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Genetic diveristy and breeding of larch (*Larix decidua* Mill.) in Romania

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Abstract. Although it is one of the most important coniferous species, the larch (*Larix decidua* Mill.) covers a restricted area in Romania, only 0.3% of the forest area, most being represented by artificial stands. The natural area of the larch is fragmented, being concentrated into five genetic centers: Ceahlău, Ciucaş, Bucegi, Lotru and Apuseni. The speed of growth, high productivity, high wood quality and resistance to adversities resulted in initiating research on the genetic variability of the larch. In 1978 and 1982 respectively, by means of international collaboration, two series of comparative trials were installed, with Romanian and foreign provenances of larch, totalizing 6 experimental areas. Selection of seed stands followed, and among them, more than 300 plus trees. There have been installed 26 orchards (134 hectares) of which 3 (15 hectares) are interspecific seed orchards (*Larix x eurolepis*). This paper presents the results obtained over the last years, regarding the continuing larch breeding program; genetic variation of the main characters in multisite provenances comparative trials planted in 1982, evaluation of the genetic parameters in a clonal seed orchard in order to advance to the second generation seed orchard, and a study of the genetic diversity by means of primary biochemical markers. The provenance tests include 24 populations of larch, 13 Romanian and 11 from the following countries: Germany (4), Austria (2), Belgium (1) and the Czech Republic (4). The studied characters are total height, diameter at 1.30 meters, volume/tree, branches characters, stem straightness and survival. At the age of 25 years from planting, the variance analysis reveals significant interpopulation genetic variation for all the studied characters. The expected genetic gain and the genetic parameters, estimated in the Adâncata larch seed orchard, shows that selection on the clones level, in the first generation seed orchard, can be successfully applied, being effective from both genetic and economic point of view. The broad-sense heritability coefficients for diameter breast height (h^2 = 0.773), branch thickness (h^2 = 0.759), angle of insertion (h^2 = 0.775) and stem form (h^2 = 0.591) indicate a strong genetic control for these characters. The expected genetic gain by means of selection of the best 10 clones ranges between 16 to 36%, depending on the character considered. The study of genetic diversity, based on the izoenzymatic markers in provenances comparative trials of larch indicates a high level of intrapopulation genetic diversity, especially for populations located in the South of Eastern Carpathians. Regarding the interpopulation genetic diversity, the provenance Ceahlău is significantly different from other surveyed populations. The obtained results provide important information in terms of long-term breeding, conservation of forest genetic resources, and also for reconsideration of this species in forestation works.
Keywords: provenances, genetic diversity, phenotypic correlation, heritability, expected genetic gain

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Introduction

In Romania, the larch (*Larix decidua* Mill.) has a natural discontinuous range, being concentrated into 5 genetic centers: Ceahlău Mountains (1400-1450 m altitude), Ciucaş Mountains (1000-1450 m), Bucegi (1200-1500 m), Lotru Mountains (670-1900 m) and Apuseni Mountains (600-1200 meters).

In the Romanian Carpathians, as well as in the Alps, the larch is situated at the upper limit of vegetation, where it forms pure stands or mixed stands with spruce, Swiss stone pine or even with fir, beech, ash and birch. The total area of natural larch is approximately 4500 ha (Enescu 1975, Stănescu 1984), representing 0.3% of the national forest area.

Since the end of the eighteenth century, the larch has been expanded far beyond its natural area of vegetation. In 2007, the area of the artificial stands of larch was 12500 ha (National Institute of Statistics, 2008), with 3500 ha less than during the years 1960-1970 (Haralamb 1963). In the most old plantations, the origin of the seeds is Austrian, from low altitudes in the Alps Mountains, or of unknown origin.

Ecologically, Romanian larch is a high-altitude race, with high light requirements, but less heliophile than the larch of Sudeti. Generally, natural larch grows on skeletal soils, on limestone and conglomerates (Stănescu 1984).

The speed of growth, high productivity, high quality wood and resistance to adversity led to the initiation of research on larch genetic variability. Between 1978 and 1982, through international collaboration, there were 2 series of comparative trials with Romanian and foreign larch provenances installed, totaling 6 experimental areas. The selection of seed stands and over 300 plus trees followed then. There were installed 26 orchards (134 ha) of which 3 (15 ha) are interspecific hybridization (*Larix x eurolepis*) seed orchards. A broad genetic base for future research was thus created.

This paper presents the results obtained over the last two years regarding the continuing larch breeding program: genetic variation of the main traits in multi-location provenances comparative trials settled in 1982, evaluation of the genetic parameters in a clone seed orchard in order to advance to the second generation seed orchards, and studying genetic diversity by means of primary biochemical markers.

Materials and methods

Study materials consist of 24 larch provenances, 13 Romanian and 11 foreign, from the following countries: Germany (4), Austria (2), Belgium (1) and Czech Republic (4) (Table 1). The tested populations come from natural stands, artificial stands and larch seed orchards.

In this test series there were installed 3 provenances comparative trials, the experimental design was square grids: 5 x 5 trees, with 3 repetitions. Experimental trials have been installed outside the natural area of larch in our country, the specific conditions of each test being presented in Table 2.

The studied larch seed orchard was installed in 1983 within the Adâncata Forest District, Suceava Forest Direction, and consisted of 50 clones from 4 provenances: Fântânele, Naruja, Păltinoasa and Ceahlău.

To investigate the interpopulational genetic variability of the measured characteristics, a simple analysis of the variance was used, with two tests of significance (*F* test and the multiple t test). It was thus possible to separate total phenotypic variance into variation categories, according to their causes: genetic and environmental.

Evaluation of the genetic parameters broad sense heritability and expected genetic gain, among the Adâncata seed orchard, were performed using the formulas of Nanson (2004) and Falconer and Mackay (1996):

$$h^2 = \sigma^2 c / \sigma^2 c + \sigma^2 e$$

$$\Delta G = i\, h^2\, T_P$$

where: $\sigma^2 c$ - genotypic variance
σ^2_P - phenotypic variance
$\sigma^2 e$ - error variance
i - selection intensity

To reveal possible relationships between the studied characters and between them and the ecological gradients of the place of origin, simple correlation coefficients (Pearson) were cal-

Table 1 Provenances of *Larix decidua* in the experiment of 1982

Code	Provenance, Country	Lat.N	Long.E	Altit.
30	Harbker, Hanau 11, seed orchard, Germany	-	-	-
31	Hanau 11, seed orchard, Germany	-	-	-
32	Berkel seed orchard, Germany	-	-	-
33	Reichenau/Kärnten, Austria	46°51′	13°58′	1550
35	Wienerwald-Lammerau, seed orchard, Austria	-	-	-
45	Haldensleben Oberforsterei Bischofswald, Germany	52°20′	11°15′	105
47	Halle seed orchard, Belgium	-	-	-
50	Reghin, Romania – artificial stand	46°40′	24°50′	450
51	Mihăeşti, Furnicoşi seed orchard, Romania	-	-	-
52	Bacău, Hemeiuşi I seed orchard, Romania	-	-	-
53	Bacău, Hemeiuşi II seed orchard, Romania	-	-	-
54	Bicaz, Romania – artificial stand	46°49’	25°52’	1100
55	Bicaz, Romania – natural stand	46°57’	25°58’	1500
56	Baia de Criş, Romania – natural stand	46°25’	23°30’	1100
57	Comăneşti, Romania – artificial stand	46°15’	26°12’	710
58	Latoriţa, Romania – natural stand	45°12’	23°55’	1100
59	Sinaia, Romania – artificial stand	45°15’	25°30’	690
60	Sinaia, Romania – natural stand	45°20′	25°30′	1600
61	Sinaia, Romania – natural stand	45°10’	25°40’	1300
62	Braşov, Romania – artificial stand	45°30’	25°30’	670
63	Zilina-Hradok, Czech Republic	49°00’	19°56’	650
64	Poprad-Hranovnica, Czech Republic	48°59’	20°11’	900

Table 2 Experimental characteristics and site conditions of larch provenances comparative trials settled in 1982

No.	Forest Direction Forest District P.U., m.u.*	Lay-out	No.of provenances	Region of provenance**	Lat. N	Long. E	Altit. m	Altitudinal plant layer	Soil type
1.	Reşiţa Băuţar U.P. VIII, u.a. 1A	4 x 4 3 repet.	16	D230	$45^{0}29'$	$22^{0}40'$	650	mountain European beech stand	brown forest soil
2.	Arad Radna U.P. IV, u.a. 9A	4 x 4 3 repet.	16	E150	$46^{0}10'$	$21^{0}50'$	300	sessile oak stand	brown forest soil
3.	Suceava Adâncata U.P. VI, u.a. 45 C	4 x 4 3 repet.	16	G160	$47^{0}30'$	$26^{0}20'$	350	oak mixed stand	brown forest soil

* P.U.- production unit and m.u.- management unit of forest district
** - The codes of regions of provenances, edition 2006

culated. Data from observations and field measurements were processed and statistically interpreted using the program Excel and SPSS.

Electrophoresis procedures and designations of gene loci and alleles follow the method of Konnert (2004). The following 10 enzymes encoded for 20 loci were assayed (Enzymes Commission number and abbreviations in parentheses): glutamate-oxaloacetate transaminase (E.C.2.6.1.1, 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), shikimate dehydrogenase (E.C.1.1.1.25, SKDH).

Data were analyzed using POPGENE (Yeh 1997). For each provenance, percentage of polymorphic loci (*P*), the average number of alleles per locus (*A/L*), the effective number of alleles per locus (*ne*) (Crow & Kimura 1970), mean of observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated (Nei 1978). Genetic differentiation was estimated using Nei's genetic distances *D* (Nei 1972) and *F*-statistics (Nei 1978). The pattern of differentiation was highlighted by a UPGMA dendrogram based on Nei's genetic distance.

Results

Interpopulational genetic variability

The results of the variance analysis within the 3 provenance trials are shown in Table 3. For all the studied characters, the differences among provenances are very significant, revealing the existence of high genetic variability for the tested provenances.

Volume per tree.
According to the average value per experiment, the highest volumes per tree have been recorded in the Băuțar`s trial, situated at the mountain European beech layer, and the lowest volumes have been recorded in the Radna trial, situated at the oak stand layer. The range of the localities, according to the general average/experiment is the following: Băuțar (x = 245.52 dm^3), Adâncata (x = 204.88 dm^3) and Radna (x = 148.10 dm^3).

The highest variability of volume/tree for the tested provenances was recorded at the Băuțar`s trial, having a variation range between 311.16 dm^3, the provenance 32 - Berkel seed orchard (Germany), and 168.05 dm^3, the provenance 61 - Sinaia (natural stand). The least variation was recorded at the Adâncata trial (49.84 dm^3), where significant differences were not obtained at the provenance level for this characteristic.

The output of timber which may be obtained by selecting the most productive provenances greatly vary from one trial to another, having values between 52% at Radna and 22% at Adâncata. At the level of Romanian provenances this is 51% at Radna, 45% at Băuțar and 17% at Adâncata.

In the Băuțar`s trial (Fig. 1), there are 6 Romanian provenances in the top range of volume/tree: 51 - Furnicoşi (*Larix x eurolepis*) seed orchard, 53 - Hemeiuşi seed orchard, 55 - Bicaz (natural stand), 58 - Latorița, 56 - Baia de Criş, 54 - Bicaz (artificial stand), together with the following foreign provenances: 32 - Berkel seed orchard (Germany) and 63 - Zilina

Table 3 Variance analysis of some studied characters in the larch provenances comparative trials

Source of variation	D.F.	S^2						
		Volume/ tree	Branches diameter	Branches number	Angle of insertion	Stem straightness	Saber butt	Survival
Băuțar trial								
Provenances	15	46662.996***	0.438	1.215**	0.616*	2.031***	892.9***	373.582***
Repetitions	2	2308.551	0.352	0.152	0.652	0.315	82.2	424.502
Error	432	16698.322	0.287	0.445	0.309	0.422	81.1	38.564
Radna trial								
Provenances	15	17651.681***	0.708***	1.862***	1.356***	2.380***	319.5***	511.141***
Repetitions	2	30241.910	0.540	1.065	0.415	1.327	635.9	105.506
Error	432	4168.889	0.231	0.362	0.211	0.344	92.8	69.204
Adâncata trial								
Provenances	15	5566.457	-	1.228	0.622	1.621***	-	214.936*
Repetitions	2	14408.950	-	3.884	0.749	0.811	-	49.096
Error	432	5658.300	-	0.768	0.379	0.297	-	107.131

*p < 0.05; **p < 0.01; ***p < 0.001

Hradok (Czech Republic). The lowest wood production was seen in the following provenances: 61 - Sinaia (natural stand), 30, 31 - seed orchards from Germany, 57 - Comăneşti (artificial stand) and 62 - Braşov (artificial stand).

In the Radna trial (Fig. 1), the following Romanian provenances showing the best volumes were 51 - Furnicoşi seed orchard, 59 - Sinaia (artificial stand), 56 - Baia de Criş, 57 - Comăneşti and 55 - Bicaz (natural stand), along with 30, 31 - seed orchards (Germany) and 63, 65 - Czech Republic. The lower volume per tree results were obtained from the following provenances: 61 - Sinaia (natural stand) and 54 - Bicaz (artificial stand).

In the comparative trial at Adâncata significant differences were not obtained for average volume per tree and diameter at 1.30 m, but only for the total height. The following provenances had the highest values for the total height: 47 - Halle seed orchard (Belgium) and 31 - Hanau seed orchard (Germany). Except for provenance 59 - Sinaia (artificial stand) and 50 - Reghin (artificial stand), situated above average/experiment, the Romanian provenances were below average/experiment for height.

Some Romanian and foreign larch provenances show genetic superiority at all localities, including 51 - Furnicoşi seed orchard, 55 - Bicaz (1500 m), 56 - Baia de Criş (1000 m), 59 - Sinaia (690 m), 63 - Zilina Hradok (Czech Republic) and 32 - Berkel seed orchard (Germania).

Branch characteristics

Considerable genetic variation has been found at the level of provenance for branches thickness, number of branches and angle of insertion (Table 3). Among the Romanian provenances, the thickest branches are constantly formed by 53 - Hemeiuşi seed orchard, 51 - Furnicoşi seed orchard, 55 - Bicaz, 56 - Baia de Criş, and among foreign provenances: 64 and 63 - Czech Republic. The thinnest branches were produced by the following Romanian provenances: 61 - Sinaia (natural stand), 58 - Latoriţa (natural stand), 54 - Bicaz (artificial stand), 62 - Braşov (artificial stand).

Provenances can be grouped into several classes, based on the angle of insertion. At the present stand age, angles of insertion are less than or close to 90°. The smallest angles of insertion are generally shown by the provenances with the slowest height and diameter growth.

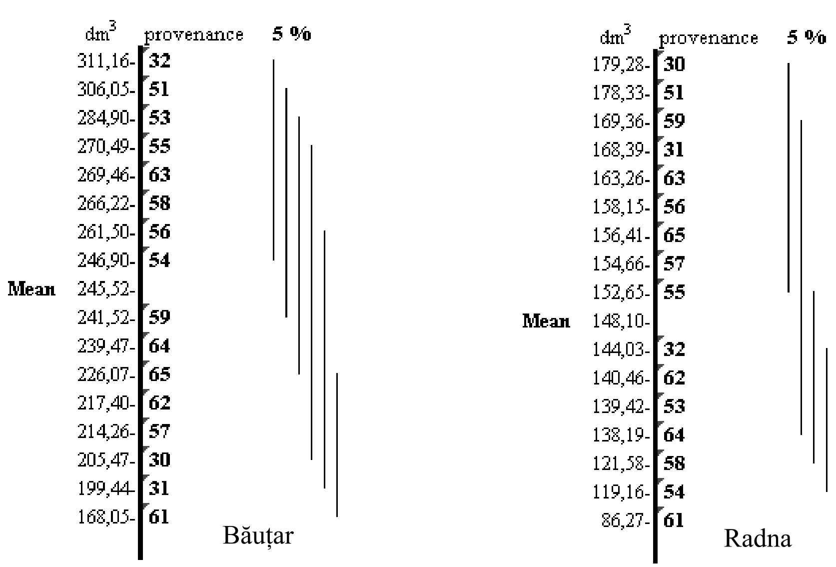

Figure 1 Variation of volume/tree in larch provenances comparative trials at 25 years old (multiple "t" test)

The following provenances produced the largest number of branches: 56 - Baia de Criş (Romania), 32 - Berkel seed orchard (Germany) and 64 - Czech Republic. The fewest branches were produced by the following provenances: 30 - seed orchard Germany, 58 - Latorița, 59 - Sinaia and 57 - Comăneşti.

Stem straightness

In all the comparative trials, the variance analysis revealed the existence of strong genetic control of stem straightness. The stem straightness was assessed by indices as follows: 1 - straight stem, 2 - minor defects, 3 - sinuous stem. Most defects were recorded in the Băuțar and Adâncata trials. Provenances showing the most stem defects, in all comparative trials, are the followings: 59 - Sinaia, 55 - Bicaz, 62 - Braşov, 56 - Baia de Criş (Romania) and 64, 65 - Czech Republic (Fig.2). The fewest defects are generally in: 32 - seed orchard (Germany), 61 - Sinaia, 57 - Comăneşti, 51 - Furnicoşi seed orchard.

Saber butt of the stem

The saber butt of the stem or the curvature at the stem basis was observed only in the trials at Băuțar and Radna. In the Adâncata trial situated on relatively level ground, this defect has not occurred. The highest percentages of trees with saber butt of the stem were recorded at Băuțar - 39.1% versus 6.95% at Radna.

Besides the influence of the location significant differences among provenances were seen in each trial for saber butt (Table 3). The greatest percent of trees with saber but was generally found in Romanian provenances: 56 - Baia de Criş, 55 - Bicaz, 59 - Sinaia, 62 - Braşov. Provenances from Czech Republic and Germany were about the average per experiment or below average. From the Romanian provenances, the lowest percentages of saber butt trees are recorded for: 61 - Sinaia (1300 m), 54 - Bicaz (1100 m) and 58 - Latorița (natural stand).

Survival

At the age of 25 years of planting, percent survival varies a lot between experimental fields, and between provenances as well. The best survival percentage was recorded at Adâncata (60.94%) and the lowest at Radna (34.62%) (Fig. 3).

Provenances with the best survival in all trials are the following: 62 - Braşov, 54 - Bicaz, 59 - Sinaia, 57 - Comăneşti, 53 - Hemeiuşi, 58 - Latorița, 63 - Czech Republic. The lowest survival in all localities was found for provenances: 61 - Sinaia, 32 - seed orchard Ger-

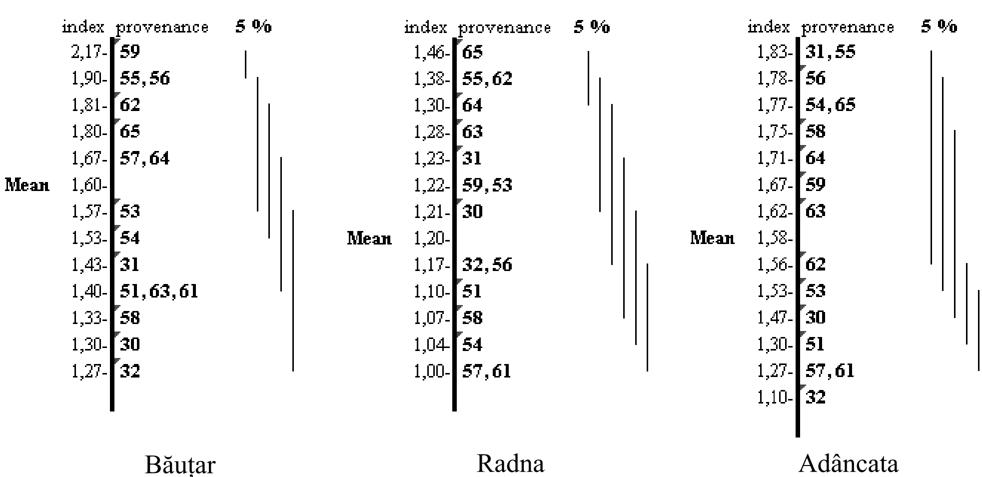

Figure 2 Variation of stem straightness in larch provenances comparative trials at 25 years old (multiple "t" test)

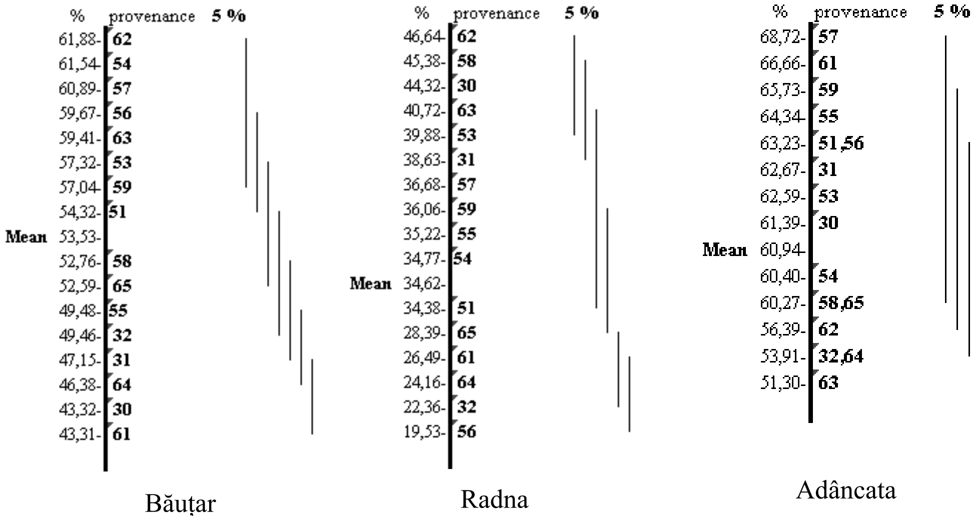

Figure 3 Variation of survival in larch provenances comparative trials at 25 years old (multiple “t" test)

many, 64 - Czech Republic.

In all comparative trials there are provenances that have both high survival and high wood production: 54 - Bicaz, 56 - Baia de Criş, 53 - Hemeiuşi, 51 - Furnicoşi, 63 - Zilina Hradok at Băuțar and 30 - Hanau, 63 - Zilina Hradok, 57 - Comăneşti, 59 - Sinaia, 55 - Bicaz.

Phenotypic correlations between the measured characteristics and between these and the origin ecological gradients

Data analysis showed the evidence of significant correlations of theoretical and practical interest between the studied characters. In all three provenance comparative trials there were positive significant correlations between diameter, volume and branches diameter (Table 4). No significant correlations were obtained for the stem straightness and saber butt. Few correlations were observed between traits measured and geographic origin gradients (Table 5). Only at Radna were significant negative correlations obtained between height and altitude of origin. Correlations between branch diameter and the latitude and longitude of origin were obtained at Băuțar, and between the insertion angle and altitude, only at Adâncata. Regarding survival, a significant correlation was obtained with latitude and a significant correlation between saber butt and the longitude was also noted. The lack of correlation with ecological gradients may be explained by the fact that many artificial larch provenances of unknown origin are in these trials.

Estimating genetic parameters and expected genetic gain

Broad sense heritability coefficients for the studied characters were calculated in a larch seed orchard at Adâncata (Table 6). They allow an assessment of the possible success when applying selection at the clone level. The highest values of heritability were obtained for: diameter at 1.30 m (h^2 = 0.773), branches thickness (h^2 = 0.759), branches length (h^2 = 0.792) and insertion angle (h^2= 0.775), which indicates high genetic control for these characters. The lowest values of heritability were obtained for branches number and for stem straightness. These results are comparable to those obtained by other authors regarding larch: h^2 = 0.69 (Paque 1992) and h^2 = 0.31 (Kowalczyk 2002) for total height, h^2 = 0.08 for stem straightness (Kowalczyk 2002).

The genetic gain expected by selecting the best 10 clones is 24% for diameter at 1.30 m

Table 4 Matrix of phenotypic correlations among studied characters in the Radna provenances comparative trial

Character	Volume/ tree	Diameter at 1.30 m	Branches diameter	Stem straightness	Survival	Saber butt
Total height	0.850**	0.784**	0.636**	0.142	-0.029	0.134
Volume / tree	-	0.969**	0.779**	0.169	0.182	0.163
Diameter at 1.30m	-	-	0.815**	0.251	0.075	0.204
Branches diameter	-	-	-	0.137	0.054	0.034
Stem straightness	-	-	-	-	0.124	0.155
Survival	-	-	-	-	-	0.177

*p < 0.05; **p < 0.01; ***p < 0.001

Table 5 Matrix of phenotypic correlations among studied characters and geographic gradients in the provenances comparative trials

Geographic gradients	Trial	Total height	Diameter at 1.30 m	Branches diameter	Branches number	Angle of insertion	Stem straightness	Survival
Latitude N	Adâncata	0.212	0.160	-0.151	-0.477	0.141	-0.313	-0.856**
	Băuțar	-0.103	0.241	0.606*	0.459	0.519	-0.099	-0.083
	Radna	0.445	0.416	0.469	-0.059	0.259	0.419	-0.287
Longitude E	Adâncata	0.025	0.002	0.145	0.553*	-0.088	-0.345	-0.298
	Băuțar	-0.057	-0.227	-0.664*	-0.373	-0.379	0.214	0.182
	Radna	-0.500	-0.211	-0.282	0.152	-0.493	-0.232	0.240
Altitude	Adâncata	-0.058	-0.515	-0.589*	0.329	-0.582*	0.166	-0.298
	Băuțar	0.156	0.011	-0.232	0.183	-0.311	-0.195	-0.562
	Radna	-0.557*	-0.501	-0.315	0.463	-0.374	0.081	-0.236

*p < 0.05; **p < 0.01; ***p < 0.001

Table 6 Variance components, phenotypic standard deviation (σ_{Ph}), broad - sense heritability (h^2) and expected genetic gain (ΔG), in the Adâncata larch seed orchard

Character	σ^2_C	$\sigma^2 e$	σ^2_P	σ_P	h^2	Δ G (%)	
						10 clone	15 clone
Diameter at 1.30m	29.009	8.543	37.552	6.128	0.773	24	18
Branches diameter	1.599	0.508	2.107	1.452	0.759	25	19
Branches length	1.050	0.276	1.326	1.152	0.792	24	18
Branches number	0.850	0.547	1.397	1.182	0.609	22	17
Angle of insertion	0.540	0.157	0.697	0.835	0.775	36	27
Stem straightness	0.186	0.129	0.315	0.561	0.591	31	23
Saber butt	59.119	59.119	118.238	10.874	0.500	16	12

and 31% for stem straightness. This means that clone selection can be successfully applied, being efficient both from a genetic and an economic point of view.

Evaluation of genetic diversity

Isoenzymatic analysis of 20 loci in 5 Romanian provenances of *Larix decidua* indicated the presence of 37 alleles. A maximum number of alleles per locus was found for PGM-B (5 alleles). Some loci were monomorphic for all investigated provenances. The proportion of polymorphic loci ranged from 40 % (58 - Latorița and 56 - Baia de Criş) to 80 % (55 - Bicaz) with a mean value 53.30% (Table 7).

The mean number of alleles per locus range from 1.53 in 56 - Baia de Criş to 2.33 in 55 - Bicaz. Across all provenances the mean number of alleles per locus was 1.82.

The heteozygosity level varied considerably among provenances. The lowest level of heteozygosity was observed in provenance 56 - Baia de Criş from western part of Carpathian Mountains (0.088) and the highest heterozygosity was found in provenance 60 - Sinaia 2 (0.161). The mean value of observed heterozygosity (H_o) was 0.119 for all provenances.

Genetic differentation between provenances was analyzed with the help of Wrigth's *F*-statistics and Nei's genetic distances coefficient. On the basis of most polymorphic loci studied we quantified differentiation by describing the correlation between uniting gametes among provenances (F_{IS}), between provenances (F_{ST}) and for the species as a whole (F_{IT}) (Table 8).

Values of F_{IS} ranged between 0.016 (for MDH-C, GOT-A, GOT-B and LAP-A) and 0.453 (6-PGDH-B), with a mean of 0.079, which indicates a 7.9 % deficiency of heterozygotes. Also, the positive F_{IT} mean value (F_{IT}= 0.170) indicates a deficiency of observed heterozygotes in relation to the Hardy-Weinberg ratio. The average F_{ST} value was equal to 0.098 which means that 9.8% of the total variation is between populations.

Genetic differentiation among provenances is great. A dendrogram constructed using the weighted pair group method of cluster analysis (UPGMA) shows the degree of similarity of provenances studied (Fig. 4).

Discussion

The results show the existence of large genetic variability among the tested provenances. Across all the tests, the following provenances have the greatest capacity for volume growth at the age of 25 years of planting: 51 - Furnicoşi seed orchard, 55 - Bicaz (1500 m, natural stand), 56 - Baia de Criş (1000 m, natural stand), 59 - Sinaia (690 m artificial stand), 63 - Zilina Hradok (Czech Republic) and 32 - Berkel seed orchard (Germany). Although few significant correlations were found between the studied characters, there are provenances showing both high growth capacity and a higher stem quality (thin branches, straight stem). Across all plantings, these are: 32 - seed orchard Germany, 61 - Sinaia, 57 - Comăneşti, 51 - Furnicoşi seed orchard, 53 - Hemeiuşi seed orchard, 58 - Latorița, 54 - Bicaz.

Although, in general, fast growing prove-

Table 7 Genetic diversity in Romanian *Larix decidua* provenances

No/Cod	Provenances	A/L	n_e	P(%)	H_o	H_e
1/56	Baia de Criş	1.53	1.23	40.00	0.088	0.116
2/58	Latorița	1.66	1.27	40.00	0.098	0.136
3/61	Sinaia	1.67	1.23	46.67	0.121	0.121
4/55	Bicaz	2.33	1.34	80.00	0.131	0.155
5/60	Sinaia 2	1.93	1.31	60.00	0.161	0.142
	Mean	**1.82**	**1.27**	**53.33**	**0.119**	**0.134**

A/L-number of alleles per locus; n_e-effectiv number of alleles per locus; *P*- percentage of polymorphic loci ($p<0.05$); H_o- observed heterozygosity; *He*-expected heterozygosity.

Table 8 Wright F-statistics for Romanian *Larix decidua* provenances

Locus	F_{IS}	F_{IT}	F_{ST}
SKDH	0.242	0.397	0.205
6-PGDH-A	-0.022	-0.017	0.005
6-PGDH-B	0.453	0.457	0.007
PGM-A	0.123	0.199	0.087
PGM-B	-0.113	-0.021	0.083
MDH-A	-0.086	-0.039	0.044
MDH-C	-0.016	-0.003	0.013
MDH-D	0.591	0.613	0.053
GOT-A	-0.016	-0.003	0.013
GOT-B	-0.016	-0.003	0.013
GOT-C	-0.342	-0.338	0.003
LAP-A	-0.016	-0.003	0.013
LAP-B	-0.028	-0.016	0.011
Mean	**0.079**	**0.170**	**0.098**

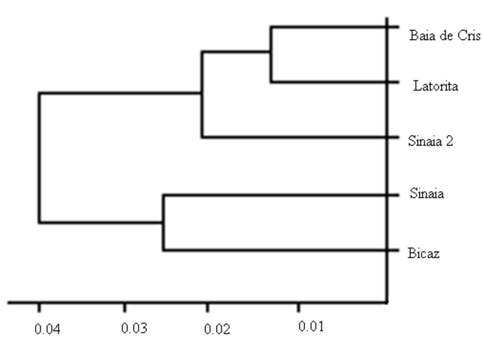

Figure 4 Dendrogram based on Nei's genetic distances

nances presented the highest mortality, in each trial there are provenances that have both high survival and high volume production. Most valuable in this regard are the following provenances: 51 - Furnicoşi, 53 - Hemeiuşi, 55 - Bicaz (natural stand), 63 - Zilina Hradok (Czech Republic).

Seedlings from the two larch seed orchards, Furnicoşi-Mihăeşti and Hemeiuşi-Bacău and from the provenance 55 - Bicaz (Ceahlău Mountains), showed outstanding genetic superiority over the other natural and artificial larch populations from Romania. These seed orchards were established in the years 1965 - 1968, based on phenotypic selection of the most valuable trees (plus) from the most valuable natural and artificial stands of larch in Romania. They probably combine the rapid growth of the Austrian provenances, introduced at the end of the eighteenth century and the better quality of the stem of the Romanian provenances. Also, the results of the izoenzymatic analysis confirm the hypothesis that the larch of the Ceahlău Mountains has a different origin from the other Romanian tested populations, probably from Poland.

Among foreign provenances, seed orchards from Germany and source 63 - Zilina Hradok from the Czech Republic have shown good results.

The absence of the significant correlations between the studied characters and the geographic gradients of source origins suggests little clinal variation. Only for branches thickness is there a positive correlation with latitude, and negative correlation with longitude and altitude. The lack of a correlation is probably due to the fact that these experimental trials tested many artificial provenances of unknown origin.

There is also significant variation from one trial to another suggesting that site conditions have a large influence on phenotypic expression of the studied characters. Differences between the best performing and the poorest performing Romanian provenances range between 51% at Radna and 17% at Adâncata for volume per tree. This suggests that important genetic gains can be obtained in timber production by selecting the most productive provenances.

These three provenances trials were planted outside the native larch area in Romania, thus the provenances that are superior for growth, stem straightness and survival can be considered valuable sources for forest reproductive material for Romania. According to national and international regulations regarding forest reproductive material, the most valuable provenances are designated tested seed sources for the regions where the comparative trials were planted.

The broad sense heritability assessed indicate a high genetic control for growth characters and for branches characters. The expected genetic gain from selecting the best 10 clones is 24% for the diameter at 1.30 m and 31% for the stem straightness. The results of this study are much better, in comparison to a larch clone test in France, where gains of 11% and 8%, respectively, were obtained for the same characters (Paque 1992).

The results are in accord with provenance research of other countries (Giertych 1979, Lines 1967, Pâques 1992, 1996, Weiser 1992). Levels of genetic diversity and differentiations observed for Romanian's provenances of *Larix decidua* are relatively similar with other studies reported in *Larix* sp. (Lewandowsky & Mejnartowicz 1991, Muller-Starck et al. 1992, Jaquish & El-Kassaby 1998, Semerikov & Lascoux 1999, Lewandowski & Burczyc 2000). The approximately 91% of observed genetic variation among populations is greater than the values obtained in a study of European populations of larch by Lewandowsky & Mejnartowicz, (1991). Also, genetic distance values found in our study are greater but comparable to values reported in other studies. Differences between 61 - Sinaia and 62 - Sinaia2 show an anthropogenic influence.

Conclusions

The results of this study indicate the existence of valuable genetic centers of larch in Romania. Provenances 55 - Bicaz (Ceahlău Mountains), 58 - Latorița (Lotrului Mountains) and 56 - Baia de Criș (Apuseni Mountains) show superior growth performance and stem quality. Also, the seeds from the larch seed orchards appear the outstanding superiority compared with those from natural and artificial larch populations.

The genetic variability, highlighted in this study, expresses the adaptive strategy of this species, being characteristic for the species with a fragmented native area. The most valuable provenances in terms of timber production stem quality and survival, will be designated tested seed sources for the provenance regions where the trials are situated.

The results provide important information for long-term breeding, preserving of forest genetic resources, and also for further consideration of this species in reforestation works.

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