

gains were also computed. There were significant differences between and within families with respect to all characters under study at 1% level of significance.

Narrow sense heritability (half-sib family, within-family and individual tree basis) was worked out for all the characters. Maximum heritability on half-sib family basis was observed for height, whereas tracheid length exhibited maximum heritability on within-family as well as individual tree basis. Minimum heritability on half-sib family, within-family and individual tree basis was noticed in the surface area of the spur (Table 1).

Genetic gain at family and within-family level was also computed (Table 1). Maximum genetic gain of family (Gf) and within family (Gw) selection was exhibited by the current year growth whereas the minimum total genetic gain (Gt) was noted in the case of specific gravity of wood. Genetic gain of family selection contributed less to the total genetic gain because of the high selection intensity (90%).

Discussion

In the massive afforestation drive in the Himalaya, chir pine well known for its timber and resin production, has outnumbered all other species because of its high commercial value and its wide range of adaptability to soil and climatic conditions. The present investigations were carried out to evaluate the half-sib progenies of 58 trees of chir pine selected from the state of Himachal Pradesh, India.

All the characters under study exhibited significant differences (between-families and within-families) at 1% level of significance. In general, most economic characteristics of special value in forest trees have a large amount of individual tree variability that will be available to the tree breeder. This is true even for the characteristics that are complex. An occasional tree species, such as *Pinus resinosa* (red pine) will show only a small amount of tree to tree genetic variation (FOWLER

and MORRIS, 1977) but these cases are exception rather than the rule. Specific gravity of the branch wood exhibited highest heritability on a half-sib family basis (60.5%) whereas tracheid length exhibited highest heritability on a within-family and individual tree basis followed by height of the plant. It has been reported that specific gravity and tracheid length in conifers are under strong genetic control (JACKSON and GREENE, 1958; ZOBEL, 1961, 1964; BYRAM and LOWE, 1988), therefore such variation and a high estimated value of heritability indicated that genetic improvement can be made by selection for specific gravity, tracheid length and height of the plant. The heritability estimates, however, indicate only the effectiveness with which selection of genotype can be based on the phenotypic performance, but fails to indicate the real genetic progress (JOHNSON et al., 1955). Therefore, high heritability need not always be accompanied by greater genetic progress. Specific gravity, tracheid length and height of the plant recorded high heritability estimates but showed only moderate genetic gain (Table 1), whereas the current year growth recorded moderate heritabilities but high genetic gain. Therefore, the current year growth with moderate heritabilities along with high genetic gain (Gt) can be considered for selection purposes.

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Genetic Structure of *Picea abies* Trees Tolerant and Sensitive to Industrial Pollution

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Summary

Comparison of the genetic structure of 2 groups of trees of *Picea abies* tolerant and sensitive to industrial pollution, has shown that there are differences in frequencies of some alleles and genotypes and also in the level of genetic variation. In average, for 10 loci in the tolerant group of trees a higher heterozygosity (H_o) and higher genotypic polymorphism index (P_g) (ca. 25%) were observed. The greatest differences in heterozygosity level are noted at G6PDH, Fest, ShDH and GOTC loci.

Key words: *Picea abies*, genetic structure, industrial pollution.
FDC: 165.3; 425.1; 174.7 *Picea abies*.

Introduction

In south-western Poland a progressing decline of the coniferous forest is observed especially dramatically so in the Sudety Mts. This is caused by heavy industrial emission, mainly of SO_2 and NO_x from Germany and the Czech Republic as well as from local emitters. In the area of Beskid Śląski Mts., individual trees of *Picea abies* with different extent of injuries

to the needles are observed. Hitherto data about the genetic structure of groups of trees differing in sensitivity to air and soil pollution pointed to a genetic background for the differentiated response of individual plants to these factors (MEJNARTOWICZ, 1983; MEJNARTOWICZ and PALOWSKI, 1989; SCHOLZ and BERGMANN, 1984; MÜLLER-STARCK, 1985; GEBUREK et al., 1987; PRUS-GŁOWACKI and NOWAK-BZOWY, 1989, 1992; PRUS-GŁOWACKI and GODZIK, 1991). We have assumed therefore that the comparison of the genetic structure of the tolerant and sensitive trees of *Picea abies* and the possible differences between them, would indicate the direction of adaptive processes in this species. In order to define the character of adaptive processes we undertook a study of the genetic structure of sensitive and tolerant to industrial pollution *Picea abies* trees, the most important forest tree species in this geographical region. Understanding the process of adaptation under anthropogenous pressure is fundamentally important for the protection of the genetic pools of forest tree populations exposed to potential risk of loss of unique alleles which occur in local populations and consequently the loss of the adaptive ability of these populations (KARNOSKY et al., 1989).

Materials and Methods

Dormant buds, collected in February 1991, were taken for isoenzymatic analysis from the Brenna Forest Range, Beskid Śląski Mts. – southern Poland. Plant material was collected by sampling pairs of neighbouring individuals (45 trees for each group strongly and slightly damaged by industrial pollution) to minimize the influence of environmental (soil) variation on the genetic structure of the 2 groups of studied trees (GREGORIUS, 1989). The trees were divided in 2 groups depending on the extend of injuries to the needles. The first group (33 trees) consisted of individuals where needles were less than 30% injured and this group was regarded as tolerant (T) to industrial pollution. In the second group of 39 sensitive (S) trees, more than 50% of the needles were damaged. The difference in the number of individuals in sensitive (S) and tolerant (T) set of trees was caused by difficulties in interpretation of electrophoregrams (weak enzymatic activity in some of studied loci). These trees were excluded from further analysis. Variability at the following 10 enzymatic loci was analyzed: fluorescent esterase (Fest) 3.1.1.1, glucose-6-phosphate dehydrogenase (G6PDH) 1.1.1.49., malate dehydrogenase (MDH – 2 loci) 1.1.1.37., isocitrate dehydrogenase (IDH) 1.1.1.42., glutamate dehydrogenase (GDH) 1.4.1.3., shikimate dehydrogenase (ShDH) 1.1.1.25., glutamate-oxaloacetic-transaminase (GOT – 2 loci) 2.6.1.1., and diaphorase (Diaf) 1.6.4.3., using the procedures of isoenzyme electrophoresis and specific staining as described by MUONA et al. (1987) with minor modifications.

Calculations of the following genetic parameters in the studied groups of trees were made: observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F), genotypic polymorphism index (Pg) and genetic distances (DN and DH) according to KAHLER et al. (1980), HEDRICK (1974), JAIN and WORKMAN (1967), NEI (1987), using the GEN computer program (NOWAK-BZOWY and BZOWY, unpublished). Statistical significance of differences in the allele and genotype frequencies in the studied groups of trees was evaluated using a homogeneity chi-square test, assuming their equal frequency in both groups (ELANDT-JOHNSON, 1971).

Results

Pollution

The level of sulphur, the main pollutant in the studied area, in the needles of *Picea abies* trees was on the average 20.0 mg

per 1 g of dry matter. In the Zielonka Forest near Poznań, in a region regarded as not so polluted the content of this element was ca 1.03 mg per 1 g of dry needles.

The concentration of SO_2 in the air was 32.0 mg/m^{-3} in the winter in the Brenna region and 14.8 mg/m^{-3} in the Zielonka Forest while 12 mg/m^{-3} and 4.24 mg/m^{-3} respectively in the growing season. Deposition of sulphur in the Brenna population was 7.3^{-1} kg/ha monthly and the pH of rain falls varied from 3.7 to 7.36. The most frequent pH was 4.5 (GODZIK, 1990).

As can be seen, air pollution in the Brenna region is several times higher than in the region regarded as not so polluted.

Gene and genotype frequencies

Frequencies of alleles and genotypes in tolerant (T) and sensitive (S) trees are shown in tables 1 and 2. Distinct differences in frequency of alleles between groups S and T are noted for loci Fest and G6PDH, alleles Fest 2 and G6PDH1 and G6PDH2. However statistical significance of these differences failed to be confirmed by chi-square test. Differences in the frequency of some alleles and genotypes in the studied groups of plants are presented in the figure 1.

Table 1. – Allelic frequencies for 10 loci in 2 groups of studied individuals of *Picea abies*. S – sensitive, T – tolerant.

Locus	Alleles	S n=39	T n=33	Locus	Alleles	S n=39	T n=33	
FEst	1	0.962	0.803	IDH	1	1.000	1.000	
	2	0.013	0.152		GOT B	1	0.561	0.565
	3	0.025	0.045			2	0.394	0.348
G6PDH	1	0.684	0.531	3		0.046	0.087	
	2	0.250	0.438	GOT C	1	0.529	0.575	
	3	0.026	0.000		2	0.457	0.400	
	4	0.040	0.031		3	0.014	0.025	
ShDH	1	0.974	0.925	GDH	1	1.000	0.985	
	2	0.013	0.050		2	0.000	0.015	
	3	0.013	0.025	Diaf	1	0.974	0.964	
MDH A	1	1.000	1.000		2	0.026	0.036	
	MDH C	1	0.923	0.985				
		2	0.077	0.015				

Table 2. – Genotypic frequencies for 10 loci in 2 groups of studied individuals of *Picea abies*. S – sensitive, T – tolerant.

Genotypes	S n=39	T n=33	Genotypes	S n=39	T n=33	
FEst	11	0.923	IDH	11	1.000	
	12	0.026		GOT B	11	0.273
	13	0.051			22	0.091
G6PDH	11	0.474	12		0.546	
	22	0.053	13	0.030		
	12	0.342	23	0.061		
	13	0.053	GOT C	11	0.200	
	14	0.026		22	0.143	
	24	0.053		12	0.629	
ShDH	11	0.947	13	0.029		
	12	0.026	GDH	11	1.000	
	13	0.026		12	0.000	
MDH A	11	1.000	Diaf	11	0.949	
	MDH C	11		0.923	12	0.051
22		0.077				
12		0.000				

*) significant differences

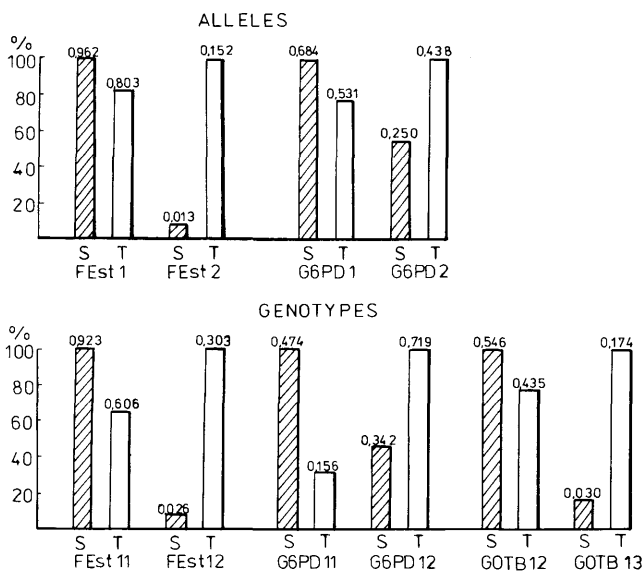


Figure 1. – Differential frequencies of selected alleles and genotypes in both groups of trees; S – sensitive, T – tolerant to industrial pollution. As 100% – the maximum frequency of a particular allele or genotype at the given locus.

Most distinct differences in genotype frequencies were noted for Fest 11 and Fest 12. The homozygote Fest 11 is more frequent in the group of sensitive trees (S) while the heterozygote Fest 12 in the group of tolerant (T) individuals. A similar trend is observed for homozygote G6PDH 11 and heterozygote G6PDH 12. The heterozygote GOT B 13 is also more common in the tolerant group. In these 2 cases statistical significance of differences in frequencies were confirmed by the chi-square test (Table 2 and Fig. 1). In contrary, the heterozygotes GOT B 12 and GOT C 12 are less common in the latest group.

As far as the mean number of genotypes per locus is concerned, there are some differences in the tested groups of trees but not in the number of alleles. In the S and T groups there are 23 alleles and 28 to 27 genotypes respectively. This gives 2.3 alleles and 2.8 and 2.7 genotypes per locus (Table 3).

Table 3. – Numbers of alleles (A) and genotypes (G) in the studied groups of tolerant (T) and sensitive (S) individuals. Average numbers of alleles and genotypes per locus are given in brackets.

Locus	A		G		Locus	A		G	
	S	T	S	T		S	T	S	T
FEst	3	3	3	3	G6PDH	4	3	6	5
ShDH	3	3	3	3	IDH	1	1	1	1
MDH A	1	1	1	1	GDH	1	2	1	2
MDH C	2	2	2	2	DIAF	2	2	2	2
GOT C	3	3	4	4	GOT B	3	3	5	4
					Together	23	23	28	27
						(2.3)	(2.3)	(2.8)	(2.7)

Heterozygosity

The data on heterozygosity (Ho and He) are presented in table 4. Among 10 analysed loci, in 9 of them there is a higher level of heterozygosity (from 28.2% Diaf, to 80.7% Fest) in the tolerant (T) group of trees than in the sensitive (S) one. How-

ever for 2 loci of GOT a slightly lower heterozygosity in this group of individuals (T) is observed. On the average, for the 10 loci the tolerant (T) group of trees has a heterozygosity ca 25% higher than the sensitive (S) one. If the 4 most differentiated loci in heterozygosity (Ho) between sensitive and tolerant set of trees (Fest, G6PDH, ShDH and Diaf – Table 4) are taken into consideration, the difference in heterozygosity between S and T groups reaches ca. 50%.

Table 4. – Observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F) and genotypic polymorphism index (Pg) in the sensitive (S) and tolerant (T) groups of trees.

Locus	Popul.	He	Ho	F	Pg
FEst	S	0.0747	0.0769	-0.0298	0.1446
	T	0.3302	0.3939	-0.1931	0.5326
G6PDH	S	0.4671	0.4737	-0.0141	0.6496
	T	0.5253	0.7813	-0.4871	0.4530
ShDH	S	0.0516	0.0526	-0.0208	0.1010
	T	0.1412	0.1500	-0.0619	0.2650
MDH A	S	0.0000	0.0000	-	0.0000
	T	0.0000	0.0000	-	0.0000
MDH C	S	0.1420	0.0000	1.0000	0.1420
	T	0.0299	0.0303	-0.0122	0.0588
IDH	S	0.0000	0.0000	-	0.0000
	T	0.0000	0.0000	-	0.0000
GOT B	S	0.5285	0.6364	-0.2041	0.6152
	T	0.5520	0.6087	-0.1027	0.6956
GOT C	S	0.5114	0.6571	-0.2849	0.5436
	T	0.5088	0.5500	-0.0811	0.6350
GDH	S	0.0000	0.0000	-	0.0000
	T	0.0299	0.0303	-0.0122	0.0588
Diaf	S	0.0499	0.0513	-0.0279	0.0973
	T	0.0689	0.0714	-0.0374	0.1326
Mean values	S	0.1825	0.1948	-0.0673	0.2293
	T	0.2186	0.2616	-0.1965	0.2831

Genotype polymorphism

Similar tendencies as for heterozygosity (Ho) are noted for genotype polymorphism (Pg). A higher Pg index is observed in the group of tolerant trees (T) for most loci (Fest, ShDH, GOTB and GOTC, and also for Diaf) (Table 4). Only for 2 loci (G6PDH and MDHC) an opposite result was obtained.

Fixation indices

The mean fixation index (F), illustrating whether the studied groups of individuals are in a HARDY-WEINBERG equilibrium, showed an excess of heterozygotes in both groups of trees. However in the case of the sensitive group (S), the excess of heterozygotes is lower than in the group of tolerant (T) trees. The most distinct differences in the fixation indices (F) for T and S trees are noted for loci MDHC, G6PDH and Ffest. Two loci of GOT are showing the reverse tendency – a higher number of heterozygotes is noted in the group of trees sensitive to industrial pollution (Table 4).

Genetic similarities

A comparison of genetic similarity indices based on the frequency of alleles (SN) and frequency of genotypes (SH) indicated that the studied groups of trees are most different for the G6PDH locus. These indices are SN = 0.942 and SH = 0.725. For the remaining loci these indices vary from 0.984 to 0.999. The mean value for 10 loci is 0.922 for SN and 0.965 for SH (Table 5).

Table 5. – Indices of genetic similarity based on allelic (SN) and genotypic (SH) frequencies of tolerant and sensitive groups of trees *Picea abies*.

Locus	SN	SH	Locus	SN	SH
FEst	0.984	0.864	G6PDH	0.942	0.725
ShDH	0.999	0.990	MDH A	1.000	1.000
MDH C	0.997	0.995	IDH	1.000	1.000
GOT C	0.994	0.967	GOT B	0.996	0.944
Diaf	0.999	0.999	GDH	0.999	0.999
Mean for 10 loci				0.992	0.965

Discussion and Conclusions

The presented results show distinct differences in the genetic structure of the studied groups of trees of *Picea abies*. These differences are expressed as various frequencies of alleles and genotypes in the groups of tolerant (T) and sensitive (S) trees. Clear differences are also noted for the level of heterozygosity and genotypic polymorphism indices, both higher in the group of trees tolerant to industrial pollution. These data indicate the direction of adaptive processes in the studied population of *Picea abies*.

Changes of genetic structure expressed as an increased level of heterozygosity in plant populations, appear connected with the increase spatial and temporal variation of environment caused by multiple factors originating from industrial pollution. More heterozygotic individuals are better adapted in such conditions (LERNER, 1954; HEDRICK, 1976, 1978; LEWONTIN, 1974; ZAURUS and FOULUS, 1987). However, changes of genetic structure of populations could be also connected with a decreased level of heterozygosity of individuals existing in extreme environmental conditions, because only special genotypes can survive in such environments. Examples of both kinds of adaptation of forest trees to environments changed by industrial activity were reported by several authors (MEJNARTOWICZ, 1983; SCHOLZ and BERGMANN, 1984; MÜLLER-STARCK, 1985; GEBUREK et al., 1987; PRUS-GŁOWACKI and NOWAK-BZOWY, 1989, 1992; PRUS-GŁOWACKI and GODZIK, 1991).

Higher level of heterozygosity (Ho) and genotypic polymorphism (Pg) indicate a higher survival ability of these trees, and more generally – of populations with a higher genetic variability. Decreased genetic variation level caused by elimination of the part of the population sensitive to industrial pollution, including also carriers of unique alleles which the population acquired during long term microevolutionary processes, constitutes an erosion of the gene pool and can reduce of adaptability to local specific environmental conditions.

Decisively higher heterozygosity in the group of tolerant trees was noted for FEst, G6PDH and ShDH loci. GOT loci are an exception, the group of sensitive individuals (S) having more heterozygotes than the T group. These differences in allele frequencies for the same loci (G6PDH, GOT) as we found were also observed by other authors (BERGMANN and SCHOLZ, 1985, 1987, 1989; GEBUREK et al., 1986). We are fully aware of the fact that it is difficult directly to connect all particular studied enzymatic loci with mechanisms of tolerance of *Picea abies* trees to industrial pollution, but one might concluded that studied loci are the suitable markers for studying changes in the genetic structure caused by industrial pollution acting on this species.

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