

GENETIC DIVERSITY OF FORESTS IN BOSNIA AND HERZEGOVINA *

Genetička raznolikost šuma u Bosni i Hercegovini

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Abstract:

U ovom radu su prikazana istraživanja genetičke varijabilnosti drveća iz šuma Bosne i Hercegovine. Analizirane su tri vrste (obična jela (*Abies alba* Mill.), smreka (*Picea abies* Karst.) i jasen (*Fraxinus excelsior* L.)) različitim metodama, a od analiziranih vrsta najdetajnije je obrađena obična jela. Sve primjenjene metode su pokazale visoki stupanja prisutnog genetičkog diverziteta.

Ključne riječi: Bosna i Hercegovina, genetički varijabilitet, obična jela, smreka, jasen.

Izvod

This paper presents research of genetic variability of forest trees in Bosnia and Herzegovina. Three species have been analyzed (Silver fir (*Abies alba* Mill.), Norway spruce (*Picea abies* Karst.) and European ash (*Fraxinus excelsior* L.)) using different methods. The most detailed analyses have been preformed on Silver fir. All applied methods have demonstrated high degree of genetic diversity.

Keywords: Bosnia and Herzegovina, genetic variability Silver fir, Norway spruce, ash.

INTRODUCTION - Uvod

Geographically, Bosnia and Herzegovina belongs to countries of South-Eastern Europe and the Balkan Peninsula. It is situated between 42°26' to 45°15' of northern latitude, and 15°44' to 19°41' of eastern longitude. It belongs to the group of relatively small countries, of about 5,000,000 ha, but in that small area it has large diversity of all natural resources. Bosnia and Herzegovina is predominantly a mountainous country with 42% area of mountains, 5% of plains, 24% of hills, and 29% of bare land ground which can be categorised in all three previously mentioned

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categories. Middle altitude is around 500 m, and the altitude goes from 0 to 2387 m at the highest mountain top.

Total area of forest surfaces is 2,501,000 ha, of which 1.130.000 ha is covered by high forests, 841,000ha by low forests and coppices, and 530,000 ha by bare lands (MATIĆ ET AL. 1971).

Specificity of our forests and forest land is their richness with forest tree and shrub species, as well as surface flora. Thus, there are 3,800 flowering plants, 60 types of fernery, 250 types of moss, 250 lichens, and numerous types of animals, birds, insects, etc. in Bosnia and Herzegovina. Special value is the fact that 93% of total forest areas are natural forests.

When it comes to environmental conditions, the area of Dinarides is very specific, because there is a diversity of ecological, edaphic, orographic (STEFANOVIĆ ET AL. 1983) and other conditions in such a small area, which have a direct effect on genetic differentiation within different species.

Large number of experts has written about and investigated diversity of central Dinarides, but so little genetic researches were conducted on the species. The reasons for that were very expensive researches and high-sophisticated equipment required for this type of analyses.

Many experts believe that species of forest trees from Dinarides showed large variability in relation to same species from the north (GÖMÖRY ET AL. 2004, SLADE ET AL. 2008, BILELA & BALLIAN 2008, VENDRAMIN ET AL. 1999). So, when comparing obtained results it can be seen that there is a large genetic variability on Dinarides (Bosnia and Herzegovina) noticed on Fir (KONNERT & BERGMANN 1995), Spruce (BOŽIČ ET AL. 2003), Ash (HEUERTZ ET AL. 2001) and Oak (SLADE ET AL. 2008), which can be confirmed by researches conducted in Bosnia and Herzegovina by BALLIAN (2003a, b) and BALLIAN ET AL. (2007a, b).

Results obtained from these researches can significantly facilitate processes of conservation or preservation of genetic diversity *in situ* or *ex situ* of some population, which sometimes can cause many problems.

The objective of this paper is to partially show genetic richness of forests in Bosnia and Herzegovina through only four economically important species of trees. Obtained results have large significance in forests management, and above all in preparatory actions and enrichment of certain species, control of origin of forest reproductive material which is important for process of artificial rehabilitation of degraded forest populations, as well as in activities relative to forming of gene banks and gene archives using methods *in situ* and *ex situ*.

MATERIALS AND METHODS - *Materijal i metode rada*

Only the natural populations of Silver Fir, Spruce and Ash in Bosnia and Herzegovina have been subject of research. Ten of nineteen selected populations for individual species have been analyzed. All analyzed populations were from different plant habitats and ecological conditions and they successfully reflected the conditions

in place in Bosnia and Herzegovina. During the winter, branches bearing dormant buds have been collected for all species. These were used for analyses. During the collection of samples, attention has been paid to insure that distance between trees is at least 50 to 100 meters in order to exclude the possibility of family relationship. Also, attention has been paid to insure that the trees are not older than 80 years. Based on the form on the analyses, 50 trees were selected per population for isoenzyme and 20 to 24 trees for DNA analyses.

Based on the genetic structure of Silver Fir and Spruce, standard isoenzyme generic markers were used. Further, zymograms were interpreted in line with the protocol issued by the KONNERT (2004). Results obtained for Fir were processed in a statistical program SAS (Statistical Analysis System), and for Spruce in a statistical program GSED (GILLET 1998), and the genetic distance between populations has been calculated in line with GREGORIUS (1987) and NEI (1972, 1978).

For Silver Fir originating from Bosnia and Herzegovina analyses of haploid types were conducted by use of three base pairs (P30141F, P30141R, P30249F, P30249R, P71936F, P71936R) for chloroplast (cpDNK) (VENDRAMIN ET AL. 1999) along with the application of the statistical program Arlekin 1.1, and with the utilization of above *nad5-4* mitochondrial marker (mtDNK) (GÖMÖRY ET AL. 2004).

Genetic analyses of Common Ash and determination of its genetic diversity have been performed by use of six pairs of micro satellite markers (FEMSATL), as well as the procedure utilized earlier by HEUERTZ ET AL. (2001). Analyses of the obtained data have been performed with the aid of the following softwares: Genepop 3.4 (RAYMOND & ROUSSET 1995), Fstat 2.9.3. (GOUDET 1995), Gendist 3.6 (FELSENSTEIN 1986) and Neighbor 3.6 (FELSENSTEIN 2004), in order to assess the level of inter population variations and genetic diversity.

RESULTS AND DISCUSSION – *Rezultati i diskusija*

Silver Fir (*Abies alba* Mill.) – *Obična jela*

The research has been conducted several times on nineteen populations of Silver Fir on the central Dinarides (Bosnia and Herzegovina) (BALLIAN 2003a, 2008, 2009; BILELA & BALLIAN 2008). The results indicate that geographical distance between populations is in correlation with the genetic distance. The average number of allele of the researched population ranged from 1.56 to 2.25, the percentage of polymorphic loci ranged from 68.75% to 81.25%. Multi loci diversity (v_{gen}) ranged from 13.2290 to 42.5977 while the gene pool diversity (v_p) ranged from 1,1358 to 1,2263, and differential (Dj) from 0.0911 do 0.1621. The actual heterozygosity (H_o) amounted from 0.1313 to 0.1725 and the expected heterozygosity (H_e) ranged from 0.1195 to 0.1844. The higher values of heterozygosity of the researched populations, compared to those of Western Europe, indicate that the researched populations did not lose much of their genetic potential on adaptation, as they were not far away from their glacial resort. We can therefore assume that researched populations have a higher resistance

compared to the populations from Central Europe as they pose significant amount of generic variability. This will be further confirmed in the coming periods. By way of usage of *nad5-4* mitochondrial marker we separated populations of Silver Fir in BiH onto two haploid types and we determined their boundaries (GÖMÖRY ET AL. 2004). Based on the use of three pairs of chloroplast primers we registered a significantly higher variability than that in other parts of Europe (VENDRAMIN ET AL. 1999), in total height populations 116 haploid types (BALLIAN 2003b).

All variables observed by different methods are significant for further work on conservation and cultivation of Silver Fir, and in the process of forest regeneration (reforestation and sowing of seed), as well as for foundation of gene banks and archives by *in situ* and *ex situ* methods.

Norway Spruce (*Picea abies* Karst.) - Smreka

Researches carried out on spruce showed large genetic variability in natural populations in Bosnia and Herzegovina (BALLIAN ET AL. 2007a, b), and determined interrelation and origin of researched populations. Results obtained showed that 15 gene loci out of 20 analyzed have large polymorphism that was proved based on relative gene frequencies. Average number of alleles was from 1.80 to 2.60, the percentage of polymorphic loci was from 75% to 90%, the multi-locus diversity (v_{gen}) was from 27.50 to 37.00, and gene pool diversity (v_p) was from 1.277 to 1.380. Value of actual heterozygosity (H_o) was from 0.1840 to 0.2400 and expected heterozygosity (H_e) was from 0.1690 to 0.2180. Average differentiation showed low value $D_j = 1.91$, which implies a high proportion of total diversity (that can be estimated on about 98%), and that can be attributed to genetic variability between individuals within populations (BALLIAN ET AL. 2007a, b). Statistically significant differences between analyzed populations have been registered as well, and obtained variability showed cline character. Spruce that originated from frosty population showed specificities in content of rare alleles (BALLIAN ET AL. 2006). Population Vlašić confirmed its allochthonous origin (BALLIAN 2007) with its genetic structure.

Reproductive material should not be used in any form until new analyses are carried out in this area, and further spreading of unwanted gene pool should be prevented by special management measures. Higher value of heterozygosity of analyzed populations in contrast to populations of Spruce from Central and Western Europe showed that analyzed populations did not lose much of its genetic potential for adaptation because they are not too distant from its resort. Hence, those populations are more resistant than populations originating from western areas because they have sufficient genetic variability. That means that future researches should be focused in that direction. Management processes should be done constantly in regards to rehabilitation of Spruce, and directed towards sustainability of autochthonous genetic diversity of local populations. Moreover, many experiments with different provenances should be carried out in order to investigate ecological and physiological traits of Spruce. These activities should complete the researches carried out so far.

Common Ash (*Fraxinus excelsior* L.) – Bijeli jasen

HEUERTZ ET AL. (2004) have found two different haploid types for Common Ash in the area of Bosnia and Herzegovina. One of them is unique for Central Europe and other of Balkan, which implies that this is the border area characterized by two haploid type gene pools. This can be the fact because of direct connection with the phenomena of high polymorphism, which is registered in population of Bosnia and Herzegovina (BELLETTI ET AL. 2004; BALLIAN ET AL. 2008).

Investigated populations of Common Ash from Bosnia and Herzegovina show genetic differences between them, and that each of them represents specific population (BALLIAN ET AL. 2008). Average number of alleles per locus was from 7.5 to 12, and actual heterozygosity (H_o) was from 0.4330 to 0.6370. Inbreeding ration (F_{IS}) was rather high and went from 0.159 to 0.444. Average genetic diversity in populations was between 0.7610 and 0.8300, and size of differentiation showed low value $D_j = 2.3 \%$. Obtained high and positive values of inbreeding can be compared with those obtained by MORAND ET AL. (2002) during the research on Common Ash populations in the area of northern France. Positive values that are gained for inbreeding ration can be connected with low number of species, as well as with weak gene flow and probably the tendency of Common Ash to inbreeding among relatives. Individuals that form populations give specific presentation of analyzed populations that can be fact due to constant anthropogenic influence in populations. Obtained value of differentiation between populations is much lower than that obtained in research carried out by HEUERTZ ET AL. (2001) in Bulgaria (8.7%). Values obtained in research carried out on Common Ash imply that there are certain barriers in gene flow according to AUSTERLITZ ET AL. (2000), which directly influenced to different process of postglacial re-colonization of Common Ash.

CLOSING OBSERVATIONS – *Zaključne napomene*

Based on the presented genetic parameters, differences between populations or within a population are visible and clear. Possible causes to this diversity, apart from natural selection and anthropogenic effects, are the development factors or specific processes of adaptation to the set ecological conditions, viewed through historical development of the species.

If the methodological effects could be excluded (for instance the number of units in the sample), development and anthropogenic factors, observed differences between populations would indicate that the adaptation processes on each of the investigated populations may have a significant role. Therefore, the following observations may be stated based on the obtained results:

- The noted variability is not that characteristic in Western, Central, and Eastern Europe. Rather, it is more or less typical for western, and central Balkans, or more specifically to the area of central Dinarides. When the size

of the territory is taken into consideration than it can be said that there is a significant genetic variability on a small territory.

The obtained grouping of populations, specific for each of the species, indicates that there is a grouping within species in line with specific ecological conditions. Also, area of Dinarides Mountains is specific as a small territorial space with high rate of change in climate, and with this change there is a change in forest communities with an abundance of species (STEFANOVIĆ ET AL. 1983).

Based on the obtained results a conclusion can be made that with all investigated species there are significant differences between populations. It can also be assumed that very often there is a genetic differentiation within these species (Silver Fir, Spruce and Common Ash) that caused a different process of postglacial migration, or adaptability of specific genotypes on certain habitats where there are specific selection processes in place, and, more than likely, anthropogenic influences in the past 2000 years.

Based on the obtained results and the discussion a recommendation can be made how to conserve and preserve our genetic diversity in forests of Bosnia and Herzegovina. Therefore, in light of the genetic wealth, when managing forests particular care should be paid in order not to disrupt this valuable genetic structure of natural forests. A recommendation can be made that processes related to regeneration of forests through natural regeneration, should be initiated. This will entail managing forests in line with the principles of suitability. While working on regeneration attention to genetic structure should be paid, in line with the age groups. In the conducted research RUETZ ET AL. (1996) results indicate differences between age stages of fir population, and the regeneration should be monitored by occasional genetic and molecular research (BEHM & KONNERT, 1999; KONNERT 2006), in order to monitor genetic structure and to intervene in needed in order to preserve it by applying appropriate growth measures. This will insure successful preservation of indigenous genetic potential.

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Sažetak

Bosna i Hercegovina pripada zemljama jugoistočne Europe, smještena je na sjeverozapadnom dijelu Balkanskog poluotoka između 42° 26' do 45° 15' sjeverne zemljopisne širine i 15° 44' do 19° 41' istočne zemljopisne dužine, sa površinom od nešto preko 5.000.000 ha. Pretežno je planinska zemlja sa 42 % planina, 5 % su ravnice, a 24% na brežuljkasto područje, te 29% kraške goleti.

Inače prema inventuri šuma od proje 45 godina ukupna površina šumskih zemljišta je 2.501.000 ha, od čega na visoke šume dolazi 1 130 000 ha, niske šume i šikare 841.000 ha, te na goleti 530.000 ha. Sa velikim bogatstvom vrsta, posebno endema, te velikom raznolikošću biljnih zajednica, i 93% prirodnih i poluprirodnih šuma.

Kako se stoljećima nalazila na margini europskih kretanja, to su se šume uspjele da očuvaju svoju genetičku strukturu. Tako prije 100 godina počinje razvoj intenzivnog šumarstva. Tada se počinje sa introdukcijom šumskih vrsta, ali u većini bez uspjeha, te je sad Bosna i Hercegovina jedna od rijetkih zemalja koja nije opterećena genetičkim zagađenjem i izmijenjenom genetičkom strukturom glavnih ekonomskih vrsta drveća (jele, smreka, bukva, hrast kitnjak).

Kako je područje Dinarida vrlo je specifično, tako na malom prostoru imamo veliku raznolikost ekoloških uvjeta, a to je ostavilo traga na genetičku raznolikost svih vrsta.

Mnoga genetička istraživanja sa šumskim drvećem iz centralnih Dinarida pokazuju veliku varijabilnost, u odnosu na iste vrste srednje, zapadne i sjeverne Europe.

Genetičkim istraživanjima dobivena velika varijabilnost kod obične jele (*Abies alba* Mill.), kod smreke (*Picea abies* Karst.) su nađeni rijetki aleli. Kod listača, običnog jasena (*Fraxinus excelsior* L.) također je registrirana specifična genetička varijabilnost.

Istraživanja su provođena u cilju olakšavanja kontrole podrijetla sjemena i sadnog materijala, kao i za poslove vezane na osnivanju banki i arhiva gena metodama *in situ* i *ex situ*.