

LEAF MORPHOMETRIC CHARACTERISTICS VARIABILITY OF DIFFERENT BEECH PROVENANCES IN JUVENILE DEVELOPMENT STAGE

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The taxonomic status of beech from the Balkan Peninsula is not yet clearly defined. There is no agreement among different authors about the morphological characteristics discriminating between the Balkan and European and/or Eastern beech. For most characteristics, the mean values are different but the ranges of variation overlap considerably. Provenance trial of beech established in Serbia, at the locality Debeli Lug, has provided an opportunity for research of interprovenance variability at the level of leaf morphometric characteristics in juvenile development stage. Research included 10 provenances originating from the Western Balkans (Serbian provenance 36 and 38; Croatian provenance 24 and 25; Bosnian provenance 30 and 32) and from Central Europe (German provenance 47 and 49; Austrian provenance 56 and Hungarian provenance 42), where following morphometric characteristics were analyzed: leaf length (Ll), leaf width (Lw), petiole length (Pl), leaf base width on 1 cm (Blw), number of veins – left (Vl), number of veins – right (Vr), distance between 3rd and 4th vein – left (Dv 3-4). The results of this research show existence of clear differentiation among provenances from the Western Balkan and from Central Europe, from the point of leaf dimensions, number of veins and leaf base width.

Key words: Balkan beech, variability, leaves, juvenile development stage

INTRODUCTION

In all Balkan countries beech is one of the most represented indigenous tree species. Except for small isolated populations of Eastern beech (*Fagus orientalis* Lipsky), in south-

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eastern Bulgaria, eastern Greece and the European part of Turkey, it is generally denoted as common or European beech, *Fagus sylvatica* L. (BECKER, 1981). However, local authors consider it mostly to be a separate species *Fagus moesiaca* (Domin, Maly) Czezcott (GÖMÖRY *et al.*, 1999).

Balkan beech was firstly described as a separate taxon by Josef Karel Maly in 1911. The description of this taxon was later completed by CZEZCOTT (1933). Opinions regarding the taxonomical status of this taxon are varied. Frequently, it is described as a separate unit (CZEZCOTT, 1933; FUKAREK, 1954).

MIŠIĆ (1957) considers it a phylogenetical link between *F. sylvatica* and *F. orientalis*. Sometimes, it is considered a hybrid between both species morphologically closer to *F. sylvatica* (BECKER, 1981), a mixture of *F. sylvatica* and *F. orientalis* with the occurrence of transition forms dominated by characters of one of the two species (STOYANOFF, 1932), an ecotype (STĂNESCU, 1979) or identical with the Crimean beech *Fagus taurica* Popl. (DIDUCH, 1992).

The morphological description of *F. moesiaca* is rather vague. There is no agreement among different authors about the morphological traits discriminating between the Balkan and European and/or Eastern beech. For most characters, the mean values are different but the ranges of variation overlap considerably.

In comparison with pure *F. sylvatica*, *F. moesiaca* should have larger leaves with more lateral veins, larger beechnuts and longer cupule peduncle (CZEZCOTT, 1933; MIŠIĆ, 1957; STĂNESCU, 1979). In addition to the morphology, *F. moesiaca* differs from *F. sylvatica* by a high sprouting capacity and a considerably higher frequency of seed years, as well as ecological requirements (MIŠIĆ, 1957). The description of the distribution range of Balkan beech is not unequivocal as well. The main part of the range seems to be the former Yugoslavia (Bosnia, Serbia, Montenegro, Macedonia), Albania, Bulgaria and Greece (FUKAREK, 1954; MIŠIĆ, 1957), but isolated occurrences have been reported from south-eastern Romania, Hungary and even Poland and the former Czechoslovakia (FUKAREK, 1954; STĂNESCU, 1979). Croatian and Slovenian populations are generally considered *F. sylvatica* (GÖMÖRY *et al.*, 1999).

MATERIALS AND METHODS

Networks of European beech provenance trials, which were established in six rounds so far, were aimed at research of genetic variability of populations originating from different regions, of the possibility of adaptation of different provenances at local site conditions and the study of the main environmental factors that determine the degree of adaptability of a population to specific habitat conditions (WUEHLISCH, 2004).

Experimental fields

One of two beech provenance trials in Serbia, located at Debeli Lug (N 44° 19' 34.01", E 21° 52' 20.39") was established in spring of 2007, in the framework of COST Action E52: "Evaluation of the Genetic Resources of Beech for Sustainable Forestry". This site is characterized with altitude of 742 m, East aspect, ridge of uniform slope and humus-siliceous soil. The tests were established by planting 2 and 3 years old seedlings of 22 provenances in a spacing of 2 m between rows and 1 m between plants (WUEHLISCH *et al.*, 2010; ŠIJAČIĆ-NIKOLIĆ *et al.*, 2009; ŠIJAČIĆ-NIKOLIĆ *et al.*, 2010; ŠIJAČIĆ-NIKOLIĆ *et al.*, 2011; STOJNIĆ *et al.*, 2010, STOJNIĆ *et al.*, 2011, VILOTIĆ *et al.*, 2011).

The site where the test is established is characterized by a humid continental climate with cold winters and humid chilly summers. The average annual air temperature is 9.3° C, average maximum temperature is 15.1° C, while the average minimum temperature is 4.1° C. The highest maximum temperature measured in Debeli Lug, in period from 1994-2004 was 41.3° C (2012). Average growing season temperature was 15.3° C, which indicates that the area is favorable for the development of forest vegetation.

Average number of days with precipitation (less than 0.1 mm per day) was 118.1 days/year, and average annual precipitation is 829 mm. During the growing season (April – September) 455 mm of rain fall (54% of the total amount of annual rainfall). The most prevalent wind is south-east (21%) and the north-west wind (19%), wind power is low and rarely exceeds 3 m/sec.

Plant material

Analysis of morphometric traits of leaves of different beech provenances was conducted in order to assess inter-provenance variability in the juvenile development phase (plant age of seven years).

Research included 10 provenances originating from the Western Balkans (Serbian provenance 36 and 38; Croatian provenance 24 and 25; Bosnian provenance 30 and 32) and from Central Europe (German provenance 47 and 49; Austrian provenance 56 and Hungarian provenance 42).

Characteristics of ten analyzed provenances of beech are shown in Table 1.

Table 1. Basic data on studied beech provenances

No	Provenance	Country of origine	Longitude	Latitude	Altitude (m)	Exposition	Year everage temp. (°C)	Year everage precip. (mm)	Year of seed colec.
24	Sjeverni Dilj Cagliński	Croatia	18° 01'	45° 17'	350	NE	10.8	779	2003
25	Vrani kamen 12a, 15b	Croatia	17° 19'	45° 37'	600	NS	8.5	470	2003
30	Tajan, Žepče	B&H	18° 03'	44° 23'	700	N	10.3	804	2003
32	Crni Vrh	B&H	17° 59'	44° 33'	500	N	9.6	1069	2003
36	Fruška Gora	Serbia	19° 55'	45° 10'	370	N-NE	11.2	782	2003
38	Kopaonik	Serbia	20° 50'	43° 10'	510	/	/	/	2003
42	Valkonya 19A	Hungary	16° 45'	46° 30'	300	S	9.5	800	2003
47	Schelklingen, IX 23-25 81023	Germany	10° 00'	48° 00'	650	/	6.0	840	2003
49	Hasbruch 81001	Germany	08° 26'	53° 08'	35	NS	8.6	760	2003
56	Scharstein, Mitterndorf	Austria	13° 58'	47° 54'	480	W	7.4	1263	2003

Morphometric characteristics of leaves

The research were conducted on a sample of ten randomly selected trees from each provenance. At the end of the growing season, 20 leaves were collected from each tree (200 leaves per provenance). Herbaria material was used for measuring of the following parameters: leaf length (Ll), leaf width (Lw), petiole length (Pl), leaf base width on 1 cm from petiole base (Blw), number of veins – left (Vl), number of veins – right (Vr), distance between 3rd and 4th vein - left (Dv 3-4), Figure 1.

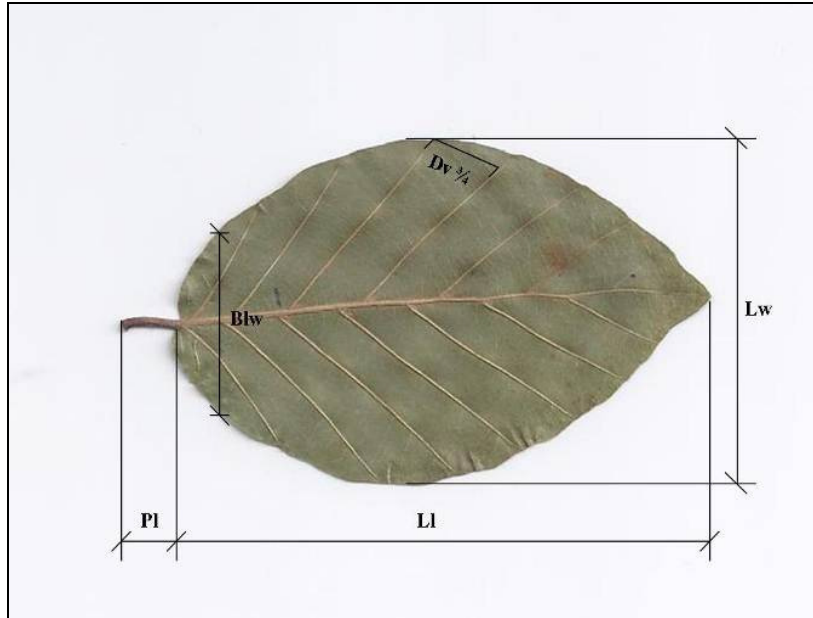


Figure 1. Analyzed morphometric characteristics of leaves

Statistical analysis

For measured morphometric characteristics of leaves, 14.000 data in total, the following statistical parameters and statistical analysis were applied: min and max value, average value, standard deviation and LSD-test.

Cluster analysis was performed based on data for seven morphometric characteristics of leaves, with the aim of determining the genetic similarity, that is, distance among the analyzed provenances. The data were processed by the software package „Statistica“.

RESULTS

Variability of beech provenances leaf traits (min and max value, average value and LSD-test) is shown in Table 2.

Table 2. Descriptive statistics and LSD-test for different morphometric characteristics of leaves

Leaf length (mm)				Leaf width (mm)			
Pr.	X _{min} X _{max}	Average	Homogenous groups	Pr.	X _{min} X _{max}	Average	Homogenous groups
56	35-66	50.13	X	56	22-47	30.71	X
49	36-70	53.79	X	30	22-43	31.23	X
30	37-70	53.88	X	42	23-45	33.08	X
42	42-78	54.73	XX	49	20-51	34.00	XX
47	41-72	56.40	X	47	25-45	34.60	XX
38	42-77	60.65	X	38	23-47	36.02	X
36	45-88	62.26	XX	25	26-53	38.94	X
24	45-77	62.42	XX	36	30-50	39.14	X
25	46-79	63.13	X	24	29-54	39.35	X
32	52-91	65.89	X	32	30-61	42.17	X
Petiole length (mm)				Base leaf width on 1 cm (from petiole base)			
30	3-7	4.73	X	30	12-32	22.09	X
56	3-8	5.19	X	56	15-40	22.51	X
42	3-10	5.59	X	42	15-35	24.55	X
36	3-9	5.71	X	38	15-35	24.62	X
49	3-10	5.87	XX	47	16-38	25.47	XX
25	3-11	6.16	XX	49	8-43	26.39	XX
32	4-8	6.30	X	36	18-38	27.50	XX
47	4-8	6.46	X	25	17-40	27.92	X
24	4-10	6.47	X	24	20-44	28.63	XX
38	4-10	7.12	X	32	15-52	29.63	X
Number of veins – left				Number of veins – right			
47	6-9	7.55	X	47	6-9	7.56	X
56	6-9	7.62	X	56	6-9	7.64	X
24	6-9	7.62	X	24	6-9	7.66	X
30	6-9	7.74	XX	30	6-9	7.73	XX
42	6-9	7.78	XX	42	6-9	7.77	XX
49	5-10	7.90	XX	49	6-10	7.90	XX
32	6-10	7.94	XX	25	7-10	7.92	XX
36	5-10	7.95	XX	36	5-9	7.94	XX
25	6-10	7.96	XX	32	6-10	7.95	XX
38	6-10	8.06	X	38	7-10	8.06	X

Table 2. Descriptive statistics and LSD-test for different morphometric characteristics of leave (continue)

Distance between 3 rd and 4 th vein - left			
56	4-9	6.10	X
42	5-10	6.28	X
30	4-8	6.33	X
38	5-10	6.65	X
49	3-9	6.65	X
47	5-8	6.77	X
25	5-8	7.15	X
24	6-9	7.2	X
36	5-9	7.23	XX
32	5-10	7.47	X

According to the results, it can be concluded that minimum leaf length is detected for Austrian (56) and German (49) provenance and it is 35 (36) mm, while maximum leaf length of 91 mm is recorded for Bosnian provenance (32).

Average values of leaf length are between 50.13 mm (Austrian provenance 56) and 65.89 mm (Bosnian provenance 32). Leaf width is less variable trait comparing to the leaf length. Minimum value of this trait (20-22 mm) is recorded for German (49), Austrian (56) and Bosnian (30) provenance. Maximum leaf width is recorded for Bosnian provenance (32) and it has a value of 61 mm.

Average values of leaf width are between 30.71 mm for Austrian provenance 56 and 42.17 mm for Bosnian provenance 32. Petiole length is a trait with average values between 4.73 mm (Bosnian provenance 30) and 7.12 mm (Serbian provenance 38).

Average values of leaf base width are between 22.09 mm (Bosnian provenance 30) and 29.63 mm (Bosnian provenance 32). The highest average value of number of veins, on both leaf sides, is recorded for Serbian provenance 38 (8.06), and the lowest values of this trait is recorded for German provenance 47 (7.44).

Average values of the distance between third and fourth vein – left are between 6.10 mm (Austrian provenance 47) and 7.47 mm (Bosnian provenance 32).

Table 3. Analysis of variance for different morphometric characteristics of beech leaves

Between provenances			
Morph-metric characteristics of leaves	Mean Square	F-ratio	P-Value
Leaf length (mm)	2689,87	45,35	0
Leaf width (mm)	1477,34	55,33	0
Petiole length (mm)	48,05	29,19	0
Leaf base width on 1 cm (from petiole base)	643,23	25,37	0
Number of veins – left	3,06	4,27	0
Number of veins – right	2,68	4,12	0
Distance between 3 rd and 4 th vein - left	21,56	27,23	0

Variability of beech leaves from different provenances is shown in Figure 2.

Results of the analysis of variance for different provenances are statistically significant for all seven analyzed traits (P-Value < 0,05).

According to the diagram of cluster analysis (Diagram 1), it can be concluded that the shortest linkage distance is recorded between Serbian (36) and Croatian (24 and 25) provenances, and two German provenances (47 and 42).

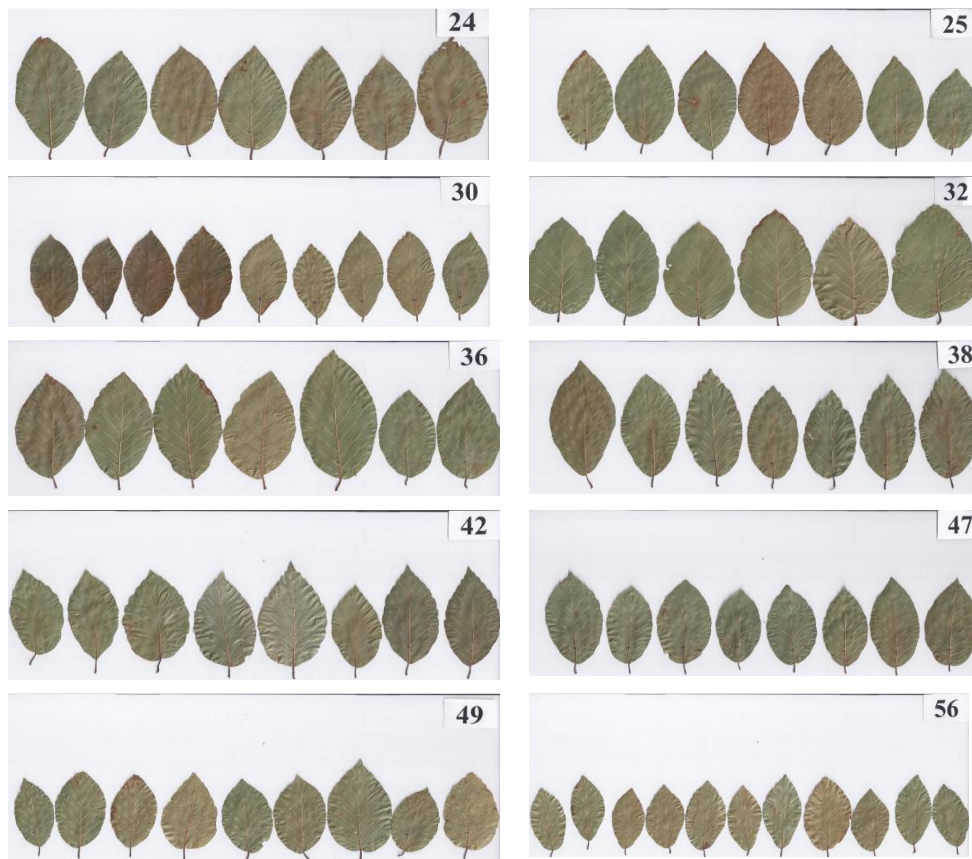


Figure 2. Variability of beech leaves from different provenances

Two homogeneous groups can be defined from all analyzed provenances: the first one is consisted of Croatian provenances (24 and 25), Serbian provenance (36) and provenance from Bosnia and Herzegovina (32) and the second one includes German provenances (47 and 49),

Austrian provenance (56), Hungarian provenance (42), Bosnian provenance (30) and Serbian provenance (38).

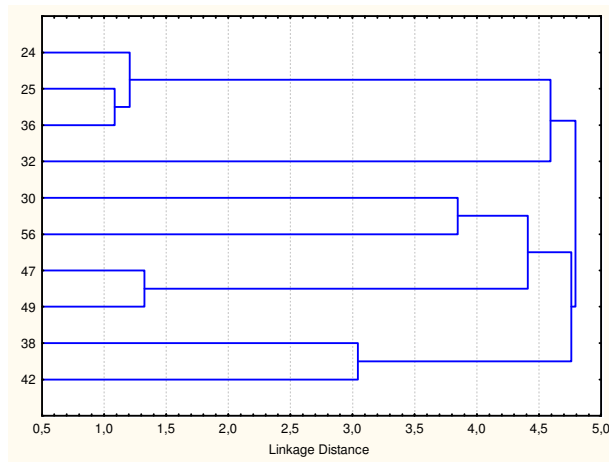


Diagram 1. Cluster analysis diagram based on the analysis of 7 morphometric foliar parameters

DISCUSSION

Shape and size of leaves is an attribute frequently used in taxonomical or ecological research (ZOLKOŠ and WŁODZIMIERZ, 2009). It may be used in estimation of negative impact of environmental factors on trees and their conditions (PREMOLI, 1996; MOLLER, 1999; HODAR, 2002; BLACK-SAMUELSSON and ANDERSSON, 2003; FREENAM *et al.*, 2005). Leaves within a tree crown are differentiated by the shape, size and thickness of leaf blade may be changed by many factors, connected with light intensity which is different in various parts of tree crown and is related to the position of tree in tree stand (BARNA, 2004) as well as to the density of stand (JACK and LONG, 1991) or crowns (FRAZER *et al.*, 2000). If they don't be collected randomly for biometric studies, the results may lead to wrong interpretations due to omitting the intra-individual diversity (COWART and GRAHAM, 1999).

Based on the foregoing, the examination of leaf traits variability in the juvenile stage of development within the provenance trial has advantages such as uniform habitat conditions, a relatively small canopy in which all leaves are exposed to same amount of light and consistent position of all trees, bearing in mind their age.

In this way the influence of environmental factors is uniform and the variability is, for the most part, the specificity of the gene pool of each provenance. Bearing in mind that the dimensions of beech leaves change during ontogeny (MIŠIĆ, 1957) initiated research is necessary to be continued in the future.

CONCLUSION

According to the results of conducted research of variability of beech leaf morphometric traits from ten provenances originating from the western Balkans and Central Europe following conclusions can be brought:

- Calculated differences between average values of analyzed leaf morph-metric traits are statistically significant;
- There is a clear differentiation between provenances from the Western Balkans and Central Europe from the point of leaves dimensions;
- Provenances from the Western Balkans, from Bosnia and Herzegovina (32), Croatia (provenances 24 and 25) and Serbian (provenance 36) are characterized with higher values of leaves traits that provenances from Austria (56), Germany (47 and 49) and Hungary (42);
- There is a clear differentiation between provenances from the Western Balkans and Central Europe from the point of the number of leaf veins;
- Provenances originating from the Western Balkans, Serbian provenances (36 and 38), Bosnian provenance (32) and Croatian provenance (25) show higher values for the average number of veins on both leaf sides comparing with provenances from Germany (47) and Austria (56);
- There is a clear differentiation between provenances from the Western Balkans and Central Europe from the point of the distance between the third and fourth leaf vein;
- Provenances from the Western Balkans, Bosnian (32), Serbian (36) and Croatian (24 and 25) provenances show higher average values for the distance between the third and fourth leaf vein comparing to the provenances from Austria (56), Hungary (42) and Bosnia and Herzegovina (30);
- Bosnian provenance 30 shows significantly lower values of analyzed leaf morphometric traits comparing to the rest of provenances from the Western Balkan.

The results of this research are in accordance with previously assessed variability of morphometric traits of beech leaves (CZECZOTT, 1933; MIŠIĆ, 1957; STĂNESCU, 1979) and with genetic differentiation defined applying isoenzyme markers for 57 beech populations originating from a wide geographical area from the foothills of the Eastern Alps over the Balkan Peninsula (GÖMÖRY *et al.*, 1999).

Taxonomical status of Balkan beech (*Fagus moesiaca* Domin, Maly/Czeczott.) which is, according to JOVANOVIĆ (2000), a separate species or separate taxon, still stays an open question, according to GÖMÖRY *et al.* (1999).

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VARIJABILNOST MORFOMETRIJSKIH KARAKTERISTIKA LISTA RAZLIČITIH PROVENIJENCIJA BUKVE U JUVENILNOJ ETAPI RAZVOJA

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Izvod

Taksonomski status bukve sa Balkanskog poluostrva još uvek nije jasno definisan. Ne postoji saglasnost autora o razlikama morfoloških karakteristika između Balkanske i Evropske i/ili Istočne bukve. Provenijenični test bukve, osnovan u Srbiji, na lokalitetu Debeli lug, pružio je mogućnost za istraživanje međuprovenijenične varijabilnosti na nivou morfometrijskih karakteristika lista. Istraživanje se odnosilo na juvenilnu etapu razvoja i obuhvatilo je 10 provenijencija sa područja Zapadnog Balkana i centralne Evrope. Analizirana su sledeća morfometrijska svojstva lista: dužina i širina lista, dužina peteljke, širina osnove lista (na 1 cm udaljenosti od baze peteljke), broj bočnih nerava – levo i desno (u odnosu na glavni nerv) i razmak između trećeg i četvrtog nerva – levo. Rezultati istraživanja pokazuju da postoji jasna diferenciranost između provenijencija sa područja Zapadnog Balkana i centralne Evrope, u pogledu dimenzija lista, širine lisne osnove i broja bočnih lisnih nerava.

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