

ORIGINAL ARTICLE

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Genetic structure of Norway spruce [*Picea abies* (L.) Karst.] provenances tested in IPTNS-IUFRO 1964/68 experiment in Krynica

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ABSTRACT

The results of previous studies have shown high breeding values of Beskidian spruce. The aim of the study was to assess the genetic structure of seventeen Norway spruce provenances from the Beskidy Mts. tested in IPTNS-IUFRO 1964/68 experiment in Krynica, which survived after massive wind damage on the plots. Polymorphism of five isozyme systems encoded in five loci was determined. The highest value of genetic diversity parameters: mean number of alleles per locus and observed heterozygosity was noted for progeny of spruce from the Eastern Beskidy Mts. ($N_a = 1.47$, $H_o = 0.15$), and the lowest – for the provenance from the Western Beskidy Mts., Babia Góra massif and the Beskid Sądecki Mts. ($N_a = 1.27$, $H_o = 0.12$). Mean genetic distance between analyzed spruce provenances was equal to 0.027.

KEY WORDS

Norway spruce, isozymes, polymorphism, seed source

INTRODUCTION

The Norway spruce (*Picea abies* (L.) Karst.) is a widely distributed forest species, particularly important in the north-eastern part of Europe. Its genetic diversity, closely associated with the glacial history, and also with the human impact, has been the aim of numerous studies (Konert 2009; Kraj 2002; Nowakowska 2009). The above-mentioned knowledge allows for creating programs and long-term strategies concerning conservation of spruce stands on the basis of genetics (Sabor 2010). Nowadays,

this tree species is especially important because more than 30% of spruce population in Europe has been affected by the highest level of foliar damage (Forest Condition in Europe 2004). The short-sighted 19th century forest economy as well as negative impacts of industrialization caused current significantly weakened condition of spruce trees. This is a consequence of the promotion of spruce monocultures designated for reforestation of regions where the fir or beech were exhausted.

Massive dieback of spruce stands due to the so called spiral disease (Barszcz and Małek 2006;

Bruchwald and Dmyterko 2010) has been observed in Poland, particularly in the Beskidy Mts. General weakness and symptoms of increased deadwood have been recorded also at the IUFRO 1964/68 experimental plots in Krynica. As a result a huge part of the experimental area was damaged by massive windbreaks during the period 1999–2002. In the Wojkowa Forest Range, two from three blocks disappeared and one (block 06) retained 50% of trees (Masternak et al. 2009). In the section 14 in the Kopciowa Forest Range (No. 01, 03, 04, 05, 07, 08, 09 and 11) an extent of damage was smaller, but also oppressive.

During annual observations of spruce trees at the experimental plot, a steadily decreasing number of live spruces has been observed, which is a serious threat to the representativeness of the entire experiment. Taking into consideration spruce state in the Beskidy Mts., the value of provenance tests, including the experiments established by IUFRO, is greater than ever. They play the major role in the evaluation of breeding characteristics of spruce stands, and also constitute *in vivo* gene banks.

The results of provenance tests showed a high value of Beskidian spruce forests (Bałut and Sabor 2001; Giertych 1997; Sabor 1996, 2010). Beskidian spruce populations are characteristic of rapid growth and high plasticity, particularly in the conditions of Western and Central Europe. Few studies, however, have provided information on genetic structure of provenances from this region (Polak-Berecka and Perchlicka 2007). The aim of the present study was to determine, using isozyme markers, the genetic structure of selected Beskidian provenances of the Norway spruce which have survived at the site of IUFRO 1964/68 experiment.

MATERIAL AND METHODS

The study was conducted on 17 populations of Norway spruce from the Beskidy Mts. tested in IPTNS-IUFRO 1964/68 experiment in Krynica. Depending on the origin of parent stands, the analyzed provenances were classified, in accordance with Krutzsch (1968), into three out of 96 geographical regions (fig. 1).

The provenances tested differed with respect to the size of population from which seeds were collected: from at least 10 trees (collection 2), one forest stand (collection 3), from several forest stands (collection 4).

The description of seed source including characteristics of tested spruce provenances is presented in tab. 1.

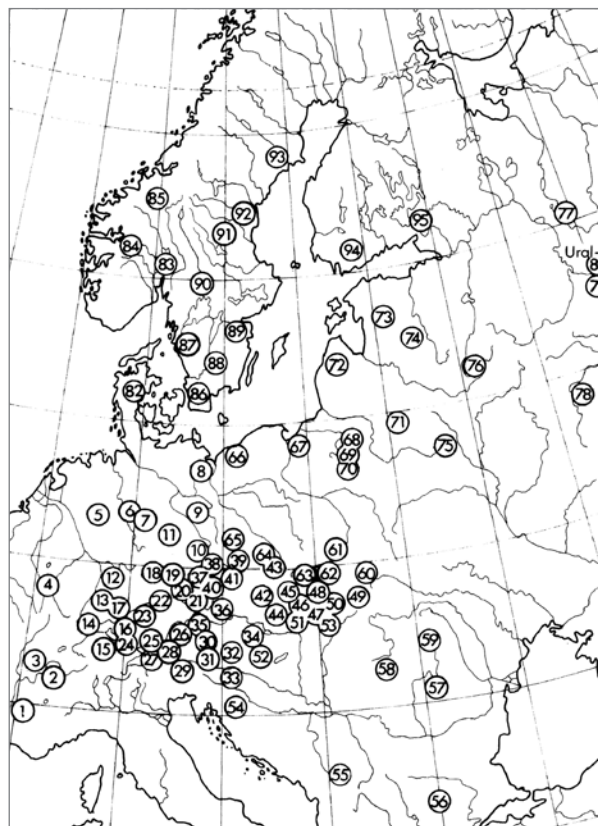


Fig. 1. Distribution of spruce provenances including region classification according to Krutzsch (1968). Black circles depict spruce provenances analyzed in the present study

Genetic diversity of 17 provenances of spruce from the Beskidy Mts. was studied using five enzyme systems encoding five loci (tab. 2). Dormant buds were used as study material. Samples were collected from five trees for each provenance in early spring 2003. Collecting larger number of samples was not possible due to the windblow disaster that took place in the period 1999–2002, and significantly reduced the number of trees on the experimental plot.

Protein separation was carried out by electrophoresis on 12% starch gel (Conkle et al. 1982). The evaluation of obtained zymograms was performed using the protocols developed by Konnert and Maurer (1995).

The genotypes of trees were determined and frequency of individual alleles in tested isozyme loci was calculated. To estimate genetic variation, the following

Tab. 1. Characteristics of Norway spruce parent stands included in the present study (Bału and Sabor 2001)

No. according to IUFRO	Provenance name	Geographic coordinates		Altitude	Type of seed source	Name of region (numbers according to Krutzsch (1968))
		latitude	longitude			
25	Tarnawa	49.0	22.5	700	2	Eeastern Beskidy Mts. 60
73	Jasina	48.3	24.3	700	4	
96	Jasina	48.3	24.3	1100	4	
61	Lipowa	49.7	19.1	700	4	Western Beskidy Mts. Babia Góra Beskid Sądecki 62
78	Bystra	49.7	19.7	700	4	
99	Orawa	49.3	19.3	950	2	
28	Istebna	49.3	18.5	650	4	Beskid Śląski Beskid Żywiecki 63
36	Rycerka	49.3	19.0	700	4	
42	Węgierska Górka	49.6	19.2	700	4	
48	Rycerka	49.3	19.1	560	4	
52	Ujszoły	49.5	19.1	500	4	
55	Rycerka, Kiczora 59 A	49.3	19.0	600	3	
65	Ujszoły	49.6	18.8	670	4	
74	Wisła, 54 A	49.7	18.9	615	3	
86	Węgierska Górka	49.6	19.1	700	4	
33	Liptovský Mikuláš. Kalište 157 A	49.1	19.9	920	4	Low Tatras

Type of seed source: 2 – seeds collected from at least 10 trees; 3 – seeds collected from a single spruce stand; 4 – seeds collected from several spruce stands.

parameters were used: mean number of alleles per locus (N_a) (Bergmann and Gregorius 1979), effective number of alleles per locus (N_e), observed (H_o) and expected heterozygosity (H_e) (Nei and Roychoudhury 1974). Additionally, fixation index F (Wright 1978), Nei's genetic distance (1972) and genetic differentiation between populations (F_{ST}) were determined. Based on Nei's ge-

netic distance, a dendrogram was constructed by un-weighted pair-group method using arithmetic averages (UPMGA) (Sneath and Sokal 1973).

Correlation coefficients between measures of genetic diversity and altitude of parent stands as well as the type of seed source used to set up the experiment were computed.

For calculating spruce genetic diversity, Pop-Gen v.1.31 software was applied (Yeh et al. 1999). Correlations between traits were estimated using Statistica ver. 8.0 software (StatSoft Inc. 2006).

Tab. 2. Summary of analyzed enzyme systems

No.	Enzyme name	E.C. number	Tested locus
1	Glucose-6-phosphate dehydrogenase (G-6-PDH)	1.1.1.49	G-6-Pdh-A
2	Glutamate dehydrogenase (GDH)	1.4.1.2	Ggh-A
3	NADH dehydrogenase (NADH)	1.6.99.3	Ndh-B
4	Sorbitol dehydrogenase (SBDH)	1.1.1.14	Sbdh-A
5	Diaphorase (DIA)	1.6.4.3	Dia-A

RESULTS

Genetic analysis of five isozyme systems showed that all tested loci were polymorphic. The highest level of genetic variation was observed for *Dia-A* locus, while the lowest – for *Gdh-A* and *Sbdh-A*.

Mean and effective number of alleles per locus calculated for 17 spruce populations from the Beskidy

Tab. 3. Characteristics of spruce genetic diversity measures of at provenance and regional level (region classification according to Krutzch)

Region of provenance according to Krutzch	Number of provenance	Provenance name	Measures of genetic diversity				
			N _a	N _e	H _o	H _e	F
Eastern Beskidy Mts. 60	25	Tarnawa	1.60	1.18	0.16	0.14	−0.157
	73	Jasina	1.40	1.29	0.20	0.16	−0.225
	96	Jasina	1.40	1.11	0.10	0.09	−0.143
		<i>Mean</i>	<i>1.47</i>	<i>1.20</i>	<i>0.15</i>	<i>0.13</i>	<i>−0.175</i>
Western Beskidy Mts. Babia Góra Beskid Sądecki Mts. 62	61	Lipowa	1.40	1.19	0.16	0.12	−0.270
	78	Bystra	1.20	1.14	0.12	0.08	−0.429
	99	Orawa	1.20	1.08	0.07	0.06	−0.200
		<i>Mean</i>	<i>1.27</i>	<i>1.14</i>	<i>0.12</i>	<i>0.09</i>	<i>−0.299</i>
Beskid Śląski Mts. Beskid Żywiecki Mts. 63	28	Istebna	1.80	1.38	0.32	0.24	−0.270
	33	Liptovský Mikuláš. Kalište 157 A	1.60	1.23	0.20	0.16	−0.217
	36	Rycerka	1.40	1.09	0.08	0.07	−0.111
	42	Węgierska Górka	1.60	1.23	0.20	0.16	−0.204
	48	Rycerka	1.20	1.09	0.00	0.06	1.000
	52	Ujsoly	1.40	1.09	0.08	0.07	−0.111
	55	Rycerka. Kiczora 59 A	1.20	1.09	0.08	0.06	−0.250
	65	Ujsoly	1.40	1.09	0.08	0.07	−0.111
	74	Wisła. 54 A	1.40	1.88	0.08	0.13	0.375
	86	Węgierska Górka	1.60	1.13	0.12	0.11	−0.111
	98	Istebna	1.40	1.09	0.08	0.07	−0.111
		<i>Mean</i>	<i>1.45</i>	<i>1.22</i>	<i>0.12</i>	<i>0.11</i>	<i>−0.011</i>
<i>Mean</i>			<i>1.42</i>	<i>1.20</i>	<i>0.12</i>	<i>0.11</i>	<i>−0.091</i>

Mts. ranged from 1.20 to 1.80 and from 1.08 to 1.88, respectively (tab. 3). The highest values of observed and expected heterozygosities were noted for the progeny of Istebna spruces, while the lowest – for Orawa and Rycerka provenances. Wright's inbreeding coefficient (F) varied from −0.429 for Bystra provenance to 1.000 for the progeny of Rycerka spruces. The mean value of this parameter was −0.091 indicating heterozygote excess within individuals included in the study.

Regional-level analysis of obtained values of genetic diversity revealed that the highest mean number of alleles per locus was characteristic for the Eastern Beskidy Mts. provenances (N_a = 1.47) and the lowest for Middle Carpathian provenances from Region 62 (Western Beskidy Mts., Babia Góra, Beskid Sądecki Mts.) – N_a = 1.27. The highest effective number of alleles per locus was noted for Western Carpathian provenances (N_e = 1.22) from Beskid Śląski and Beskid Żywiecki

regions, while the lowest for the progeny of population from Region 62 (Western Beskidy Mts., Babia Góra, Beskid Sądecki Mts.) – N_e = 1.14.

In the case of observed and expected heterozygosity, the highest values of the parameters investigated were obtained for Eastern Beskidy Mts. population (H_o = 0.15, H_e = 0.13) and the lowest for Region 62 population (Western Beskidy Mts., Babia Góra, Beskid Sądecki Mts.) which were equal to H_o = 0.12 and H_e = 0.09, respectively. Progeny of spruces from Region 63 (Beskid Śląski and Beskid Żywiecki) was characterized by the highest value of Wright's inbreeding coefficient equal to −0.011. The lowest value of this parameter (−0.299) was noted for the progeny of spruces from Region 62 (Western Beskidy Mts., Babia Góra, Beskid Sądecki Mts.).

The values of genetic differentiation F_{ST} varied from 0.104 in *G-6-pdh* locus to 0.226 in *Dia-A* locus.

The mean value of F_{ST} for analyzed spruce populations was 0.146.

Genetic distance between tested spruce provenances from the Beskidy Mts. was equal to 0.027. Most of the populations were divided into several small clusters, separated by small distances both between and inside formed units. Only in the case of Region 73 Jasina, a significant distinction from remaining provenances was noted which was indicated by large genetic distance equal to 0.23 (fig. 2).

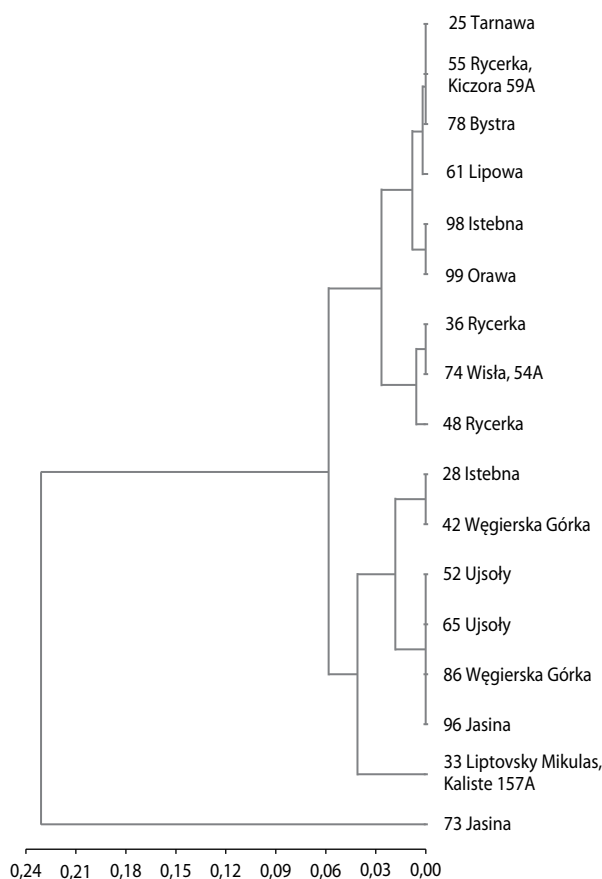


Fig. 2. Dendrogram constructed for Norway spruce populations from the Beskidy Mts. based on Nei's genetic identity (1978) by unweighted pair-group method using arithmetic averages (UPGMA)

No significant correlations were found between genetic diversity measures and altitude of parent stands as well as the type of seed source used to set up the experiment.

DISCUSSION

Genetic diversity of spruce population from the Beskidy Mts., expressed as mean number of allele per locus ($N_a = 1.42$) and observed heterozygosity ($H_o = 0.12$), is significantly lower from spruce polymorphism based on 7 loci, noted by Bergmann and Gregorius (1979) ($N_a = 2.14-3.14$, $H_o = 0.36-0.45$). Differences in the estimated values of genetic diversity can result from the number of analyzed loci and a level of polymorphism as well as from the type of the object used in the study. The highest consistency of results was obtained when compared with studies on selected spruce provenances tested in IPTNS-IUFRO 1964/68 experiment, where mean number of alleles per locus was 1.41 and observed heterozygosity – 0.11 (Masternak et al. 2011).

High values of the parameters tested were calculated for Istebna spruces (provenance 28), which confirms data previously presented by Polak-Berecka and Perchlicka (2007). Comparable results were found in the study conducted by Modrzyński and Prus-Głowacki (1998) which indicated high polymorphism (80%) and genetic diversity expressed as mean number of alleles (2.40) and observed heterozygosity (0.228) for Istebna provenance. Breeding characteristics of spruces from Istebna region have been proven in numerous provenance experiments (Giertych 1984). The analysis of the values of selection parameters tested on IUFRO 1964/68 conservation area in Krynica, revealed very high quality of spruces from the Beskid Śląski Mts. which was expressed by high survival rate, tree height corresponding to the 1st site class and, in most cases, late flushing and resistance to spring frosts. Spruce forests from the Beskid Śląski Mts. are assigned to a group of provenances for which growth characteristics improve with age (Sabor 1996). The value of F_{ST} coefficient calculated for 17 spruce provenances from the Beskidy Mts. was equal to 0.146 indicating that almost 15% of variability was explained by interpopulation differentiation. The obtained value is significantly higher that previously described in other works on coniferous species of wide distribution in which 90% of variability was attributed to variation within population (Ledig 1986). The high value of F_{ST} coefficient can result from low gene flow between analyzed populations according to the rule saying that the lower level of gene flow the smaller intrapopulation variation is observed being

accompanied by higher variation between populations (Burczyk 1998).

Genetic distance between spruces from the Beskidy Mts. was equal to 0.027 and similar to the value obtained by Lewandowski and Burczyk (2002) for spruce populations from the southern region of north-east Poland. The results presented in our work also confirm those obtained by Lewandowski et al. (1997). In the latter work, the highest individuality (0.008) was found between a group of populations from the Sudety Mts. and north-east Poland, while the lowest – between populations from the Beskidy Mts. and the north-eastern part of Poland (0.005). Obtained values of genetic distances for spruce populations from the Beskidy Mts. are lower than those for Swedish (0.084), Belarusian (0.081), Russian (0.073) and Ukrainian (0.077) provenances (Krutovskii and Bergmann 1995). At the same time, they exceed values noted for provenances from the entire range of distribution (0.007) (Langercrantz and Ryman 1990) as well as for Slovakian (0.016) (Paule et al. 1990) and Italian spruces (0.019) (Giannini et al. 1991).

In this study no differences in spruce genetic structure dependent on the type of seed source used to set up the experiment were documented. Studies conducted by Giertych (2002) and Skråppa (1994) further indicate that phenotypic selection does not affect a level of genetic variation in spruces. Evaluation of the influence of selection on genetic structure of forest trees requires additional research with the greatest possible number of individuals as well as applying various biochemical and molecular markers.

Provenance experiments are important objects of research that allow not only for gaining knowledge about phenotypic and genetic value of progeny tested stands, but they also constitute *in vivo* genetic banks. This is especially important in the face of disaster and the loss of valuable parent's stands when tested material can be used for reconstituting the population (Chałupka et al. 2008). History of the spruce provenances, tested in IPTNS-IUFRO 1964/68 experiment in Krynica showed, however, that sometimes valuable material may be irretrievably lost.

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