GENETIC DIFFERENTIATION IN SEED STANDS OF EUROPEAN BEECH (*Fagus sylvatica* L.) IN PART OF BOSNIA AND HERZEGOVINA

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Beech (*Fagus sylvatica* L.) is one of the most important forest trees in Bosnia and Herzegovina in both economic and environmental terms. The total area of forest in which beech is present is approx. 1,652,400 ha. There is a proportionate need to plant new forests and produce genetically high quality seed and saplings.Biochemical analysis of the genetic structure of eight populations of beech using ten enzyme systems from 16 isoenzyme gene loci revealed significant differences between the populations analyzed. Variance levels were high in some gene loci, while in some populations monomorphism was recorded only for individual gene loci. The average number of alleles per locus ranged from 2.1875 to 2.5625, and the average number of genotypes per locus varied from 2.6875 to 3.2500. The multilocus genetic diversity at the population level ranged from 63.276 to 162.001, and the genofund diversity varied from 1.2708 to 1.3416.The average differentiation value obtained for all populations was fairly low (Dj=5.81), indicating a percentage of overall variance of about 94.194%.

Key words: European beech (*Fagus sylvatica* L. s.l.), seed stand, variability, isoenzyme, differentiation, Bosnia and Herzegovina

INTRODUCTION

The use of seed from forest tree species of known origin is explicitly prescribed by European Union regulations – 1999/105/EC (ANONYMOUS 1999). These set out the importance of demarcation of regions of provenance to control the movement of forest reproductive material and to avoid its being used in unsuitable environmental conditions (BALLIAN 2011). Seed regions are thus the first and fundamental level in the management of genetic resources earmarked for

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the production of forest reproductive material. Here one should primarily take into account the need for the most effective management of genetic resources in regions where there is greater diversity, and for the indigenous genofund to be protected from unnatural introgressions that could alter or destroy the genetic structure of indigenous populations of particularly high value.

On the basis of these principles, most European countries have applied the latest geno-environmental research methods to define regions of provenance for the most important tree species. In Serbia, analysis of regional genetic diversity using RAPD markers – 28 primer from 27 natural populations of beech led to the identification of six regions of provenance (IVETIĆ *et al.* 2012). Also, some of the physiological tests were carried out indicating difference in transpiration and efficiency of water utilisation (STOJNIĆ *et al.* 2012). Analysis of seed and seedlings traits showed significant difference (ŠIJAČIĆ-NIKOLIĆ *et al.* 2007) In Croatia, five seed zones for beech were identified within two districts, which were further subdivided into 12 regions (GRAČAN 2003). Heading south, in Greece, morphological traits and gene markers revealed a possible influence of *F. orientalis* on the east side of Rodopi and at the low elevations, while characters resembling *F. sylvatica* were observed mainly on the western part of the mountains and in higher altitudes. It can be considered as the end of natural area of Fagus sylvatica (PAPAGEORGIOU *et al.* 2007).

Beech (*Fagus sylvatica* L.) is the species with the most extensive range of Bosnia and Herzegovina now found in 60% of the country's forest area (LOJO and BALIĆ 2011), constituting 46% of all tall forest in mixed woodlands with fir, or fir and spruce, or about 1,652,400 ha.

The Dinaric region is very distinctive in both morphology and climate, which is the basic reason why this area contains a major centre of plant and genetic diversity, which has also left its mark on beech (BRUS 1999, GÖMORY *et al.* 1999; BALLIAN *et al.* 2012).   This has been confirmed by research into beech conducted by BRUS (1999), GÖMORY *et al.* (1999) and BALLIAN *et al.* (2012), which included a number of populations from Bosnia and Herzegovina. These studies yielded high variance values, which is not characteristic of central Europe but is typical of the Balkans (ŠIJAČIĆ-NIKOLIĆ *et al.* 2013) It is found over a wide altitudinal range in a variety of plant communities, which has affected its adaptive genetic variance. Beech populations in Bosnia and Herzegovina are contiguous or overlap with beech populations in Serbia which several authorities describe as of a separate species (*Fagus moesiaca* /Domin, Maly/Czeczott) (ISAJEV 2005) or as a subspecies of European beech, *Fagus sylvatica* subsp. *moesiaca* /Maly/ Czeczott (GÖMÖRY *et al.* 1999, KAJBA 2003).   Where areas are contiguous, spontaneous hybridization is constantly taking place, which results in hybrids with specific characteristics that further complicate the genetic study of beech in Bosnia and Herzegovina, and thereby the principles of transfer of reproductive material.

In recent decades, as a result in particular of changing forest management habits as well as increasing climate change, beech is gaining in importance year on year, which is reflected in the creation of an indigenous seed base. Thus far 13 seed objects have been selected, which are also objects of prime importance for the preservation of the indigenous gene pool. As a result of the very poor structure in many natural beech forests, which are at various stages of degradation, we shall have to resort in future to artificial regeneration. For this, the genetic count of all potential sources of reproductive material is required. This research will thus form the basis for the future planned use of beech reproductive material, the preservation of its indigenous genofund, and the definition of regions of provenance.

MATERIALS AND METHODS

Using the recognized methodology for isoenzyme analysis, studies were conducted in six natural populations of European beech in Bosnia and Herzegovina (Table 1).

Table 1. Basic data on the beech populations studied in Bosnia and Herzegovina

Population	Lokaliti	Altitude (m)	Longitude	Latitude	Meteo station (for the population) and altitude in m	Distance betwen meteostation and population (aproxim.)	Area	Region	Average Temperature in ºC		Average perticipation in mm		Vegetation period in days
									Year	Betw. IV. – IX.	Year	Betw. IV. – IX.	
Bihać	Risovac	657	44° 43' 11"	15° 59' 01"	Bihać (246)	10 km	Cazinska krajina	-	10,7	16,8	1347	586	204
Bugojno	Skrta nišan	729	43° 58' 24"	17° 30' 36"	Bugojno (562)	9 km	Srednje bosansko	Vranički	8,8	14,8	826	383	174
Busovača	Tisovac	624	44° 03' 11"	17° 50' 59"	Fojnica (584)	10 km	Srednje bosansko	Vranički	8,2	14,1	1234	522	165
Kakanj	Brnjic	646	44° 12' 09"	18° 04' 35"	Zenica (344)	25 km	Srednjo bosansko	Sarajevsko - zenički	10,3	16,6	804	416	197
Olovo	Duboštica	959	44° 16' 50"	18° 18' 39"	Maoča (335)	12 km	Zavidovičko-tesličko	-	9,8	15,8	1183	642	183
Prenj	Rakov laz	1158	43° 32' 24"	18° 01' 09"	Konjic (280)	12 km	Submediteransko planinsko	-	11,1	16,9	1404	496	205

Isoenzyme markers were used to analyze the genetic structure, while the interpretation of the zymogram followed the protocol provided by KONNERT (2004) (Table 2).

Ten enzyme systems were analyzed, revealing the polymorphic nature of the gene loci (Table 2). A statistical analysis of the genotypes and allele frequency for all 17 gene loci studied was conducted for each of the six populations studied. The degree of genetic variability within and between populations was determined using a variety of genetic parameters, such as:

- genetic multiplicity: A/L = average number of alleles per locus and G/L = average number of genotypes per locus

- genetic diversity: (v_p) = gene pool diversity; and v_{gam} = multilocal diversity (GREGORIUS 1978);

- heterozygosity (H_{st}= true H_{te}= theoretical heterozygosity (NEI 1978);

- fixation index

- subpopulation differentiation (Dj.), GREGORIUS and ROBERDS (1986).

Table 2. Enzyme systems, E.C. referential number, scored loci and number of alleles

Enzyme systems	E.C. Number	Scored loci	Number of alleles
Phosphoglucose isomerase	5.3.1.9	*Pgi –B*	3,
Glutamate oxalacetate transminase	2.6.1.1	*Got –A, –B,*	3, 3,
Acotinase	4.2.1.3	*Aco – A, -B,*	3, 4,
Phosphoglucomutase	2.7.5.1	*Pgm –A,*	4,
Menadion reductase	1.6.99.2	*Mnr –A,*	5,
Isocitrate dehydrogenase	1.1.1.42	*Idh –A,*	3,
Malatdehidrogenase	1.1.1.37	Mdh –B, -C.	5, 2,
6Phosphogluconate dehydrogenase	1.1.1.44	*6-Pgdh –A, –B, -C,*	3, 2, 4,
Shikimate dehydrogenase	1.1.1.25	*Sdh –A,*	3,
Peroxidase	1.11.1.7	*Per –A, -B,*	2, 3,
Total 10	-	16	52

The statistical analysis was conducted using SAS macros software (MACGEN – STAUBER and HERTEL 1997: http://www.mol.schuttle.de/wspc/genetik1. htm<).

RESULTS AND DISCUSSION

Allele variability

Allele frequency in all 16 gene loci analyzed revealed a certain degree of polymorphism, though in certain populations gene locus *Pgi* demonstrated monomorphism, as in the case of the Bihać, Bugojno, Busovača and Kakanj populations. The allele frequencies for the gene loci analyzed are set out in Table 3.

Table 3 also shows that rare alleles appear in the populations. The rare allele *Pgi-B1* appears in the Olovo population, while in the research by BALLIAN *et al.* (2012) it was found in the Drvar population. Allele *Aco-A1* appears only in the Prenj population, but was found in the Olovo population in a previous study (BALLIAN *et al.* 2012). It is worth noting here the allele *Aco-A3*, which appeared infrequently; according to BALLIAN *et al.* (2012) it features principally in the populations of western Bosnia by the Croatian border, and in the northern Bosnian population of Tešanj, while in this study it appeared in the Bugojno and Busovača populations. Other rare alleles found were *Mdh-B2* in the Kakanj population, *6-Pgdh-A1* in the Bihać population, and *Sdh-A2* in the Bugojno and Olovo populations; allele *Sdh-A1* was not recorded in this study. Another specific feature is the monomorphism of gene locus *Mnr – A* in the Bugojno population.

We should not omit a reference here to the presence of rare alleles in gene locus *Idh-A*. These rare alleles and specific monomorphism may serve as identifiers for beech seed objects in Bosnia and Herzegovina.

Table 3. Allele frequencies

Gene loci	Allels	Bihać	Bugojno	Busovača	Kakanj	Olovo	Prenj
Pgi	*B1*	0.0000	0.0000	0.0000	0.0000	0.0100	0.0000
	B2	1.0000	1.0000	1.0000	1.0000	0.9600	0.9900
	B3	0.0000	0.0000	0.0000	0.0000	0.0300	0.0100
Got	*A1*	0.0500	0.2100	0.0918	0.0900	0.0600	0.2813
	A2	0.9500	0.7900	0.9082	0.9100	0.9400	0.6979
	A3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208
Got	*B1*	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
	B2	0.2300	0.1800	0.3100	0.3200	0.3500	0.3600
	B3	0.7700	0.8200	0.6900	0.6700	0.6500	0.6400
Aco	*A1*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0100
	A2	1.0000	0.9688	0.9490	0.9700	0.9800	0.9500
	A3	0.0000	0.0104	0.0204	0.0000	0.0000	0.0000
	A4	0.0000	0.0208	0.0306	0.0300	0.0200	0.0400
Aco	*B2*	0.1400	0.1300	0.2347	0.1900	0.1300	0.1400
	B3	0.8500	0.8200	0.7653	0.7900	0.8100	0.8500
	B4	0.0100	0.0500	0.0000	0.0200	0.0600	0.0100
Pgm	*A2*	0.1500	0.0600	0.0500	0.0600	0.2700	0.0800
	A3	0.8500	0.9400	0.9500	0.9400	0.7300	0.9200
Mnr	*A1*	0.0200	0.0000	0.0000	0.0100	0.0300	0.0100
	A2	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
	A3	0.9100	1.0000	0.9800	0.9400	0.9200	0.9000
	A4	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
	A5	0.0700	0.0000	0.0200	0.0300	0.0500	0.0900
Idh	*A2*	0.3400	0.5000	0.3500	0.3000	0.2000	0.2700
	A3	0.6600	0.5000	0.6500	0.6900	0.7900	0.7300
	A4	0.0000	0.0000	0.0000	0.0100	0.0100	0.0000
Mdh	*B1*	0.0100	0.0300	0.1400	0.0600	0.0600	0.0200
	B2	0.0000	0.0000	0.0000	0.0300	0.0000	0.0000
	B3	0.9000	0.9100	0.6500	0.7800	0.8000	0.9100
	B4	0.0900	0.0600	0.2100	0.1300	0.1400	0.0700
Mdh	*C1*	0.3600	0.1531	0.2100	0.1900	0.2100	0.0816
	C2	0.6400	0.8469	0.7900	0.8100	0.7900	0.9184
6-Pgdh	*A1*	0.0300	0.0000	0.0000	0.0000	0.0000	0.0000
	A2	0.7400	0.9300	0.9100	0.9500	0.9184	0.9900
	A4	0.2300	0.0700	0.0900	0.0500	0.0816	0.0100

6-Pgdh	B1	0.1100	0.1400	0.1100	0.0700	0.1100	0.0500
	B2	0.8900	0.8600	0.8900	0.9300	0.8900	0.9500
6-Pgdh	C1	0.7200	0.8900	0.8500	0.9100	0.8600	0.8700
	C3	0.0500	0.0100	0.1100	0.0100	0.0000	0.0600
	C4	0.1400	0.1000	0.0400	0.0500	0.1100	0.0400
	C5	0.0900	0.0000	0.0000	0.0300	0.0300	0.0300
Sdh	A2	0.0000	0.0200	0.0000	0.0000	0.0100	0.0000
	A3	1.0000	0.9700	0.9500	0.9700	0.9800	0.9700
	A5	0.0000	0.0100	0.0500	0.0300	0.0100	0.0300
Per	A1	0.2917	0.4359	0.4796	0.4762	0.3111	0.4400
	A2	0.7083	0.5641	0.5204	0.5238	0.6889	0.5600
Per	B1	0.1735	0.1500	0.2000	0.0600	0.1400	0.1500
	B2	0.7551	0.8500	0.7900	0.9400	0.8200	0.7700
	B3	0.0714	0.0000	0.0100	0.0000	0.0400	0.0800

Genetic diversity

The values obtained for intra-population genetic variability are set out in table 4. The median number of alleles per locus varied from 2.1875 in the Bugojno and Busovača populations to 2.5625 in the Kakanj population. This high value for the Kakanj population cannot be linked to the number of individuals analyzed, which was the same for all populations (50 trees per population). It is of interest that the average number of alleles per locus in the Kakanj population is probably directly associated with its origin, which is indigenous, located in the central Dinaric Alps shading into the Pannonian foothills of northern Bosnia and Herzegovina. The other populations in this study also showed high values for this trait. The average number of genotypes in a locus was fairly high in all except the Busovača population, which may be related to its location and the specific action of the selection process that prevails in the central Bosnian zone with its distinctive geological structure, with the Vranica massif, but above all by past human action.

Table 4. Average number of alleles and genotypes in a locus, true heterozygosity (H_{st}) and theoretical heterozygosity (H_{te}), fixation index

Population	Multiplicity		Heterozygosity		Fiksation index
	A/L	G/L	H_{st}	H_{te}	
Bihać	2.5000	3.0625	0.2276	0.2546	0,0992
Bugojno	2.1875	2.7500	0.1988	0.2131	0,0395
Busovača	2.1875	2.6875	0.2905	0.2525	-0,1155
Kakanj	2.5625	3.0625	0.2093	0.2153	0,0420
Olovo	2.5000	3.2500	0.2387	0.2453	0,0324
Prenj	2.5000	3.0000	0.2188	0.2243	-0,0031

True and theoretical heterozygosity were also analyzed. True heterozygosity varied from 0.1988 in the Bugojno population to 0.2905 in the Busovača population. These values are significantly higher than in the research conducted by BALLIAN *et al.* (2012), indicating the very good genetic structure in the seed objects that were analyzed. The theoretical heterozygosity values were greater than the true values in four populations, indicating positive values for the fixation coefficient and the presence of inbreeding in the populations studied (table 4). In the Busovača population, theoretical heterozygosity was lower than true, suggesting the presence of inbreeding in that and the Prenj population with a low negative value.

The heterozygosity value of populations from south-eastern Europe, using various genetic markers, ranged from 0.2 to 0.4 (PAULE *and* GÖMÖRY 1999), while the median number of loci was about 2.5, which corresponds to the interval of our investigations. The average heterozygosity value (H_0) for 13 beech populations in Slovenia ranged from 0.220 to 0.273 (BRUS 1999).

Table 5. Diversity and differentiation values

Population	Diversity		Differentiation	Subpopulation differentiation
	V_{gem}	$V_p(n_e)$	δ_T	Dj (%)
Bihać	162.001	1.3416	0.2572	8.28
Bugojno	63.276	1.2708	0.2154	5.05
Busovača	158.198	1.3378	0.2551	5.59
Kakanj	65.639	1.2712	0.2155	4.36
Olovo	116.196	1.3250	0.2478	6.14
Prenj	82.645	1.2891	0.2266	5.41

The genetic variability parameters indicate the presence of significant differences in allele frequency among six beech seed stands (populations) in Bosnia and Herzegovina. The greatest diversity was found in the Bihać population, which is also the westernmost (V_{gem}=162.001 and V_p=1.3416) (table 5), while in the research by BALLIAN *et al.* (2012) the highest value was found in a population from eastern Bosnia (Igrište). A very low diversity value was recorded in the Bugojno population, well below the average of the other populations studied (V_{gem}=63.276 and V_p=1.2708), though a higher value was expected, given its location. The other populations had diversity values greater than the V_{gem}=65.639 recorded in the Kakanj population, to 158.198 in the Busovača population (table 5), unlike earlier studies (BALLIAN *et al.* 2012).

Studies of the polymorphism of the chloroplast genome of European beech from 85 populations, over almost the entire range of beech, using the PCR method (DEMESURE *et al.* 1996), indicated a high degree of genetic differentiation (Gst=0.831). These results are contrary to those of the nuclear differentiation by isoenzymes, suggesting that very little exchange of genetic material by seed has taken place. PAULE and GOMORY (1997) did not obtain high values for interpopulation variation; 95% of variability was intrapopulation.

Analyzing the genetic differentiation results (δ_T) given in table 5 reveals that differentiation within populations ranges from 0.2154 in the Bugojno population to 0.2572 in the Bihać population, the highest range in the populations studied. The greatest mean differentiation of the genofund within populations (subpopulations) (Dj) was that of the Bihać population, with

8.28% (table 5), and the least was that of the Kakanj population, with 4.36%, which are greater values than those found in the research conducted by BALLIAN et al. (2012). It is interesting to note, however, that in both bases the western populations showed the greatest differentiation. The median value is Dj 5.81%, indicating the interpopulation differentiation in this study. The result obtained in this study demonstrates that 5.81% of total genetic diversity can be ascribed to differentiation between populations, and the balance of 94.19% to allele variance between individuals in a given population.

The results of studies of the genetic markers of beech and their dependence on the distribution of the populations vary. BELLETTI and LANTERI (1996) studied ten isoenzyme loci in beech stands in north-western Italy which did not indicate any relationship between genotypes and geographical distance, with just one old stand (Palanfrè) differing from the others. At the same time, LEONARDI and MENOZZI (1995) found significant differences between Italian provenances from north to south. COMPS et at. (1998) studied populations from the Alps to the Pannonian plain in Hungary, finding significant interpopulation differences among the

BRUS (1999) conducted comprehensive studies of genetic diversity using isoenzymes in beech populations from central and south-eastern Europe. Though the genetic differentiation was low, it increased from north-west to south-east.

The results of earlier studies and those set out in this paper indicate that when conducting artificial regeneration or replanting of beech forest, the following factors should be taken into account:

- the genetic structure of the seed, which should not be poor, and must correspond to the local populations identified on the basis of seed regions (regions of provenance), as proposed for Croatia by GRAČAN et al. (1999), while in Bosnia and Herzegovina these studies and the environmental vegetational regionalization provided by STEFANOVIĆ et al. (1983) should be observed;
- the genetic basis for the adaptation of European beech populations at various altitudes. The presence of different genetic structures among beech populations has been identified, and the frequency of certain alleles changes significantly from lower to higher altitudes. The results of these studies show that reproductive material from beech stands from lower altitudes must be tested when transferring to higher altitudes, and vice versa (ISAJEV 2006);
- the optimal number of plants in the regeneration process that will represent the genetic structure of the population in later (mature) stadia ZIEHE et al. (1989);
- the use of beech seed collected from as many trees as possible evenly distributed in the population and collected in different years. This would reduce the potential for the harmful effects of inbreeding, which is found in every population to a greater or lesser degree (HADŽISELIMOVIĆ 2005). It would thus better preserve the genetic structure of the population, as it could be that for one reason or another some of the trees in a given collection year do not flower or are infertile (KONNERT 1996), or that fertilization is unidirectional as a result of the wind always blowing from the same quarter;
- maintaining the greatest possibility heterogeneity in the plantations, using minimal propagation techniques, cultivation measures or other works in the regenerated beech population. This means that it is desirable to allow natural selection to

eliminate as many genotypes as possible that are unsuited to a given habitat (MUONA *et al.* 1988);

- the need to encompass all alleles and genotypes regardless of which method is chosen to regenerate beech woodlands. In countries or regions such as Bosnia and Herzegovina, therefore, with extremely diverse environmental conditions in a very small area (STEFANOVIĆ *et al.* 1983) and many beech forest communities, the method of multiple population enrichment should be applied (BALLIAN and KAJBA 2011).

The fundamental premise in the artificial regeneration of beech populations in Bosnia and Herzegovina, however, should be to pay particular attention to the local populations or those from a given region. Since beech demonstrates great plasticity and adaptive potential, the genetic basis of these features in Bosnia and Herzegovina should be tested by setting up a series of field studies and provenance tests, as has happened in other countries in the region (GRAČAN 2003, STOJNIĆ 2012). The first steps towards this have already been taken near Kakanj by initiating an international test of beech provenance (BALLIAN and ZUKIĆ 2011).

CONCLUSION

The values obtained for the number of alleles and genotypes per locus, theoretical and true heterozygosity, fixation index and subpopulation differentiation are within the range of those obtained in earlier studies. In some gene loci the variability was high, but in others only certain gene loci displayed monomorphism. True and theoretical heterozygosity demonstrated the presence of inbreeding in many of the populations analyzed. Rare alleles were recorded at gene locus *Idh-A* which could serve as identifiers for beech seed objects in Bosnia and Herzegovina. The genetic variability parameters revealed considerable diversity in allele frequency between six seed stands (populations) of beech in Bosnia and Herzegovina, which constitutes the basis for further activity to define the limits of transfer of forest reproductive material within the borders of Bosnia and Herzegovina.

REFERENCES

ANONYMOUS (1999): Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material. Official Journal L 011 (15/01/2000): 17-40.

BALLIAN, D. (2011): Basic principles of zoning of seed zones on genetic principles. Croatian academy of science and arts, Works: 12/13: p. 18-41.

BALLIAN, D., F. BOGUNIĆ, O. MUJEZINOVIĆ, D. KAJBA (2012): Genetic diferentiation of beech (*Fagus sylvatica* L.) in Bosnia and Herzegovina. Journal of Forestry: 11/12: p. 589-598.

BALLIAN, D., D. KAJBA (2011): Forest tree breeding and conservation. University book p. 299

BALLIAN, D., N. ZUKIĆ (2011): Analysis of the growth of common beech provenances (Fagus sylvatica L.) in the international experiment near Kakanj. aPpaer of Faculty of foresty Sarajevo *41* (2):75-91.

BELLETTI. P., S. LANTERI (1996): Allozyme variation among European beech (*Fagus sylvatica* L.) stands in Piedmont, North-Western Italy. Silvae Genet., *45* (1): 33-37.

BEUS, V. (1997): Forest communities. Government of Federation of B&H, Department for education, science, culture and sport. Sarajevo-Publishing, Sarajevo.

BRUS, R. (1999): Genetic variation of beech (Fagus sylvatica L.) in Slovenia and comparison with its variability in Central and South-Eastern Europe: PhD thesis, Ljubljana, p:130.

COMPS, V., CS. MÁTYÁS, J. LETOUZEY, T. GEBUREK (1998): Genetic variation in beech populations (*Fagus sylvatica* L.) along the Alp Chain and in the Hungarian Basin. For. Genet., *5*(1): 1-9.

DEMESURE, B., B. COMPS, R. PETIT (1996): Phylogeography of the common beech (*Fagus sylvatica* L.) in Europe inferred by restrictions studies of PCR-amplified chloroplast DNA fragmets. Evolution, *50* (6): 2515-2520.

GÖMÖRY, D., L. PAULE, R. BRUS, P. ZHELEV, Z. TOMOVIĆ, J. GRAČAN (1999): Genetic diferentiation and phylogeny of beech on the Balkan penisula, J, Evol, Biol, *12*: 746-754,

GRAČAN, J., A. KRSTINIĆ, S. MATIĆ, Đ. RAUŠ, Z. SELETKOVIĆ (1999): Regions of provenances (units) in Croatia, Forest Research Institute, Jastrebarsko, Works: *34* (1): 55-93.

GRAČAN, J. (2003): Achievements in breeding of beech in Croatia. pp:278-303. in: Matić, S (eds.). European beech (*Fagus sylvatica* L.) in Croatia. Academy of forests science and Croatian forests.

GREGORIUS, H.R. (1978): The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math Biosci. *41*:253–271.

GREGORIUS, H.R., J.H. ROBERDS (1986): Measurement of genetical differentiation among subpopulations. Theor.App. genet. *71*, 826-834.

HADŽISELIMOVIĆ, R. (2005): Bioantropologija – Biodiverzitet recentnog čovjeka. INGEB, Sarajevo.

IVETIĆ V., V. ISAJEV, A. NIKOLIĆ, M. KRSTIĆ, D. RISTIĆ, M. KOSTADINOVIĆ (2012): Delineation of beech provenance regions in Serbia by spatial analysis of genetic diversity. Genetika, Vol *44*, No. 1, 101 - 108.

ISAJEV, V. (2005): Beech variability and breeding in Serbia. The chapter in monograpy: Beech *(Fagus moesiaca /Domin, Mally/Czeczott. in Serbia*. Asociation of forest engineers and forest technician in Serbia, Belgrade, p: 139-177.

KAJBA, D. (2003): Intrapopulation and interpopulation variability of beech. pp: 247-263. in: Matić, S (eds.). European beech (*Fagus sylvatica* L.) in Croatia. Academy of forests science and Croatian forests.

KONNERT, M. (1996): Beeinflussen Nutzungen einzelner Bäume die genetische Struktur von Beständen? Die Wald, *23*: 1284-1291.

KONNERT, M. (2004): Handbook for Isoenzyme analyse. www.genre.de/fgrdeu/blag/iso-handbuecher.

LEONARDI, S., P. MENOZZI (1995): Genetic variability of *Fagus sylvatica* L. in Italy: The role of postglacial recolonization. Heredity, 75 (1): 35-44.

LOJO, A., B. BALIĆ (2011): Forest area and forest soils. In: Lojo,A., B. Balić, M. Hočevar, S. Vojniković, Ć. Višnjić, J. Musić, S. Delić, T. Treštić, A. Čabaravdić, S. Gurda, A. Ibrahimspahić, M. Dautbašić, O. Mujezinović: The condition of forests and forests solis in Bosnia and Herzegovina after the second taxation survay between 2006 - 2009 (in print)

MUONA, O., A. HARJU, K. KÄRKKÄINEN (1988): Genetic comparison of natural and nusery grown seedlings of *Pinus sylvestris* using allozymes, Scand. J. of Forest. Res., 3:37-46.

NEI, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:- 583-590.

PAULE, L., D. GÖMÖRY (1997): Genetic diversity of beech populations in Europe. in: Turok, J., Kremer, A, de Vries, S. (eds.): First EUFORGEN Meeting on Socal Broadleaves, 23-25. October 1997. IPGRI, Bordeaux, p:152-163.

STAUBER, A., H. HERTEL (1997): MACGEN. http://www.mol.schuttle.de/wspc/genetik1. htm<.

STEFANOVIĆ, V., V. BEUS, Č. BURLICA, H. DIZDAREVIĆ, I. VUKOREP (1983): The environmental and vegetational regionalization of Bosnia nad Herzegovina, Faculty of Forestry in Sarajevo, Special edition:No, 17, Sarajevo, p. 51.

STOJNIĆ S., S. ORLOVIĆ, A. PILIPOVIĆ, D. VILOTIĆ, M. ŠIJAČIĆ-NIKOLIĆ, D. MILJKOVIĆ (2012): Variation in leaf physiology among three provenances of European beech (Fa*gus sylvatica* L.) in provenance trial in Serbia. - Genetika, Vol *44*, No. 2, 341-353.

ZIEHE, M., H.R. GREGORIUS, H. GLOCK, H.H. HATTEMER, S. HERZOG (1989): Gene resources and gene conservation in forest tres: General concept. In: Scholc, F., Gregorius, H. R., Rudin, D. (ed.): Genetic Effects of Air Pollutants in Forest Tree Populations, Springer-Verlag, Berlin, 173-185.

PAPAGEORGIOU, A. C. , A. VIDALIS, O. GAILING, I. TSIRIPIDIS, S. HATZISKAKIS, B. STEFANOS BOUTSIOS, S.GALATSIDAS, and REINER FINKELDEY (2008): Genetic variation of beech (*Fagus sylvatica* L.) in Rodopi (N.E. Greece), European Journal of Forest Research 127, p:81-88.

ŠIJAČIĆ-NIKOLIĆ, M., V. IVETIĆ, R. KNEŽEVIĆ, and J. MILOVANOVIĆ (2007): Analysis of seed and seedliings traits of different provenances of beech, Acta Herbologica Vol. *16*, No 1, p: 15-27

GENETIČKA DIFERENCIJACIJA SJEMENSKIH SASTOJINA OBIČNE BUKVE (*Fagus sylvatica* L.) U DIJELU BOSNE I HERZEGOVINE

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Izvod

Provedenom biohemijskom analizom genetičke strukture sjemenskih sastojina (populacija) bukve, uz upotrebu 16 izoenzimskih genskih lokusa dokazane su značajne razlike. Varijabilnost kod nekih genskih lokusa je velika, dok je kod nekih populacija samo za neke genske lokuse registrovan monomorfizam.Prosječan broj alela po lokusu kretao se od 2,1875 kod populacije Bugojno i Busovača do 2,5625 kod populacije Kakanj, dok se prosječni broj genotipova u lokusu kretao od 2,6875 kod Busovača do 3,2500 kod populacije Olovo. Kada je u pitanju heterozigotnost, najveća je u populaciji Busovača, a najmanja u populaciji Bugojno.Neki od registrovanih alela predstavljaju rijetke alele, kao što je Pgi-B1, što je veoma cijenjeno kod kasnijih utvrđivanja porijekla sjemena i sadnog materijala, te predstavljaju specifične markere tih sastojina. Pored važnosti za određivanje porijekla reproduktivnog materijala, jako je bitno i za uspješno provođenje mjera gazdovanja.Negativne vrijednosti fiksacijskog indeksa u istraživanim sjemenskim sastojinama su pokazatelj da posjeduju dovoljno genetičke varijabilnosti, te da se može slobodnije gazdovati istim. Pro tome ne bi izgubile mnogo od svoga genetičkog potencijala za adaptaciju. Ovo je registovano prije svega u populacijama Busovača i Prenj.Genetska multilokusna raznolikost kretala se između 63,276 i 162,001, a najveća je u populaciji Bihać, dok se raznolikost genofonda kretala od 1,2708 do 1,3416.Dobijena je srednja veličina diferencijacije za sve populacije, koja je prilično niska (Dj=5,81). Ova veličina pokazuje i udio ukupne raznolikosti, koji se može procijeniti na oko 94,194%. To je zbog genetske raznolikosti međupopulacijama i unutarpopulacija, te varijabilnosti i slabe diferencijacije međupopulacijama. Najveću diferencijaciju pokazuje populacija Bihać (8,28) što ukazuje na veliku stabilnost i homogenost te populacije spram drugih u ovom istraživanju.Kako je ovim istraživanjem samo dana djelomična genetička struktura šest od 13 sjemenskih sastojina bukve u Bosni i Hercegovini, potrebno je naglasiti potrebu daljih istraživanja u bilju genetičke rejonizacije ove vrijedne vrste. U tim poslovima posebnu pažnju treba posvetiti razgraničenju provenijencija (sjemenskih sastojina), kao i na eksperimentalnu rejonizaciju temeljenu na ogledima provenijencija, kroz istraživanja ekološko - fizioloških svojstava.Tokom ispitivanja utvrđeno je postojanje visoko značajne pozitivne genetičke korelacije između početka cvjetanja i dužine klasa, početka cvjetanja i mase 1.000 sjemena, broja generativnih izdanaka i prinosa sjemena po biljci i dužine klasa i mase 1.000 sjemena.