



Genetic variation and early selection in *Larix decidua* Mill. from progeny test in Romania

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Abstract

• **Key message** High genetic variation and determinism for quantitative traits of *Larix decidua* Mill. were found in a progeny test established at its south-eastern limit of distribution area. The top range families are from both native populations and artificial stands of unknown origin. Recurrent selection at the nursery stage based on total height can reduce the generation time and increase the genetic gain.

• **Context** Despite the economic and ecological importance of *Larix decidua* in Europe, little information is available regarding its genetic variation and genetic parameters, particularly in the south-eastern part of its distribution area.

• **Aims** The objectives of this study were to investigate genetic variation, to estimate genetic parameters and to predict genetic gain in three alternative selection strategies for quantitative traits of *L. decidua* in a progeny experiment at the nursery stage.

• **Methods** Genetic variation of growth, branch traits and bud flushing were assessed in an open-pollinated progenies from 56 plus trees of *L. decidua*. Plus trees were selected from two natural populations (native to the Romanian Carpathians) and four artificial stands of unknown origin (most likely from the Austrian Alps). Analysis of variance was performed at three levels: provenance region, family and within family. The variance components, coefficients of heritability, genetic and phenotypic correlations, and genetic gain were also determined.

• **Results** Large genetic variation in quantitative traits was found among *L. decidua* open-pollination families. Results highlight high genetic control for growth traits and bud flushing and support the premise that the strategy based on recurrent selection will bring the greatest genetic gain in the next breeding generation.

• **Conclusion** Results complete the knowledge concerning genetic parameters of some traits important for *L. decidua* breeding programmes in Europe. Further analysis is necessary in order to determine the origin of the genitors involved in the next breeding cycle, given that the best families come from both natural populations and artificial stands, which are very most likely from the Tyrolean Alps.

Keywords *Larix decidua* · Quantitative traits · Genetic variation · Heritability · Genetic correlations · Genetic gain · Early selection · European larch

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Contributions of the co-authors Conceived and designed the experiment: GM; performed the experiments: GM, IM; acquisition and analysis of the data: GM, AA, IM; wrote the paper: GM

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1 Introduction

European larch (*Larix decidua* Mill.) is an ecologically and economically important forest tree species in Europe due to its fast-growing capacity, high adaptability and durable wood. Similar to the rest of Europe, the distribution area of European larch in Romania is discontinuous and more reduced compared with other coniferous species. It covers only 0.3% of the forest area, most being represented by artificial stands (NFI 2018). The natural distribution area of the European larch is concentrated in Ceahlau, Ciucas, Bucegi, Lotru and Apuseni Mountains. Romanian larch belongs to the Carpathian subspecies (*L. decidua* Mill. subsp. *carpatica*

Sim.) and is an ecotype of altitude, growing at over 1,200 m a.s.l. with Swiss stone pine (*Pinus cembra* L.) and Norway spruce (*Picea abies* L. Karst). Only in the Apuseni Mountains does it descend between 600 and 1,200 m. Some authors considered that Romanian larch belongs to subspecies *polonica*; however, it differs to that subspecies due to its mountainous and subalpine habitat, stem straightness, branches of average thickness and resistance to European larch canker (*Lachnellula willkommii*) (Siman 1943; Svoboda 1953; Dumitriu–Tataranu et al. 1970).

In the middle of the nineteenth century, the European larch from the South-Eastern Carpathians were affected by an intense seed transfer from other areas, particularly from the Tyrolean Alps (Rubtov 1965; Raffl et al. 2018). Many plantations of unknown origin established in Transylvania, Bucegi Mountains and Eastern Carpathians, are assumed to be from Alpine seed sources.

In Romania, European larch represents an important forest tree species and therefore its genetic improvement programme began in the 1960s. The main objectives of the larch breeding programme were to increase yield and quality of wood and improve resistance to biotic and environmental factors. Selection of seed stands and plus trees according to growth, stem straightness and crown habitus, and establishing of seed orchards were the first steps in the breeding programme. Over 500 plus trees were selected in natural and artificial stands. Twenty-six seed orchards (134 ha) were installed (Enescu 1972) and are currently the main sources for producing genetically improved seeds in Romania.

Long-term tree breeding involves repeated cycles of recombination and selection (Danusevicius and Lindgren 2001). Although, in Romania, the first cycle of the larch breeding programme was initiated 55 years ago, this programme has yet to be completed. Candidates for the next breeding population have to be tested in progeny trials. Information regarding the genetic parameters and pattern of variation of the traits for which selection will be made will be used in determining the optimal breeding strategy.

The assessment has focused on growth traits, branch characters and bud flushing, all of which are among the most important traits in breeding programmes. Growth traits and branch characters influence the quality and value of timber. The phenology of bud flushing is fundamental for survival and growth of the forest species. Thus, autochthonous Romanian larch begins its growing season very early and late spring frosts often cause injuries to young shoots if the forest reproductive material is poorly adapted to the climate of the planting site.

At the European level, larch breeding programmes were initiated in nearly all countries but with different degrees of intensity (Pâques 2009). Results regarding the geographic variability in both IUFRO and national experiments were the subject of numerous publications but the most comprehensive

syntheses for international experiments where those of Giertych (1979), Schober (1985), Weisgerber and Sindelar (1992). Results demonstrated that there is high genetic variability between European larch populations and broad adaptability of central European provenances. The Sudetan populations obtained the highest growth in almost all testing sites, good adaptability and resistance to canker but poor stem form (Giertych 1979; Schober 1981; Lacaze and Birot 1974; Pâques 1996; Rozkowski 2000). The provenances from the south-eastern Alps had moderate growth but excellent stem form. The central Poland and Alpine populations should be preferred for high wood density compared to Sudetan ones (Pâques and Rozenberg 1995). The populations from the inner Alps had a low adaptation and should not be used beyond their local conditions (Kulej 1995; Chmura and Rozkowski 2002). However, there are some contradictory results; for example, Kulej (2002) reported high heterogeneity for the Sudetan populations and high variation within a single forest district for populations from central Poland. Based on 7 allozyme loci, Maier (1992) found that the most homogeneous populations regarding the larch canker susceptibility and growth rate are those from Eastern Europe (Poland, Sudetan region and Tatra Mountains). Also, substantial differences in growth rate between western and eastern Alpine provenances have been pointed out.

In Romania, the results from three provenance trials established in 1978 showed the best growth performances at 30-year-old recorded provenances Hinterburg (East Tyrol), Kreith, Sudtirol, Obsteig (South Tyrol) and native populations Sinaia and Voineasa. Provenances Hinterburg and Sudtirol highlighted also good adaptability (Mihai 2009).

However, despite the economic importance of European larch, information of genetic parameters of the traits are available only for some populations of *sudetica* and *polonica* varieties (Gracan 1973; Dietze 1974; Sindelar 1992; Weiser 1992; Frydl and Sindelar 2002; Kowalczyk 2002; Pâques and Jacques 2002) and very few for alpine (Hering et al. 1989) and Romanian Carpathians larch (Mihai and Teodosiu 2009). The results demonstrated that there were large differences in growth traits, wood quality and adaptability among progenies of the same provenances.

The objectives of this study were (1) to investigate genetic variation in growth, branch characters and bud flushing in a European larch nursery test in Romania; (2) to estimate variance components and heritability; (3) to evaluate the genetic and phenotypic correlations; and (4) to estimate the genetic gains.

Additionally, this study aims to provide data for early selection from the first-generation progeny in order to select the elite parents and to establish the second generation of seed orchards.

This information will complete the knowledge concerning inheritance of some traits of European larch and will be useful

for those interested in breeding of this species in Europe. Also, such information will be necessary to formulate optimum strategies for this species and to conserve the valuable populations (both natural and artificial), from which the plus trees come from, as forest genetic resources for European larch in Romania.

2 Materials and methods

2.1 Genetic material and mating design

In 2009, there was a good fructification in European larch seed orchards in Romania. To evaluate the breeding value of plus trees from first-generation seed orchards and to produce progeny for field tests, open-pollinated (OP) seeds from 56 clones were collected from two seed orchards. These seed orchards are situated in the Ploiesti and Horezu forest districts, in the oak and beech layer, respectively. The seed orchards are very well isolated and are composed of vegetative copies of 78 plus trees selected from five provenance regions (Parnuta et al. 2010).

To maintain high genetic diversity in future generations, clones involved in open-pollinated families come from all five provenance regions of plus trees (Fig. 1). Plus trees were selected in the 1960s from two natural and four artificial stands. The natural stands are located at high altitude (in Sinaia and Voineasa forest districts (Bucegi Mountains and Lotru Mountains)) and are native to the Romanian Carpathians. The artificial stands are located at lower altitude in Anina, Brasov, Sacele and Sinaia forest districts and are of

unknown origin. The geographic coordinates of the plus trees tested in this experiment are presented in Table 1.

2.2 Nursery design and assessments

Open-pollinated seeds were sown in nursery beds for germination in spring 2010. At the age of 2 years, the seedlings were planted in polyethylene bags filled with humus in a randomised complete block design with three replications in a single experiment, in the Sinaia nursery. The nursery is situated in the mountain beech zone, at 45° 29' N latitude, 25° 59' E longitude and at 695 m a.s.l. In each OP family, ten seedlings/plot in each of the three repetitions were evaluated.

Traits evaluated included the following: total height at the ages of 2, 3, and 4 (HT2, HT3, HT4) in centimetres; current height increment at ages 3 and 4 (h3, h4) in centimetres; root collar diameter at the ages of 3 and 4 (DRC3, DRC4) in centimetres; branch length at the ages of 3 and 4 (LB3, LB4) in centimetres; number of branches at the ages of 3 and 4 (NRB3, NRB4); and bud flushing (BB).

The assessment of bud flushing (BB) was made in the spring of 2013. The bud flush survey involved recording only the terminal bud because the lateral buds could be at a different stage, using 4 index, starting from 1 = unbroken bud to 4 = needles completely open and fully elongated (Ducci et al. 2012). This simplified interpretation of the data by ranking the family average to a scale of 1 = late to 4 = early. The observations were made in a single day and only once, on 25 April. The primary dataset is available at <https://doi.org/10.5281/zenodo.2633792> (Mihai et al. 2019).

For the progeny test, air temperature data were recorded using a logger installed at the nursery. Also, the geographic

Fig. 1 Location of European larch plus trees and nursery experiment

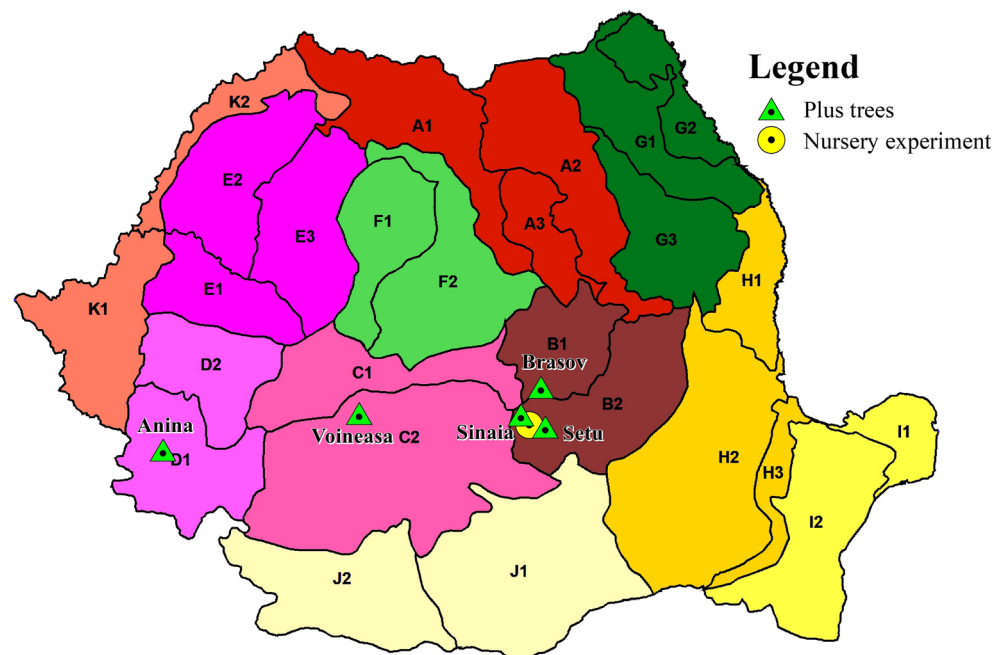


Table 1 Location of plus trees tested in progeny experiment. Provenance region by Pamuta et al. (2010)

Nr. crt	Clone number	Altitude (m)	Lat. N	Long. E	Forest district	Provenance region	Type of stand
1	1–9, 23	1290–1475	45°21′	23°53′	Voineasa	C2	Natural
2	13–22	700	45°10′	22°20′	Anina	D1	Plantation
3	10–22, 33–48, 56	650–730	45°66′	25°30′	Brasov, Sacele	B1	Plantation
4	24, 27, 28, 32, 49–55	1050–1220	45°33′	25°56′	Sinaia	B2	Natural
5	25–26, 29–31	875	45°16′	25°25′	Sinaia	B2	Plantation

parameters of the parents' origin were determined in a GIS system (LAT = latitude; LONG = longitude; ALT = elevation, in metres a.s.l.).

2.3 Statistical analysis

The statistical analysis was based on individual seedling measurements using the following mathematical model (Nanson 2004—Eq. 1):

$$Y_{kijl} = \mu + P_k + R_i + F_j + FR_{ji} + e_{ijkl} \quad (1)$$

where Y_{kijl} = performance of the l^{th} seedling in the k^{th} provenance region, the j^{th} family and the i^{th} repetition; μ = overall mean; P_k = effect of the k^{th} provenance region; R_i = effect of the i^{th} repetition; F_j = effect of the j^{th} family; FR_{ji} = interaction of the j^{th} family and i^{th} repetition; and e_{ijkl} = random error associated with $kijl^{\text{th}}$ seedlings.

Data were first examined for the assumption of homogeneity of variance using the SPSS programme (version 19). We assessed homogeneity visually using plots of residuals vs. predicted values. The additional assumption that experimental errors are normally distributed was not required because it does not influence estimates of variance components (Hallauer and Miranda 1981). Analyses of variance were performed for each trait using GLM procedure (SPSS version 19). The total amount of variation was divided into provenance regions, families, repetitions components and interaction between them. All effects were considered to be random except for the provenance region, which was considered to be fixed. The expected components of variance were performed using the R Core Team (Version 3.5.1, 2018). For the estimates of the genetic parameters to be valid, parents must be a random sample from a random mating population. The open-pollinated families used in this experiment were generated in two seed orchards composed of clones from different regions and most likely of different origins. Studies carried out in both populations and seed orchards of European larch demonstrate that the outcrossing rate is relatively low, indicating that self-fertilization is an important component of its mating system and there is a little inbreeding other than selfing (Kosinski 1986; Lewandowski and Burczyk 2000; Slobodnik 2002). Paule and Gomory (1992) found that the multilocus estimate of outcrossing rate in a seed orchard was $tm = 0.852$, while

Lewandowski and Burczyk (2000) found $tm = 0.675$ in a natural population. When open-pollinated families are considered true half-sib families, $\sigma^2_F = 1/4$ of σ^2_A . We considered OP families as not true half-sib families and, consequently, a coefficient of 2.5 instead of 4 was used, which reflects possible 30% selfing in open-pollinated families (Squillace 1974). This calculation avoids overestimation of the variance components and the genetic parameters (Eq. 2):

$$\sigma^2_A = 2.5\sigma^2_F \quad (2)$$

where σ^2_A = additive genetic variance and σ^2_F = family variance.

The narrow-sense individual (h^2_i) and half-sib family heritabilities (h^2_{HS}) were calculated by the following formulas (Nanson 2004—Eqs. 3 and 4):

$$h^2_i = \sigma^2_A / \sigma^2_{Ph1} = 2.5\sigma^2_F / (\sigma^2_F + \sigma^2_{FB} + \sigma^2_e) \quad (3)$$

$$h^2_{HS} = \sigma^2_F / \sigma^2_{Ph2} = \sigma^2_F / (\sigma^2_F + \sigma^2_{FB}/r + \sigma^2_w/rn) \quad (4)$$

where σ^2_{Ph1} and σ^2_{Ph2} = phenotypic variances; σ^2_A = additive genetic variance; σ^2_F = family variance; σ^2_{FB} = family \times repetition interaction variance; $\sigma^2_e = \sigma^2_p + \sigma^2_w / n$; σ^2_p = variance among plots; σ^2_w = within-plot variance; n = number of seedlings per plot; and r = number of repetitions.

Standard errors for heritability were estimated according to Delta's method (Lynch and Walsh 1998).

The genetic gains were calculated both at the family and individual levels for different breeding strategies (Falconer and Mackay 1996). Genetic gains from mass selection ΔG_1 (forward selection) and from family selection ΔG_2 (backward selection) were estimated as

$$\Delta G_1 = ih^2_i \sigma_{Ph1} \quad (5)$$

$$\Delta G_2 = ih^2_{HS} \sigma_{Ph2} \quad (6)$$

If the parents of the best OP families will be mated, estimation of the genetic gain was obtained by doubling i :

$$\Delta G_3 = 2ih^2_{HS} \sigma_{Ph2} \quad (7)$$

Selection intensity i was 1.953 and 1.671 for half-sib family selection (select top 5% and 10% families) and 1.369 and 0.929 for individual selection (select top 5% and 10% individuals within each half-sib family) (Becker 1967).

To compare the level of additive genetic variance in each trait independent of their means, the additive genetic coefficient of variation (CVA, %) was calculated following Cornelius (1994):

$$\text{CVA} = (\sigma_A/\mu) 100 \quad (8)$$

Genetic correlations were calculated from the additive genetic covariance of two traits, X and Y, in the numerator and the square root of the product of their additive genetic variances in the denominator (Falconer and Mackay 1996).

Pearson's correlations based on family means were also calculated to examine phenotypic correlations and relationships between traits and geographic coordinates of the parents' origin (LAT = latitude; LONG = longitude; ALT = elevation, in metres a.s.l.).

3 Results

3.1 Genetic variation and variance components

The plots of residuals vs. predicted values are presented in Fig. 3 (Annex) and it can be observed that the dots are spread out fairly evenly across the line (mean on experiment), indicating that the assumption of homogeneity of the variances is valid.

The analysis of variance for growth traits, branch characters, and bud flushing is presented in Table 2. Highly significant differences among OP families were found for all studied traits ($p < 0.001$). The differences among regions of provenances were also significant. The families which exhibited the best growth traits at age 4 years were from B2 provenance region both from natural and artificial stands, while the poorly performing families were from B1 and D1 provenance regions (Fig. 2). Differences from the mean on experiment of total height at the age of 4 for open-pollinated families of European larch are presented in Fig. 4 (Annex). The top range families were from natural (families 50, 4, 49) and artificial stands too (families 46, 56, 31, 21), located in Bucegi Mountains, Lotru Mountains and Brasov Depression. From the total of 20 OP families belonging to natural populations, 12 were situated above the mean on experiment and eight under the mean. From families of unknown origin, the best growth performances obtained those from Sinaia forest district (lower altitude in Bucegi Mountains). The weakest growth performances were obtained for family 5 from the Lotru Mountains and some families from Brasov Depression.

Differences among OP families regarding bud flushing were also highly significant. Bud flushing evaluation was performed on 25 April, when 81% of seedlings had their buds in

the stages 3 and 4, and only 3% of them were unbroken. At this time, air temperature was already higher than 10 °C for eight consecutive days. The earliest families were from B1 (Brasov Forest District) and D1 provenance regions (Anina Forest District), with all buds burst and 34% of them in stage 4 (average index 3.21 and 3.15, respectively). The latest families were from the C2 provenance region (Voineasa Forest District), which had 10% of buds still unbroken at the evaluation time. The next tardive families were from the B2 provenance region, Sinaia Forest District—natural stand.

Variance component estimates are listed in Table 3. The results indicated that the contribution of additive genetic variance (σ^2_A) to the total phenotypic variance (σ^2_{Ph}) varied between 4% for NRB4 and 87% for HT3. Among quantitative traits, total height had the largest values of additive genetic variance, while the smallest values were obtained for number of branches. The additive genetic variance was greater for height than for root collar diameter of seedlings in all measurement years, ranging from 53 to 87% for height and 63–66% for diameter. An increasing trend with age of the additive genetic variance was observed for root collar diameter, current height increment and branch length.

The level of additive genetic variance of each trait was revealed by CVAs (Table 3). The CVAs ranged from 7 to 25%, depending on the analysed trait. Current height increment had higher CVA at this age while NRB3 and NRB4 presented the weakest values. The phenotypic coefficients of variation were also calculated. The CVs were higher than CVAs for all traits and displayed different variation among traits compared with CVAs. Thus, the most variable traits according to CVs values were h3, h4, NRB3, NRB4, LB3 and LB4. Both CVAs and CVs displayed an increasing trend with age for all traits.

3.2 Heritability

The values of the heritability coefficients are listed in Table 3. Narrow-sense individual heritability estimates ranged from 0.04 for NRB4 to 0.87 for HT3, while the family heritability ranged from 0.20 for NRB4 to 0.91 for HT4. The highest values of heritabilities were obtained for total height (between 0.53–0.87 for h^2_i and 0.78–0.91 for h^2_{HS}) and the weakest values for number of branches. Bud flushing exhibited high heritability values that indicate strong genetic control.

3.3 Genetic and phenotypic correlations

Genetic and phenotypic correlations between analysed traits are shown in Table 4. High additive genetic correlations were obtained between growth traits. Branch traits were correlated

Table 2 Analysis of variance for quantitative traits of European larch

Source of variation	DF	Variance (s ²)					
		HT2	HT3	HT4	DRC3	DRC4	h3
Region of provenance (P)	4	235.185***	1148.209***	3548.789***	30.766***	63.776***	594.936***
Family (F)	55	320.370***	554.045***	2375.104***	13.041***	47.056***	175.750***
Repetition (R)	2	150.640	19.312	66.333	0.006	1.696	69.226
Interaction F × R	110	71.775	66.958	218.846	1.964	7.026	46.506
Error	1512	26.028	26.587	156.139	0.995	3.355	24.248
Source of variation	DF	Variance (s ²)					
		h4	NRB3	NRB4	LB3	LB4	BB
Region of provenance (P)	4	1467.388***	7.967***	3.799***	363.390***	137.025	12.636***
Family (F)	55	967.420***	1.334***	1.268***	248.422***	463.575***	3.923***
Repetition (R)	2	26.168	2.262	2.287	162.123	86.371	0.188
Interaction F × R	110	121.269	0.709	1.015	72.918	75.717	0.068
Error	1512	74.520	0.400	0.489	64.309	47.376	0.471

Level of significance is represented as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

HT2, HT3, HT4 total height at ages 2, 3 and 4; h3, h4 current height increment at ages 3 and 4; DRC3, DRC4 root collar diameter at ages 3 and 4; LB3, LB4 branch length at ages 3 and 4; NRB3, NRB4 number of branches at ages 3 and 4; BB bud flushing

only with total height and current height increment. The genetic correlations between bud flushing and growth traits were negative but statistically non-significant.

Phenotypic correlations were also strong. We found positive highly significant correlations among the growth traits and between them and branch traits. The phenotypic correlations between bud flushing and growth traits were negative and significant too.

Correlations among growth traits and geographical coordinates of the location of origin of the plus trees were not found. Bud flushing, instead, was negatively correlated with altitude ($r = -0.450$ ***) and positively correlated with latitude ($r = 0.300$ *) of the parents'

origin (Table 5). Correlations with longitude were positive but non-significant.

3.4 Genetic gain

The genetic gain was calculated for growth traits and bud flushing at the ages of 4 years as the deviation (%) from population means (Table 6). Genetic gain varied with selection method, selection intensity and analysed traits. Regardless of the selection method, genetic gain was, generally, higher for total height than for root collar diameter. Genetic gain from family selection was higher than genetic gain from individual tree selection for all traits. If the best 10% of the best parents from first generation seed orchards will be mated, a genetic gain of 31–40% for total height, 30–39% for diameter and 39–49% for current height increment could be achieved.

4 Discussion

Results revealed high genetic variability at both provenance region and family levels for studied traits. The best growth performances were obtained for the progenies of plus trees selected in the natural populations from Bucegi Mountains in one of the five genetic centres of European larch in Romania. The weakest performances were obtained for the OP families of some plus trees from Brasov Depression. Also, significant differences were found between these two groups (native and artificial) in terms of phenology. Results are in agreement with a recent study regarding the anthropogenic transfer of European larch

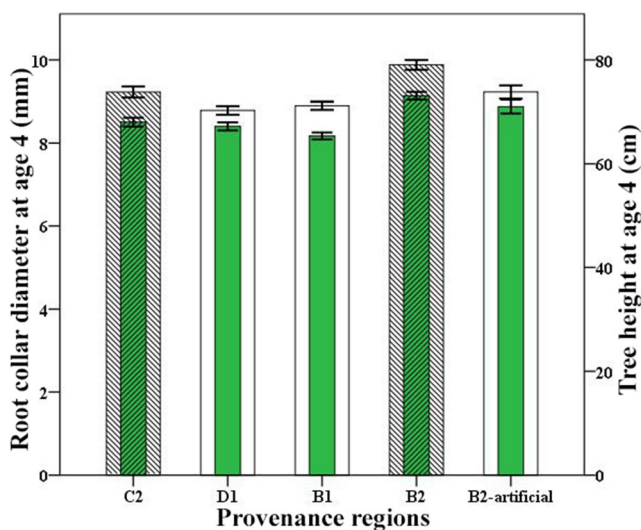


Fig. 2 Variation of the total height and the root collar diameter on provenance regions. Total height was marked with green colour and root collar diameter with white. Native populations were lined

Table 3 Genetic parameters for studied traits at different ages

Traits	Mean	CV	CVA	σ^2_A	σ^2_{Ph}	h^2_{HS}	h^2_i
HT2	25.32 (0.15)	25	18	20.72 (53)	38.89	0.78 ± 0.078	0.53 ± 0.108
HT3	40.49 (0.17)	17	16	40.59 (87)	46.86	0.88 ± 0.052	0.87 ± 0.127
HT4	68.20 (0.37)	22	20	179.69 (77)	234.28	0.91 ± 0.040	0.77 ± 0.114
DRC3	6.32 (0.03)	19	15	0.92 (63)	1.46	0.85 ± 0.061	0.63 ± 0.109
DRC4	9.16 (0.05)	25	20	3.34 (66)	5.06	0.85 ± 0.060	0.66 ± 0.112
h3	15.17 (0.14)	37	22	10.77 (35)	30.78	0.74 ± 0.088	0.35 ± 0.082
h4	33.87 (0.25)	31	25	70.51 (66)	107.40	0.87 ± 0.053	0.66 ± 0.108
NRB3	1.73 (0.02)	39	13	0.05 (12)	0.45	0.47 ± 0.120	0.12 ± 0.049
NRB4	2.14 (0.02)	35	7	0.02 (4)	0.55	0.20 ± 0.117	0.04 ± 0.042
LB3	23.43 (0.21)	36	16	14.63 (21)	71.02	0.71 ± 0.106	0.21 ± 0.053
LB4	24.11 (0.19)	33	24	32.32 (51)	63.14	0.84 ± 0.065	0.51 ± 0.096
BB	3.07 (0.02)	26	18	0.32 (50)	0.64	0.82 ± 0.022	0.50 ± 0.085

Proportion of additive genetic variance in phenotypic variance and standard error of means are given in brackets CV phenotypic coefficient of variation, CVA additive genetic coefficient of variation, σ^2_A additive genetic variance, σ^2_{Ph} phenotypic variance, h^2_i narrow-sense individual heritability, h^2_{HS} half-sib family heritability

from the Alpine region (Tyrol) to the Carpathians (Raffl et al. 2018). The authors found a strong affiliation of the Romanian population near Brasov (R3) to Tyrolean populations.

The contribution of additive genetic variance to the total phenotypic variance was high for growth traits and bud flushing and increased moderately from age 2 to 4. The large values of both narrow-sense and family heritability demonstrate that there is sufficient chance of success when using different selection methods in a breeding programme. It should be noted that these high values of heritability could be biased due to maternal effects (given the young age of the seedlings), common environmental effects in the nursery and by dominant effects as a result of inbreeding. At this age, the total height is the most heritable trait in European larch. Similar results

were reported by Frydl and Sindelar (2002) and Kowalczyk (2002) for some populations from the Sudetan Mountains. Pâques (2009), summarizing trends in narrow-sense heritabilities for European larch, demonstrated that the largest values were obtained for phenology and stem form and then for growth traits; branching appears as the less-heritable trait.

Results indicate that growth traits of this species at the nursery stage are phenotypically as well as genetically correlated. Both traits can be included in the breeding programme because both are heritable and positive genetically correlated. A single index that combines both height and diameter can be used for selection. The negative correlations between growth and bud flushing show that there are chances to select individuals with high growth performances and later bud flush,

Table 4 Trait-trait genetic (above line) and phenotypic correlations (below line)

	HT2	HT3	HT4	DRC3	DRC4	h3	h4	NRB3	NRB4	LB3	LB4	BB
HT2	–	0.394**	0.339*	0.232	0.263	0.063	0.269*	0.098	0.084	0.184	0.230	–0.159
HT3	0.829***	–	0.367**	0.282*	0.267*	0.547***	0.276*	0.192	–0.016	0.193	0.250	–0.186
HT4	0.724***	0.834***	–	0.256	0.294*	0.242	0.400**	0.079	0.040	0.310*	0.342	–0.147
DRC3	0.479***	0.618***	0.571***	–	0.307*	0.004	0.195	0.152	–0.002	0.068	0.159	–0.166
DRC4	0.544***	0.588***	0.657***	0.665***	–	0.683***	0.267*	0.010	0.100	0.222	0.219	–0.116
h3	0.122	0.656***	0.503***	0.451***	0.310*	–	0.163	0.045	–0.027	0.124	0.001	–0.011
h4	0.564***	0.616***	0.908***	0.428**	0.586***	0.332*	–	0.070	0.028	0.324*	0.344**	–0.081
NRB3	0.150	0.313*	0.130	0.244	0.015	0.354**	–0.116	–	0.110	0.179	0.010	–0.222
NRB4	0.084	–0.018	0.043	–0.094	0.103	–0.144	0.029	0.087	–	0.153	0.003	–0.003
LB3	0.347**	0.387**	0.631***	0.198	0.439***	0.218	0.648***	–0.261	0.148	–	0.387**	–0.038
LB4	0.471***	0.545***	0.758***	0.341*	0.470***	0.332*	0.748***	–0.015	–0.084	0.757***	–	–0.061
BB	–0.370**	–0.459***	–0.369**	–0.403**	–0.282*	–0.317*	–0.199	–0.499***	–0.024	–0.020	–0.190	–

Table 5 Phenotypic correlations among traits and geographic parameters

	ALT	LAT	LONG
HT4	0.133	-0.203	0.033
DRC4	0.145	-0.081	0.109
h4	0.068	-0.009	0.170
NRB4	0.085	0.248	0.432**
LB4	0.071	-0.061	-0.006
BB	-0.450***	0.300*	0.152

which would lead to fewer spring frost injuries. A similar correlation between growth and bud flushing was also noted by Malm and Stener (2002). Correlations with geographical coordinates of parents' origin indicate a population pattern, namely that trees from lower altitudes and southern latitudes open their buds earlier than trees from northern latitudes and higher altitudes. Genetic differences between populations from different parts of the range regarding bud flushing suggest that the transfer of forest reproductive materials could have serious consequences if maladapted provenances will be translocated.

High genetic gains can be obtained at this young age, through selection of total height and annual height increment at both individual and family levels. In Germany, Dietze (1976) estimated a genetic gain of approximately 5–10% for height by selecting the best plus-tree progenies at age 13 years. Pâques (2009) reported genetic gains of around 20–40% for stem volume at age 10 by selection of the best 30 clones (out of 157) in a population of polonica larch. Our estimations are higher than those listed above and can be biased due to some maternal effects or selfing and this question will be clarified in the future. These results are also

Table 6 Genetic gain (%) by selection of best 5% or 10% families (ΔG_1 , ΔG_2), individuals within families (ΔG_3 , ΔG_4) and parents (ΔG_5)

Character	Family		Individual		Parent
	ΔG_1	ΔG_2	ΔG_3	ΔG_4	ΔG_5
HT2	20	17	18	12	33
HT3	18	16	20	14	31
HT4	23	20	24	16	40
DRC3	17	15	17	11	30
DRC4	23	19	22	15	39
h3	23	20	18	12	39
h4	29	25	28	19	49
BB	21	18	18	12	35

important for nursery practice. Thus, the selection of seedlings in nurseries should be based on height and not root collar diameter, as recommended by current nursery guidelines.

The quantitative genetic parameters provide important information for breeding programmes and to plan the breeding strategy of this species. Our results indicate the pertinence of the recurrent selection method and phenotypic selection on the basis of growth traits to select the elite parents and then to recombine selected entries in the second generation of seed orchards. Recurrent selection at nursery stage can reduce the generation time and increase the gain per year.

5 Conclusions

The experiment reported here reveals large genetic variability for studied traits at both seed source and progeny levels, information which can be used in breeding programme and forest regeneration work. Results highlight high genetic control for growth traits and bud flushing of European larch and support that the strategy based on recurrent selection from the first-generation progeny at the juvenile age will produce the greatest genetic gain in the next breeding generation. Genotyping and paternity analysis are necessary to determine the origin of the genitors and the precise relationships among sibs of the same families for the next breeding cycle, given that the best families come from both natural populations and artificial stands, most likely from the Tyrolean Alps. Results complete the knowledge concerning genetic parameters of the European larch and are extremely important both for breeding programmes and conservation of forest genetic resources under climate changes.

Consequently, first-generation seed orchards are not only a *qualified* source for producing forest reproductive material, but also constitute a large genetic base for breeding, even if they were not established primarily for this purpose.

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Data availability Primary data set generated and/or analysed during the current study are available in the Zenodo repository (Mihai et al. 2019) at <https://doi.org/10.5281/zenodo.2633792>.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

ANNEX

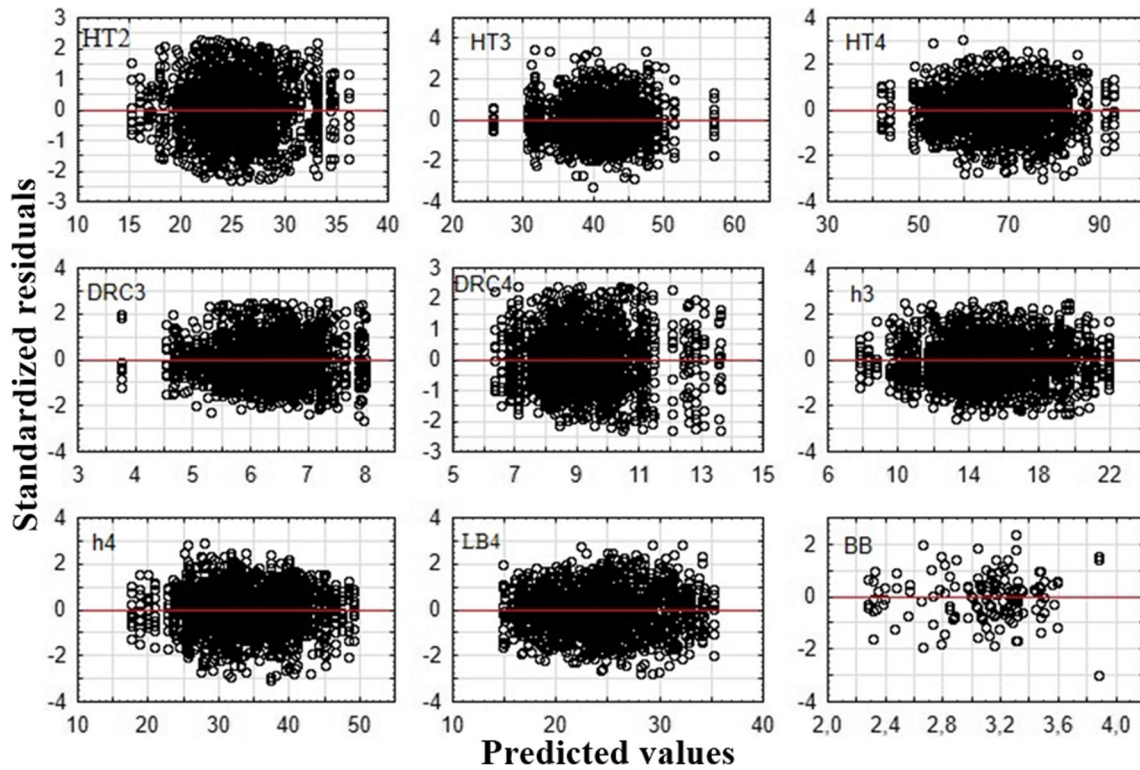


Fig. 3 Plot of observed standardized residuals vs predicted values for studied traits

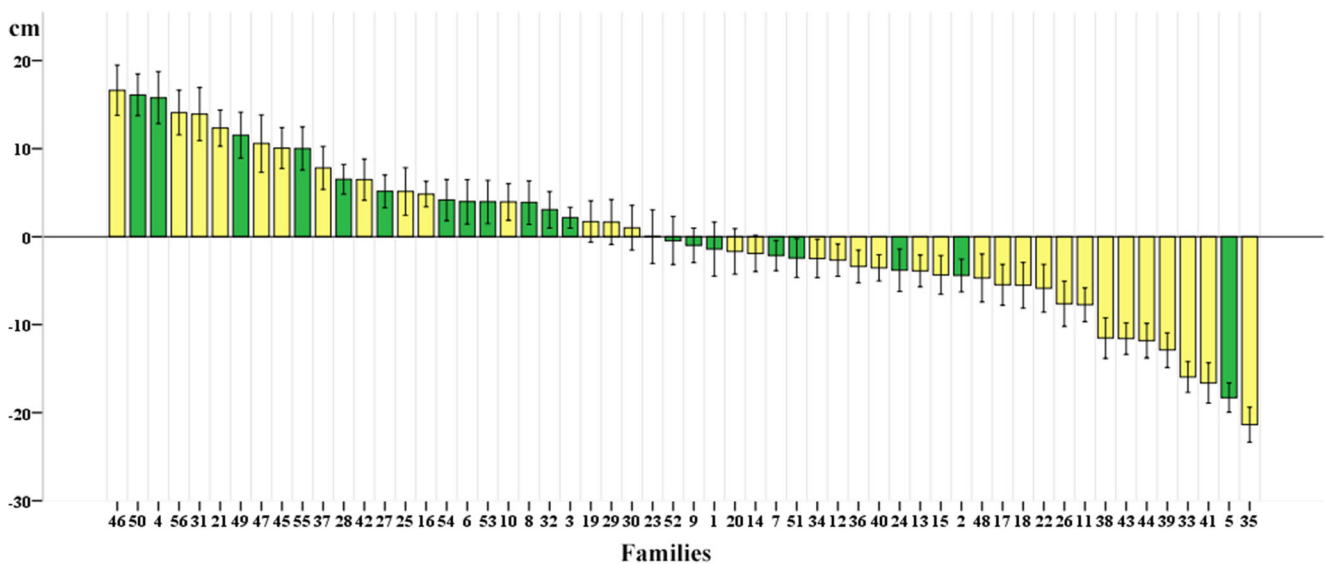


Fig. 4 Differences from the mean on experiment of total height at age of 4 for open-pollinated families of European larch. Native populations are marked with green colour and artificial stands with yellow colour

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