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Diallel crossing (10x10) in Swiss stone pine. Juvenile-adult correlations and genetic gain for predicting forward selection

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Abstract Swiss stone pine (*Pinus cembra*) is a viable solution for afforestation in the high mountain zone, at the upper limit of the forests, due to the species adaptation ability to the limiting climatic conditions. The aim of this study was to analyse the genetic variability, inheritance rate and juvenile-adult correlations, in a 26 years full-sib (10x10 complete mating design) trial, in order to predict the genetic gain of forward selection. In the spring of 2023, measurements and evaluations were carried out for all the existing trees, for Survival rate (Sr), growth (Diameter as breast height- Dbh, Trees' height- Th, Trees' volume- Tv) and quality traits (Forks, stem slenderness- Ss, branches' diameter- Bd, branches' finesse- Bf, defoliation- Def). The 90 cross-pollinated (CP) families registered 50% of Sr whereas the other 10 self-cross-pollinated (consanguineous) registered just 11% of Sr, and the majority of those trees are dominated and face extinction in the next years. Juvenile-adult correlations were highly significant ($p<0.001$), indicating that early selection (at the age of 14 years) might be efficient. At the trial age, based on the medium to high heritabilities, the forward selection of the best 10% individual trees from the top 20 of the 90 CP families, for Dbh, could be applied, which ensure at least 9.2% of genetic gain.

Keywords: age-age correlations; breeding strategy; forward selection; heritability; *Pinus cembra*.

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Introduction

Numerous researchers recommend the use of Swiss stone pine (*Pinus cembra*) for afforestation in the high mountain zone (Holzer 1975, Hoff et al. 1980, Holtmeyer 1994, Kronfuss & Havranek 1999, Paulsen et al. 2000, Bussotti 2002, Li & Yang 2004, Ulber et al. 2004, Blada & Popescu 2012, Caudullo & de Rigo 2016), at the upper limit of the forests and subalpine area, due to the ability of the species to adapt to the limiting climatic conditions and the favorable effects they generate, by: reducing the intensity of avalanches and torrents, increasing the resistance of stands to windfalls and snow breaks (both in pure stands and in mixtures with spruce), and resistance to *Cronartium ribicola*, a fungus that causes canker and drying of 5-needle pines.

Molecular genetic analyses of Swiss stone pine populations in the Alps and the Carpathians indicated low genetic diversity, especially in the Tatra Mountains (Dzialuk et al. 2014), while in the Carpathians, the level of genetic diversity was slightly higher (Belokon et al. 2005, Höhn et al. 2009). The two areas represented distinct glacial refuges (Gugerli et al. 2023) of this very old tree species (Dauphin et al. 2021). The high level of inbreeding recorded in the Tatra Mountains has also been recorded in other fragmented or isolated populations of Swiss stone pine (Lewandowski & Burczyk 2000, Politov et al. 2008, Salzer & Gugerli 2012), a fact that requires an increase in the number of genetically improved seed sources, constituted both *in situ* and *ex situ*, after a genetic diversity analysis (Saura et al. 2008, Funk et al. 2012, Wojnicka-Półtorak et al. 2015).

In Romania, a breeding program on Swiss stone pine were coordinated by Blada (1987, 2015), as a result of which the afforestation of 50 ha in Pietrosul Rodnei mountain (Eastern Carpathians) was achieved, and also nine comparative trials (3 half-sib, 2 full-sib, 3 of provenances and one with clones) has been established in the 1979-2004 period (Blada 2019). Outcomes from these experiments were extensively published (Blada 1994, 2003; Blada & Popescu, 2007, 2008, 2012), which confirmed the value of these experiments and the need to continue and expand the research.

The aim of this study was to analyse the genetic variability, inheritance rate and juvenile-adult correlations, in a 26 years full-sib (10x10 complete mating design) trial, in order to predict the genetic gain of forward selection.

Materials and Methods

In 1989, ten Swiss stone pine parental trees were randomly selected in the Southern Carpathians (Retezat mountain, Gemenele population, figure 1), at an altitude of 1850 m (a.s.l.), and a 10x10 diallel mating design was established (Blada 1999), according to Griffing (1956). Controlled pollination took place in July 1989, while the seeds were collected in September 1990 (Blada & Popescu 2008).

In the autumn of 1996, when the seedlings grown in the nursery reached the age of 6 years, a field trial was established in the Cibinului mountains (Southern Carpathians, near Păltiniş, in Sibiu County, Figure 1), at 1650 m altitude, 45˚63' north latitude, and 23˚92' east longitude (WGS 84 coordinate system). The 100 families (90 from out-crossing and 10 from self-pollination) were planted in a complete randomized block design with four replicates, 15 trees per unitary plot, and 2.5 x 2.5 m spacing (Blada & Popescu 2008).

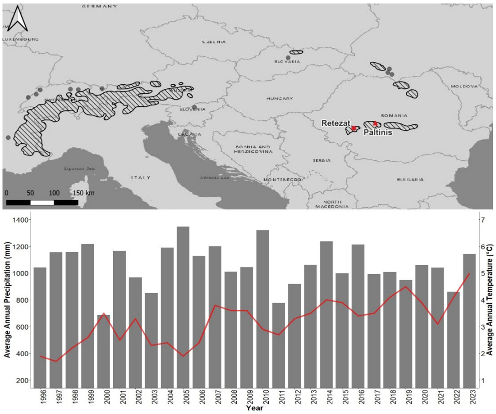

Figure 1 Location of the Păltiniş field trial (red triangle) and provenances origins (red circle) on the *Pinus cembra* distribution map (Caudullo et al. 2017). Natural species distribution is hatched. Regional climate trends (botom) in the mean annual temperature (red) and total precipitation (grey) for the Păltiniş trial during the last 28 years were computed using Climate downscaling tool (B4EST 2024).

Currently, the trial is administrated by forest district Răşinari, being located in the production unit V Onceşti, plots 89P and 90P, on an area of 3.7 ha (Photo 1). The trial is located on a higher, undulating slope, with a sunny (southern) exposition and 7-degree inclination. The biotope is represented by a mountain spruce forest of low to medium productivity and the soil is a typical Prepodzoil.

Photo 1 Aspects of *Pinus cembra* from Păltiniș full sib trial (credit photo: Dan Pepelea).

The natural forest type is a normal spruce with *Vaccinium* flora (Anonymous 2022). The average annual temperature was 3.2°C, and the sum of annual precipitation was 1062 mm (B4EST 2024). Whereas the precipitation showed no clear trend within the last century, the mean temperature has increased in the last 20 years (Figure 1).

After 26 growing seasons, in the spring of 2023, measurements and evaluations were carried out for all the existing trees, for growth and quality traits, as follows: a) diameter at breast height (Dbh), using a forest calliper; b) tree height (Th) and the fork height (Fh) measured using a Vertex V instrument; c) average branch diameter (Bd), in the whorl situated at 1.3 m from the ground, measured with electronic callipers; d) the fork (F) was evaluated on a scale of 7, according to Treebreedex protocol (Ducci et al. 2012), as follows: 1= more forks in the lower third of the stem, 2= one fork in the lower third of the stem, 3= more forks in the middle third of the stem, 4= one fork in the middle third of the stem, 5= more forks in the higher third of the stem, 6= one fork in the higher third of the stem, 7= no fork; e) the stem straightness (Ss) was evaluated on a scale of 5, according to Treebreedex protocol (Ducci et al. 2012), as follows: 0= vertical and rectilinear stem, 1= 1-2 small curves, 2= three or more small curves, 3= 1-2 big curves, 4= three or more big curves; f) the defoliation (D) was evaluated on a scale of 5 classes, according to ICP protocol (Eichhorn et al. 2020), as follows: 0= healthy tree (0-10% defoliation), 1= slightly damaged tree (11-25% defoliation), 2= moderately damaged tree (26-60% defoliation), 3= severely damaged tree (61-99% defoliation), 4= dead tree.

The trees' volume (Tv) was also calculated using the regression equation method (Giurgiu et al. 2004) and the branches' finesse was estimated with the following formula:

$$\mathrm{Bf} = \frac{\mathrm{Bd}}{\mathrm{Dbh}} \times 100$$

Also, the survival rate (Sr) was calculated at the family level as a percentage between the number of trees that had survived until the age of 26 years and the number of planted trees.

Statistical analysis

A diallel mating design, according to Griffing's (1956) Method 3, model 1, was used, where one set of F1's and reciprocals but not the consanguineous were included. The comparisons of cross-pollinated (CP) with self-cross-pollinated (SP, consanguineous) were made in Statistica (StatSoft 2010) and R software's (R Core Team 2023). The mathematical model for diallel analysis was taken from Wilcox (1976), and is a combination of Hayman (1954) and Griffing's (1956) models, as:

$$x_{kij} = u + g_i + g_j + s_{ji} + m_i + n_{ij} + e_{kij}$$

where: x_{kij} = the value of the kth progeny from the cross between ith female and jth male; u = the general mean; g_i and g_j = the general combining ability effects for the ith and jth parents, respectively; m_i = the maternal effect of the ith parent; s_{ij} = the specific combining ability effect for the cross between the ith and jth parents; n_{ij} = the interaction effect between the ith and jth parents, such that $r_{ij} = - r_{ji}$ (reciprocal effect not accounted for by maternal or paternal effects); e_{kij} = random error (Blada & Popescu 2008).

The standard errors (Se) of the variance component estimates were calculated according to Anderson and Bancroft (1952). The additive variance was used to estimate the variance of GCA among all of the parents, and was estimated as $1/4\sigma^2_A$, assuming that all epistatic genetic variances were irrelevantly small. Also, the estimated variance of SCA was an estimator of $1/4\sigma^2_D$ (Kriebel et al. 1972, Becker 1984, Blada & Popescu 2008).

For breeding strategy, two types of heritabilities were calculated, and, consequently, two genetic gains were determined, as follows:

- broad-sense family mean heritability (h^2_1):

$$h^2_1 = (2\sigma^2_{GCA}+\sigma^2_{SCA}) / \sigma^2_{Ph1} = (2\sigma^2_{GCA}+\sigma^2_{SCA}) / (2\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_{Mat}+\sigma^2_{Mat\text{-}Int}+\sigma^2_e/k)$$

where: σ^2_{GCA}, σ^2_{SCA}, σ^2_{Mat}, $\sigma^2_{Mat\text{-}Int}$, σ^2_e and σ^2_{Ph1} = general, specific, maternal, maternal-interaction, error and phenotypic variances, respectively, and k = number of blocks = 4.

According to Falconer (1981), the genetic gain (G_1) was estimated as:

$$\Delta G_1 = i_1 \bullet h^2_1 \bullet \sigma_{Ph1}$$

where: i_1 is the selection intensity Becker (1984) and σ_{Ph1} is the family mean phenotypic standard deviation.

- narrow-sense family mean heritability (h^2_2):

$$h^2_2 = (2\sigma^2_{GCA}) / \sigma^2_{Ph1} = (2\sigma^2_{GCA}) / (2\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_{Mat}+\sigma^2_{Mat\text{-}Int}+\sigma^2_e/k)$$

The genetic gain (G_2) was estimated as:

$$\Delta G_2 = i_1 \bullet h^2_2 \bullet \sigma_{Ph1}$$

The standard error of heritability was calculated in breedR package (Munoz & Rodriguez 2016) of R programme (R Core Team 2023), using Delta's method (Lynch & Walsh 1998). All genetic gains were expressed in comparison with the trial mean. To estimate the genetic gain, two selection thresholds were chosen: the first in favour of the most valuable ten CP families to maximize the genetic gain, and the second in which the number of selected CP families was doubled, to ensure a higher level of genetic diversity. Finally, the optimal variant was adopted.

Results

Phenotypic variability

The *Survival rate (Sr)* average of the 90 cross-pollinated (CP) families was 49.8%, with the highest value for the trees reported to the mother 50 (55.8%), while the lowest Sr (42.1%) was registered by mother 209 (Figure 2). Significant influences ($p<0.05$) of replications and mothers were registered, while the fathers had an insignificant influence ($p>0.05$). Only 53 of the planted 474 consanguineous (self-cross-pollinated, SP) trees combinations have survived, most of them being dominated and having a major risk of extinction in the coming years.

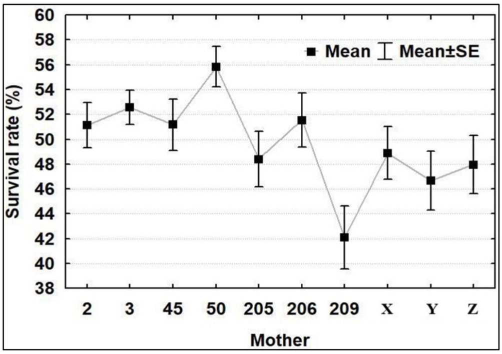

Figure 2 Survival rate variation among the mothers, for CP combinations, in the Păltiniș trial.

In case of the principal *growth traits* (Th and Dbh), the CP families registered 34% and 42% higher mean values than SP, which generated 2.3 times higher Tv. The best mother trees (X) for Tv, was superior with 27% than the trial average (Table 1). The best families, in terms of Tv, were Xx209 and Xx205, with a 45% higher than the trial average. Highly significant variances of GCA ($p<0.001$) were registered for all growth traits, while variances of SCA, maternal and replication were significant to highly significant, especially for Th (Table 2). Of the total number of 1804 trees, 54.5% present *fork* (F), which is located, on average, slightly above the middle of Th (Table 1).

Regarding *branches* (Bd, Bf), we can observe, from table 1, that the branches of the CP families, although they are thicker with 14%

than SP, reporting to their stem, these are 19% finer (Table 1). A significant influence of family ($p<0.05$) was registered only for Bf, while the replication factor had a significant (for Bd) to highly significant ($p<0.001$, for Bf) influences. At the same time, GCA had a highly significant effect on both branches' traits (Table 2).

Table 1 Parent performances of CP and average SP at 26 years (trial age).

Parent	Th (m)	Dbh (cm)	Tv (m^3)	Fork index	Fh (m)	Bd (cm)	Bf (%)	Def	Ss index
2	6.0	16.1	0.055	5.3	3.3	2.35	14.9	0.09	0.68
3	5.7	15.8	0.052	5.4	3.0	2.41	15.6	0.12	0.71
45	5.7	15.6	0.050	5.9	3.4	2.45	16.2	0.10	0.67
50	6.0	15.7	0.053	5.3	3.3	2.39	15.5	0.14	0.57
205	5.6	16.3	0.053	5.5	3.0	2.45	15.4	0.18	0.47
206	6.0	16.0	0.056	5.8	3.2	2.39	15.4	0.26	0.26
209	5.9	16.4	0.058	4.3	2.9	2.45	15.4	0.10	0.75
X	6.2	17.7	0.070	4.2	3.2	2.50	14.3	0.16	0.69
Y	6.1	16.0	0.056	4.8	3.3	2.27	14.3	0.13	0.96
Z	5.9	16.6	0.060	4.3	2.9	2.31	14.3	0.09	1.13
Mean CP	**5.9**	**16.2**	**0.056**	**5.1**	**3.1**	**2.4**	**15.1**	**0.14**	**0.69**
Mean SP	**4.4**	**11.4**	**0.024**	**5.9**	**2.7**	**2.1**	**18.7**	**0.26**	**1.28**
Mean Trial	**5.9**	**16.1**	**0.055**	**5.1**	**3.1**	**2.4**	**15.3**	**0.14**	**0.70**

Note: Th= trees' height, Dbh= diameter at breast height, Tv= trees' volume, Fh= fork height, Bd= branches' diameter, Bf= branches' finesse, Def = defoliation, Ss= stem straightness.

Table 2 ANOVA results of the Păltiniş trial.

Factor	DF	MS_{Dbh}	MS_{Th}	MS_{Bd}	MS_{Bf}
Replication	3	40.7*	1.36***	0.04*	15.53***
GCA	9	23.68***	1.56***	0.33***	16.78***
SCA	35	5.40*	0.32***	0.07	3.68
REC	45	3.26	0.18	0.07	2.92
•Mat	9	4.35	0.40**	0.07	6.00*
•Mat-int	36	2.99	0.13	0.06	2.15
Error	267	3.33	0.15	0.07	2.76

Note: DF = degrees of freedom [statistical model according to Wilcox 1976: Replication: k-1, GCA: p-1, SCA: p(p-3)/2, REC: p(p-1)/2, Mat: p-1, Mat-interaction: (p-1)(p-2)/2, Error: (k-1)(p2-p-1). k=replications=4, p=parents=10]. MS = mean square. Significant influences, * at $p<0.05$, ** at $p<0.01$, *** at $p<0.001$, according to Fisher test. Dbh, Th, Bd and Bf as in table 1. GCA and SCA= general and specific combining abilities. REC= reciprocal effect. Mat and Mat-int= maternal and maternal-interaction effects.

Both CP and SP families registered good results for Defoliation (Def) and Stem straightness (Ss), respectively a healthy tree, with vertical and rectilinear stems, with 1-2 small curves on some trees (Table 1).

Trait-trait and juvenile-adult correlations

The phenotypic correlations (Table 3) between the analysed traits indicated that not all traits can be improved simultaneously. The growth traits were positively and highly significantly correlated with each other (r= 0.75***–0.95***), but, unfortunately, also significantly correlated with branches diameter (r= 0.37***–0.63***) and the fork presence (r= -0.29*** to -0.39***, with increasing of growth traits, the fork index it decreases, from 7- no fork, to 1- more forks in the lower third of the stem).

In case of the CP families, highly significant juvenile-adult correlations (Table 3) were registered, both for Th (r= 0.72***) and Dbh (r= 0.71***), which suggests that, in the future, forward selection could be practiced even from the juvenile stage, at the age of 14 years. Both for Th and Dbh, at age 26 and 7 (after trial establishment), the best performing mother tree was X, followed by Y combination for Th, and Z for Dbh. At the opposite pole, the mother 45, registered the lowest result for both traits at the two analysed ages.

Age-age trends differ significantly for CP and SP families. Thus, if at the age of 8 years (after seeded) the average values for Th and Dbh were almost identical, at 14 years, CP was superior by 34.5% and 53%, for Th and Dbh, respectively. The difference was maintained until the age of 33 years (after seeded, 26 growing seasons after trial establishment) for Th, while for Dbh, the difference has been slightly reduced, the average result recorded by CP being superior to the consanguineous with 42% (Figure 3).

Table 3 Trait-trait (up) phenotypic correlations, and juvenile-adult (down) correlations for the CP families.

N=360	Th	Tv	Fork	Bd	Bf	Def	Ss
Dbh	0.75***	0.95***	-0.38***	0.63***	-0.59***	-0.27***	-0.04
Th	-	0.83***	-0.29***	0.37***	-0.61***	-0.15**	-0.13*
Tv		-	-0.39***	0.54***	-0.62***	-0.21***	-0.04
Fork			-	-0.23***	0.23***	0.04	-0.36***
Bd				-	0.20***	-0.29***	0.04
Bf					-	0.08	0.08
Def						-	-0.05
Ss							-

N=10	Th14	Dbh33	D14
Th33	0.72***	x	0.47*
Th14	-	0.50*	x
Dbh33		-	0.71***
D14			-

Note: Dbh, Th, Bd, Bf, Def, and Ss as in table 1. Th33 and Dbh33 are the current values, while Th14 and D14 are the juvenile values, at the seedlings age of 14 years. Significant correlations: * at p<0.05, ** at p<0.01, *** at p<0.001.

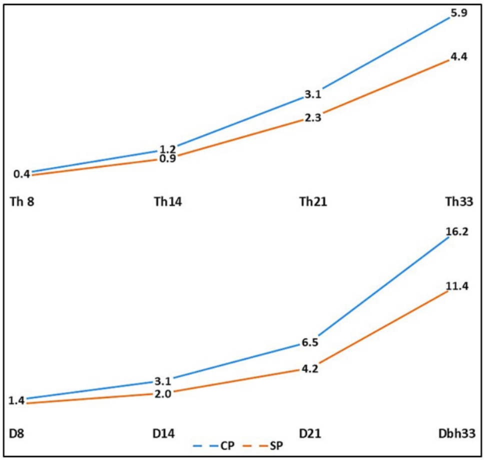

Figure 3 Age trend in Th (up) and Diameter (down) for CP and SP progenies, in the Păltiniş trial. (D8 and D14 are collar diameters, at 8 and 14 years after seeded, respectively 1 and 7 years after trial establishment; D21 was measured at ½ of Th; Dbh33 and Th33 are the average values determined in the spring of 2023, at 33 years after being seeded and 26 growing seasons in Păltiniş Trial)

Genetic parameters and forward selection

Variance components

A highly significant influences ($p<0.001$) of all analysed traits were registered for GCA, while SCA and maternal effects were statistically significant only for some traits, Dbh and Th, respectively, for Th and Bf (Table 2). The variance component estimates, standard errors and dominance ratios were presented in table 4. For the analysed traits, the genetic variance participation ranged between 21.9% and 52.2%, with the highest inheritance rate for Th. Also, Th was the only trait for which the SCA variance participation was higher than GCA (Table 4).

GCA and SCA effects

The ANOVA test (Table 2) shows highly significant variance in GCA for all four studied traits. Consequently, GCA effects were

Table 4 Genetic (S^2_G) and environment (S^2_E) variances estimates (±standard errors) and dominance ratios (%).

Components	Dbh		Th		Bd		Bf	
S^2_{GCA}	0.286±0.16	7.4%	0.019±0.01	9.8%	0.004±0.002	5.6%	0.205±0.11	6.6%
S^2_{SCA}	0.259±0.16	6.7%	0.021±0.009	10.8%	0.001±0.002	1.4%	0.115±0.11	3.7%
S^2_{Mat}	0.017±0.02	0.4%	0.003±0.002	0.0%	0.0001±0.00	0.2%	0.048±0.03	1.5%
$S^2_{Mat-int}$	0	-	0	-	0	-	0	-
Total S^2_G	**0.52**	**13.5%**	**0.040**	**20.6%**	**0.0051**	**7.2%**	**0.368**	**11.8%**
S^2_E / K	3.33	86.5%	0.154	79.4%	0.0659	92.8%	2.763	88.2%
Total S^2_P	**3.85**	**100.0%**	**0.194**	**100.0%**	**0.071**	**100.0%**	**3.131**	**100.0%**
S^2_{SCA}/ S^2_{GCA}	0.91		1.062		0.216		0.56	

Note: GCA, SCA, Mat, Mat-int, Dbh, Th, Bd and Bf as in table 1. Negative variance estimates of S^2Mat-int were considered zero and their standard errors were not calculated.

calculated for each parent, with positive and negative, non-significant, significant, and highly significant effects identified (Table 5). The significance of GCA effects was determined using the t-test at probabilities of 5%, 1%, and 0.1%.

Table 5 General combining ability (GCA) effects for each parent.

Parent/ trait	Dbh	Th	Bd	Bf
2	-0.376	-0.023	-0.068	-0.168
3	0.006	-0.045	0.030	0.123
45	**-0.676**	**-0.167**	0.037	**0.974**
50	-0.466	0.080	-0.047	0.106
205	0.161	**-0.235**	0.083	0.372
206	0.039	0.070	-0.030	-0.181
209	0.343	-0.049	0.048	0.085
X	**1.158**	**0.256**	0.066	**-0.707**
Y	-0.489	0.086	**-0.112**	-0.301
Z	0.301	0.026	-0.007	-0.304
SE	**0.645**	**0.139**	**0.091**	**0.588**
Trait average	*16.2*	*5.9*	*2.4*	*15.1*
LSD 5%	0.632	0.136	0.089	0.576
LSD 1%	0.832	0.179	0.117	0.758
LSD 0.1%	1.061	0.228	0.149	0.967

Note: The significance of GCA effects was determined using the t-test at probabilities of 5%, 1%, and 0.1% (highlighted in bold).

Positive GCA effects were recorded in half of the parents for all four analysed traits, indicating a considerable variation around the mean value. Highly significant positive GCA values were recorded for parent X, for both Dbh and Th traits. Positive GCA effects for Dbh were also recorded for parents Z and 209, but these were not statistically significant. Negative significant GCA effects for the Dbh and Th traits were noted for parent 45. The fact that parents exhibit both positive and negative effects, whether significant or not, for the analysed traits indicates that the population from which the parents were selected is relatively homogeneous with high variability. To achieve remarkable genetic gains, the most valuable parents should be carefully selected from the base population based on the extrapolated experimental results.

Heritability

The inheritance rate, for the analysed traits, was medium to high, with the highest heritability being recorded for Th, followed by Dbh. Broad-sense family heritability (h^2_1) ranged from 0.35 (Bd) to 0.60 (Th), while the narrow-sense heritability (h^2_2) increases from 0.32 (Bd) to 0.39 (Th). The Dbh registered a medium inheritance rate (Table 6). These results, indicating a medium to high level of heritability, allow us to carry out the forward selection strategy of Swiss stone pine, for growth and branches traits.

Genetic gain

The genetic gain that could be achieved for the four analysed traits was estimated at the family level (Table 6), with the mention that, if an individual selection will be made within the most valuable families, the progress will be even greater. If the multiplication takes place vegetatively, a genetic gain (ΔG_1) of 3.1% (Bd) to 13.4% (Dbh) will be achieved. At the same intensities, if the multiplication takes place sexually, the genetic gains (ΔG_2) ranged between 2.8%, for Th and Bd, and 9.2%, for Dbh (Table 6). Therefore, the best genetic gain (without a significant reduction of genetic diversity) will be obtained by the selection of the best 20 families for Dbh, action doubled by the selection of the best individuals within them, also depending on Dbh (situation in which Th and Bd will also be improved because those quantitative traits are strongly correlated). However, because among the 20 most valuable families there are only nine different mothers, in the next generation of the breeding program they will be combined with provenances from other experiments, in order to avoid the reduction of genetic diversity.

Table 6 Estimates of heritability (h^2) and genetic gains (ΔG%) based on family selection.

Parameter / Trait	h^2_1	h^2_2	ΔG_1 -best 10 families-	ΔG_1 -best 20 families-	ΔG_2 -best 10 families-	ΔG_2 -best 20 families-
Dbh	0.51 ± 0.100	0.35 ±0.096	13.4	9.2	4.6	3.6
Th	0.60 ± 0.105	0.39 ± 0.097	5.4	4.3	3.5	2.8
Bd	0.35 ± 0.092	0.32 ± 0.086	3.9	3.1	3.5	2.8
Bf	0.44 ± 0.095	0.35 ± 0.094	5.4	4.2	4.2	3.3

Note: h21, h22, ΔG1, ΔG2 are the broad-sense and narrow-sense family heritability (±standard error), and the specific genetic gains. Dbh, Th, Bd and Bf as in Table 1.

Discussion

Phenotypic variability

A significant genetic and phenotypic variabilities among families and also inside of each family, for all of the analysed traits were registered, which favours the breeding programme. This finding is in accordance with the previous study of Blada & Popescu (2008), where it was concluded that, at the age of 14 years, although the Swiss stone pine is a slow-growing species, the level of variability among and within families is very high. This fact is encouraging if we refer to the molecular genetic analyses, performed in the Alps and the Carpathians, which indicated a low genetic diversity and an increased risk of inbreeding depression (Politov et al. 2008, Salzer & Gugerli 2012, Dzialuk et al. 2014). At the same time, other research reported a higher differentiation and genetic diversity in Swiss stone pine populations from Carpathians than the Alps populations (Belokon et al. 2005, Höhn et al. 2009).

In the present research, comparisons of mean performances of CP and SP, for the analysed growth (Th, Dbh, Tv) and quality (Bf) traits, showed highly significant differences in favours of CP (34%, 42%, and 19% for Th, Dbh, and Bf, respectively), which is in accordance with the previous findings (Blada & Popescu 2008). Only 11% of the consanguineous trees have reached the trial age of 26 years (33 years after seeded), and they showed an underdeveloped state of vegetation being at major risk of disappearance in the coming years. Similar results were recently reported for Norway spruce (Budeanu et al. 2019).

Trait-trait and juvenile-adult correlations

The trait-trait significant to highly significant positive phenotypic correlations between the analysed growth traits are in accordance with previous research, on numerous tree species, like Norway spruce (Apostol & Budeanu 2019), European beech (Besliu et al. 2024), as well as for the species studied in the present article (Blada & Popescu 2008, 2012).

Juvenile-adult growth traits correlations were very high, indicating that early selection might be efficient. Because of that, in the future, selection could be practiced even from the juvenile stage, at the age of 14 years. Earlier, Blada & Popescu (2008) recommended an even lower age, of 6 years, for growth traits selection.

Genetic parameters and forward selection

The high level of genetic variability registered for all of the analysed traits, as well as the high number of families involved in this experiment, ensured the success of the genetic breeding program. The Swiss stone pine is a characteristic tree species of the Carpathian subalpine ecosystems. In this context, the breeding program for Swiss stone pine should not focus on increasing the productive or qualitative capacity of the wood, but rather on improving the protective characteristics of the species, such as growth, trunk diameter, crown density, and the size of first-order branches. These characteristics are particularly important as they contribute to reducing the time required for artificial establishment in sparse forests, increasing the resilience of spruce stands at the altitudinal limit of the forest, enhancing precipitation retention capacity, reducing soil erosion, and stabilizing avalanche corridors. The obtained results can support a breeding program for Swiss stone pine in the Romanian Carpathians, based on the creation of genetic resources and the phenotypic selection of plus trees. To achieve the highest possible genetic gains within populations, without reduction of genetic diversity, 10-20% of the specimens exhibiting the most vigorous growth will be selected, while also having well-developed first-order branches (large thickness), a characteristic that provides resistance and protection against strong winds, precipitation retention, and avalanche resistance.

In Păltiniş field trial, the narrow-sense were smaller than broad-sense heritabilities, for all growth traits, in accordance with the previous findings (Blada & Popescu, 2008). For growth traits, after an increased trend of heritability from 2 to 7 years after planting, in the last 19 years the values were maintained. For Dbh, the broad-sense heritability slightly increased, from 0.50 to 0.56, in the first 7 years after planting (Blada & Popescu 2008), after which it decreased slightly, to 0.51. The narrow-sense Dbh heritability increased from 0.27 to 0.38 in the first 7 years after planting (Blada & Popescu 2008), after which it decreased slightly in the last 19 years (in 2023, the Dbh h^2_2 was 0.35). The same upward trend in the first years was also recorded for Th, after which it decreased from 0.74 and 0.65, at the trial age of 7 years (Blada & Popescu 2008), to 0.60 and 0.39, currently, for broad-sense and narrow-sense heritabilities, respectively. An increasing trend of heritability in the first 8 years for growth traits was also found for *Pinus taeda* (Xiang et al. 2003), while for *Pinus contorta* an opposite trend in the first years after planting was registered (Xie & Ying 1996). An intermediate situation between the two previously mentioned, respectively a constant trend in time of family heritability for growth traits, was registered for *Pinus pinaster* (Kusnandar et al. 1998).

For Dbh, the expected genetic gain increased in the last 19 years. Therefore, if the most valuable 20 of the 90 families were selected, the genetic gains increased from 6% and 4% (Blada & Popescu 2008), to 13.4% and 9.2% in present. For Th, the genetic gain decreased significantly in the same period, from 11% and 10%, at 7 years after plantation time (Blada & Popescu 2008), to 4.3% and 2.8% in present, especially due to the reduction of the annual height growth in recent years. The best genetic gain, 13.4%, is expected from the selection of the best 10 of the 90 CP families, according to Dbh (Table 6).

Therefore, based on the results of the current research, we propose the forward selection of the best 10% individual trees from the best 20 of the 90 CP families (Figure 4), for Dbh, which ensures at least 9.2% genetic gain. However, considering that among the 20 most valuable families (which have an average Dbh 10% above the average of the experiment) there are only nine different mothers (only mother 50 is missing), in the next generation of selection they need to be combined with provenances from other experiments (which exist in the Romanian Carpathians and will be analysed in the next period), in order to avoid the reduction of genetic diversity.

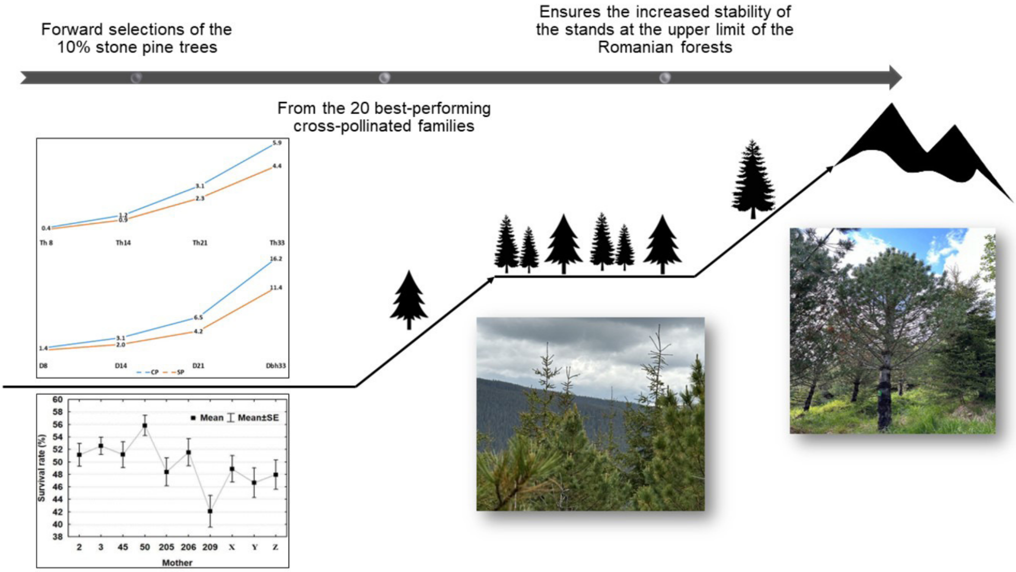

Figure 4 Graphical abstract of the breeding strategy.

A breeding strategy for this slow growing species is necessary because of its major importance for the afforestation in the upper altitudinal layer of the Carpathians and Alps forests, where it can be mixed with Norway spruce (contributing to the reduction of wind damage suffered by the spruce stands), and, to the same extend, representing a solution for the reforestation of the gaps in the subalpine phytoclimatic layer (Gugerli et al. 2023). Additionally, Swiss stone pine is a source of resistance to blister rust caused by *Cronartiun ribicola*, presents valuable wood for furniture and sculpture, it is a beautiful ornamental species, and its seeds (known as pine nuts) are edible and have many food and pharmaceutical uses (Blada & Popescu 2012).

Conclusions

The high level of genetic variability, inheritance rate, trait-trait and age-age correlations, registered for all of the analysed traits (especially for growth traits), as well as the high number of families involved in this experiment, ensured the success of the breeding program. Self-pollination was unfavourable and leads to a reduced survival and low values of growth traits compared to cross-pollinated (CP) material. Furthermore, most of consanguineous trees are dominated and presents a major risk of extinction in the coming years.

Juvenile-adult growth traits correlations were very high, indicating that early selection, even from the juvenile stage (at the age of 14 years), might be efficient. Based on the medium to high heritabilities and the estimated genetic gain, the forward selection of the best 10% individual trees from the top 20 of the 90 CP families for Dbh, could be applied, which ensures at least 9.2% genetic gain.

Research must be continued up to 1/2 of the rotation period of the Swiss stone pine, and also diversified, through genomic and transcriptomic analyses, investigation of the species' resilience to environmental factors, phenological observations and assisted migration evaluation, in order to expand knowledge about this species.

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Conflicts of Interest

The authors declare no conflict of interest.

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