

# Effect of seed collection and site altitude on the growth and genetic variability of early and late flushing provenances of Norway spruce tested in the IPTNS-IUFRO 1964/68 site in Poland

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## ABSTRACT

The growth characteristics and the genetic variability of 23 population of Norway spruce tested in the largest international comparative experiments IPTNS-IUFRO 1964/68 in Krynica were analysed. The studied populations belong to early and late flushing provenances from Alpine, Carpathian and north-eastern range of occurrence of species. The height, diameter at breast height (DBH) and genetic diversity of 79 trees were examined using random amplified polymorphism DNA (RAPD) technique. The mean spruce height at the age of 45 years was 17.5 m and the DBH 20.4 cm. The average and the effective number of alleles per locus calculated for all studied populations was 0.90 and 1.20, respectively. The expected heterozygosity was 0.10. The obtained results show statistically significant relationship of the parameters of genetic variability of Norway spruces and the type of seed collection from which the IUFRO experience was established. It was found that the genetic variability of the studied population depends on the longitude and height above sea level of mother stands. No significant correlation was found between the type of seed collection and location of mother stands and height and DBH of Norway spruce. Also the growth characteristics (height and the DBH) of trees do not depend on their genetic variability.

## KEY WORDS

*Picea abies*, RAPD, genetic polymorphism, selection

## INTRODUCTION

Provenance experiments are great source of knowledge on plasticity and diversity of forest trees. Progeny testing in sub-populations in similar habitat conditions allows to eliminate the influence of the environment and gives the opportunity to investigate genetic variability and search for plastic provenance of high strength of

adaptability. Provenance experiments, apart from the possibility of observation of phenotypic traits, give an opportunity to conduct genetic analyses of progeny of population under study, often very geographically distant.

Undoubtedly, the most important role in understanding the genetic variability of Norway spruce has been played by the International Union of Forest Re-

search Organisations (IUFRO). On its initiative, a series of experiments testing the sub-populations of this species has emerged: UFRO 1938/39, IUFRO 1964/68 and IUFRO 1972. The largest of the above-mentioned studies is IPTNS-IUFRO 1964/68, an international inventory test, where on 20 areas, one can find 1096 spruce provenances from the entire range of the species, including 91 from the Polish territory. Efforts put in preparation and maintenance of experiment on such a large scale have resulted in many studies reporting the variability of adaptive (Sabor 1984; Sabor 1989; Żółciak et al. 2009) and growth (Bałut, Sabor 2002; Masternak et al. 2011; Sabor and Stanuch 2009) traits, as well as genetic variability of this species (Masternak et al. 2011; Masternak and Sabor 2013; Masternak 2015). An additional advantage of this experiment is collections of trees of each provenance, which were used to establish experimental areas (Krutzsch 1968). The lack of such information often hampers interpretation of the results (Konnert and Ruetz 2001).

The main objective of the study was to evaluate the growth characteristics (height and diameter at breast height (DBH)) and the genetic polymorphism (analysed using random amplified polymorphism DNA (RAPD) technique) of selected origins of early and late flushing spruce within the IPTNS-IUFRO 1964/68 experiment. The study attempts to determine how the observed variability is affected by high altitude location of maternal forest stands and diverse number of trees used as input material.

## MATERIAL AND METHODS

### The plant material

The study included progeny of 23 selected early and late flushing provenances of Norway spruce from Alpine, Carpathian and north-eastern range of the occurrence of species, which are tested in the international inventory experiments IPTNS-IUFRO 1964/68 (Tab. 1). Each population was represented by 10 randomly selected trees. The examined provenance was included by Krutzsch (1968) to 12 regions of the occurrence of the spe-

**Table 1.** Characteristics of the seed source and average values of growth and genetic variability of the studied populations of Norway spruce

Region	Name of populations	Krutzsch regions	Seeds collection	Spring flushing	Geographical coordinates		Height above sea level	Growth parameters		RAPD			
					Latitude	Longitude		Height	DBH	N <sub>a</sub>	N <sub>c</sub>	H <sub>c</sub>	I
Alpine populations	0417 – Stanz-Kindtal-Allerheiligen	32–Styria (N–E) 1; Austria	6	E	47.5	15.5	750	17.1	19.2	0.848	1.160	0.096	0.145
	0441 – Deutschlandsberg	33–Styria (S–E); Austria	6	E	46.8	15.2	700	15.9	18.6	1.025	1.205	0.117	0.173
	0451 – Seewiesen, Seereith	32–Styria (N–E) 1; Austria	3	E	47.6	15.3	875	14.6	15.6	1.000	1.163	0.095	0.143
	0735 – Knittelfeld	32–Styria (N–E) 1; Austria	6	E	47.2	14.8	1300	17.0	20.0	0.696	1.085	0.052	0.079
	0761 – Liembergwald/Zell Am See, 59	28–Tyrol–Salzburg; Austria	5	E	47.3	12.8	875	16.4	20.8	0.152	1.005	0.004	0.006
	0765 – Stuebing/Gamskogel	32–Styria (N–E) 1; Austria	6	E	47.3	15.6	640	15.1	17.8	1.177	1.261	0.151	0.223
	0986 – Foelz, Mayerberg	32–Styria (N–E) 1; Austria	3	E	47.5	15.2	950	17.3	18.2	0.886	1.132	0.073	0.107

Carpathian populations	0340 – Cimpeni, XV Valea Mare 24	58–BihorMts, Transylvania; Romania	2	L	46.3	23.0	1260	17.5	20.5	0.911	1.182	0.103	0.151
	0439 – Dorna Cindreni, II Rosia, 50A	59–East Carpathians; Romania	2	L	47.4	25.4	975	18.4	21.4	1.101	1.219	0.130	0.197
	0487 – Cucureasa, 65	59–East Carpathians; Romania	1	L	47.3	25.0	1260	17.8	19.7	0.380	1.069	0.040	0.058
	0700 – Cosna, Cucureasa 4A	59–East Carpathians; Romania	2	L	47.3	25.2	1025	16.9	20.5	0.823	1.186	0.104	0.150
	0749 – Cucureasa, 65	59–East Carpathians; Romania	1	L	47.3	25.0	1410	17.8	21.0	0.684	1.075	0.043	0.068
	0922 – Cucureasa, 65	59–East Carpathians; Romania	1	L	47.3	25.0	1100	18.7	22.2	1.063	1.202	0.114	0.170
	0925 – Tarnawa	60–East Beskids (Tarnawa); Poland	2	L	49.1	22.8	700	17.6	20.3	0.342	1.087	0.046	0.066
	0111 – Bricalovic	75–Belarus	4	L	53.3	28.7	160	19.8	24.7	1.215	1.219	0.126	0.188
	0146 – Puszcza Białowiecka	70–Białowieża Primeval Forest; Poland	4	L	52.6	23.6	130	17.8	21.0	1.19	1.210	0.120	0.179
	0326 – Knyszyn	69–Augustów Lakeland, Podlasie; Poland	4	L	53.3	22.9	150	18.4	22.9	1.025	1.181	0.105	0.156
North – eastern populations	0351 – Ukmerges	71–Vilnius Lakeland, Belarus Lakeland; Lithuania, Belarus	6	L	55.3	24.7	73	18.7	23.6	0.987	1.235	0.132	0.192
	0447 – Borki Knieja	68–MasurianLakeland; Poland	2	L	54.1	22.1	160	18.6	21.8	0.962	1.136	0.079	0.119
	0834 – Molvotitsk	76–East Russia (Valdai Hills); Russia	4	L	57.5	32.5	200	17.0	18.8	1.203	1.246	0.143	0.213
	0841 – Ignalino	71–Vilnius Lakeland, Belarus Lakeland; Lithuania, Belarus	6	L	55.3	26.2	185	15.6	17.5	1.076	1.271	0.154	0.226
	0856 – Roslavi	78–Russia 2 (Central Russian Upland, Smolensk–Moscow Heights)	4	L	54.0	33.0	270	18.4	19.9	1.177	1.295	0.166	0.241
	0917 – Mikaszowka	69–Augustów Lakeland, Podlasie; Poland	4	L	53.9	23.4	120	18.5	21.2	0.646	1.141	0.081	0.120
	1147 – Mogilevskoje Oblast	75–Belarus	6	L	53.7	30.0	150	17.9	22.7	1.228	1.264	0.148	0.218
						Mean		17.5	20.4	0.900	1.20	0.100	0.100
						Standard deviation		1.2	2.1				
						Coefficient of variability		7.15	10.17				

E – early flushing provenances; L – late flushing provenances;  $N_a$  – average number of alleles per locus;  $N_e$  – effective number of alleles per locus;  $H_e$  – expected heterozygosity.

cies. The studies were conducted on 230 spruce trees grown from six seed collections: from one tree, collection 1; from at least 10 different trees, collection 2; from a single tree stand, collection 3; from several stands, collection 4; from the stands recognised by the local forest administration, collection 5; and from one forest district area, collection 6 (Sabor 1977).

### Growth features

The total height of trees was measured by Vertex system with an accuracy of up to 0.1 m. DBH at 1.3 m was measured by caliper with an accuracy of up to 0.1 cm. Growth parameters of trees were characterised by arithmetic mean, standard deviation and coefficient of variability.

### RAPD analysis

Genomic DNA was extracted by means of the commercial Qiagen DNeasy Plant Mini Kit. Each amplification reaction was performed in a 10- $\mu$ l reaction mixture consisting of 10 $\times$  concentrated reaction buffer, 25 mM magnesium chloride, 10 mM deoxynucleotide, 100 mM primer, 0.5 U Taq polymerase and 20 ng of genomic DNA. The sequences of the primers that were used are given in Table 2. Amplifications were conducted in a thermocycler 'T-Personal' from Biometra programmed for 40 cycles, which consisted of denaturation (94°C, 30 s), annealing (42°C, 30 s) and elongation of DNA (68°C, 2.5 min). These cycles were preceded by a 5-min initial denaturation at 94°C and ended with a 5-min elongation of the products formed at 68°C. After each PCR cycle, the amplification products were separated using agarose gel (1.5%) electrophoresis. The results were examined under ultraviolet (UV) light and stored by the Syngen company system for visualisation of gels, and the computer program Scion Image. The electrophoretic images were obtained after the section of the PCR products were analysed for the presence or absence of product in a particular position in the gel with regard to the 1-kb DNA fragment length standard (Fermentas Company).

### Data analysis

On the basis of the RAPD analysis, the average number of alleles per locus ( $N_a$ ) was determined. The distribution of allelic variants in populations of spruce was based on the effective number of alleles at the lo-

cus ( $N_e$ ) (Bergman and Gregorius 1979). The expected heterozygosity ( $H_e$ ) was determined according to Nei and Roychoudhry (1974). The calculation of the level of the interpopulation differentiation was based on Shannon's index (I) (Brown and Weir 1983). On the basis of the genetic distances between early and late flushing population of Norway spruce, the principal coordinate analysis (PCoA) method was used. The parameters of genetic variation were calculated using GeneAlex ver. 6.41 (Pekall and Smouse 2006).

The distribution of the compatibility of the studied growth traits with a normal distribution was checked using the Shapiro–Wilk test. The homogeneity of variance of the spruce growth parameters was verified by Levene's test. The effects of origin of population on the growth characteristics of spruce were evaluated using analysis of variance (ANOVA). The relationship between the growth traits, the genetic parameters and the type of seed collections, which was established by the experience and location of mother stands, was analysed using the nonparametric Spearman correlation method. The calculations were performed using Statistica ver. 9.0 (Stat.Soft. Inc. 2010).

## RESULTS

### Growth variability of phenological forms of Norway spruce

The highest value of parameters of growth characteristics (height and DBH) were shown by spruce trees from late flushing population 0111 – Bicalovic. The lowest height and DBH were recorded in early flushing population 0451 – Seewiesen, Seereith. Compared to the height of trees, the DBH has a higher variability, which was demonstrated by higher value of the coefficient of variation (Tab. 1). ANOVA showed no significant effect of population on the height and DBH of spruce at the age of 45 years.

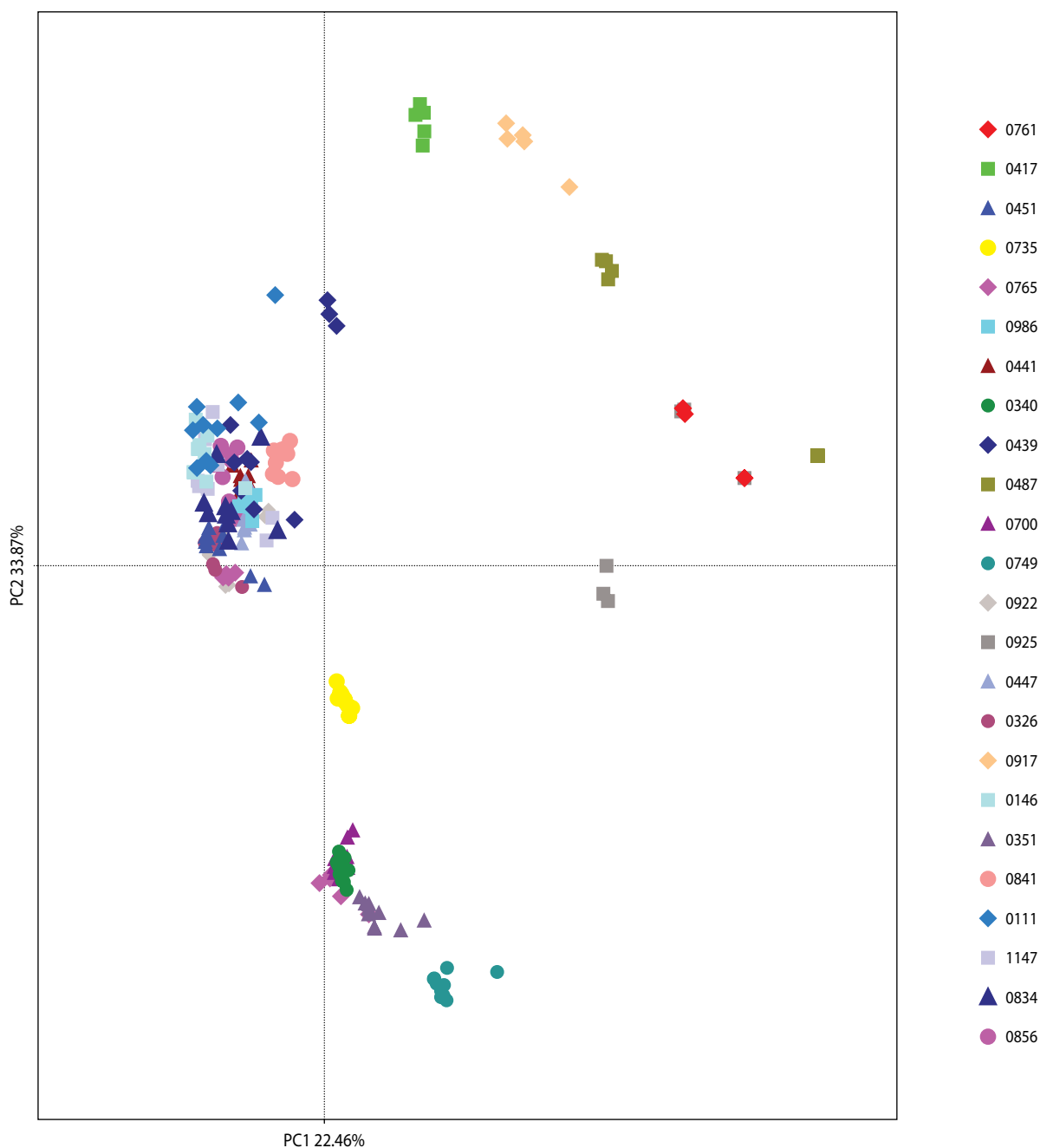
### Genetic variability of phenological forms of Norway spruce

The highest mean number of alleles per locus ( $N_a$ ) was exhibited by spruces from population 1147 – Mogilevskoje Oblast, and the effective number of alleles per locus ( $N_e$ ) provenance was from 0856 – Roslavi. The lowest value of these parameters was observed in the

population 0761 – Liembergwald/Zell Am See, 59. The Shannon index ( $H'$ ) and the expected heterozygosity ( $H_e$ ) ranged from 0.006 to 0.241 and from 0.004 to 0.166, respectively, as shown in Table 1.

The PCoA method allowed to graphically represent the distribution of spruce variation in a two-dimension-

al system, where the first two components accounted for more than half of the total variation (58.32%). Most of the late flushing trees were located in the first quadrant chart, whilst late provenances were found mainly in the fourth quarter (Fig. 1).



**Figure 1.** The result of principal coordinate analysis (PCoA). The numbering of the population according to Table 1

**Table 2.** The characteristic of RAPD primers (Operon Company) and the number of generated products

Name of a primer	Sequence (5'→3')	Number of generated products
Opa 10	GTGATCGCAG	12
Opj 6	TCGTTCCGCA	12
Opa 4	AATCGGGCTG	11
Opg 10	AGGGCCGTCT	11
Opc 9	CTCACCGTCC	10
Ope 9	CTTCACCCGA	10
Opa 9	GGGTAACGCC	7
Ope 8	TCACCACGGT	6

### Statistical analysis

In this study, there was no effect of location of maternal forest stands or type of seeds collection on height and DBH of Norway spruce at the age of 45. Moreover, growth features were not dependent on genetic variability of trees. Significant positive correlation was observed between the values of all of genetic variability parameters and longitude as well as between effective number of alleles at locus, expected heterozygosity and longitude. It indicates a continuous variability of genetic polymorphism parameters from southwest to northeast (Tab. 3).

There is a strong negative relationship between genetic variability and the height above sea level of maternal forest stands. The obtained result indicates narrowing of the gene pool of mountain origins compared to lowland populations. Additionally, positive correlation between the type of seeds collection and effective number of alleles at single locus, expected heterozygosity

and the Shannon index indicates that the greater the number of trees for which one collected seeds for experimental purposes, the greater variability is attributed to their progeny (Tab. 3).

## DISCUSSION

### Variability of growth features and genetic polymorphism

The mean value of expected heterozygosity of Norway spruce tested in IPTNS-IUFRO 1964/68 experiment in Krynica was almost two times lower ( $H_e = 0.14$ ) compared to the previously estimated for the Baltic-Nordic ( $H_e = 0.283$ ), Hercine-Carpathian ( $H_e = 0.279$ ) and Alpine (0.288) provenances (Collignon et al. 2002) as well as for the Polish populations ( $H_e = 0.298$ ) (Nowakowska et al. 2006). The observed differences may probably result from the analysis of different loci and may result from the specific nature of the investigated population (provenience experiment analysing the progeny of spruce from the entire range of the species). It can also be assumed that the result obtained by the author was affected by subjective, phenotype-targeted selection of trees for the analysis. First, from the same experiment of Norway spruce of IPTNS-IUFRO 1964/68, studied provenances were used to identify markers correlated with the late flushing (Masternak 2015).

The present studies it was aimed at determining the genetic variability of spruce and its relationship with maternal forest stands location and the type of seeds collection. The PCoA also indicated a relationship between genetic structures and the time of vegeta-

**Table 3.** The correlation coefficients between the type of seed collection, growth traits and parameters of genetic variation of spruces

Traits	Growth traits		Parameters of genetic variability			
	H	DBH	Na	Ne	He	I
Seeds collection	-0.235	-0.145	0.368	0.481*	0.514*	0.495*
Height above sea level	-0.353	-0.341	-0.456*	-0.493*	-0.502*	-0.474
Longitude	0.375	0.336	0.479**	0.570**	0.540**	0.545**
Latitude	0.336	0.203	0.368	0.424*	0.422*	0.398
Height	—	0.872***	0.104	0.014	0.02	0.022
DBH	0.872***	—	0.133	0.036	0.038	0.037

Significant at \* 0.05 level, \*\* 0.01 level and \*\*\* 0.001 level.

tion period start. Differences in DNA of provenance characterised by different phenotype were confirmed in the study conducted by Collignon et al. (2002) who divided the origin of Norway spruce into two main groups based on the RAPD analysis – Northern and Central Europe – and the obtained results were consistent with the variability estimated based on the phenological characteristics. Moreover, results of the studies obtained for other species of forest trees demonstrate a significant diversity of genetic structure in populations characterised by different phenological forms (Kraj and Sztorc 2001).

### **The effect of seed collection on the growth and genetic polymorphism**

The significant impact of the type of seed collection obtained from the parental stands on the genetic variability of progeny tested in the IPTNS-IUFRO 1964/68 experiment in Krynica has been proved. However, research by Hosius et al. (2006) showed that the cultivation treatments had no influence on the genetic structure of young stands of spruce, pine, oak and beech. Similar conclusions were reached by Skrøppa (1994), who found no differences in the genetic structure of spruce populations from natural and artificial regeneration. According to Giertych (2002), the removal of defective stands and target diameter harvesting is carried out on the basis of the observed phenotype and both do not change the effects of natural selection because they do not impoverish the genetic structure but only accelerate the process of natural tree removal. Giertych (1989) argues that the intensity of natural selection is much higher than for human-directed selection. In nature, each tree should be replaced by a single individual. The author points out, however, that a change in the intensity is related to a change in the direction of the selection. Populations selected by man will be characterised by good growth and health but not necessarily by the ability to adapt, such as the populations selected by nature. On the basis of SSR markers, Nowakowska (2007) found no significant impact of the number of trees (15 and 65 individuals) on the genetic diversity of Scots pine.

Gömöry (1992), studying the genetic structure of six enzyme systems of spruce in the primary forest and in a forest formed from natural and artificial renewal, found no differences in the forest resulting from natu-

ral regeneration and confirmed a clear depletion of the genetic structure of forest stands resulting from artificial renewal. This is supported by studies, led by Tröber and Brandes (2005), on the genetic structure of natural regeneration in Germany, taking into account the number of trees that were involved in the regeneration. The analysis of 10 isoenzymatic loci revealed that the genetic structure of the progeny of small groups or individual trees was characterised by a gentle decline in the genetic diversity, compared to the renewal of the solid stands.

Analysing the impact of the selection on the structure and variability of artificially renewed forest stands, it should be remembered that this is the result of many factors. Undoubtedly, one of the major issues is the seed lot used for the production of reproductive material. The genetic composition of seed from the same tree could be different each year. The level of genetic variation comes not only from the choice of provenances but also from the procedures used for seed harvesting, the selection of trees and the quality of the harvested seeds (Giertych 2002). Harvesting seeds from deformed or stunted trees, randomly or outside the forest, can effectively deform the gene pool designed to breed a new generation (Giertych 1989). In addition, research by Konnert and Ruetz (2003) showed that the method used for sorting seedlings in the plant nursery may have a minor influence on the genetic structure of populations.

### **Effect of the height above sea level on growth features and values of genetic variability**

Many authors indicate that populations of lowlands have decreased number of polymorphic loci in comparison with spruce populations in mountain regions (Prus-Głowacki and Modrzyński 2003; Prus-Głowacki et al. 2007), explaining their observations by Marshall and Allard (1970) hypothesis, which states that heterozygotes exhibit greater ability to adapt to stress conditions. Similar results were obtained during evaluation of the impact of pollution on genetic variability of spruce, where the trees resistant to air pollution and thus characterised by high adaptability, exhibited higher level of genetic variability (Prus-Głowacki and Godzik 1991).

In the present study, we obtained significant differences in the genetic variability of progeny tested depending on the height above sea level of maternal forest



stands, demonstrating decreased genetic variability of mountain provenance in comparison with the population of lowland. Similar results were obtained by Bergmann and Gregorius (1979) as well as Modrzyński and Prus-Glowacki (1998), who showed that adaptation to the mountain conditions is correlated with gene pool narrowing. A similar opinion was raised by Maghuly et al. (2008), who found that adaptation to mountain conditions is associated with a significant reduction in heterozygosity based on spruce analysis using SSR markers of nuclear DNA. In turn, Lewandowski and Burczyk (2002), who analysed polymorphism of isoenzymes of Polish spruce, did not observe any significant differences between the lowland and mountain populations. Moreover, Polak-Berecka (2000) denotes that selection processes together with adaptation may affect the genetic structure of spruce population; however, the author indicates that at the current stage of research, one cannot determine the direction of possible changes.

#### **Impact of geographic location of the source of seeds' origin on growth features and genetic variability parameters**

The study demonstrated the existence of a continuous variation of genetic variability parameters of spruce. With increasing longitude, one observed increased effective number of alleles at a single locus and expected heterozygosity as well. In contrast, longitude was positively correlated with all analysed parameters of genetic variability. The obtained results are consistent with the Wright hypothesis (1976), who found that many physiological, morphological and incremental characteristics of forest trees exhibit cline variability, which usually runs from north to south, from higher to lower positions and from dry to moist habitats. Sabor (1998) adds that these trends mentioned are the result of adaptation of trees to environmental conditions. So far, the cline variability has been proven for many phenotypic traits, for example, the bud set (Danusevičius and Gavriliavičius 2001; Sjøgaard et al. 2008), growth rates (Holzer 1968; Modrzyński 1995), tolerance to UV-B radiation (Pukacki and Modrzyński 1998) or drought (Modrzyński and Erikson 2002) of Norway spruce.

#### **Relationship between growth features and genetic polymorphism**

In the previous studies, a significant relationship between growth features and genetic polymorphism of trees was found only for birch (Wang 1996) and maritime pine (Durel et al. 1996). In the present study, as in the studies conducted by many other authors (Bush and Smouse 1992), such relationship was not reported. However, with no doubts, such relationships may be found; however, as reported by Polak-Berecka (2000), it relates only those loci that are involved in metabolic pathways important for the organism's metabolism. In terms of spruce, up to now, significant correlations between genetic variability and, amongst others, environmental stress have been observed (Bergmann and Scholz 1985).

#### **CONCLUSIONS**

Norway spruce is characterised by high genetic variability, which results mainly from the wide range of occurrence of this species. This variability can be studied through the provenance tests, where the progeny of the particular population of the species is placed in one location. In these experiments, the relationship between various traits can be examined, which was the aim of the study in this paper.

In our study, we proved the relationship between the genetic variability of the population and their geographical location (longitude, height above sea level). This indicates that natural selection causes the adaptation to local environmental conditions. Uncontrolled transfer of populations between different regions or from lower to higher height above sea level can cause disintegration of stands. Therefore, it is necessary to use a seed regionalisation. In our study, it was also found that if the seeds are collected from a large number of mother trees, the progeny was characterised by greater genetic variability. This is an argument for the correctness of the selection in Poland, because population selection constitutes a basic direction of selection, whilst individual selection is its complement.



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