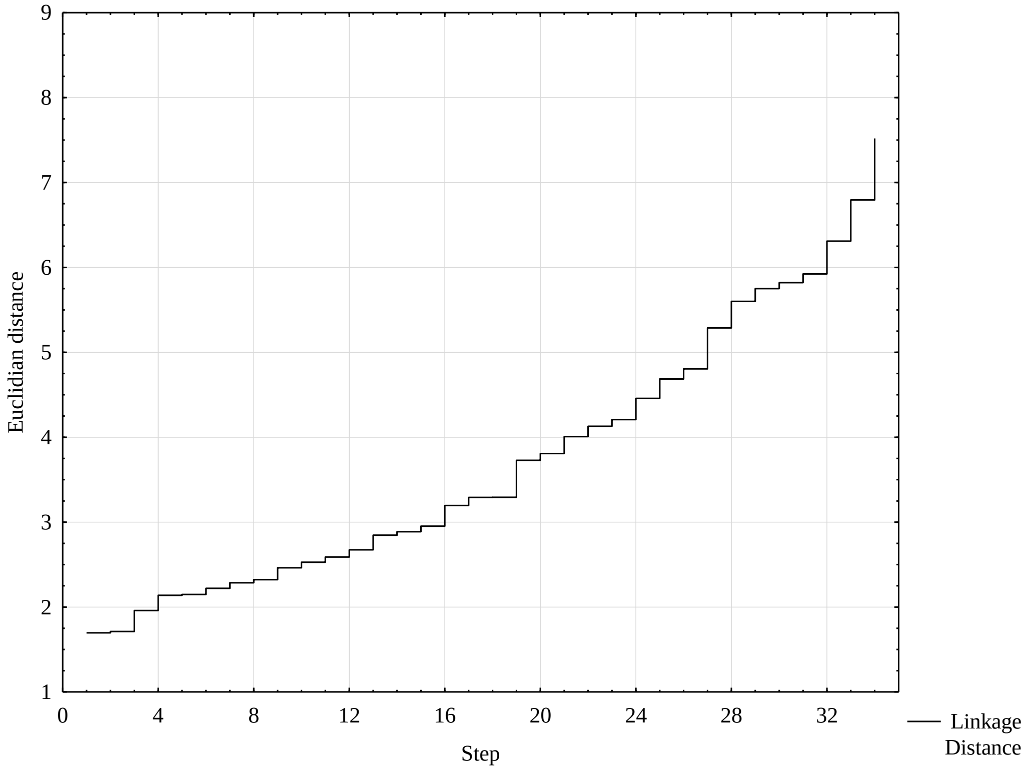

Figure 5. Amalgamation schedule (Scree test) of UPGMA method of agglomeration for leaf morphometric characters of examined *Juglans regia* half-sib progenies.

According to the forward stepwise discrimination analysis based on mean values half-sib progenies, traits lt/LP and lt/LL are sufficient to discriminate these clusters. The model based on lt/LP correctly allocated progenies in clusters by 78,6%, while the model that included both traits achieved 100% of correct allocation (data not shown). Regarding the position of mother trees in the area (KOVAČ *et al*., 2021), it seems that main difference between clusters 3 and 1 is that their mother trees were close to creeks that are tributaries to river Danube in valleys sheltered from strong winds, which was not the case with parent trees of the first cluster. The parent trees of progenies of cluster 2 were also in riverbeds as it were trees of progenies of cluster 3. It seems that progenies from cluster 2 and 3 represent variability within similar ecological conditions, and the origin of their variability is still to be studied.

The data in this study suggest relatively moderate differences between half-sib progenies originating form Đerdap gorge, although most of examined traits are under significant effect of differences between half-sib progenies and all of them under effect of differences between plants within half-sib progenies. Variation between plants within half-sib progenies is mostly higher than variation between half-sib progenies, and only five measured leaf parameters (most of them, except l-1, closely related) had contribution of expected variation between half-sib progenies and between plants within half-sib progenies smaller than residual variation. This suggest that other parameters, morphological and molecular, should be included in more precise differentiation between examined half-sib progenies.

Although that leaf morphological parameters as important in taxonomical and population studies in Persian walnut, majority of recent studies in this field are based on molecular markers (BERNARD *et al*., 2018). The main reason is that leaf quantitative traits could not be regarded as markers, since they are inherited by many QTLs that interact in various ways among them and with environmental agents. KOVAČEVIĆ (2014) cited MARCET (1961) and YING *et al.* (1976), who had suggested that leaf size and shape of a population could be related to the specificities of the environment in which that population grew and evolved. The relation of morphological traits and molecular markers is still questionable. BURSTIN and CHARCOSSET (1997) emphasized that correlation between marker and phenotypic distances decrease with increase of QTLs involved in the variation of traits. However, MALVOLTI and FINESCHI (1994) found association of leaf morphometric distances with matrix of genetic distances as well as of matrix of distances based on fruit morphological traits for twenty-one *Juglans regia* populations from Central Italy. There was no association between genetic distance matrix and matrix of distances based on fruit morphological traits. Half-sib tests are a prerequisite for the mainteanance of collections in order to preserve genetic source of species (LITTVAY, 2011). Considering all of this, it seems that, leaf morphometric parameters could still be important in bringing additional information in population studies beside widely used genetic markers, especially with implementation of multivariate methods. In that sense, the variability studies in natural stands with *Juglans regia* should be continued, as part of an effort for conservation and restoration of endangered species (KOVAČ *et al*., 2021; PILIPOVIĆ *et al*., 2011) as well as of efforts in mitigation of the effects of expected climate changes (KOSTIĆ *et al*., 2019) and improvement of ecosystem services (ZORIĆ *et al*., 2019).

ACKNOWLEDGEMENTS

This paper financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Project No. 451-03-68/2022-14/200117 and 451-03-68/2022-14/200197

Received, December 07th, 2021

Accepted September 28th, 2022

REFERENCES

AKCA, Y., S.M., SEN (2001): A study on the genetic variability and selection of superior walnut (*Juglans regia* L.) trees within seedling population of around of Van lake. Acta Horticulturae, *544*: 119-124.

ARZANI, K., H., MANSOURI-ARDAKAN, A., VEZVAZVAEI, M.R., ROOZBAN (2008): Morphological variation among Persian walnut (*Juglans regia*) genotypes from central Iran. New Zealand Journal of Crop and Horticultural Science, *36*: 159–168.

ATTAR, S.K., K., KUMAR, S.K., JHA (2014): Diversity analysis in persian walnut (*Juglans regia* L.) trees of Shimla hills. Indian Forester, *140*(8): 789-792.

BARTLETT, M.S. (1936): The Square Root Transformation in Analysis of Variance. Supplement to Journal of the Royal Statistical Society, *3*:68–78.

BERNARD, A., F., LHEUREUX, E., DIRLEWANGER (2018): Walnut: past, and future of genetic improvement. Tree Genetics & Genomes, *14*: 1.

BURSTIN, J., A., CHARCOSSET (1997): Relation between phenotypic and marker distances: theoretical and experimental investigations. Heredity, *79*: 477-483.

CEROVIĆ, S., B., GOLOŠIN, J., NINIĆ TODOROVIĆ, S., BIJELIĆ, V., OGNJANOV (2010): Walnut (*Juglans regia* L.) selection in Serbia. Horticultural Science (Prague), *37*: 1-5.

EBRAHIMI, A., A., ZAREI, R., FATAHI, M., GHASEMI VARNAMKHASTI (2009): Study on some morphological and physical attributes of walnut used in mass models. Scientia Horticulturae, *121*: 490–494.

EBRAHIMI, A., R., FATAHI, Z., ZAMANI (2011): Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia* L.) using morphological traits and SSRs markers. Scientia Horticulturae, *130*: 146–151.

FADY, B., F., DUCCI, N., ALETA, J., BECQUEY, R., DIAZ VAZQUEZ, F., FERNANDEZ LOPEZ, C., JAY-ALLEMAND, F., LEFÈVRE, A., NINOT, K., PANETSOS, P., PARIS, A., PISANELLI, H., RUMPF (2003): Walnut demonstrates strong genetic variability for adaptive and wood quality traits in a network of juvenile field tests across Europe. New Forests, *25*: 211–225.

LESLIE, C.E., G.H., MCGRANAHAN (1988): Native population of *Juglans regia* L. International Conference on Walnuts, 19–23 Sept 1988, Yalova, Turkey. Ataturk Central Horticultural Research Institute: 111-124.

FERNÁNDEZ LÓPEZ, J., N., ALETÀ, R., ALIA (2001): Long term conservation strategies: *Juglans regia* L. genetic resources conservation strategy. In: Noble Hardwoods Network: Fourth and Fifth Meetings, 4-6 September 1999 in Gmunden, Austria and 17-19 May 2001 in Blessington, Ireland. EUFORGEN: 38-43.

IANOVICI, N., A., LATIȘ, A., RĂDAC (2017): Foliar traits of *Juglans regia*, *Aesculus hippocastanum* and *Tilia platyphyllos* in urban habitat. Romanian Biotechnological Letters, *22*(2): 12400-12408.

JAFARI, S.M.H., M.M.R., MARVI, H., SOBHANI, J., MOZAFARI (2006): Morphological leaf characteristics of Persian walnut in Iranian population. Iranian Journal of Forest and Poplar Research, *14* (23):1-19.

KOVAČ, M., B., KOVAČEVIĆ, S., ORLOVIĆ (2021): Inventorying of Persian walnut (*Juglans regia* L.) trees in natural stands in "Đerdap" national park. Šumarstvo, 2021(1-2): 159-172. [In Serbian]

KOVAČEVIĆ, B. (2014): Variability of leaf morphometric characters in *Populus nigra* populations in the basin of river Danube. In: Šiler B., Škorić M., Mišić D., Kovačević B., Jelić M., Patenković A., Kurbalija Novičić Z.: Variability of European Black Poplar (*Populus nigra* L.) in the Danube Basin. Zoran Tomović, Ivana Vasić (Eds.), Public Enterprise "Vojvodinašume": 52-85.

KOSTIĆ, S., T., LEVANIČ, S., ORLOVIĆ, B., MATOVIĆ, D.B., STOJANOVIĆ (2019): Pedunculate and turkey oaks radial increment and stable carbon isotope response to climate conditions through time. Topola, *204*: 29-35.

LITTVAY, T. (2011): Phenotypic stability and adaptability of families of common walnut (*Juglans regia* L.) in progeny tests. Šumarski list-special edition, 38-45.

MALVOLTI, M.E., S., FINESCHI (1994): Morphological Integration and Genetic Variability in Juglans regia L. Journal of Heredity, *85*: 389-394.

MEDAREVIĆ, M. (2001): Šume Đerdapa. Public Enterprise Nacionalni park "Đerdap"

MIKIĆ, T., D., BALLIAN, B., KOVAČEVIĆ, S., PILIPOVIĆ, M., MARKOVIĆ (2012): Preliminary studies on the variability of wild cherry (*Prunus avium* L.) in some populations in Serbia. Proceedings of International Scientific Conference „Forest in future: Sustainable use, Risks and Challenges“, 4th-5th October 2012, Belgrade, Republic of Serbia: 151-160.

MIKIĆ, T., S., ORLOVIĆ, B., KOVAČEVIĆ, M., MARKOVIĆ A., PILIPOVIĆ (2008): Variability in wild pear (*Pyrus pyraster* Burgsd.) populations in Serbia based on leaf morphological characteristics. Proceedings of International Scientific Conference „Forestry in Achieving Millennium Goals“, 13-15 November 2008, Novi Sad: 359-365.

NOROUZI, R., S., HEIDARI, M.A., ASGARI-SARCHESHMEH, A., SHAHI-GARAHLAR (2013): Estimation of phenotypical and morphological differentiation among some selected Persian walnut (*Juglans regia* L.) accessions. International Journal of Agronomy and Plant Production, *4*(9): 2438-2445.

PILIPOVIĆ, A., S., ORLOVIĆ, S., STOJNIĆ, V., GALOVIĆ, M., MARKOVIĆ (2011): Inventarization of wild cherry (Prunus avium) genefond in Serbia in the aim of directed genetic potential utilization. Topola, *187/188*: 53-63.

ROSS-DAVIS, A., Z., HUANG, J., MCKENNA, M., OSTRY, K., WOESTE (2008): Morphological and molecular methods to identify butternut (*Juglans cinerea*) and butternut hybrids: relevance to butternut conservation. Tree Physiology, *28*:1127–1133.

SOROKOPUDOV, V.N., T.A., KUZNETSOVA, S.N., SHLAPAKOVA, T.C., NGUYEN (2015): Morpho-anatomical and ecological features of the species of the genus Juglans L. in the conditions of Belgorod region. Theoretical & Applied Science, *23*: 72-82.

SHARMA, O.C., S.D., SHARMA (2001): Genetic divergence in seedling trees of Persian walnut (*Juglans regia* L.) for various metric nut and kernel characters in Himachal Pradesh. Scientia Horticulturae, *88*(2): 163-171.

TIBCO SOFTWARE INC. (2020). Data Science Workbench, version 14. http://tibco.com.

VASTAG, E., B., KOVAČEVIĆ, S., ORLOVIĆ, L., KESIĆ, M., BOJOVIĆ, S., STOJNIĆ (2019): Leaf stomatal traits variation within and among fourteen European beech (*Fagus sylvatica* L.) provenances. Genetika, *51*(3): 937-959.

ZORIĆ, M., I., ĐUKUĆ, LJ., KLJAJIĆ, D., KARAKLIĆ, S., ORLOVIĆ (2019): The possibilities of improvement of ecosystem services in Tara National park. Topola, *203*: 53-63.

VARJABILNOST NEKIH SVOJSTAVA ORAHA (*Juglans regia* L.) U TESTIRANIM LINIJAMA POLUSRODNIKA POREKLOM IZ ŠUMA ĐERDAPA

Milica KOVAČ[1*], Branislav KOVAČEVIĆ[2], Saša ORLOVIĆ[1,2]

[1]Poljoprivredni fakultet, Univerzitet u Novom Sadu, Srbija
[2]Institut za nizijsko šumarstvo i zaštitu životne sredine, Univerzitet u Novom Sadu, Srbija

Izvod

U okviru napora za zaštitu i obnavljanje varijabilnosti *Juglans regia* L. u Đerdapskoj klisuri , Srbija, gde raste kao autohtona reliktna vrsta, u ovom radu utvrđena je varijabilnost deset izmerenih i devet izvedenih morfometrijskih parametara listova, linija polusrodnika potomaka domaćeg oraha poreklom iz šuma Đerdapske klisure.Prema doprinosu ukupnoj očekivanoj varijansi, parametri na koje su najviše uticale razlike između polusrodnih potomaka su širina lista (LW), dužina dominantne bočne liske (lL) i duživa vršne liske (lt) (20-30 %). Koristeći opterećenja sa prve četiri rotirane glavne komponente, koje opisuju 90,1% ukupnog varirannja, svi parametric su grupisani u četiri grupe, gde su parametric LW, lL i lt su bili u prvoj grupi, što ukazuje na multikolinearnost između njih. Ovi parametric su takođe imali relativno visoka opterećenja sa prvom kanoničkom promenljivom iz kanoničke diskriminacione analize. Samo jedan od parametara (lt) je odabran naprednom stepenastom diskriminacionom analizom, gde je model se četiri odabrana parametra lista dostigao 26,5 % tačne alokacije, dok je model sa svim proučavanim parametrima ostvario 62,4% tačne alokacije. Potomci polusrodnika su aglomoreirani u tri klastera, pri čemu dva mala potiču od stabala koja se nalaze u blizini zaštićenih dolina malih pritoka Dunava, nasuprot potomaka majčinskim stablima prvog klastera, koja su izložena dominantnim vetrovima.

Primljeno 07.XII.2021.
Odobreno 28. IX 2022.