GENETIC DIVERSITY AND DIFFERENTIATION IN SWISS STONE PINE (*Pinus cembra* L.) PROVENANCES FROM ROMANIA

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Abstract

Seven provenances of Swiss stone pine (*Pinus cembra* L.) from the main range area of the Romanian Carpathians were analysed using ten isozymes systems (GDH, GOT, IDH, MDH, MNR, 6-PGDH, PGI, PGM, LAP, SKDH). For the investigated provenances, the average, the expected heterozygosity was 0.046, the polymorphic loci per populations were 31.8 %, the number of alleles per locus was 1.11. Analysis of *F*-statistics has showed a deficiency of heterozygotes per population, in reference to the Hardy-Weinberg ratio. Analysis of FST has proved that over 93% of the genetic variation is located at the level of populations whereas 7% of genetic variation is located in between. The dendrogram obtained by means of genetic distances has indicated that there is no clear differentiation between the Northern and Southern provenances from the Romanian Carpathians. Amongst all, Stâna de Râu provenance is the most distinctly differentiated, with the highest level of genetic diversity.

Keywords: *Pinus cembra*, provenance, isozyme system, genetic variation, genetic distance

Rezumat

DIVERSITATEA ŞI DIFERENȚIEREA GENETICĂ LA PROVENIENȚE DE PIN CEMBRA DIN ROMÂNIA

Şapte proveniențe de pin cembra din întregul areal din România au fost analizate prin intermediul a 10 sisteme enzimatice (GDH, GOT, IDH, MDH, MNR, 6-PGDH, PGI, PGM, LAP, SKDH). Pentru aceste proveniențe, heterozigoția a fost în medie de 0.046, proporția locilor polimorfici la nivel de populație a fost de 31.8%, iar numărul alelelor per locus de 1.38. Indicele *F* a arătat o deficiență de heterozigoți la nivel de populație, în raport cu proporția Hardy-Weinberg. Analiza FST arată că peste 93% din variația genetică se găseşte la nivel intrapopulațional, iar 7% interpopulațional. Dendrograma obținută prin intermediul distanțelor genetice a indicat faptul că nu există o diferențiere clară între proveniențele din nordul şi sudul Carpaților românești, proveniența Stâna de Râu diferențiindu-se net de celelalte şi având cel mai ridicat nivel al diversității genetice.

Cuvinte cheie: *Pinus cembra*, proveniență, sistem izoenzimatic, variație genetică, distanță genetică

1. INTRODUCTION

Knowledge of the genetic structure of populations is an important step for genetically satisfactory gene conservation (Eriksson & Ekberg, 2001). During the last decades, isozymes became an useful tool in the investigation of genetic structure of -forest tree populations (Lundkvist & Rudin, 1977). These markers can contribute, also, to the delineation of provenance region (Geburek, 1997), or offers valuable information about autochthony of populations. For example, an isozyme gene locus (GDH) was useful as diagnostic marker for Alpine autochthony of populations (Bergmann, 1984).

The Swiss stone pine (*Pinus cembra* L.), a locally important species in Europe, with multiple uses over centuries (Klumpp & Stefsky, 2004), is naturally distributed in Austria, France, Germany, Italy, Poland, Romania, Slovakia, Switzerland, and Ukraine (Blada & Popescu, 2004). In Romania, the species occurs at timberline of Carpathians, especially in mountains with glacial phenomena – i.e. Rodna, Călimani, Bucegi, Făgăraş, Lotru, Retezat, Parâng, Godeanu, Ţarcu (Şofletea, 2001) - where could be identified in pure stands or mixed, with *Picea abies* and *Larix decidua*, according with the altitudinal gradient. A facilitation factor for gene exchange among populations is the seeds dispersal by birds (*Nucifraga caryocatactes* L. – the nutcracker). Following that, at high elevation, the competitiveness of *Pinus cembra*, including the mutualism with the nutcracker, may lead to a more effective preservation of the gene pool. In contrast, at lower elevation, the populations may have suffered a reduction of genetic diversity due to regular management activities and a limited activity of nutcrackers in such dense forest (Klumpp & Stefsky, 2004). The main threats for *Pinus cembra* is the areal fragmentation and environmental conditions specific for high altitudinal zone that can due a lose of genetic informations. (Ulber et.al., 2004)

Different isozyme studies were conducted in *Pinus cembra* populations, the first results being reported by Szmidt (1982), on the genetic structure of isolated populations (Belokon et al., 2005). For Alpine natural populations of Swiss stone pine, Bergmann and Hattemer (1995) reported a low level of genetic diversity. Also, Bulletti & Gullace (1999) studied genetic diversity and differentiation among five populations of *Pinus cembra* from the Italian Alps, by means of isozyme variation at 15 loci and contrasted with five *Pinus sylvestris* populations (Blada & Popescu, 2004). Klumpp & Stefsky (2004) analysed the genetic variation by isozyme gene markers of *Pinus - cembra* along an elevation transect in Austria (mature stands + natural recruitment at three elevations levels - subalpine, high and middle mountainous zone).

According to the putative survival areas during the last glaciation, isozyme variation indicates a low degree of genetic differentiation between a Swiss stone pine population from Carpathians Mountains and the populations from the Alps (Ulber et. al., 2004). Another comparative analysis, including Alpine and Eastern Carpathians populations, shows that the Carpathians populations seem to have a higher level of genetic diversity than those from the Alps (Belokon et al., 2005). For the Romanian populations, the study of Höhn et al. (2005), based on chloroplast microsatellites markers (cpSSR), investigated the genetic diversity and the differentiations of four

populations from Călimani and Retezat Mountains.

The aim of the paper was to analyse the genetic diversity and differentiation in provenances of *Pinus cembra* from main parts of the Romanian's species range, as a tool for *in situ* conservations.

2. MATERIALS AND METHODS

Seven provenances of Swiss stone pine (*Pinus cembra*) were investigated (table 1, fig. 1). Buds were collected from 25 trees per provenance and electrophoresis was carried out on bud tissue collected during the winter of 2006.

Bud tissues were homogenized in extraction buffer (0.1m Tris-HCl, pH 7.2, PVP-40 and 0.07 m *β*-Mercaptoethanol). The following 10 enzyme systems were used to assess genetic diversity (in parentheses is the Enzyme Commission number and abbreviations for each enzyme): Glutamate-oxaloacetate transaminase (E.C. 2.6.1.1,

Table 1. Geographical characteristics of the analysed provenances (after Blada, 1996)
Caracteristicile geografice ale provenienţelor analizate (după Blada, 1996)

Provenance	Lat. (^{0}N)	Long. (^{0}E)	Alt. (M)
Gemenele (Ge)	$45^0$35’	$22^0$50’	1780
Pietrele (Ptr)	$45^0$23’	$22^0$52’	1650
Stâna de Râu (StR)	$45^0$25’	$23^0$03’	1680
Călimani (Cal)	$47^0$07’	$25^0$17’	1650
Valea Lalei (La)	$47^0$33’	$25^0$05’	1520
Pietrosul (Pet)	$47^0$37’	$24^0$40’	1770
Păpuşa-Iezer (Pap)	$45^0$29’	25^0 05’	1480

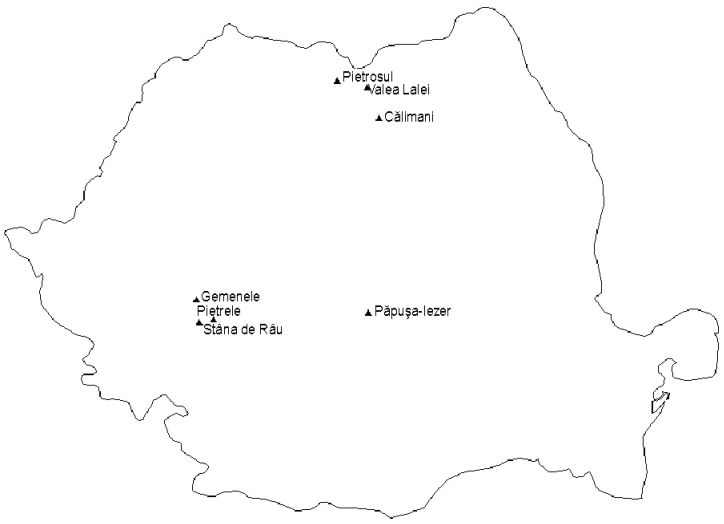

Fig. 1. Map of the sampling provenances
Harta provenienţelor eşantionate

GOT), isocitrate dehydrogenase (E.C. 1.1.1.42, IDH), malate dehydrogenase (E.C. 1.1.1.37, MDH), Menadione reductase (E.C. 1.6.99.2, MNR), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44, 6-PGDH), phosphoglucose isomerase (E.C. 5.3.1.9, PGI), phosphoglucomutase (E.C. 2.7.5.1, PGM), glutamate dehydrogenase (E.C. 1.4.1.2, GDH), Leucine aminopeptidase (E.C. 3.4.11.1, LAP) and shikimate dehydrogenase (E.C. 1.1.1.25, SKDH). These enzyme systems are encoded for *Pinus cembra* by 24 loci. Electrophoretic conditions were those mentioned by Hertel et al. (2004) and are presented in table 2. Information concerning inheritance and descriptions of analyzed enzyme systems are described in literature (Bergmann et al., 1995; Belokon et al., 2005).

Genetic diversity was estimated using the following parameters: (i) the percentage of polymorphic loci (*P*). A locus was designated as polymorphic if the most common allele had a frequency of less than 95%; (ii) the average number of alleles per locus (*A/L*); (iii) the effective number of alleles per locus (n_e), which was calculated following Crow and Kimura (1970); (iv) the expected proportion of heterozygotes H_e at each locus was calculated according to Nei (1975); (v) deviation from Hardy-Weinberg expectations was tested by calculating Wright's fixation index (*F*) (Wright, 1965).

Population genetic differentiation was estimated using Nei's genetic distances *D* (Nei, 1972) and *F*-statistics (Nei, 1987). F_{IS} and F_{IT} represents the correlation between united gametes within subpopulations and for the populations as a whole respectively. F_{ST} describe differentiation level between populations. Nei's genetic distance was used for clustering of populations using UPGMA method modified from Neighbour procedure of PHYLIP version 3.5. Statistical analyses were performed using POPGENE Version 1.31 (Yeh et al., 1997).

3. RESULTS

3.1 Genetic diversity

The seven investigated provenances of Swiss stone pine have a low variability. Of the 19 investigated loci, 42.1% were polymorphic. For all loci 32 alleles were detected. Relative frequency of alleles is presented in table 3.

Regarding to the distribution of the rare alleles detected in the same loci, GDH-

Table 2. Buffer systems for the analysed enzymatic systems
Sisteme tampon pentru sistemele enzimatice analizate

Buffer systems	Buffers		Enzyme systems
	Gel buffer	Electrode buffer	
Tris-Citrat	0.15mTris-citrat pH 7.5	0.02mTris-citrat pH 7.5	MDH, IDH, SKDH, 6-PGDH,
Ashton	0.2m LiOH-borate pH 8.1	0.05m Tris-citrat pH 8.1	PGI, LAP
Poulik	0.3m NaOH-borate pH 8.2	0.075m Tris-citrat pH 8.7	GDH, MNR, GOT

A3 and IDH-A4 were detected only in the provenances Gemenele and allele LAP-B5 was found only in the provenance Păpuşa-Iezer.

The average number of alleles per locus (*A/L*) ranged from 1.21 (Călimani) and 1.47 (Stâna de Râu and Valea Lalei). Mean value calculated was 1.38 (Table 4). The mean effective number of alleles per locus was 1.11. Pietrosul provenance had the highest value (n_e=1.15), while the Călimani provenances had the lowest one (n_e=1.09). The mean expected heterozygosity per population ranged from 0.058 (Călimani) to 0.080 (Gemenele). Wright's fixation index *F* indicate an excess of homozygotes for the Northern Romanian Carpathians populations Călimani and Pietrosul, and an excess of heterozygotes for the Southern provenances Gemenele and Pietrele. For provenances Stâna de Râu and Valea Lalei *F* indices are 0.00.

Table 3. Alleles relative frequencies for the investigated loci in seven *Pinus cembra* provenances from the Romanian Carpathians
Frecvenţele relative ale alelelor pentru locii analizaţi la şapte proveniențe de *Pinus cembra* din Carpaţii româneşti

Loci	Allele	Provenances						
		Gemenele	Pietrele	Stana de Rau	Călimani	Valea Lalei	Pitrosul	Păpuşa-Iezer
GOT-A	A2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GOT-B	B2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GOT-C	C2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	A1	-	0.08	0.060	0.040	-	-	-
GDH-A	A2	0.960	0.920	0.920	0.960	0.980	1.000	1.000
	A3	0.040	-	0.020	-	0.020	-	-
IDH-A	A2	0.980	1.000	1.000	1.000	1.000	1.000	1.000
	A4	0.020	-	-	-	-	-	-
LAP-A	A2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B2	0.680	0.680	0.060	0.660	0.700	0.660	0.740
LAP-B	B3	0.060	-	0.920	-	0.100		0.040
	B4	0.026	0.320	0.020	0.340	0.180	0.340	0.180
	B5	-	-	-	-	0.020	-	0.040
MNR-A	A2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MNR-B	B2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MNR-C	C2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-A	A2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-B	B2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-C	C1	-	0.040	0.020	-	0.080	0.060	-
	C2	1.000	0.960	0.980	1.000	0.920	0.940	1.000
	D1	0.380	0.220	0.020	0.060	0.160	0.040	0.080
MDH-D	D2	0.062	0.780	0.980	0.940	0.800	0.940	0.920
	D3	-	-	-	-	0.040	0.020	-
6PGDH-A	A2	0.940	0.980	0.900	1.000	1.000	1.000	1.000
	A3	0.060	0.020	0.100	-	-	-	-
6PGDH-B	B2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6PGDH-C	C2	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	A1	0.040	0.080	0.020	-	-	0.180	-
	A2	-	-	-	-	-	-	-
SKDH-A	A3	0.780	0.780	0.760	0.640	0.760	0.400	0.480
	A4	0.180	0.120	0.200	0.360	0.220	0.380	0.480
	A5	-	0.020	0.020	-	0.020	0.040	0.040
SKDH-B	B2	1.000	1.000	1.000	1.000	1.000	1.000	1.000

3.2. Genetic differentiation

F-statistics were calculated considering that all provenances behave as a unique one with a mean F_{IT}. The mean value of F_{IT} was 0.102 (table 5), and the average value of F_{IS} is 0.034, indicating also a deficiency of heterozygotes.

For individual loci, F_{ST} varied from 0,017 for IDH-A to 0,112 for MDH-D with a mean value 0.070. Analysis of F_{ST} show that about 93% of the genetic variation resides within each populations and 7% of genetic variation is between populations.

Matrix of Nei's genetic distances (table 6) shows that the maximum value was found between provenances Stâna de Râu and Păpuşa-Iezer and also between Stâna de Râu and Gemenele; the minimum value was found between Valea Lalei and Pietrele. A dendrogram (fig. 2) show the results of the cluster analysis, based on the Nei's genetic distances (UPGMA methods after Phylip Version 3.5).

4. DISCUSSION

The parameters of genetic variation presented in this study are comparable to the values reported for other Swiss stone pine populations, in studies in wich more than 15 isozyme loci were analysed (Goncharenko et al., 1992, Klumpp & Stefsky, 2004; Belokone et al., 2005). It is necessary to take into account that the individual investigations are base on different sample size (6 to 148 trees per populations) or different collected samples (provenance experiment or natural populations).

The mean percentage of polymorphic loci (*P* = 31,8 %) is higher than the calculated by Klumpp & Stefsky (2004) for mature stands and their recruitments along an elevation transect in Austria (*P* = 25 %) or Belokon et al. (2005) for five populations of the Alps and the Easthern Carpathians (Ukraine)(*P* = 26,67%).

Table 4. Intrapopulation genetic diversity parameters
Parametrii diversității genetice intrapopulaționale

Group	Provenance	Multiplicity	Diversity	Heterozygosity (%)		Fixation
		A/L	n_e	observed	expected	index *F*
Southern Carpathians	Gemenele	1.42	1.13	0.090	0.080	-0.125
	Pietrele	1.42	1.11	0.088	0.074	-0.189
	Stâna de Râu	1.47	1.10	0.067	0.067	0.000
	Păpuşa-Iezer	1.31	1.10	0.048	0.058	0.173
Eastern Carpathians	Călimani	1.21	1.09	0.050	0.057	0.123
	Valea Lalei	1.47	1.11	0.071	0.071	0.000
	Pietrosul	1.36	1.15	0.046	0.070	0.343

Table 5. Genetic differentiation estimated with *F*-statistics
Diferențierea genetică estimată cu testul *F*

Loci	LAP-B	IDH-A	GDH-A	MDH-C	MDH-D	6-PGDH-A	SKDH-A	Mean
F_{IS}	-0.083	-0.020	0.183	-0.063	-0.127	-0.084	0.236	0.034
F_{IT}	-0.028	-0.002	0.207	-0.029	-0.0005	-0.026	0.298	0.102
F_{ST}	0.050	0.017	0.030	0.032	0.112	0.053	0.081	0.070

Table 6. Matrix of Nei's genetic distances
Matricea distanţelor genetice Nei

Povenienţa	Gemenele	Pietrele	Stâna de Râu	Călimani	Valea Lalei	Pietrosul	Păpuşa-Iezer
Gemenele	***						
Pietrele	0.002	***					
Stâna de Râu	0.012	0.006	***				
Călimani	0.007	0.004	0.004	***			
Valea Lalei	0.003	0.001	0.008	0.003	***		
Pietrosul	0.013	0.008	0.009	0.002	0.007	***	
Păpuşa-Iezer	0.010	0.008	0.012	0.002	0.005	0.002	***

For the parameters *A/L* (mean number of alleles per locus), the value 1.38 is lower than that obtained in other studies: for example, Lewandowski et al. (2000) found a mean number of alleles per locus of 1.57 in high elevation populations from the Alps. Similar results were obtained for n_e (effective number of alleles per locus) and H_O (observed heterozygosity).

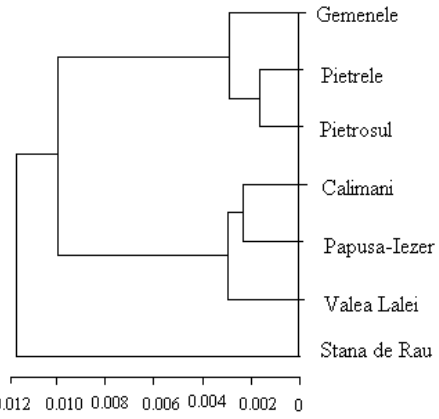

Fig. 2. Dendrogram of the genetic distances
Dendrograma distanţelor genetice

Regarding fixation indices (*F*), an excess of homozygosity was found for Easthern Romanian Carpathians provenances and an excess of heterozygosity for provenances originary from Retezat Mountain (Southern zone). For the assemblage of analysed provenances, the Wright's fixation index indicated an excess of homozygotes ($F = 0.046$). An excess of homozygotes seems to be specific for Swiss stone pine populations, according to similar results observed by other authors (Lewandowski et al. 2000, Belokon et al., 2005). The observed excess of homozygotes could be caused to self-fertilization and mating between related trees, neither can be excluded the hypothetical action of natural selection acting against heterozygotes at certain developmental stages (Karkkainen et al., 1996 after Lewandowski et al. 2000).

Within provenances, the genetic differentiation was relative low ($F_{ST} = 0.070$), which means that about 93 % of the genetic variation is accounted by intrapopulational diversity, and only 7% is explained by interpopulational variability. For five Alpine and Eastern Carpathians populations, Belokon et al., (2005) founded for F_{ST} a value of 0.040.

Dendrogram of genetic distances did not show a clear differentiation between Northern and Southern populations, and that the overall differentiation between provenances is low. Stâna de Râu provenance is distinguished from all and represents a valuable gene poole, while Călimani provenance is characterised by a low level of genetic diversity. Analyses performed with cpSSR markers (Höhn et al., 2005) show

also a low level of genetic diversity for a provenance originating from Călimani Mountains (Mureş area), a possible explanation offered being related to the small number of trees, scatterly distributed over a large area, together with an increasing of the anthropogenic pressure (i.e. grazing), also a threat for the Carpathians gene pool.

For conservation purposes, valuable gene pool regarding genetic diversity, like Stâna de Râu (with special emphasis) and Gemenele should be preserved, while special management measures should be applied for Călimani and Păpuşa-Iezer provenances. In the case of Călimani population, exists the risk of genetic diversity diminution, especially due to the human pressure and disturbances; in the future, will be necessary supplementary evaluations for the valuable gene pools identification.

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REFERENCES

BELOKON, M.M., BELOKON, YU.S., POLITOV, D.C., ALTUKHOV, Yu.P., 2005. Allozyme polymor phism of Swiss Stone Pine (*Pinus cembra* L.) in Mountainous Populations of the Alps and the Eastern Carpathians. Russian Journal of Genetics 41(11): 1268-1280.

BERGMANN, F., HATTEMER, H.H., 1995. Isozyme gene loci and their allelic variation in *Pinus sylvestris* L. and *Pinus cembra* L. Silvae Genetica 44 (5-6): 286-289.

BLADA, I., 1997. Stone Pine (*Pinus cembra* L.) provenance experiment in Romania. I. Nursery Stage at Age 6. Silvae Genetica 46(4): 197-200.

BLADA, I., POPESCU, F., 2004. Genetic research and development of five-needle pines (*Pinus* subgenus *Strobus*) in Europe: An Overview, pp. 51-59. In: Sniezko, R. et al.(Eds), Breeding and genetic resources of five-needle pines: growth, adaptability and pest resitence, IUFRO W.P.2.02.15, Int. Conf. Medford, Oregon, USA, July, 2001, 259p.

CROW, J.F., KIMURA, M., 1970. Introduction to Population Genetics Theory, vol. 6. Harper & Row/Kluwer Academic Publishers, New York/Dordrecth, 1990.

ERIKSSON, G., EKBERG, I., 2001. An Introduction to Forest Genetics. SLU Repro, Uppsala, 2001, 166 p.

GEBUREK, Th., 1997. Isozymes and DNA markers in gene conservation of forest trees. Biodiversity and Conservation 6, 1639-1654.

GONCHARENKO, G.G., PADUTOV, V.E., SILIN, A.E., 1992. Population structure, gene diversity, and differentiation in natural populations of Cedar pines (*Pinus* subsect. *Cembrae, Pinaceae*) in the USSR. Pl. Syst. Evol. 182:121-134.

KLUMPP, R.TH., STEFSKY, M., 2004. Genetic variation of Pinus cembra along an elevation transect in Austria. In: Sniezko, R.A., Samman, S., Schlarbaum, S E., Kriebel, H.B. (eds.), Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance, pp. 136-140.

YEH, F., 1997. PopGene. Web: http://www.ualberta.ca/¬ fyeh. Accesat: iunie 2007.

HERTEL, H., KONNERT,M., MAURER, W., 2004. Anleitung für Isoenzymuntersuchungen bei Kiefer

(*Pinus sylvestris*). Web: http://www.genres.de/fgrdeu/handbuecher/kiefer-arbeitsanleitung.pdf. Accesat: mai 2007.

HÖHN, M., ABRAN, P., VENDRAMIN, G.G., 2005. Genetic Analysis of Stone Pine Populations (*Pinus cembra* L. subsp. *cembra*) from the Carpathians Using Chloroplast Microsatellites. Acta Silv. Lign. Hung. 1: 39-47.

LEWANDOWSKI, A., BURCZYK, J., 2000. Mating system and genetic diversity in natural populations of European larch (*Larix decidua*) and Stone pine *(Pinus cembra*) located at higher elevations. Silvae Genetica 49(3): 158-161.

LUCĂU-DĂNILĂ, A., 1995. Studii de variabilitate genetică la brad (*Abies alba* Mill.) realizate cu aju torul marcherilor enzimatici şi terpenici. Teză de doctorat, Universitatea Transilvania, Braşov. 137 p.

LUNDKVIST, K., RUDIN, D., 1977. Genetic variation in eleven populations of *Picea abie*s as determined by isozyme analysis. Hereditas 85: 67-74.

NEI, M., 1972. Genetic distance between populations. Am. Nat., 106: 283-292.

NEI, M., 1978: Estimation of average heterozygosity and genetic distance from a small number of-individuals. Genetics. 89: 583-590.

NIKOLIC, D., TUCIC, N., 1983: Isoenzyme variation within and among populations of European Black Pine (*Pinus nigra* Arnold). Silvae Genetica 32(3-4): 80-89.

SZMIDT, A.E., 1982. Genetic variation in isolated populations of Stone pine (*Pinus cembra*). Silva Fennica 16: 196-200.

ŞOFLETEA, N., CURTU, L., 2001. Dendrologie. Ed. Pentru Viaţă. Braşov, 300 p.

ULBER, M., GUGERLI, F., BOZIC, G., 2004. EUFORGEN Technical Guidelines for genetic conservation and use for Swiss stone pine (*Pinus cembra*). International Plant Genetic Resources Institute, Rome, Italy, 6 p.

WRIGHT, S., 1965. The interpretation of population structure by *F*-statistic with special regard to systems of mating. Evolution 19: 395-420.

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