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ISOZYME POLYMORPHISM OF NORWAY SPRUCE (*Picea abies* Karst.) IN SLOVAKIA

I. GENETIC STRUCTURE OF ADJACENT POPULATIONS

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ABSTRACT

Isozyme polymorphism was studied in 9 indigenous populations of *Picea abies* Karst. (Norway spruce) from Slovakia. A total of 9 enzyme systems were analyzed, comprising 15 allozyme loci. Four pairs of adjacent populations were studied with the aim to determine their genetic similarity.

From among 9 investigated enzyme systems Aco, Dia, Pgi and Est were used for the first time to assess genetic variation in *Picea abies*. Much polymorphism was found at intra-population level. The values of mean heterozygosity in macrogametophytes (mother trees) varied between 0.217 and 0.267.

Seven loci were characterized by heterozygosity values higher than 0.2. Using the hierarchical model of the heterozygosity partitioning we found that on average 2.88 % of gene diversity was due to the differences among populations, 1.45 % was due to the differences on the regional level and the remaining 95.76 % was due to the differences within populations.

Genetic distances were used to analyze genetic differences among populations. We did not find any distinct relationship between geographical location of individual populations and the genetic distance among them. The possible explanation of this phenomenon is that some populations are not indigenous. In addition, seed material used in this study could include limited number of individuals which would further obscure inferences concerning differences among populations.

Key words: *Picea abies* Karst., isozyme polymorphism, genetic structure of populations

1 INTRODUCTION

In recent decades, studies of the genetic structure of forest tree populations using genetic markers were characterized by a considerable diversity of applications. They enabled not only investigation of the genetic structure of populations, but also studies of the processes operating on population level

such as e.g. mating system, gene flow, etc. From among the genetic markers of biochemical character isozymes are often considered to be among the most suitable to investigate the processes mentioned above.

Conifers are characterized by two types of tissues i.e. haploid macrogametophyte and diploid embryo, which enables parallel studies of the genetic properties on the parental as well as on the progeny level.

Genetic structure of *Picea abies* Karst. populations in Europe is being investigated by isozyme analyses from the beginning of the seventies. These earlier studies, concerned polymorphism on inter-population as well as on intra-population levels. Minor differences in gene structure were found in populations originating from distant places in Europe (Bergmann 1973). No differences were found among more adjacent populations (Tigerstedt 1974). In addition, Lundkvist (1974) did not find any distinct relation between heterozygosity and altitude of population origin.

From the evolutionary point of view, the variability of some loci is considered as selectively neutral. However, for some allozyme loci, differences which may be due to selection were found (Tigerstedt 1974). Also Bergmann (1975, 1978) found that the polymorphism of acid phosphatase may be related to mean temperature of the environment. Bergmann (1978) suggested that variation of acid phosphatases may be parallel to bud-set variation pattern. However, Tigerstedt (1974) did not detect clinal variation pattern in phosphatases, but Lundkvist and Rudin (1977) found certain geographical patterns in acid phosphatase variation.

Bergmann (1985) analyzed extensive material from all-European natural range of *Picea abies* and explained the observed geographical variation pattern in relation to the postglacial distribution of *Picea abies* in Europe. Bergmann and Gregorius (1979) used several indices of gene diversity to prove that marginal populations of *Picea abies* are characterized by low gene diversity.

The aim of this paper was to investigate genetic variation in 9 *Picea abies* populations from Slovakia using allozyme markers in order to determine: (i) genetic structure of indigenous *Picea abies* populations from Slovakia; (ii) variation pattern and genetic structure of adjacent population using the hierarchical model — trees within the populations, populations within the regions, regions; (iii) genetic distances between the individual populations.

2 MATERIAL

Seed samples originating from 9 indigenous populations of *Picea abies* from Slovakia were used as an experimental material. The populations were situated in four geographical regions and from each region one pair of adjacent populations was selected to investigate the variation pattern within and between regions. The seed samples originated from the commercial seed collections and were supplied by the Seed Extraction Plant in Liptovský Hrádok. Basic information on idividual populations is given in Table 1.

About 150 seeds, originating from bulk samples were analyzed in each population. One sample from population from the Tatra National Park (TANAP)

Table 1

List of indigenous *Picea abies* populations from Slovakia used for the investigation of isozyme polymorphism

Population	Stand	Age	Altitude	Category	Latitude	Longitude
1 Liptovský Hrádok Kráľova Lohota	118 a	90	850	IIA	48°57′	19°47′
2 Liptovský Hrádok Malužiná	95 b	110	1150	IIB	48°57′	19°47′
3 Beňuš Závadka	427 n	90	900	IIB	48°52′	19°49′
4 Beňuš Červená Skala	117	100	950	IIB	48°44′	20°06′
5 Čierny Balog Šaling	7 b	90	650	IIA	48°41′	19°43′
6 Čierny Balog Krám	115 d_1	90	800	IIA	48°40′	19°41′
7 Krásno nad Kysucou Nová Bystrica	44	80	800	IIB	49°26′	18°56′
8 Oravský Podzámok Zakamenné	384	90	780	IIA	49°23′	19°06′
9 TANAP Tatranská Kotlina	1194	120	850	IIB	49°12′	20°15′

represented seed collected from 40 individual trees. In this case 10 seeds per tree were analyzed.

3 METHODS

ISOZYME ANALYSES

Seeds were stored at —18°C. Before analyses the seeds were pre-germinated for 3 to 5 days on moist filter paper. Individual macrogametophytes extracted from the seeds were homogenized in Tris-glycine buffer pH 8.7 containing 0.06 (v/v) mercaptoethanol. Isozyme separation was carried in 12 % starch gels. The following three buffer systems were used: Tris-borate buffer pH 8.1 (Ashton and Braden, 1961) for Lap, GDh, Got, FEst; Tris-versene-borate buffer pH 8.0 (Linhart et al., 1981) for Pgm, Pgi, Dia and Tris-citrate buffer pH 7.0 (Shaw and Prasad, 1970) for Aco, MDh separation. Recipes for buffers and staining solutions are given elsewhere (Wang et al. in preparation). A total of 15 allozyme loci were scored for each individual macrogametophyte.

STATISTICAL ANALYSES

For statistical analyses of our data set (9 populations containing 1743 seeds), a computer program GENSTRUCT (Paule, 1984) was used. The calculation of variances and intra-locus variances was done according to Nei and Roychodhury (1974).

Table 2

List of isozyme systems used for the analyses of *Picea abies* isozyme polymorphism

Isozyme system	Abbreviation	Ec-code	No. of loci
Aconitase	Aco	EC 4.2.1.3.	1
Diaphorase	Dia	EC 1.6.4.3	2
Fluorescent esterase	FEst	EC 3.1.1.1	1
Glutamate dehydrogenase	GDh	EC 1.4.1.3	1
Glutamate oxaloacetate transaminase	Got	EC 2.6.1.1	2
Leucine aminopeptidase	Lap	EC 3.4.11.1	2
Malate dehydrogenase	MDh	EC 1.1.1.37	2
Phosphoglucose isomerase	Pgi	EC 5.3.1.9	2
Phosphoglucomutase	Pgm	EC 2.7.5.1	2

4 RESULTS

ISOZYME INHERITANCE

From among nine isozyme systems studied, five systems i.e. Lap, Got, GDh, MDh and Pgm were earlier investigated and tested for inheritance. The remaining four systems (FEst, Aco, Dia and Pgi) were used without prior testing of inheritance and the segregation was presumed on their previous application in other conifers (e.g. in Scots pine Yazdani et al. 1983; Szmidt and Yazdani 1983).

List of isozyme systems studied including their EC code is given in Table 2 and the individual allelic variants are shown in Fig. 1.

ALLELIC FREQUENCIES

Due to the space reason the allelic frequencies based on the macrogametophyte analysis of populations are not given. Relatively high variation of individual allelic variants was found. The differences in allelic frequencies among individual populations were tested using G-test. No significant differences among populations were found even with respect to highly polymorphic loci such as Aco, Dia C, FEst and Got B.

As regards the loci with low heterozygosity values the observed differences were mainly due to the occurrence of rare alleles. Using these rare alleles the populations can be differentiated. For example, at locus Dia B, allele B1 was found only in the population Malužina, while at the locus GDh the allele A1 was found only in the population TANAP.

HETEROZYGOSITY OF POPULATIONS

The heterozygosities at individual loci and populations as well as mean heterozygosities for individual populations are given in Table 4. From the entire comparison the loci Pgm A and Pgm B which were not analyzed in all populations were excluded.

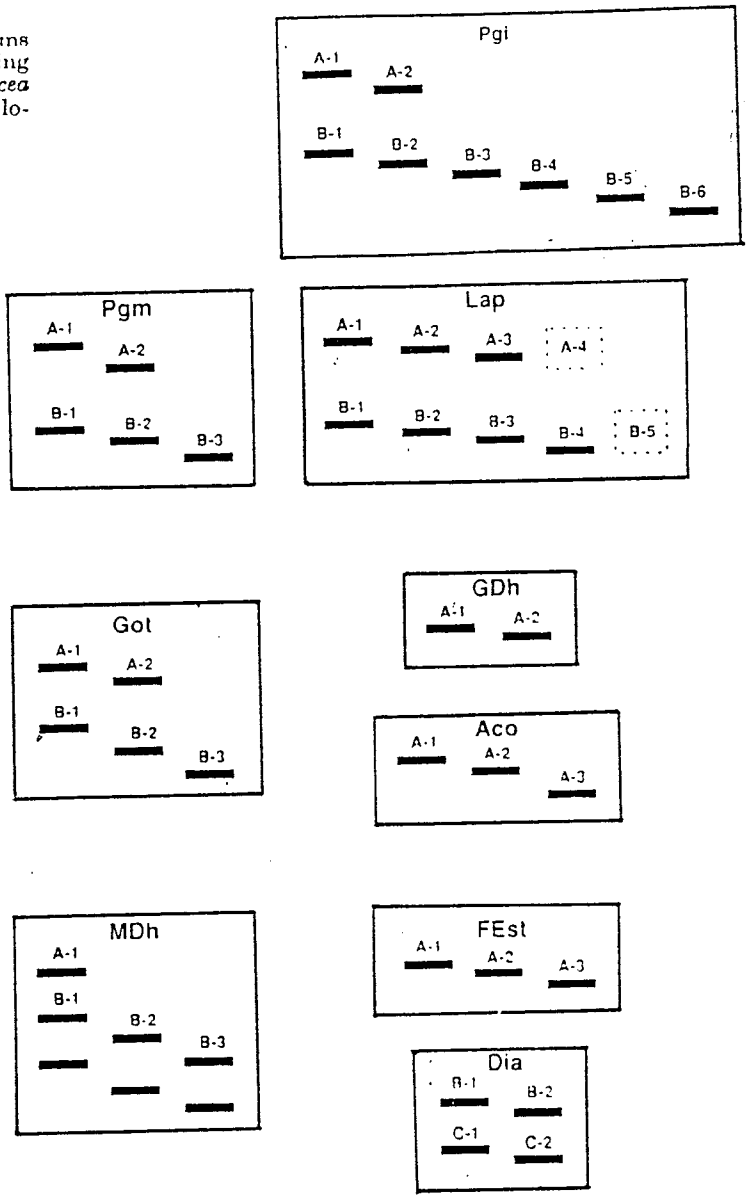

Fig. 1. Line diagrams of isozyme staining phenotypes of *Picea abies* showing allozymes

Usually those loci which are characterized by the highest heterozygosity values are at the same time characterized by low variability among individual populations. Conversely, the loci with low values of heterozygosity at the populations (e.g. Dia B, Pgi A, Got A and MDh A).

Mean heterozygosity computed for all 13 loci varied between 0.2171 (Oravský Podzámok — Zakamenné) and 0.267 (Liptovský Hrádok — Králova Lehota). In the case of two adjacent populations originating from Liptovský Hrá-

Table 3

Mean observed heterozygosities in *Picea abies* populations from Slovakia

Locus	Populations								
	1	2	3	4	5	6	7	8	9
Aco	0.352	0.464	0.378	0.377	0.435	0.330	0.438	0.394	0.420
Dia B	0.036	0.000	0.191	0.039	0.043	0.000	0.034	0.000	0.039
Dia C	0.500	0.480	0.484	0.499	0.500	0.500	0.489	0.497	0.500
FEst	0.114	0.100	0.065	0.064	0.079	0.143	0.069	0.099	0.078
Pgi A	0.069	0.000	0.013	0.013	0.000	0.000	0.016	0.000	0.000
Pgi B	0.534	0.485	0.664	0.529	0.486	0.540	0.516	0.496	0.579
GDh	0.012	0.029	0.040	0.026	0.023	0.087	0.069	0.013	0.076
Got A	0.047	0.044	0.077	0.077	0.265	0.040	0.096	0.051	0.039
Got B	0.503	0.517	0.508	0.507	0.511	0.503	0.459	0.511	0.526
Lap A	0.451	0.140	0.163	0.065	0.101	0.144	0.161	0.190	0.282
Lap B	0.263	0.254	0.263	0.360	0.220	0.331	0.308	0.292	0.306
MDh A	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDh B	0.558	0.536	0.555	0.539	0.473	0.320	0.270	0.271	0.078
H	0.267	0.238	0.256	0.226	0.243	0.226	0.227	0.217	0.226
V_h	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
V_H	0.063	0.063	0.061	0.060	0.059	0.058	0.057	0.057	0.061

Table 4

Standard genetic distances among *Picea abies* populations

Population	Population							
	1	2	3	4	5	6	7	8
2	0.0104							
3	0.0071	0.0038						
4	0.0244	0.0048	0.0076					
5	0.0185	0.0025	0.0051	0.0029				
6	0.0209	0.0052	0.0065	0.0002	0.0030			
7	0.0263	0.0084	0.0087	0.0018	0.0049	0.0033		
8	0.0241	0.0044	0.0093	0.0006	0.0026	0.0005	0,0021	
9	0.0322	0.0114	0.0154	0.0031	0.0077	0.0302	0.0032	0.0013

dok the difference in mean heterozygosities is 0.029, in populations originating from Beňuš the difference represents 0.067 and in populations originating from Čierny Balog this difference represents 0.016 and finally in populations originating from Beskydy region this difference represents only 0.0100.

As regards variation coeficients of heterozygosities in individual populations, most homogenous single locus heterozygosities were found in the population TANAP ($v_x = 29$ %) and the most heterogenous are the populations Malužiná and Červená Skala ($v_x = 39.9\%$, or 39.6%, respectively).

The standard errors of heterozygosities between populations and intra-locus standard errors are given in the two bottom lines of the Table 4. The populations with the highest and the lowest heterozygosities are characterized by the highest and lowest inter-locus errors respectively.

Regarding the fact that when considering all loci rather low mean values of heterozygosities were obtained, we computed also the heterozygosities based on the following loci: Aco, Dia C, Pgi B, Got B, Lap A, Lap B and MDh B, i.e. those loci which were represented by heterozygosity values greater than 0.1. The lowest heterozygosity values were found in the populations Zakamenné (0.377) and Krásno nad Kysucou (0.377) and population Kráľova Lehota (0.452).

GENETIC VARIATION AMONG POPULATIONS

The minimum and standard genetic distances and their standard errors were computed for each pair of populations. With regard to the fact that the results of both computations are by their character rather similar, we decided to utilize only the standard genetic distances. In this way, the results are comparable with other papers, for the utilization of standard distances is more frequent than the utilization of minimum genetic distances or other measures of genetic similarity such as e.g. Rogers' coefficient, Gregorius coeficient, Gregorius' distance. In addition, variation coefficients of both compared genetic distances and all pairs of populations are in the case of standard genetic distances greater than in minimum genetic distances, i.e. minimum genetic distances show greater variability.

In comparison of the first population i.e. Liptovský Hrádok — Kráľova Lehota with all other populations the differences were in all cases (except the distance to Beňuš — Závadka) greater than between the two adjacent populations Kráľova Lehota and Malužiná. Beskydy populations showed in all cases greater genetic distances than among adjacent populations (Zakamenné and Krásno nad Kysucou). In general, genetic distances among more geographically distant populations were smaller than the distances among adjacent populations. The population originating from the TANAP showed smaller genetic distances to both populations originating from Beskydy, than to both populations originating from Čierny Balog and Červená Skala and only then to all other populations originating from the Low Tatra (Závadka, Kráľova Lehota and Malužiná) (Tab. 4).

The second set of genetic distances is based on the allelic frequencies at only those seven loci which were characterized by the highest heterozygosities i.e. Aco, Dia C, Pgi B, Got B, Lap A, Lap B and MDh B. The results were similar to those described above, although the absolute values of genetic distances were higher than those obtained for 13 loci. The lowest values of the genetic distances were obtained in the case of the populations from Čierny Balog and from Beskydy. The values of variation coefficients of genetic distances computed for seven loci increased in both cases.

The cluster analysis served the investigation of the similarity of individual populations. The basic hypothesis i.e. the decrease of genetic distances of adjacent populations could not be confirmed. As shown in figure, we have obtained two different clusters of populations. One cluster contained populations Závadka, Čierny Balog — Šaling, while the other cluster contained all other

populations except that from Králova Lehota. The last given population differs from all other populations.

HIERARCHICAL PARTITIONING OF THE GENE DIVERSITY

Using the analysis of gene diversity in hierarchically divided populations (Nei 1973) we divided the gene diversity into the hierarchical structure i.e. trees within stands, stands within regions and regions.

The division of gene diversity at individual loci into this structure is given in Table 5. Regarding rather low heterozygosity in Dia B and MDh A it was not possible to divide the heterozygosity in these loci into hierarchical structure and due to this fact these two loci were omitted from the comparison.

As shown in Table 5 the gene diversity within populations varied from 88.66% (MDh B) to 99.16% (Aco), in average 95.76%; populations within the regions from 0.42% (Aco) to 8.49% (Lap A), in average 2.88% and between regions from 0.00% (Lap B) to 6.68% (MDh B), in average 1.45%.

From the entire comparison it appears that the greatest part of the gene diversity is due to the differences within populations (95.7%) rather than the sub-populations within the regions (2.88%) and finally the lowest ratio is represented by the variation between the regions (1.45%).

Table 5

Partitioning of genetic diversity in 9 populations of *Picea abies*

Locus	H_T	G_P	G_{PR}	G_{RT}
Aco	0.4009	99.16	0.42	0.42
Dia	0.5000	99.37	0.83	0.30
F Est	0.0933	98.60	1.06	0.34
Pgi A	0.0123	90.26	6.33	3.41
Pgi B	0.5301	98.58	1.07	0.35
G Dh	0.0436	95.55	4.01	0.44
Got A	0.0865	94.49	3.36	2.16
Got B	0.5106	99.17	0.55	0.28
Lap A	0.2110	89.90	8.49	1.61
Lap B	0.2222	99.12	0.88	0.00
MDh B	0.4809	88.46	4.66	6.68

where: H_T — total heterozygosity
G_P — diversity within populations
G_{PR} — diversity between populations within regions
G_{RT} — diversity between regions

5 DISCUSSION

The inheritance patterns of allozyme variants at individual isozyme loci in *Picea abies* was studied by Bergmann (1971, 1974), Bergmann and Gregorius (1979), Lundkvist (1979), Lundkvist and Rudin (1977).

Poulsen et al. (1983) and Tigerstedt (1974). They described many isozyme systems, of which Lap, Got, GDh, MDh, Pgm were used in our paper. Leucine-aminopeptidase (Lap) is one of the most frequently investigated isozyme systems in forest conifers. It was used in almost all papers investigating the variation at the population or geographic level.

In our paper, we have used several new isozyme markers such as Aco, Dia, Pgi and FEst. The linkage among these isozyme loci were described by Muona et al. (1987).

Bergmann (1985) compared allelic frequencies of different origins of *Picea abies* in Europe using 5 isozyme loci: Lap B, GDh A, FDh A, Sod B, G-6-PDh A. We have got a comparable results in the case of Lap B. For this locus, Bergmann (1985) combined all allelic variants except B4 into one and obtained, similarly to our results, the frequency of remaining alleles 0.97 and the frequency of B4 0.03. In the case of GDh we obtained the frequency of the allele A1 0.97, while he used to describe the allele with the highest frequency A2. With the highest probability in so wide material he has found one allele more than we could detected in our material from Slovakia.

Lundkvist (1979) investigated geographic variability of four populations by comparing 11 isozyme loci. We have obtained corresponding results with his results, for in GDh the most frequent allele detected was the one with the highest mobility. Isozyme locus MDh A was, similarly as in our case, rather monomorphic.

Comparison heterozygosities of comparable loci GDh, Got and Lap, indicated that in our material the values of heterozygosity of GDh were in all cases lower than 0.1, while in the case of Bergmann's data the heterozygosities of 16 populations varied from 0.00 to 0.495; Scandinavian populations 0.28 to 0.49; continental populations 0.00 to 0.11 and Siberian spruce 0.50 (Bergmann 1985).

In the case of Scandinavian populations Lundkvist (1979) reported the heterozygosity values 0.18 to 0.48. In the locus Lap A we obtained the values of heterozygosity per locus 0.10 to 0.45 and in Lap B 0.22 - 0.35. Lundkvist (1979) obtained in Scandinavian samples the heterozygosity values 0.44 to 0.73, or 0.37 to 0.55, respectively. In another Scandinavian material Lundkvist and Rudin (1977) reported the values of heterozygosity of 0.298 to 0.486, or 0.378 to 0.510 and Bergmann (1985) obtained for locus Lap A 0.49 to 0.63 and for Lap B 0.45 to 0.62.

Comparison of mean heterozygosities of populations obtained in this study with data reported earlier is not possible for the authors utilized different sets of isozyme loci and due to this fact they have obtained also very variable values of mean heterozygosities.

Division of the total gene diversity into hierarchical structure gave us rather surprising results. Intra-population gene diversity is rather high and represents 95.7%, between populations within regions 2.88% and 1.45% between regions. In *Pinus longaeva*, Hiebert and Hamrick (1983) obtained the intra-population diversity 96.12% and between populations 3.78%. In *Pinus monticola*, Steinhoff et al. (1983) found the proportion of intra-population diversity 85.2% and between populations 14.8%.

A review of inter-population diversities was published by Brown and Moran (1979) and these varied in *Picea abies* from 2.4% to 2.7%, in *Pinus nigra*

(subspecies) from 6.9% to 13%, in *Pinus sylvestris* 14%, in *Pseudotsuga menziesii* from 2.9% to 3%, in *Eucalyptus sp.* (self-pollinating species) from 17% to 18% etc.

It is evident that inter-population differentiation will depend on the number and the character of the loci used, as well as on the mating system in individual tree species. From this point of view the analysis of genetic structure of populations can be considered as suitable tool for measuring not only the changes of the genetic structure of populations in space and time, but also the changes in populations due to their domestication (natural vs. breeding populations).

Matrix of genetic distances obtained from the analysis of 9 *Picea abies* populations from Slovakia did not indicate any distinct relation between the geographical and genetic distances among individual populations. Approximately in the half of the investigated cases the genetic distances between individual adjacent populations were greater than between the geographically distant populations. On the one hand, this may indicate the occurrence of non-indigenous populations, but on the other, the observed allelic frequencies could have been affected by our sampling procedure.

6 CONCLUSIONS

We used for the first time for *Picea abies* four isozyme systems Aco, Dia, Pgi and FEst. In the investigated populations rather high isozyme polymorphism on intra-population level was found. The values of mean heterozygosity varied in mother trees (macrogametophytes) from 0.22 to 0.27.

From among the 15 investigated loci, the following seven ones Aco, Dia C, Pgi B, Got B, Lap A, Lap B and MDh B had higher heterozygosity values than 0.2. At these loci the variation within populations was well characterized and vice versa the remaining are suitable, with regard to the occurrence of rare alleles, for the discrimination of populations.

Using hierarchical model of the division of heterozygosity we found that on the average 1.45% of gene diversity is attributable to the differences between regions, 2.88% is due to the differences between populations within regions and remaining 95.76% is due to the differences on the inter-population level.

Genetic distances were used for the analysis of genetic similarity of populations. It was not found that geographically adjacent populations had also the smallest genetic distances. To some extent this fact can be explained by the non-indigenous populations or by the bulk character of the seed samples studied.

LITERATURE

1. Ashton, G.C. — Braden, A. W.H.: Serum δ-globulin polymorphism in mice. Australian Journal of Biological Sciences, 14, 1961, 248—253
2. Bergmann, F.: Genetische Untersuchungen bei *Picea abies* mit Hilfe von Isoenzym-Identifizierung. I. Möglichkeiten für genetische Zertifizierung von Forstsaatgut. Allgemeine Forst-und Jagdzeitung, 142, 1971, s. 278—280
3. Bergmann, F.: Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. II. Genetische Kontrolle von Esterase- und Leucinaminopeptidase-Isoenzymen im haploiden Endosperm ruhender Samen. Theoretical and Applied Genetics, 43, 1973, s. 222—225

4. Bergmann, F.: Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. III. Geographische Variation von 2 Esterase- und 2 Leucinaminopeptidase-Loci in der schweidschen Fichtenpopulation. Silvae Genetica, 22, 3, 1973, s. 63—66
5. Bergmann, F.: The genetics of some isozyme systems in Spruce endosperm *(Picea abies)*. Genetika, 6, 3, 1974, s. 353—360
6. Bergmann, F.: Genetischer Abstand zwischen Populationen 2. Die Bestimmung des genetischen Abstandes zwischen europäischen Fichtenpopulationen *(Picea abies)* auf der Basis von Isoenzym-Genhäufigkeiten. Silvae Genetica, 23, 1—3, 1974, s. 28—32
7. Bergmann, F.: Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genetica, 24, 1975, s. 175—177
8. Bergmann, F.: The allelic distribution at an acid phosphatase locus in Norway Spruce *(Picea abies)* along similar climatic gradients. Theoretical and Applied Genetics, 52, 1978, s. 57—64
9. Bergmann, F.: Genetic differentiation of Norway Spruce in Europe revealed by isozyme-gene-systems. IUFRO Working Party „Norway Spruce Provenances", Vienna, 1985, s. 9
10. Bergmann, F. — Gregorius, H. R.: Comparison of the genetic diversity of various populations of Norway Spruce *(Picea abies)*. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umeå, 1979, s. 99—107
11. Brown, A. H. D. — Moran, G. F.: Isozymes and the genetic resources of forest trees. In: Symposium on Isozymes of North American Forest Trees and Forest Insects, Berkeley, 1981, s. 1—10
12. Gömöry, D.: Vplyv obhospodarovania na genetickú štruktúru populácií smreka obyčajného (*Picea abies* Karst.) na Slovensku. VŠLD, Zvolen (dissertation thesis), 1988, s. 135
13. Hiebert, R. D. — Hamrick, J. L.: Patterns and levels of genetic variation in Great Basin bristlecone pine, *Pinus longaeva*. Evolution, 37, 2, 1983, s. 302—310
14. Linhart, Y. B. — Davis, M. L. — Mitton, J. B.: Genetic control of shikimate dehydrogenase in ponderosa pine. Biochemical Genetics, 19, 1981, s. 641—646
15. Lundkvist, K.: Inheritance of leucine aminopeptidase isozymes in *Picea abies* Karst. Hereditas, 78, 1974, s. 91—96
16. Lundkvist, K.: Allozymne frequency distributions in four Swedish populations of Norway Spruce (*Picea abies* Karst.) 1. Estimation of genetic variation within and among population, genetic linkage and mating system parameter. Hereditas, 90, 1979 s. 127—143
17. Lundkvist, K. — Rudin, D.: Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas, 83, 1977, s. 767—774
18. Muona, O. — Yazdani, R. — Lindqvist, G.: Analysis of linkage in *Picea abies*. Hereditas, 106, 1987, s. 31—36
19. Nei, M.: Analysis of gene diversity in subdivided populations (population structure, genetic variability, heterozygosity, gene differentiation). Proceedings of National Academy of Science, USA, 70, 12, 1973, s. 3321—3323
20. Nei, M.: Mathematical models of speciation and genetic distance. In: Karlin, S. — Nevo, E. (eds.): Population Genetics and Ecology. Proceedings of the International Conference on Population Genetics and Ecology, Israel, 1976, s. 723—764
21. Nei, M. — Roychoudhury, A. K.: Sampling variances of heterozygosity and genetic distance. Genetics, 76, 1974, s. 379—390
22. Paule, L.: Využitie izoenzýmových analýz pri výskume genetickej štruktúry populácií smreka. VŠLD, Zvolen (research report), 1985, s. 74
23. Paule, L. — Gömöry, D.: Comparison of different clustering methods for matrices of genetic distances based on allelic frequencies. Abstracta Botanica, 11, 1987, s. 43—51
24. Shaw, R. C. — Prassad, R.: Starch gel electrophoresis of enzymes, compilation of recipes. Biochemical Genetics, 4, 1970, s. 297—320
25. Steinhoff, R. J. — Joyce, D. G. — Fins, L.: Isozyme variation in *Pinus monticola*. Canadian Journal of Forest Research, 13, 6, 1983, s. 1122—1131
26. Szmidt, A. E. — Yazdani, R.: Inheritance of aconitase and phosphoglucose isomerase electrophoretic variants of Scots Pine (*Pinus sylvestris* L.) tissues. In: Szmidt, A. E.: Genetic studies of Scots Pine (*Pinus sylvestris* L.) domestication by means of isozyme analysis. Umeå (PhD. thesis), 1984, s. 186

27. Tigerstedt, P. M. A.: Genetic structure of *Picea abies* as determined by the isozyme approach. In: Proceedings of the IUFRO Joint Meeting on Working Parties on Population and Ecological Genetics, Stockholm, 1974, s. 282—292

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IZOENZÝMOVÝ POLYMORFIZMUS POPULÁCIÍ SMREKA NA SLOVENSKU. I. GENETICKÁ ŠTRUKTÚRA SUSEDNÝCH POPULÁCIÍ SMREKA

SÚHRN

V 9 autochtónnych populáciách smreka z územia Slovenska sme skúmali izoenzýmový polymorfizmus 9 izoenzýmových systénov: ACO, DIA, FEST, GDH, GOT, LAP, MHD, PFI, PGM, v ktorých sme detekovali 15 izoenzýmových lokusov. Výber populácií sme uskutočnili tak, že boli vybraté 4 páry susedných populácií za účelom zistenia ich genetickej príbuznosti.

Spomedzi sledovaných 9 izoenzýmových systémov boli 4 použité po prvý raz (ACO, DIA, PGI, FEST). V skúmaných populáciách sa zistil vysoký izoenzýmový polymorfizmus na vnútropopulačnej úrovni. Hodnoty priemerných heterozygotností sa pri materských jedincoch (endospermy) pohybovali v rozpätí 0.2171 — 0.2667.

Sedem lokusov (ACO, DIA, PGI, B, GOT B, LAP A, LAP B, A MHD B) sa vyznačovalo vyššími hodnotami heterozygotnosti než 0.2, teda tieto lokusy možno považovať za lokusy dobre vyjadrujúce heterozygotnosť populácií a naopak, zostávajúce lokusy sú charakterizované výskytom zriedkavých alel.

Využijúc hierarchický model rozkladu heterozygotnosti sme zistili, že v priemere 2,88 % génovej diverzity sa dá pripísať rozdielom na úrovni populácií, 1,45 % rozdielom medzi oblasťami a 95,76 % vnútropopulačným rozdielom.

Genetické vzdialenosti sme využili na analýzu genotickej príbuznosti populácií. Pri použití zhlukovej analýzy sme nezistili, že geograficky priľahlé populácie majú aj najnižšie hodnoty genetických vzdialeností. Tento jav možno vysvetliť buď nepôvodnými populáciami, alebo charakterom experimentálneho materiálu. Populačné vzorky môžu reprezentovať malý počet materských jedincov.

ИЗОФЕРМЕНТНЫЙ ПОЛИМОРФИЗМ У ПОПУЛЯЦИЙ ЕЛИ В СЛОВАКИИ I. ГЕНЕТИЧЕСКАЯ СТРУКТУРА СОСЕДНИХ ПОПУЛЯЦИЙ ЕЛИ

РЕЗЮМЕ

В 9-ти популяциях автохтонных елей на территории Словакии изучался изоферментный полиморфизм 9-ти изоферментных систем: ACO, DIA, FEST, GDH, GOT, LAP, MDH, PFI, PGM, в которых было обнаружено 15 изоферментных локусов. Для того, чтобы установить их генетическое родство, было отобрано 4 пары соседних популяций.

Из 9-ти исследуемых изоферментных систем четыре были взяты впервые (ACO, DIA, PGI, FEST). В исследуемых популяциях был обнаружен высокий полиморфизм на внутривидовом уровне. Средняя гетерозиготность материнских особей (эндосперма) колебалась в пределах 0,2171– 0,2667.

Средняя гетерозиготность семи локусов (ACO, DIA, PGI, B, LAP A, LAP B и MDH B) оказалась выше, чем 0,2, т. е. эти локусы хорошо выражают гетерозиготность популяции, и, наоборот, остальные локусы характеризуются наличием редко встречающихся аллелей.

При помощи иерархической модели разложения гетерозиготности было установлено, что в среднем 2,88 % геновых разновидностей можно объяснить различиями на уровне популяции, 1,45 % — различиями между областями и 95,76 % — внутрипопуляционными различиями.

Генетическая дальность использовалась для анализа генетического родства популяций. С помощью кластерного анализа не удалось доказать, что географически смежные популяции должны характеризоваться наиболее низкими величинами генетической дальности. Это явление можно объяснить или неавтохтонностью популяций или же характером экспериментального материала. Популяционные пробы представляют малое число материнских особей.