PRELIMINARY RESULTS ON ALLOZYME DIVERSITY AND DIFFERENTIATION OF NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) IN POLAND BASED ON PLUS TREE INVESTIGATIONS

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ABSTRACT

Genetic diversity and genetic differentiation among Polish populations of Norway spruce (*Picea abies* (L.) Karst.) were studied analyzing allozyme variation at 24 loci. Presented investigations based on 81 clones represented 6 populations from three different regions: Sudety Mts., Beskid Mts. and North-East Poland. On average, the expected heterozygosity was 0.134, 52.8% of loci were polymorphic, and the number of alleles per locus was 1.8. Our data confirm the hypothesis that a lower level of variation of *Picea abies* exists in central Europe, compared to other regions of its natural distribution. Relatively small allozyme differentiation among regions was observed ($F_{st} = 0.027$, and Nei's genetic distances ranged from 0.005 to 0.008). Reasons for the low genetic differentiation observed between Southern and Northern populations are briefly discussed.

KEY WORDS: *Picea abies*, allozymes, genetic diversity and differentiation.

INTRODUCTION

Norway spruce (*Picea abies* (L.) Karst.), besides Scots pine, is one of the most important tree species in Poland, covering more than 500,000 hectares. For many years, morphological characters of this species have been intensively investigated in many provenance-testing programs (Giertych 1979, Krutzsch 1992). Also, isoenzyme variation and differentiation between populations in Europe were rather well documented, compared to other tree species (Tigerstedt 1973, Lundkvist and Rudin 1977, Bergmann and Gregorius 1979, Altukhov et al. 1986, Lagercrantz and Ryman 1990, Giannini et al. 1991, Paule and Gömöry 1993, Goncharenko et al. 1995, Krutovskii and Bergmann 1995, Müller-starck 1995). In contrast to morphological characters (Giertych 1972, 1973, 1976), information about isoenzyme variations in Norway spruce from Poland is very scarce (Krzakowa and Korczyk 1995, Prus-Głowacki and Godzik 1995).

The present Polish distribution of Norway spruce is the result of spreading from two different refugia, located in the Carpathians and in the present day area of Moscow. Spruce from the first refugium spread to the Southern part of Poland, and from the second – to North-Eastern Poland (Schmidt-Vogt 1977). For this reason, it is very interesting to investigate the level of isoenzyme variation and differentiation between populations from these two different regions. Some of preliminary results are presented in this paper.

MATERIALS AND METHODS

Plant material

Seeds were collected in 1993 from 81 clones in a Norway spruce clonal archive established by the Institute of Dendrology in 1968-1973. The investigated clones originated from plus trees of six localities: 1. Przerwanki (4 clones), 2. Zwierzyniec Białowieski (13 clones), 3. Ustroń (3 clones), 4. Rycerka (14 clones), 5. Istebna (37 clones), 6. Stronie Śląskie (10 clones). They belong to three different regions: North-East Poland (pop. 1 and 2), Beskid Mts. (pop. 3, 4, and 5) and Sudety Mts. (pop. 6) (Fig. 1).

Electrophoretic analysis

Analyses were performed using macrogametophyte tissue from dry seeds. To investigate an individual genotype of a tree, 8 macrogametophytes were analysed. The following 12 enzyme systems encoded by 24 loci were employed: (Enzyme Commission number, locus abbreviations and number of detected alleles in each locus in parentheses): fluorescent esterase (EC 3.1.1.1., Fle,(3)), glutamate dehydrogenase (EC 1.4.1.2., Gdh,(2)), glutamate-oxaloacetate transaminase (EC 2.6.1.1., Got1,(1); Got2,(1); Got3,(3)), isocitrate dehydrogenase (EC 1.1.1.42., Idh1,(2); Idh2,(2)), leucine aminopeptidase (EC 3.4.1.1., Lap1,(4); Lap2 (3)), malate dehydrogenase (EC 1.1.1.37., Mdh1,(2); Mdh2,(2); Mdh3,(4); Mdh4,(2)), menadiene reductase (Mnr1,(3); Mnr2,(4); Mnr3,(3)), phosphoglu-

TABLE 1. Mean estimate of number of alleles per locus (Na), percentage of polymorphic loci (P, 99% criterion), observed heterozygosity (Ho), expected heterozygosity (He), and Wright's fixation index (F) with standard errors in parentheses, in the investigated regions of Norway spruce.

Region	Na		P	Ho		He		F
1. Sudety Mts.	1.4	(0.1)	41.7	0.108	(0.032)	0.124	(0.037)	+0.129
2. Beskid Mts.	2.3	(0.2)	58.3	0.127	(0.035)	0.135	(0.037)	+0.059
3. NE Poland	1.7	(0.1)	58.7	0.152	(0.041)	0.143	(0.037)	-0.063

comutase (EC 2.7.5.1., Pgm1,(3)), 6-phosphogluconate dehydrogenase (EC 1.1.1.44., 6Pgdl,(2); 6Pgdl,(3)), phosphoglucose isomerase (EC 5.3.1.9., Pgi1,(1); Pgi2,(3)), sorbitol dehydrogenase (EC 1.1.1.14., Srdh,(3)), superoxide dismutase (EC 1.15.1.1., Sod1,(2); Sod2,(1)).

Protein extraction and electrophoresis procedures have been taken from Muona et al. (1987). The interpretation of zymograms follows the inheritance models reported by Bergmann (1973), Lundkvist (1979), Muona et al. (1987), Morgante et al. (1989) and Goncharenko et al. (1995).

Statistical methods

Genetic variability was described by the average number of alleles per locus (Na), percent of polymorphic loci (P, 99% criterion) and expected heterozygosity (He). Genetic differences between populations were measured by the genetic distance index (D) of Nei (1972). Because of the small number of individuals within stands all statistical analyses were done only for the three geographical regions and not for individual samples.

RESULTS AND DISCUSSION

Among the 24 studied loci 20 were polymorphic and only four were monomorphic. The most polymorphic loci appeared to be Mdh3, Men2 and Lap1, which had four alleles per

locus. Nine loci had three alleles, and eight loci had only two variants. Parameters of genetic variations for the three geographical regions are presented in Table 1. The mean number of alleles per locus (Na) ranged from 1.4 for Sudety Mts. region to 2.3 for Beskid Mts. The highest percentage of polymorphic loci (above 58%) was observed for populations from the Beskid Mts. and NE Poland, while lower values were observed for the Sudety Mts. (P=41.7%). Expected heterozygosities were generally similar among populations from the three regions and did not deviated significantly from the observed ones. An excess of heterozygotes was observed only for populations from NE Poland (F=-0.063).

Parameters of genetic variation presented in this study are high and generally similar to those reported for other conifers (Müller-starck et al. 1992). However, they are lower than the mean parameters calculated for 70 European populations of Norway spruce by Lagercrantz and Ryman (1990). The lower variation of *P. abies* from Central Europe was already reported and it is suggested that the populations from Central Europe lost their diversity during the last glaciation (Lagercrantz and Ryman 1990).

Generally, small differences among populations from the three geographical regions were observed (Table 2). The Nei's genetic distance ranged from 0.005 between Beskid Mts. and NE Poland to 0.008 between Sudety Mts. and NE Poland. Also the Fst parameter (Fst=0.027) indicated that only 2.7% of total variation resulted from variation among populations from different regions.

Gianini et al. (1991) found that mean Nei's genetic distance among nine Italian populations of *P. abies* was 0.019, and 4.2% of the total genetic variation was due to interpopulational differentiation. The genetic distance observed for five Latvian Norway spruce populations ranged from 0.003 to 0.012, while 1.7% of total variation resulted from differences among populations (Goncharenko et al. 1995). In the light of these findings, the level of population differentiation observed in our study (where D ranged from 0.005 to 0.008 and Fst = 0.027) should be considered as low, which is rather unexpected, because of the specific migrations of *P. abies* after the last glaciation in Poland.

As it was mentioned above, Norway spruce was spread to Poland from two different refugia, located in the Carpathians and in the present day area of Moscow (Schmidt-Vogt 1977).

Fig. 1. Locations of the studied populations of *Picea abies*.

TABLE 2. Genetic distance following Nei (1972) between three regions of Norway spruce in Poland.

Region		1	2	3
1.	Sudety Mts.	–	0.006	0.008
2.	Beskid Mts.		–	0.005
3.	NE Poland			–

The different origin of North-Eastern and Southern populations should be reflected in genetic differentiation parameters. This is only partially observed in our study.

The possible explanation for this is a possibility of extensive gene flow which could have lowered differences among re-immigrated populations. Środoń (1967) suggested that the two groups of populations probably came into contact during the Atlantic period (5,000-3,000 years before present). Another reason of low observed population differentiation may result from intensive management of *P. abies* during the last 200 years, which included uncontrolled seed transfer among populations over large distances.

In general, the sample sizes are small and therefore all presented here parameters of genetic variation and differentiation between regions should be considered with caution, and treated as preliminary for further, more intensive studies.

In order to explain more precisely the small differentiation of Norway spruce observed by us in Poland, further investigations are needed which should include a larger number of populations from different regions.

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LITERATURE CITED

- ALTUKHOV Yu., KRUTOVSKII K.V., GAFAROV N.I., DUKHAREV V.A., MOROZOV G.P., 1986. Allozyme variability in a natural population of Norway spruce (*Picea abies* (L.) Karst.). I. Polymorphic systems and mechanism of their genetic control. *Genetica* 22: 2135-2151 (In Russian with English summary).
- BERGMANN F., 1973. Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. II. Genetische Kontrolle von Esterase- und Leucinaminopeptidase-Isoenzymen im haploiden Endosperm ruhender Samen. *Theor. Appl. Genet.* 43: 222-225.
- BERGMANN F., GREGORIUS H.-R., 1979. Comparison of the genetic diversities of various populations of Norway Spruce (*Picea abies*). In: *Proceedings of the Conference on Biochemical Genetics of Forest Trees*, pp. 99-107. Umea.
- GIANNINI R., MORGANTE M., VENDRAMIN G.G., 1991. Allozyme variation in Italian populations of *Picea abies* (L.) Karst. *Silvae Genetica* 40(3/4): 160-166.
- GIERTYCH M., 1972. Provenance differences in the time of spruce (*Picea abies* (L.) Karst) flushing in Poland. *Arbor. Kórnickie* 17: 169-183.
- GIERTYCH M., 1973. A contribution to the discussion on the range and origin of Norway spruce in Poland. *Sylvan* 117(10): 14-25 (In Polish with English summary).
- GIERTYCH M., 1976. Genetic variability of Polish spruce (*Picea abies* (L.) Karst.) races. *Arbor. Kórnickie* 21:189-211 (In Polish with English summary).
- GIERTYCH M., 1979. Report on the IUFRO 1938 and 1939 provenance experiments on Norway spruce (*Picea abies* (L.) Karst., Polish Academy of Sciences, Institute of Dendrology, Kórnik, pp. 179.
- GONCHARENKO G.G., ZADEIKA I.V., BIRGELIS J.J., 1995. Genetic structure, diversity and differentiation of Norway spruce (*Picea abies* (L.) Karst.) in natural populations of Latvia. *For. Ecol. Manag.* 72: 31-38.
- KRUTOVSKII K.V., BERGMANN F., 1995. Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) Karst., and Siberian, *P. obovata* Ledeb., spruce species studied by isozyme loci. *Heredity* 74: 464-480.
- KRUTZSCH P., 1992. IUFRO's role in coniferous tree improvement: Norway spruce (*Picea abies* (L.) Karst.). *Silvae Genetica* 41(3):143-150.
- KRZAKOWA M., KORCZYK A.F., 1995. Enzymatic diversity of Norway spruce (*Picea abies* (L.) Karst. from the Białowieża Forest. In: *Protection of forest ecosystems biodiversity of Białowieża primeval forest*. (eds. P. Paschalis, K. Rykowski & S. Zajęzkowski). pp. 67-80. Warszawa.
- LAGERCRANTZ U., RYMAN N., 1990. Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. *Evolution* 44(1): 38-53.
- LUNDKVIST K., 1979. Allozyme frequency distributions in four Swedish populations of Norway Spruce (*Picea abies* K.) I. Estimations of genetic variation within and among populations, genetic linkage and a mating system parameter. *Hereditas* 90: 127-143.
- LUNDKVIST K., RUDIN D., 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. *Hereditas* 85: 67-74.
- MORGANTE M., VENDRAMIN G.G., GIANNINI R., 1989. Genetics of 6PGD and SKDH in Norway spruce (*Picea abies* (L.) Karst.). *J. Genet. Breed.* 43: 67-71.
- MUONA O., YAZDANI R., LINDQVIST G., 1978. Analysis of linkage in *Picea abies*. *Hereditas* 106: 31-36.
- MÜLLER-STARCK G., 1995. Variation in high elevated populations of Norway spruce (*Picea abies* (L.) Karst.) in Switzerland. *Silvae Genetica*, 44(5-6): 356-362.
- MÜLLER-STARCK G., BARADAT P., BERGMANN F., 1992. Genetic variation within European tree species. *New Forests* 6: 23-47.
- NEI M., 1972. Genetic distance between populations. *Am. Nat.* 106: 283-292.
- PAULE L., GÖMÖRY D., 1993. Genetic structure of Norway spruce (*Picea abies* Karst.) populations from mountainous areas in Slovakia. *Lesnictvi* 39: 10-13.
- PRUS-GŁOWACKI W., GODZIK S., 1995. Genetic structure of *Picea abies* trees tolerant and sensitive to industrial pollution. *Silvae Genetica* 44(2-3): 62-65.
- SCHMIDT-VOGT H., 1977. *Die Fichte*, Band 1, Verlag Paul Parey, Hamburg, 647 pp.
- ŚRODOŃ A., 1967. The common spruce in the Quaternary of Poland. *Acta Palaeobotanica* 8(2): 3-61 (In Polish with English summary).
- TIGERSTEDT P.M.A., 1973. Studies on isozyme variation in marginal and central populations of *Picea abies*. *Hereditas* 75: 47-60.

WSTĘPNE WYNIKI BADAŃ NAD ALLOZYMOWYM ZRÓŻNICOWANIEM
ŚWIERKA POSPOLITEGO (*PICEA ABIES* (L.) KARST.) W POLSCE
NA PODSTAWIE ANALIZY DRZEW DOBOROWYCH

STRESZCZENIE

Na podstawie analizy 24 loci allozymowych badano poziom genetycznego zróżnicowania świerka pospolitego (*Picea abies* (L.) Karst.) w Polsce. Przedstawione badania oparte są na analizie 81 klonów pochodzących z drzew doborowych reprezentujących trzy odrębne regiony występowania świerka pospolitego: Sudety, Beskidy, północno-wschodnia Polska. W badanym materiale 52,8% loci było polimorficzne, średnia liczba alleli w locus wyniosła 1,8 a średnią wartość heterozygotyczności oczekiwanej oszacowano na 0,134. Wyniki naszych badań potwierdzają hipotezę o niższym poziomie zmienności genetycznej świerka pospolitego w centralnej Europie, w stosunku do innych rejonów naturalnego występowania gatunku. Jednocześnie stwierdzono stosunkowo niską wartość genetycznego zróżnicowania pomiędzy świerkami z badanym regionów (dystanse Nei'ego zawierają się w granicach od 0,005 do 0,008, a $F_{st} = 0,027$). Ewentualne tego przyczyny zostały pokrótce omówione w dyskusji.

SŁOWA KLUCZOWE: *Picea abies*, allozimy, zmienność genetyczna.