UDC 575.630
https://doi.org/10.2298/GENSR1701259C
Original scientific paper

NORWAY SPRUCE (*Picea abies* Karst.) VARIABILITY IN PROGENY TESTS IN BOSNIA AND HERZEGOVINA

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Cvjetković B., M. Konnert, B. Fussi, M. Mataruga, M. Šijačić-Nikolić, V. Daničić, A. Lučić (2017): *Norway spruce (Picea abies Karst.) variability in progeny tests in Bosnia and Herzegovina*.- Genetika Vol 49, No1, 259- 272.

Norway spruce is one of the most important economic species is Bosnia and Herzegovina. It is the species at the edge of its natural range; nevertheless it attains significant growth and yield results. The species is often used for afforestation purposes. In the previous period, 4 progeny tests of Norway spruce were established in Bosnia and Herzegovina. The offspring from 6 natural populations: Han Pijesak 1, Han Pijesak 2, Foča, Potoci, Olovo and Kneževo were build-in the progeny tests. In 2016, the samples of Norway spruce from 2 progeny tests: Srebrenica and Drinić were collected. In total, 360 samples were collected. DNA isolation was done according to Dumoline et al. (1990). For assessment of genetic differences among populations, co-dominant nSSR microsatellite system had been used. The number of effective alleles ranged from 7.78 in the population Potoci up to 15 in the population Kneževo, the average number of alleles was 13. The observed heterozygosity ranged from 0.61 for the population Han Pijesak 2 up to 0.68 for population Kneževo. The average observed heterozygosity was 0.65. Fixation index was in the range from -0.073 in the population Potoci, to 0.030 for the population Han Pijesak 2. The average value of Wright fixation index is -0.007. The average fixation index indicates the existence of a very small number of homozygotes. Concerning the variability among populations it has been concluded that the total level of genetic differentiation among populations was very low (F_{ST}= 0.026). The result of Nei's genetic distance shows that the populations Olovo and Potoci are separated from

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other populations. The results obtained by genetic markers, in addition with other, morphological and physiological markers will be the basis for the further investigation of Norway spruce adaptability and possibility for the transfer of genetic material in light of climate changes.

Key words: Norway spruce, nSSR markers, progeny tests, Bosnia and Herzegovina

INTRODUCTION

Norway spruce presents one of the most important forest tree species in Europe, as well as in Bosnia and Herzegovina. This species is the dominant species in the largest virgin forest in Europe – Perućica, and other virgin forests such as Janj and Lom, where Norway spruce attains amazing dimensions (MAUNAGA *et al*., 2005; KEREN *et al*., 2014). In Bosnia and Herzegovina, Norway spruce is at the southernmost range of the species which is at the same time disjunctive. The importance of Norway spruce in Europe and worldwide is stressed by the fact that it is one out of 4 forest tree species which the whole genome sequence is available (NYSTEDT *et al*., 2013). Many studies related to *ex-situ* conservation and the improvement of the species qualitative and quantitative traits and the resilience to the adverse environmental conditions have been carried out (SKRØPPA, 2003; RUOTSALAINEN, 2014; SKRØPPA and STEFFENREM, 2015).

Norway spruce in Europe is extensively used in afforestation and reforestation beyond the natural range (SPIECKER, 2000). The same situation is in Bosnia and Herzegovina. The share of produced seedlings in nurseries in Bosnia and Herzegovina ranges between 60% and 85% (MATARUGA *et al*., 2012), which indicates the importance of this conifer species in the country where broadleaves forests prevail.

Based on phenotypic and genotypic data Norway spruce areal is mostly is divided into three macrogeographic regions (domains): the Alpine, Hercyno-Carpathian and Baltico–Nordic domains (HUNTLEY and BIRKS, 1983; SCHMIDT-VOGT, 1986; ACHERE *et al*., 2005; CHEVARRIA, 2005). Some authors distinguish 5 different domains of Norway spruce (LAGERCRANTZ and RYMAN, 1990). COLLIGNON *et al*. (2002) and HEUERTZ *et al*. (2006) suggest the division of the area in only two domains: the Baltic-Nordic and the Alpine. Norway spruce in Bosnia and Herzegovina is part of Alpine domain of Norway spruce.

Many authors have dealt with the genetic variability of Norway spruce in Europe. It has also been stated that the differences in the genome determined by using nuclear DNA markers and/or biochemical markers indicate higher variability between the trees in the stands than between the populations within the same domain (LAGERCRANTZ and RYMAN, 1990; BERGMANN, 1991; MÜLLER-STARCK *et al*., 1992; MÜLLER-STARCK, 1995; VENDRAMIN *et al*., 1999; SPERISEN *et al*., 2001; SKRØPPA, 2003; MAGHULY, 2006; JANSSON, 2013).

The influence of altitude on the differentiation of Norway spruce was investigated by: GEBUREK, (1999), MAGHULY *et al*., (2006), BALLIAN *et al*., (2007a), KONNERT, (2009), GALOVIĆ *et al*., (2015). The diferences were significat. Differences in the genetic structure of Norway spruce were also observed in the same habitat, in different age groups, under the influence of selective cutting (WOJNICKA-POLTORAK *et al*., 2013).

TOLLEFSRUD *et al*. (2009), were investigating the genetic variability of Norway spruce and they found that Norway spruce survival in separate refugia and postglacial colonization led to the significant structuring of the genetic variation in the southern range of the species of which Bosnia and Herzegovina is a part. BUCCI and VENDRAMIN (2000) determined 16 different haplogroups in Norway spruce in Europe. In that study Norway spruce from central and western

Bosnia and Herzegovina belongs to the Alpine-Central European group, while Norway spruce in eastern Bosnia and Herzegovina belongs to the 'unclassified' group. Genetic variability of Norway spruce in Bosnia and Herzegovina by investigating 12 isozymes and analyzing 20 loci was studied (BALLIAN *et al*. 2006; BALLIAN *et al*., 2007a; 2007b; BALLIAN, 2010). They found that the populations of Norway spruce forests, although on the edge of natural area, have similar genetic diversity compared to other regions and thus are expected to have kept much of their genetic potential for adaptation. They noticed differences between Norway spruce populations from Slovenia and Bosnia and Herzegovina. MATARUGA *et al*. (2014) analyzed the genetic variability using 3 nSSR, 1 mtDNA and 1 cpDNA marker and found significant differences in the 11 natural populations of Bosnia and Herzegovina.

Progeny tests, which were established for investigating Norway spruce in Bosnia and Herzegovina, represent an opportunity to get to know the genetic potential of this particular species and different provenances or genotypes (ŠIJAČIĆ-NIKOLIĆ and MILOVANOVIĆ, 2012) and improve the quality of the reproductive material as was done for other species: Austrian pine and Serbian spruce (MATARUGA *et al*., 2011; CVJETKOVIĆ *et al*., 2013). The most improved Norway spruce reproductive material which is currently used in the world, originates from the first, second and third generation of seed orchards which were established by the reproductive material often previously tested in field trials such as progeny tests. The gain in yield is 10–25%, depending on the selection intensity of the parent trees (RUOTSALAINEN, 2014).

Some previous results, obtained by investigations of physiological and morphological markers of Norway spruce in progeny tests in Bosnia and Herzegovina, were published. The statistically significant differences concerning bud burst, height, height increment and root collar were recorded among 6 investigated populations (CVJETKOVIĆ *et al.*, 2015a; 2016) as well as differences for survival rates among and within populations (CVJETKOVIĆ *et al.*, 2015b; 2015c).

The aim of this study was to examine genetic variability of Norway spruce from 6 natural populations, by analyzing several representative half-sib lines planted in progeny tests in Bosnia and Herzegovina. Another aim was to determine whether Norway spruce from Bosnia and Herzegovina has lower genetic variability than populations from the center of its distribution due to its peripheral distribution in Bosnia and Herzegovina.

MATERIALS AND METHODS

In Bosnia and Herzegovina there are 4 Norway spruce progeny tests. Samples from two of them: Drinić and Srebrenica were investigated (Fig. 1). Both progeny tests consist of 6 Norway spruce populations from different parts of Bosnia and Herzegovina. Some of the populations are on the southern border of the natural range in Europe (Figure 1). Each population is represented by the offspring which is composed of different numbers of half-sib lines (Table 1). A total of 36 half-sib lines are incorporated in the progeny tests.

Five samples from each half-sib line and from each progeny test were collected, making a total of 10 samples per half-sib line. The sampling was based on earlier studies of survival, morphometric and physiological parameters (CVJETKOVIĆ *et al.*, 2015a; 2015b; 2015c, 2016). The samples were collected in July 2015 in both progeny tests, put in paper bags and dried until October 2015, when they were subjected to the investigations.

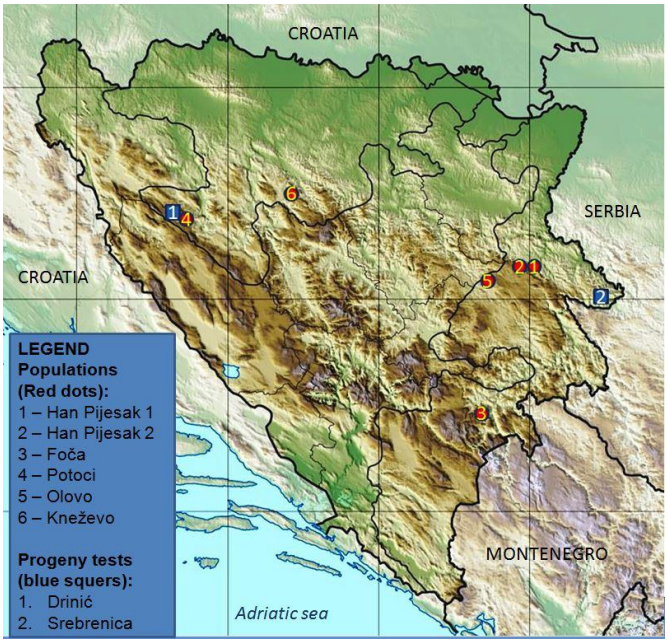

Figure 1. Locations of natural populations where seed was collected and the locations of progeny tests

Table 1. Data on populations and half-sib lines

Nº	Population	Total half-sib lines per population	Number of samples
1	Han Pijesak 1 (*local. „Kusače“*)	7	70
2	Han Pijesak 2 (*local. „Radojevac“*)	5	50
3	Foča	8	80
4	Potoci	2	20
5	Olovo	5	50
6	Kneževo	9	90

DNA isolation and PCR amplification

The genomic DNA was isolated form dried needles 0.3-0.5 g following the method by DUMOLIN *et al*. (1995). The yield and purity of the isolated DNA were determined by spectrophotometry in the solution of 95 ml of autoclaved water and 5 ml of DNA solution. For PCR reaction the genomic DNA was diluted to about 20-40 nanograms per µl and the Type-it Microsatellite PCR kit by Qiagen was used. DNA samples were amplified by using specific primers for nuclear microsatellites revealing codominant alleles for 11 polymorphic loci (Table 2).

PCR amplification was performed in the total volume of 15 µl solution: 1 µl of 20-40ng/µl genomic DNA, 7,5 µl of 2X Qiagen Multiplex PCR Master mix , 5 µl of RNase free

water and 1.5 µl of Primer Mix respectively. The targeted fragments were amplified by using the following PCR protocol:
- initial denaturation (95°C for 15 min), 29 amplification cycles (94°C for 30s, 53°C for 90s, 72°C for 30 s), 1 final elongation step (60°C for 30 min) for: WS00716.F13, WS0092.M15 and WS0022.B15.
- initial denaturation (95°C for 15 min), 29 amplification cycles (94°C for 30s, 55°C for 90s, 72°C for 30 s), 1 final elongation step (60°C for 30 min) for: WS0073.H08, and WS00111.K13.
- initial denaturation (95°C for 15 min),), 29 amplification cycles (94°C for 30s, 62°C for 90s, 72°C for 30 s), 1 final elongation step (60°C for 30 min) for: WS0023.B03, EAC1F04,WS0046.M11, Pa_47, Pa_44 and Pa_51.

Table 2. List of primers used for fragment amplification

Locus	F primer	R primer	Fragment length (bp)	Annealing temp. [°C]
WS00716.F13[1]	TCAAGTAATGGACAAACGATACA	TTTCCAATAGAATGGTGGATTT	214-320	53
WS0092.M15[1]	GATGTTGCAGGCATTCAGAG	GCACCAGCATCGATTGACTA	204-216	53
WS0022.B15[1]	TTTGTAGGTGCTGCAGAGATG	TGGCTTTTTATTCCAGCAAGA	160-220	53
WS0073.H08[1]	TGCTCTCTTATTCGGGCTTC	AAGAACAAGGCTTCCCAATG	209-256	55
WS00111.K13[1]	GACTGAAGATGCCGATATGC	GGCCATATCATCTCAAAATAAAGAA	181-237	55
WS0023.B03[1]	AGCAGCTGGGGTCAAAGTT	AAAGAAAGCATGCATATGACTCAG	162-236	62
WS0046.M11[1]	CACTAGGGCATTGGGAAGAA	ATGAGAGGCTGGGGTATGAA	231-240	62
EAC1F04[2]	TGTAAGTCTGCTTGAAGGTGG	CAGATGGGGGTTGGGTAT	181-371	62
Pa 44[3]	AAGGCAGCCAAAGTGAAGAA	CTTGGCATTCCCTAGTGAGC	271-305	62
Pa 51[3]	CAGATGTGGGCACTTGTTTG	TGGTCATGGTGGTGTTCAT	124-145	62
Pa 47[3]	ATCAATTGCCCTACCAGCAC	TGCTCAATTTCCTGCATCTG	95-125	62

1-RUGINGS et al., 2004; 2-SCOTTI et al., 2002; 3-FLUCH et al., 2011.

The preparation for fragment analysis was performed on 1 µl of PCR product by adding 30 µl of SLS and 0.5 µl of Standard 400 of BeckmanCoulter™ run on CEQ 8000and GeXP (BECKMAN COULTER CO) sequencers. The automated binning process of all nuclear simple sequence repeats was carried out by using the fragment analysis tool of Beckman Coulter.

The statistical program GenAlex ver. 6.501 (PAEKALL and SMOUSE, 2005) was used for the statistical analysis. The following parameters were calculated: the average number of different alleles (Na) per population over all loci, the number of effective alleles (Ne), the observed (Ho) and the expected (He) heterozygosity and the fixation index (F_{IS}). F_{ST} (population genetic differentiation), F_{IS} (fixation index), F_{IT} and Nm (number of migrants) were determined.

RESULTS AND DISCUSSION

Eleven nuclear microsatellite loci were studied. Locus Pa51 was monomorphic in the populations: Han Pijesak 1, Han Pijesak 2 and Potoci. In the rest of the populations this locus had 2 alleles. WS0046.M11 locus was monomorphic only in the population Potoci. These 2 loci were excluded from the calculations. The most polymorphic locus is EAC1F04 with 66 different

alleles. The total inter-population variability (F_{ST}) was only 2-5%, which is almost the same as the results obtained in the previous research (KONNERT, 2009; TOLLEFSRUD *et al*., 2009).

The mean number of alleles per locus ranged from 7.78 in the population Potoci, up to 15 in the population Kneževo, while the average number of alleles was 13 (Table 3) which is higher in comparison to other economic species in the country such as oak (BALLIAN *et al*., 2010) or common ash (BALLIAN *et al*., 2008). Mean number of effective alleles ranged from 4.73 (Potoci population) up to 8.80 (population Han Pijesak 2).

The observed heterozygosity ranged from 0.61 obtained in the population Han Pijesak 2 to 0.68 in the population Kneževo. The average observed heterozygosity was 0.65 (Table 3). Expected heterozygosity ranges between 0.63 (population Potoci) and 0.71 (population Kneževo). This results supports the earlier results obtained by BALLIAN *et al*. (2006; 2007b) for Prodac population, which is geographically closest to the population Potoci, where the above-mentioned authors found the minimal expected heterozygosity in Bosnia and Herzegovina. Norway spruce has a higher observed heterozygosity than common ash (BALLIAN *et al*., 2008), and about the same as the pediculate oak (BALLIAN *et al*., 2010) but lower expected heterozigosity.

In their research in forests in Austria, at different altitudes and at different ages of trees, MAGHULY *et al*. (2006) found that observed heterozygosity ranged from 0.40 to 0.60, while the expected heterozygositzy was in the range from 0.75 to 0.84. In Poland also high levels of expected heterozygosity (He = 0.853) and the observed heterozygosity (Ho = 0.841) were noted (NOWAKOWSKA *et al*., 2014). The average expected heterozygosity for Norway spruce in the progeny test in Bosnia and Herzegovina tests was greater than the expected heterozygosity for 6 populations in the Alps (He=0.588-0.671) (MALONE *et al*., 2007). Our study suggests that relatively high expected heterozygosity exist in Bosnia and Herzegovina and thus adaptive potential is present, which confirms the results obtained in the previous studies on isoenzymes BALLIAN *et al*. (2006).

Shannon's index ranged from 1.43 in the population Potoci up to 1.92 in the population Kneževo. Compared with Shannon's index in other countries, is determined that the value of this index in Bosnia and Herzegovina is lower than for example in Poland. In Poland, the determined value of Shannon's index was I = 2.42 for the three populations in the so-called "spruceless" zones, I = 2.90 for the population in the northeastern part of Poland, and I = 2.96 for the southern population (NOWAKOWSKA, 2009). This is clearly significantly above the levels of Shannon's index, which was found in the territory of Bosnia and Herzegovina.

Fixation index was in the range from -0.073 in the population Potoci, to 0.030 for the population Han Pijesak 2. The average value of Wright fixation index (WEIR and COCKERHAM, 1984) is -0.007. The average fixation index indicates the existence of a very small number of homozygotes. It is opposite result in comparison to the central parts of Norway spruce areal, such as the Alps, where Malone et al. (2007) observed a lack of heterozygotes. A significant positive value in stands of different ages of Norway spruce (NOWAKOWSKA *et al*., 2014), as well as other forest species in Bosnia and Herzegovina (BALLIAN *et al*., 2008; 2010) was also observed.

Concerning the variability among populations it has been concluded that the total level of genetic differentiation among populations was very low (F_{ST}= 0.026), which is smaller than in some other parts of Europe such as Poland, where F_{ST}=0.039 was determined by using 3 nSSR markers (NOWAKOWSKA, 2009). GEBUREK (1999) found the F_{ST} = 0.012 for 29 Alpine populations by using 17 isoenzyme loci. SCOTT *et al*. (2000) found that the F_{ST} = 0.118 for 8 populations along the Alpine chain using 7 SCAR (sequence characterized amplified region) loci. HEUERTZ *et al*.

(2006) found that F_{ST}-values for 7 populations along the entire natural range of Norway spruce varied between 0 and 0.289 for each loci and revealed substantial differentiation among the populations, with an overall value of F_{ST}=0.117 over all 229 SNPs. Romanian population was the most differentiated population in pairwise comparisons (0.194< F_{ST} <0.262). The populations within the Baltico–Nordic domain were significantly, though weakly differentiated (F_{ST} = 0.025), whereas the populations from the Alpine domain were not significantly differentiated (F_{ST} = 0.015). TOLLEFSRUD *et al*., (2009) found F_{ST}=0.029 for 37 populations from North Europe and Russia. Based on the comparisons of the investigations in Central and Northern Europe, we can say that Norway spruce from the progeny tests in Bosnia and Herzegovina has not lost its genetic wealth which is the basis for the process of adaptation to the new challenges posed by the environment.

Table 3. Mean number of alleles per locus (Na), mean number of effective alleles per locus (Ne), Shannon index (I), observed and expected heterozygosity (Ho resp. He) and fixation index (F)

Population		Na	Ne	I	Ho	He	F
Foča	Mean	14.33	8.03	1.84	0.64	0.69	0.012
	St. err.	3.55	2.32	0.36	0.08	0.10	0.084
Han Pijesak 1	Mean	14.78	8.09	1.90	0.66	0.70	0.013
	St. err.	3.89	2.19	0.36	0.08	0.09	0.085
Han Pijesak 2	Mean	12.78	6.90	1.78	**0.61**	0.68	**0.030**
	St. err.	3.17	1.84	0.34	0.09	0.09	0.095
Kneževo	Mean	**15.00**	**8.80**	**1.92**	**0.68**	**0.71**	-0.010
	St. err.	3.75	2.47	0.36	0.09	0.10	0.085
Olovo	Mean	14.00	7.21	1.85	0.67	0.69	-0.015
	St. err.	3.42	1.80	0.34	0.08	0.09	0.065
Potoci	Mean	**7.78**	**4.37**	**1.43**	0.65	**0.63**	**-0.073**
	St. err.	1.79	0.89	0.28	0.09	0.10	0.056
TOTAL	Mean	13.11	7.23	1.79	0.65	0.68	-0.007
	St. err.	1.34	0.80	0.13	0.03	0.04	0.031

Fixation index for each locus ranged from -0.328 to 0.387 (table 4). The average value is -0.009, which is close to zero. It may therefore be said that Norway spruce populations are almost in equilibrium with HW. The deviations from Hardy-Weinberg equilibrium were associated with the positive values of F_{IS} for loci: WS0022.B15, WS00111.K13, WS0023.B03, EAC1F04 and the negative values for WS00716.F13, WS0092.M15, WS0073.H08, Pa44, Pa47 (table 4). F_{IT} as the deviation from Hardy-Weinberg proportions in the total population was small – 0.015.

The migration coefficient (Nm) indicated high gene flow between the studied generations of Norway spruce trees but smaller, for example, than Nm in Poland (Nm =18.687).

Inter-population differentiation was estimated using Nei's genetic distance (NEI, 1972). The results are shown in Table 5. The results indicate that the lowest difference is between the populations of Han Pijesak 1 and Foča (0.038). The highest genetic difference was recorded between the populations Foča and Potoci (0.117), which was expected due to large geographical

distance between these two populations. Surprisingly, the difference between the two populations from the area of Han Pijesak was higher than the differences between other, geographically more distant populations. Although the differences exist, they are not large and range in relatively small intervals of 0.038-0.117.

Table 4. Overall inbreeding (F_{IT}), differentiation within populations (F_{IS}), differentiation among populations (F_{ST}), number of migrants (Nm)

Locus	Fis	Fit	Fst	Nm
WS00716.F13	-0.014	0.017	0.030	7.980
WS0092.M15	-0.328	-0.308	0.016	15.794
WS0022.B15	0.007	0.034	0.027	8.902
WS0073.H08	-0.047	-0.027	0.019	12.674
WS00111.K13	0.004	0.036	0.032	7.538
Pa44	-0.074	-0.054	0.019	12.972
WS0023.B03	0.387	0.407	0.033	7.439
EAC1F04	0.253	0.282	0.039	6.143
Pa47	-0.271	-0.249	0.017	14.207
Mean	-0.009	0.015	0.026	10.406
SE	0.075	0.075	0.003	1.170

The results of Nei's genetic distance presented in a PCoA shows that the populations Olovo and Potoci are separated from other populations (figure 2 and 3). Especially, population Potoci appears to be separated at all three axes. In contrast to the results obtained by BALLIAN *et al.* (2007b) which clearly point to the two groups of the populations according to geographic location: the group of populations from Western Bosnia and the groups of populations from Eastern Bosnia, the results obtained by our research, after investigating nSSR markers, did not indicate a clear differentiation of the eastern and western populations in Bosnia and Herzegovina. In the future investigations some more populations from west part of Bosnia and Herzegovina should be included.

Table 5. Pairwise genetic distance according to Nei between the investigated Norway spruce populations

Foča	**Han Pijesak 1**	**Han Pijesak 2**	**Kneževo**	**Olovo**	**Potoci**	
0.000						**Foča**
0.038	0.000					**Han Pijesak 1**
0.067	0.061	0.000				**Han Pijesak 2**
0.047	0.044	0.066	0.000			**Kneževo**
0.074	0.067	0.076	0.076	0.000		**Olovo**
0.117	0.096	0.104	0.100	0.110	0.000	**Potoci**

If we compare the results of PCoA genetic analysis with the previously established morphometric variability (CVJETKOVIĆ *et al*., 2016), the physiological parameters CVJETKOVIĆ, *et al.*, 2015a) as well as the survival of seedlings (CVJETKOVIĆ *et al*., 2015b; 2015c), some similar patterns can be observed.

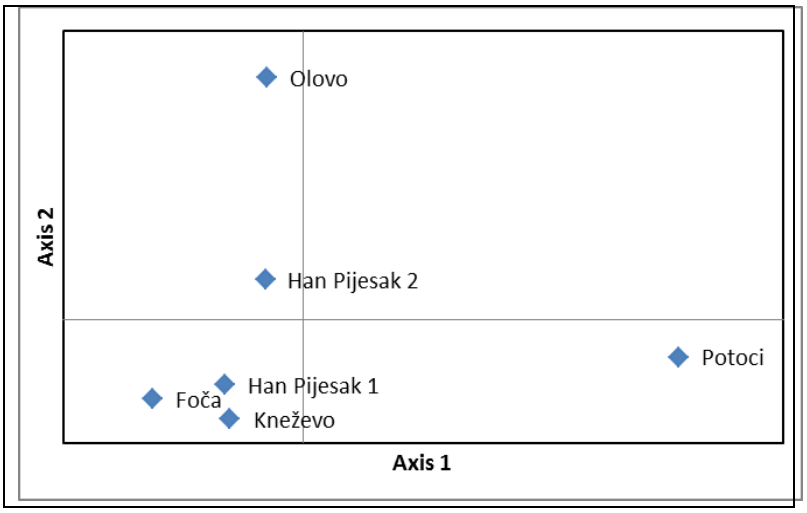

Figure 2. Principal component analysis principal coordinates 1 vs 2

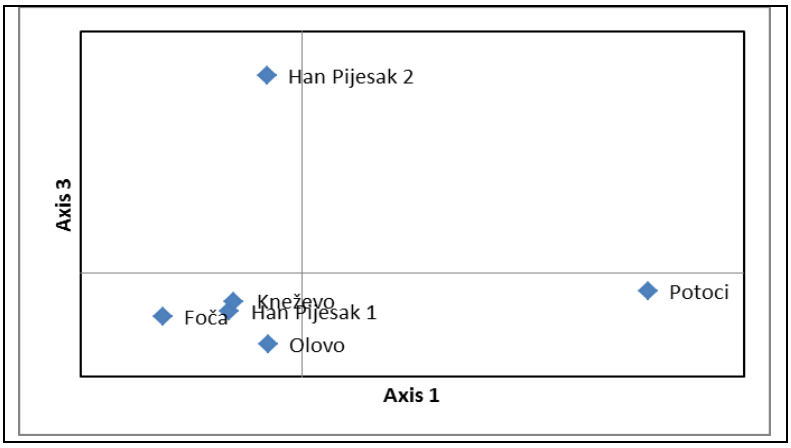

Figure 3. Principal component analysis principal coordinates 1 vs 3

CONCLUSIONS

Norway spruce in progeny tests in Bosnia and Herzegovina is the basis for further work on the breeding of this species. Norway spruce natural range in Bosnia and Herzegovina is at its south edge and disjunctive in same time. The study of genetic variability was aimed to determine

the level of genetic variability among populations as a basis for further determination of the variability of physiological and morphological characteristics. Tested populations in Bosnia and Herzegovina are characterized by a high diversity within, but a small diversity among populations which correspond with the general picture of Norway spruce in Europe.

In comparison with the previous studies indicating the allochthonous origin of Norway spruce on Mountain Vlašić (BALLIAN *et al.*, 2006; 2007b), in this study there were no significant differences in the results of the interpopulation variability of Norway spruce in progeny tests. This could point to the fact that there was no introduction of Norway spruce from other parts of Europe in Bosnia and Herzegovina to the populations covered by this research.

Due to the low value of fixation index F_{IS}, it can be considered that gene flow of Norway spruce forests in Bosnia and Herzegovina is not disturbed. F_{ST} value, indicates that Norway spruce in Bosnia and Herzegovina has low genetic differentiation, but not significantly smaller than most of the other regions. Also, the mean and effective number of alleles is equal to, or greater than the number of alleles when compared to other species investigated so far in Bosnia and Herzegovina (e.g. penducilate oak and common ash).

The studies of genetic markers nSSR of Norway spruce from progeny tests indicate a high variability of Norway spruce forests in Bosnia and Herzegovina which already show some similarity with the survival of seedlings and the morphological and physiological patterns. It could be the basis for the delineation of the region of provenances and the safe transfer of Norway spruce reproductive material from the forests in Bosnia and Herzegovina.

ACKNOWLEDGMENTS

This paper was realized as a part of the TA “Trees 4 Future” project under the title "Genetic variability of Norway spruce in progeny tests in Bosna and Herzegovina (VANSBIH)", co-funded by the European Union Seventh Framework Program FP7 under grant agreement N° 284181. The project was realized at the Bavarian office for forest seeding and planting (Bayerisches Amt für forstliche Saat- und Pflanzenzucht).

Received May 05th, 2016
Accepted December 16th, 2016

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VARIJABILNOST SMRČE (*Picea abies* Karst.) U TESTOVIMA POTOMSTVA U BOSNI I HERZEGOVINI

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Izvod

Smrča u Bosni i Hercegovini predstavlja jednu od najvažnijih ekonomskih vrsta. Vrsta se nalazi na ivici svog prirodnog areala. Postiže odlične rezultate u proizvodnom pogledu. U proteklom periodu osnovani su testovi potomstva u koje je ugrađeno potomstvo iz 6 populacija smrče iz čitave Bosne i Hercegovine. Tokom 2016. godine u dva testa potomstva, u Driniću i Srebrenici, sakupljeni su uzorci za genetičke analize. Na 6 populacija urađene su analize korišćenjem nSSR markera. Analize su pokazale postojanje vrlo malog stepena homozigotnosti što ukazuje na neometan protok gena uprkos pojavi disjunkcije areala. Stepen genetičke diferencijacije je nizak, F_{ST}= 0.026. Posmatrano u odnosu na NEI genetičku distancu, izdvojile su se dve populacije: Olovo i Potoci.Rezultati dobijeni upotrebom genetičkih markera koristiće se u daljim procesima oplemenjivanja smrče u BiH kao i za potrebe definisanja mogućnosti transfera reproduktivnog materijala ove vrste.

Primljeno 05.V 2016.
Odobreno 16. XII. 2016.