AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic ISSN 1842-4309

Not. Bot. Hort. Agrobot. Cluj 37 (2) 2009, 245-251

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Allozyme Variation of Coniferous Tree Species from Maramures Mountains, Romania

Alexandru Lucian CURTU[1)], Nicolae SOFLETEA[1)], Raul RADU[1)], Anton BACEA[1)]
Ioan Vasile ABRUDAN[1)], Anca BUTIUC-KEUL[2)], Sorina FARCAS[3)]

[1)] *Transilvania University Brasov, Faculty of Forest Sciences, 1 Sirul Beethoven, Brasov, Romania; lucian.curtu@unitbvro (corresponding author), nic.sofletea@unitbv.ro, raul.radu@geodis.ro, bacea_anton@yahoo.com, abrudan@unitbv.ro*

[2)] *Faculty of Biology and Geology, Department of Experimental Biology, Babes-Bolyai University, Cluj-Napoca 400084, Romania; alkeul@yahoo.com*

[3)] *Institute of Biological Research, Department of Taxonomy and Ecology, Cluj-Napoca 400015, Romania; soryna001@yahoo.com*

Abstract

Two coniferous tree species, Norway spruce [*Picea abies* L. (Karst)] and silver fir (*Abies alba* Mill.), from Maramures Mountains Nature Park, in northern Romania, were examined by means off allozyme markers. For Norway spruce, the genetic structure was observed at 19 enzyme coding loci in two populations situated at different elevations above the see level. Moderate levels of genetic variation within populations (on average, the number of alleles per locus was 2.14, the expected heterozygosity was 0.144) and an extremely low variation (FST=0.003) between the two populations was found. The genetic diversity was slightly higher in the low elevated population as compared to the high elevated spruce population. The estimated values for genetic multiplicity and diversity were comparable with those reported for a series of populations from the nearby Ukrainian Carpathians. For silver fir, the genetic variation was estimated at five enzyme coding loci from two enzyme systems, peroxidases and esterases, in five populations distributed throughout Maramures Mountains. Only three out of five loci were polymorphic in at least one population. The genetic diversity within populations was low (on average, expected heterozygosity was 0.093) and genetic differentiation among populations was relatively high (FST=0.106) which is consistent with their geographical position in the region. The results may contribute to a better understanding of the genetic structure in two of the most important tree species from Romania.

Keywords: *Picea abies, Abies alba*, allozymes, genetic diversity

Introduction

Allozymes, i.e. any variant form of an enzyme which had a genetic basis, known also under the more general term of isozymes, have been extensively used for estimating genetic variation in a wide range of plant and animal species. Allozyme markers are codominant in expression and reveal relatively high levels of polymorphism. Particularly in tree species, the allozyme variation seems to be much higher than in animals and in other types of plants (Hamrick *et al*., 1992). Allozymes have been used in tree species for describing geographical patterns of variation and delimiting taxa with application in gene conservation (e.g. Vicario *et al*., 1995), evaluating the impact of forest management and tree improvement on genetic diversity (e.g. Hosius, 1993; Hussendörfer, 1996).

Norway spruce [*Picea abies* L. (Karst)] and silver fir (*Abies alba* Mill.) are among the most important tree species of Europe. In Romania, *Picea abies* and *Abies alba*, occupy approximately 22% and 5% of the forest area, being the most valued coniferous species from ecological and economical point of view (Sofletea and Curtu, 2007). The northern boundary of their natural distribution in Romania is attained in the Maramures Mountains, a vast natural area, which was included on the list of 'Natura 2000' sites. Norway spruce and silver fir represent the only coniferous tree species which occur naturally in the region (Sofletea and Curtu, 2007)

As a consequence of their importance, the both species were genetically studied by means of allozyme markers (Bergmann *et al*., 1990; Breitenbach-Dörfer *et al*., 1997; Giannini *et al*., 1991; Konnert and Bergmann, 1995; Longauer, 2001; Müller-Starck, 1995). However, in spite of the large occurrence and high importance of the species there is only one study which investigated allozyme variation in five Romanian populations of silver fir (Danila, 1994). Moreover, there is no report of allozyme variation in Norway spruce populations from the Romanian Carpathians.

The aim of this study was to analyse the genetic variation estimated by means of allozyme genetic markers in populations of Norway spruce and silver fir from Maramures Mountains. Since Norway spruce is naturally occupying a large altitudinal range in the area, occurring from low altitudes (750-800 m) to high altitudes (1400-1450

m). The issue of genetic differences between low and high elevated populations was also addressed. This work is part of a larger study of biodiversity in spruce and fir forests from Maramures Mountains Nature Park.

Materials and methods

Allozyme analysis in Picea abies

A total of 161 individuals from two populations of Norway spruce were sampled. Both populations are located in 'Novicioru' massif, in the heart of 'Maramures' Mountains. One population, 'Culmea Novicioru' (47°46'25"N; 24°37'59"E), is situated at high elevation (1300-1350 m) whereas the second population, 'Valea Novicioru' (47°45'11"N; 24°38'04"E), is situated at low elevation (800-850 m), along the river with the same name. All sampled trees were mature and distributed at least 30 meters from one another in order to avoid relatedness. Until analysis in the laboratory of Brasov University, the material was stored at - 60°C.

Eleven enzyme systems were interpreted: formiat-dehydrogenase (E.C.-No. 1.2.1.2; *Fdh*), glutamate-dehydrogenase (E.C.-No. 1.4.1.3; *Gdh*), glutamate-oxaloacetate-transaminase (E.C.-No. 2.6.1.1; *Got*), isocitrate-dehydrogenase (E.C.-No. 1.1.1.42; *Idh*), leucine-aminopeptidase (E.C.-No. 3.4.11.1; *Lap*), malate-dehydrogenase (E.C.-No. 1.1.1.37; *Mdh*), menadione-reductase (E.C.-No. 1.6.99.2; *Mnr*), 6-phosphogluconate-dehydrogenase (E.C.-No. 1.1.1.44; *6-Pgdh*), phosphoglucose-isomerase (E.C.-No. 5.3.1.9; *Pgi*), phosphoglucomutase (E.C.-No. 2.7.5.1; *Pgm*) and shikimic-acid-dehydrogenase (E.C.-No. 1.1.1.25; *Skdh*). Only zones with clear and repeatable genotypes were considered. 19 enzyme coding gene loci were observed: *Fdh-A*, *Gdh-A*, *Got-A*, *Got-B*, *Got-C*, *Idh-A*, *Idh-B*, *Lap-A*, *Lap-B*, *Mdh-A*, *Mdh-B*, *Mdh-C*, *Mnr-B*, *6-Pgdh-A, 6-Pgdh-B, 6-Pgdh-C, Pgi-B, Pgm-A, Skdh-A*. Methodological details of enzyme extraction, horizontal starch-gel electrophoresis (composition of gel and electrode buffers, separation conditions), staining of gels and genotyping followed standard procedures (Konnert and Werner, 2004).

Allozyme analysis in Abies alba

For silver fir, a total of 54 individuals from five populations were sampled: 'Culmea Novicioru', 'Valea Novicioru', 'Valea Vaserului' (47°47'32"N; 24°37'37"E), 'Borsa' (47°41'45"N; 24°38'36"E) and 'Ruscova' (47°48'05"N; 24°20'61"E). Buds and needles were collected in the same year as for *Picea abies*. The analyses were performed by the laboratory of Institute of Biological Research Cluj-Napoca. Only two enzymes systems could be interpreted in all samples: peroxidases (E.C.-No. 1.11.1.7; *Per*), and esterases (E.C.-No. 3.1.1.1; *Est*). Both enzyme systems have been separated on polyacrylamide gels by isoelectric focusing (IEF) (Acquaah, 1992). Gel concentration was 5%. For gel preparation a stock solution of acrylamide/bisacrylamide mixed with ampholine A, pH=3.5-5.0/ampholine B pH=3.5-10.0 ratio 1:1, H_2O, amonium persuphate 10% and Temed. Two buffers were used in cuvettes: 20 mM NaOH/10 mM H_3PO_4; Running was performed at 120 V, one hour, with a Consort device. Histochemical identification of enzyme variants was performed according to several protocols described elsewhere (Acquaah, 1992).

Data analysis

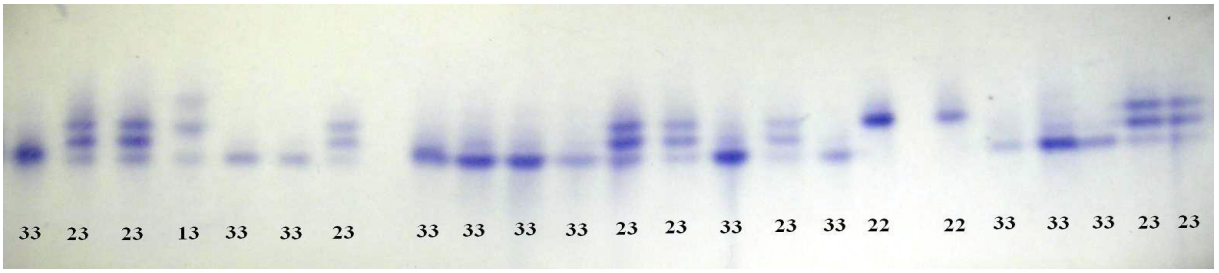
Genetic variation within populations was quantified as percentage of polymorphic loci (*PPL*), average number of alleles per locus (*A/L*), expected heterozygosity (H_e) and heterozygote deficit (F_{IS}). The deviations from Hardy-Weinberg proportions were tested with C^2 test. In addition, variation among populations within the two species was measured by F_{ST} index. The software *GenAlEx* version 6.2 (Peakall and Smouse, 2006) was used for computations.

Results and discussion

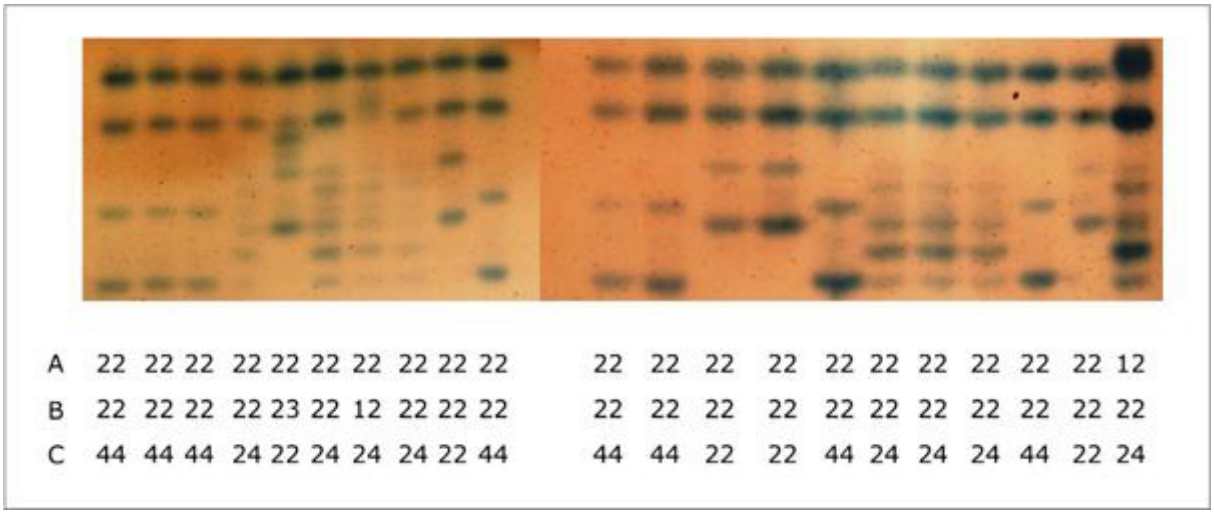
Allozyme analysis in Norway spruce

After cutting the starch gels into slides and their staining, interpretable zymograms for eleven enzyme systems were obtained (see Fig. 1, for examples of zymograms). For several systems, such as Got, two-three enzyme coding loci (e.g. Got-A, -B and -C) were observed. Allele frequencies at the 19 observed enzyme coding loci were computed for both populations (Tab. 1). A total of 40 and 41 alleles were detected in 'Culmea Novicioru' and 'Valea Novicioru' populations, respectively. More loci were polymorphic in the high elevated population, 'Culmea Novicioru', as compared with the low elevated population, 'Valea Novicioru' (Tab. 2) although the mean number of alleles was slightly higher in the valley population. The highest variation, in terms of genetic multiplicity, was observed at loci Pgi-B and Lap-B in 'Culmea Novicioru' population, and at loci Pgi-B, Lap-B and Skdh-A in 'Valea Novicioru' population, respectively. Only two loci, Mdh-A and Mdh-B, were monomorphic in all samples (Tab. 1). At Pgi- B gene locus, one rare allele (Pgi-B-3') was found, which was not described in the latest report on isoenzyme studies in Germany (Konnert and Werner, 2004).

In terms of genetic diversity, the differences between populations are very small, as shown by the estimated values of expected heterozygosity (Tab. 2). There is nearly no deficit or excess of heterozygote genotypes since the mean values of FIS are very close to zero. Moreover, only at one out of 38 loci, for both populations, there was a significant deviation ($P<0.05$) from the Hardy-Weinberg proportions (locus Idh-A, $P=0.001$) due to the presence in the sample



a



b

Fig. 1. Patterns for the enzyme gene loci *Pgi-B* (a), *Got-A*, *B* and *C* (b) as obtained from different trees of *Picea abies* from Maramures Mountains. Both enzyme systems are dimmer, i.e. heterozygotes' phenotypes show a hybrid band. The enzyme migrated from the bottom (cathode) to the top (anode). The corresponding genotypes are given at the bottom of the gel. Nomenclature is according to Konnert and Werner (2004)

of two homozygote genotypes for a relatively rare allele (frequency=0.06) in 'Culmea Novicioru' population.

The extremely low value of FST (0.003) indicates that the movement of alleles, i.e. gene flow, between the two populations is very high. Locus-specific FST values ranges between 0.000, e.g. for locus Got-C, and 0.006, for locus 6-Pgdh-A. Despite of presumable existing reproductive barriers (e.g. flowering phenology) between spruce populations situated at different elevations about the see level, the genetic exchange via pollen and seeds seems to be very effective in this area. Norway spruce pollen can be carried by wind over long distances (Pakkanen *et al.*, 2000; Paule *et al.*, 1993). A very low FST value was also reported for nine Norway spruce populations from Ukrainian Carpathians (Korshikov and Privalikhin, 2007).

When considering 'Culmea Novicioru' and 'Valea Novicioru' populations as one large population, 'Maramures' Mountains, the values of all parameters are increasing (Tab. 2) but there are still lower than those reported for the same parameters in highly elevated populations of the Swiss Alps (Müller-Starck, 1995). The considerable difference between the mean values of gene diversity (He) in Alps and Maramures comes, at least in part, from the use of different sets of enzyme gene loci. However, very similar values of gene diversity were reported for populations from Ukrainian Carpathians (He=0,160) and Italian Alps (He=0,165) using a comparable number of genetic markers (Giannini *et al.*, 1991; Korshikov and Privalikhin, 2007). Lower estimates (0.07-0.10) were computed for Norway spruce provenances originating from central and south-eastern Europe (Lagercrantz and Ryman, 1990).

The low elevated population located along the 'Novicioru' river, 'Valea Novicioru', revealed a slightly higher value of genetic diversity as compared to the high elevated population, 'Culmea Novicioru' (1300-1350 m). An explanation would be the introduction of foreign reproductive material into the valley population, hypothesis which is confirmed by the forest records. According to these, the seeds were brought from other spruce stands from the same region. In the field trip no cones with rounded scales belonging to Picea abies var. europaea were found, which is in agreement with the data from the forest inventory. By contrast, the high elevated population, 'Culmea Novicioru', is very probably pristine.

The same pattern, i.e. a slightly smaller value for the high elevated Norway spruce population (1550-1700 m), was observed along an altitudinal transect in 'Piatra Craiului' Mountains, Southern Carpathians (Curtu, 2003).

Tab. 1. Allele frequencies at 19 enzyme coding gene loci in two *Picea abies* populations from Maramures Mountains

Enzyme gene locus	Allele	Population	
		'Culmea Novicioru'	'Valea Novicioru'
Fdh-A	1	0.006	0.000
	2	0.994	1.000
Gdh-A	2	0.976	0.987
	3	0.024	0.013
Got-A	2	0.000	0.006
	3	1.000	0.994
Got-B	1	0.024	0.026
	2	0.976	0.968
	3	0.000	0.006
Got-C	2	0.470	0.474
	4	0.530	0.526
Idh-A	2	0.060	0.038
	3	0.940	0.962
Idh-B	3	0.994	1.000
	4	0.006	0.000
Lap-A	3	0.012	0.019
	4	0.982	0.968
	5	0.006	0.013
Lap-B	1	0.024	0.026
	2	0.018	0.058
	3	0.127	0.147
	4	0.831	0.769
Mdh-A	2	1.000	1.000
Mdh-B	2	1.000	0.994
	3	0.000	0.006
Mdh-C	4	1.000	1.000
Mnr-B	2	0.006	0.000
	3	0.994	1.000
6-Pgdh-A	2	0.988	1.000
	3	0.012	0.000
6-Pgdh-B	2	0.596	0.532
	5	0.404	0.468
6-Pgdh-C	2	0.470	0.513
	5	0.530	0.487
Pgi-B	1	0.018	0.019
	2	0.295	0.250
	3'	0.000	0.013
	3	0.681	0.705
	4	0.006	0.013
Pgm-A	2	0.976	0.955
	3	0.024	0.045
Skdh-A	1	0.024	0.058
	2	0.018	0.013
	3	0.958	0.923
	6	0.000	0.006

In that study the genetic diversity was based on four sequence-tagged-sites (STS) markers. However, the genetic variation was not smaller in high elevated spruce stands than in adjacent lower elevated areas in allozyme genetic

Tab. 2. Genetic variation of *Picea abies* populations in Maramures Mountains

Population	N	PPL	A/L	H_e	F_{IS}
'Culmea Novicioru'	83	78.95%	2.11	0.139	0.009
'Valea Novicioru'	78	68.42%	2.16	0.147	-0.013
'Maramures Mountains'	161	89.47%	2.37	0.144	0.003

N - sample size; *PPL* – percentage of polymorphic loci; *A/L* – mean number of alleles per locus; H_e – expected heterozygosity; F_{IS} – heterozygote deficit

inventories carried out in Alp Mountains (Müller-Starck, 1995) and in Ukrainian Carpathians (Korshikov and Privalikhin, 2007).

Allozyme analysis in silver fir

The method of isoelectric focusing on polyacrylamide gel electrophoresis ensures the separation of enzyme variants according to their isoelectric point. Three major regions could be identified in the gel: the alkaline region (in the upper part of the gel), the neutral region (in the middle of the gel) and the acidic region (in the lower part of the gel). According to this method, three loci were identified for peroxidases (see Fig. 2) and two for esterases.

In silver fir populations only three out of five gene loci were polymorphic. A total of five multilocus genotypes were identified (Tab. 3). The highest number of alleles (3) was observed at locus *Per-A*. However, no more than two alleles per population were detected. One population, 'Valea Novicioru', was monomorphic at all studied loci. The number of observed alleles is very sensitive to the sample size. In this study the sample size per population was small. Taking into account the scattered distribution of silver fir in the region, the sampling strategy was less individuals per population and more populations than in Norway spruce.

The most common allele (allele 2, frequency=0.78) at locus *Per-A* in 'Borsa' population shows a relatively similar frequency with that estimated (0.70) at the same location, 'Borsa', in a study of six silver fir populations from Romania and one from France (Lucau Danila, 1994). In the same study, the presence of allele 3 at locus *Per-C* in Borsa population is reported which is consistent with the present findings.

The mean number of alleles per population is ranging from 1.00 in 'Valea Novicioru' population to 1.60 in 'Culmea Novicioru' population (Tab. 4). The highest level of gene diversity was estimated in two fir populations, 'Culmea Novicioru' and 'Borsa', respectively. The genotypic structure at all loci and in all populations did not deviate significantly ($P<0.05$) from the proportions expected under Hardy-Weinberg equilibrium (*P* values varied between 0.43 and 0.87). The genetic differentiation among the five populations was high ($F_{ST}=0.106$). This high value is explained by the large geographical distance between some pairs of fir populations.

For Maramures Mountains, considered as one population, the expected heterozygosity is low ($He<0.10$) as compared with other investigations in the same species (Bergmann *et al*., 1990; Breitenbach-Dörfer *et al*., 1992; Konnert and Bergmann, 1995; Longauer, 2001). This result should be put in connection with the small sample size and low number of observed loci. Therefore, future work in silver fir will consider more loci and sampled individuals.

Conclusions

The present study is the first published report on allozyme variation in Norway spruce populations from Romanian Carpathians. The used set of markers revealed a high number of allelic variants and a moderate level of genetic diversity within populations, as compared to other studies. The genetic differentiation between the two

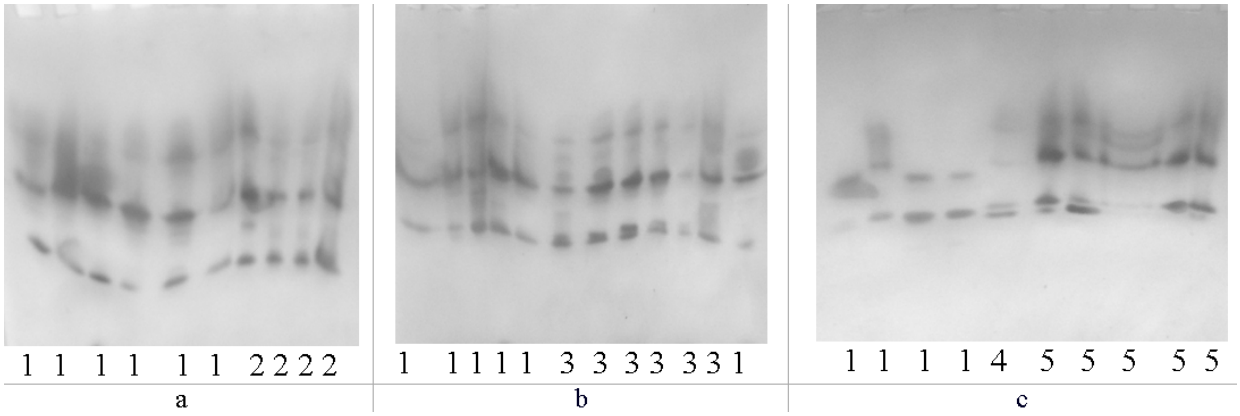


Fig. 2. Patterns for the enzyme gene loci *Per-A, Per-B* and *Per-C* as obtained from different trees of *Abies alba* from *Maramures* Mountains: (a) 'Valea Vaserului' population (b) 'Culmea Novicioru' population and (c) 'Borsa' population. The numbers indicate different multilocus genotypes.

Tab. 3. Allele frequencies at five enzyme gene loci in three *Abies alba* populations from 'Maramures' Mountains

		Population				
Enzyme coding locus	Allele	'Valea Vaserului'	'Valea Novicioru'	'Culmea Novicioru'	'Borsa'	'Ruscova'
Per-A	2	1.000	1.000	0.875	0.786	0.950
	3	0.000	0.000	0.125	0.214	0.050
Per-B	2	0.800	1.000	0.833	1.000	0.800
	3	0.200	0.000	0.167	0.000	0.200
Per-C	1	0.000	0.000	0.167	0.000	0.000
	2	1.000	1.000	0.833	0.800	1.000
	3	0.000	0.000	0.000	0.200	0.000
Est-B	1	1.000	1.000	1.000	1.000	1.000
Est-C	1	1.000	1.000	1.000	1.000	1.000

spruce populations was extremely low suggesting high levels of gene flow. The data obtained for silver fir is useful taking into account that population genetic studies in Romanian silver fir are scarce. The result is a good starting point for further investigations into the genetic structure of spruce and fir populations from the Eastern and Southern Carpathians. The correlation of the genetic results to those obtained from fossil pollen records in the area may bring new insights into the recent Quaternary history and future evolution of the two species.

Tab. 4. Genetic variation of *Abies alba* populations in Maramures Mountains

Population	N	PPL	A/L	H_e	F_{IS}
Valea Vaserului	10.0	20.00%	1.20	0.064	-0.250
Valea Novicioru	12.0	0.00%	1.00	0.000	-
Culmea Novicioru	12.0	60.00%	1.60	0.155	-0.181
Borsa	9.4	40.00%	1.40	0.131	-0.261
Ruscova	10.0	40.00%	1.40	0.083	-0.151
Maramures Mountains	53.4	60.00%	1.80	0.093	-0.086

N - sample size; *PPL* – percentage of polymorphic loci; *A/L* – mean number of alleles per locus; *He* – expected heterozygosity; *FIS* – heterozygote deficit

Acknowledgments

The authors are grateful to Costel Bucur, park manager, and Gabriel Covaza, ranger, Maramures Mountains Nature Park, Stefan Tomoioaga, chief of the Forest District Viseu, Romanian State Forest Service, Romsilva, for their support and assistance in the field, Ioan Calin Moldovan and Horia Curtu for their help during the sample collection. This work was founded by Ministry of Education and Research, Romania, ANCS-CNMP through the Partnership CONBIODIV, PNII, No. 3-015/2007.

References

Acquaah, G. (1992). Practical Protein Electrophoresis for Genetic Research. Dioscorides Press, Portland, Oregon.

Bergmann, F., H.R. Gregorius and J. B. Larsen (1990). Levels of genetic variation in European silver fir (*Abies alba*). Are they related to species decline? Genetica. 82(l):1-10.

Breitenbach-Dörfer, M., W. Pinsker, R. Hacker and F. Müller (1992). Clone identification and clinal allozyme variation in populations of *Abies alba* from the Eastern Alps (Austria). Plant Systematics and Evolution. 181(1-2):109-120.

Breitenbach-Dörfer, M., M. Konnert, W. Pinsker, R. Starlinger and T. Geburek (1997). The contact zone between two migration routes of silver fir, *Abies alba* (*Pinaceae*), revealed by allozyme studies. Plant Systematics and Evolution. 206(1-4):259-272.

Curtu, A. L. (2003). Genetic variability of Norway spruce [*Picea abies* (L.) Karst.] estimated by DNA markers (in Romanian). Revista Padurilor. 3:10-15.

Giannini, R., M. Morgante and G. G. Vendramin (1991). Allozyme variation in Italian populations of *Picea abies* (L.) Karst. Silvae Genetica. 40(3/4):160-166.

Hamrick, J. L., M. J. W. Godt and S. L. Sherman-Broyles (1992). Factors influencing levels of genetic diversity in woody plant species. New Forests. 6(1-4):95-124.

Hosius, B. (1993). Wird die genetische Struktur eines Fichtenbestandes von Durchforstungseingriffen beeinflußt? Forst und Holz. 48(11):306-308.

Hussendörfer, E. (1996). Wird "Biodiversität" durch eine künstliche Bestandesbegründung beeinflußt? p. 160-176. In: *Biodiversität und nachhaltige Forstwirtschaft* (Eds.). Müller-Starck G, Ecomed, Landsberg.

Kannenberg, N. and K. Gross (1999). Allozymic variation in some Norway spruce populations of the International IUFRO provenance-testing programme of 1964/1968. Silvae Genetica. 48(5):209-217.

Konnert, M. and F. Bergmann (1995). The geographical

distribution of genetic variation of silver fir (*Abies alba*, *Pinaceae*) in relation to its migration history. Plant Systematics and Evolution. 196(1-2):19-30.

Konnert, M. and M. Werner (2004) Isoenzymuntersuchungen bei Fichte (*Picea abies*) - Anleitungen zur Trennmethodik und Auswertung der Zymogramme, 1-22. Bayerisches Amt für forstliche Saat- und Pflanzenzucht (ASP).

Korshikov, I. I. and S. N. Privalikhin (2007). Genetic structure of populations of Norway spruce (*Picea abies* (L.) Karst.) from Ukrainian Carpathians. Russian Journal of Genetics. 43(12):1364-1372.

Lagercrantz, U. and N. Ryman (1990). Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. Evolution 44(1):38-53.

Longauer, R. (2001). Genetic variation of European silver fir (*Abies alba* Mill.) in the Western Carpathians. Journal of Forest Science. 47(10):429-438.

Lucau Danila, A. (1994). Genetic variability of silver fir (*Abies alba* Mill.) estimated by enzyme and terpene markers (in Romanian). Universitatea Transilvania Brasov, PhD Thesis.

Müller-Starck, G. (1995). Genetic variation of high elevated populations of Norway spruce (*Picea abies* (L.) Karst.) in Switzerland. Silvae Genetica. 44(5-6):356-362.

Pakkanen, A., T. Nikkanen and P. Pulkkinen (2000). Annual variation in pollen contamination and outcrossing in a *Picea abies* seed orchard. Scandinavian Journal of Forestry Research. 15(4):399-404.

Paule, L., D. Lindgren and R. Yazdani (1993). Allozyme frequencies, outcrossing rate and pollen contamination in *Picea abies* seed orchards. Scandinavian Journal of Forestry Research. 8 (1):8-17.

Peakall, R. and P. E. Smouse (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288-295.

Sofletea, N. and A. L. Curtu (2007). Dendrology (in Romanian). Ed. Universitatii Transilvania, Brasov.

Vicario, F., G. G. Vendramin, P. Rossi, P. Liò and R. Giannini (1995). Allozyme, chloroplast DNA and RAPD markers for detecting genetic relationships between *Abies alba* and the relic population of *Abies nebrodensis*. Theoretical and Applied Genetics. 90(7-8):1012-1018.