Genetic Variation in Stone Pine Half-Sib Progenies

loan Blada [1]

Abstract:--Total height, annual height growth, root collar diameter, total number of branches and total number of buds around the leader bud were recorded at age 6.

The experimental material was included 136 half-sib families originating from stone pine natural populations from the Carpathian Mountains. Population samples were included in a randomized complete block experiment with four replications and ten seedlings per family per replication. Highly significant (p<0.001) family variation for all traits was detected. Very high family heritabilities were estimated for total height ($h_f^2 = 0.968$), root collar diameter ($= 0.938$) and total number of branches ($= 0.966$). Genetic correlations between total and annual height growth and root collar diameter were high or very high, ranging between 0.804 and 0.969. These correlations indicated favorable conditions for obtaining substantial genetic gain for a combination of these traits. By selecting the best 30 to 45 families, genetic gains in total height growth and diameter between 28.8 % and 23.4 % and between 18.8 % and 15.3 %, respectively, could be achieved. Suggestions for a breeding strategy are made.

Keywords: *Pinus cembra,* half-sib progeny, growth traits, genetic variance, heritability, genetic gain.

INTRODUCTION

Stone pine *(Pinus cembra* L.) is naturally distributed at high elevations of the Alps and Carpathians, including the Tatra Mountains. (Georgescu & lonescu 1932; Beldie 1941; Critchfield *& Little* 1966; Sauermoser 1994; Konak 1994). The species is important from the following points of view: ecological (Holzer 1972; Frey 1994); silvicultural (Frey 1994; Holzer 1994; Blada 1996); industrial (Holzer, 1972; Contini & Lavarelo 1982); genetic (Bingham 1972; Holzer 1975; Blada 1994); landscaping, tourism and other recreation functions (Gordon 1994; Blada 1997 b)

Although stone pine is a very slow-growing species, it is of particular importance for forestry in the Carpathian subalpine zone. Due to a lack of improved planting material, a genetic improvement program including intra- and interspecific crosses was developed in Romania (Blada 1990). Improvement of growth traits was the main objective of the program.

This paper reports nursery-stage variation among 136 stone pine half-sib progenies.

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MATERIALS AND METHODS

Initial material and nursery progeny test

Open pollinated seeds were collected from 136 stone pine trees growing in several natural populations from the Carpathian and Alps Mountains. Only the availability of cones per tree was taken into account in parent selection. To reduce the likelihood of relatedness, the trees were separated by a minimum of 50 meters. In September 1991, two seeds were sown per polyethylene pot (22 cm x 18 cm) filled with spruce humus. After sowing, the seeded pots were placed in nursery beds where they were arranged in a randomized complete block design The second seedling, if present, was removed in the second year of growth. A 10-seedling row plot in each of four blocks represented each of the 136 families. As stone pine is a very slow-growing species, the seedlings were grown in the initial pots throughout the six-year nursery testing period.

Traits measured

Five traits (Table 1) were measured in the autumn of 1995 when the plants were six years old. Plot means comprised the basic data for statistical analysis.

Traits	Units	Symbols
Total height growth	cm	H.6
Annual height growth	cm	h.6
Root collar diameter	mm	RCD.6
Total branches	No.	TNB.6
Total buds around the terminal bud	No.	TNBAL.6

Table 1. Traits measured at age six.

Statistical analysis

Two-way ANOVA based on plot means was performed. The following mathematical model was applied:

$$X_{ik} = m + a_i + b_k + e_{ik} \qquad (1)$$

where: X_{ik} = plot average from the *i-th* open-pollinated family in the *k-th* replication; m = the general mean of the whole experiment; a_i = the random effect of the *i-th* half-sib progeny ($i = 1,2...I$); b_k = the effect of the *k-th* replication ($k = 1,2...K$); e_{ik} = the random error. Replications and families were considered to be random effects. Variance components of the random effects were estimated by equating mean squares to expected mean squares. Standard errors (SE) of the variance components were computed with the

formula given by Anderson & Bancroft (1952). Genetic coefficients of variation (GCV) were calculated with the formula:

$$GCV = (\sqrt{\acute{o}^2_f}/x)\, 100 \qquad (2)$$

where: $\acute{o}^2_f$ = the family genetic variance ; x = the population mean
Narrow-sense family heritabilities (h^2_f) were calculated as:

$$h^2_f = \acute{o}^2_f/(\acute{o}^2_f + \acute{o}^2_e/k) \qquad (3)$$

The confidence intervals (95%) were estimated for heritability by Knapp's et al. (1985) formulas. Genetic gain ($\ddot{A}G$) was calculated by Falconer's (1981) formula:

$$\ddot{A}G = ih^2_f \acute{o}_{Phl} \qquad (4)$$

where: i = intensity of selection taken from Becker (1984), and $\acute{o}_{Phl}$ = phenotypic standard deviation.

RESULTS AND DISCUSSIONS

Genetic variation

Highly significant (p<0.001) differences among the 136 families were found in all traits (Table 2, row 2). As a full table of family means cannot be presented here, Table 3 shows family means of 10 best and 10 poorest families for each trait, indicating the magnitude of family variation. Large variation among family means was found. For total height growth and root collar diameter, the poorest groups had averages (X_2) of 13.4 cm and 7.7 mm, respectively; while the averages of the best groups (X_1) were 29.6 cm and 13.0 mm, respectively, i. e. a difference (D_1) of 120.7 % and 69.3 %. At the same time, the difference between the two groups of families was 152.6 % in total number of branches and 120.3 % in total number of buds around the leader. Differences (D_2) between the top group (X_1) and the test mean (X) were smaller but still significant (Table 3, last line). As expected, differences among individual families were much greater. For example, at six years of age, the worst family was 11.5 cm tall, while the best family measured 35.4 cm, i.e. a difference of 204.3%. The genetic coefficients of variation for total height, annual height growth, root collar diameter and total number of branches was 22.0%, 25.8%, 14.6% and 26.3%, respectively (Table 2, last line). Thus, it has been demonstrated that stone pine half-sib families posses considerable genetic variation in the analysed traits, suggesting that selection for improvement will be effective.

Variance components

Analyses of the trait data yielded estimates of variance components and their standard errors presented in the lower part of Table 2. At age 6, the family additive genetic variance was 88% for total height growth, 80 % for annual height growth, 79 % for root

Source	DF	Mean squares of the traits				
		H.6	h.6	RCD.6	TNB.6	TNBAL.6
Replications (r)	3	5.3533	5.9100	5.3800	0.6767	0.7933
Families (f)	135	76.5636***	19.0947***	8.7253***	12.4602***	2.1853***
Error (E)	405	2.4094	1.1166	0.5438	0.4252	0.2839
Components						
$\acute{o}_f^2 \pm SE$		18.5386 (88) ± 2.3131	4.4945 (80) ± 0.5771	2.0454 (79) ± 0.2637	3.0088 (88) ± 0.3764	0.4754 (63) ± 0.0662
$\acute{o}_e^2 \pm SE$		2.4094 (12) ± 0.1689	1.1166 (20) ± 0.0783	0.5438 (21) ± 0.0381	0.4252 (12) ± 0.0298	0.2839 (37) ± 0.0199
$\acute{o}_{Ph}^2 = \acute{o}_f^2 + \acute{o}_e^2$		20.9480	5.6111	2.5892	3.4340	0.7593
$\acute{o}_{Ph1}^2 = \acute{o}_f^2 + \acute{o}_e^2/k$		19.1409	4.7737	2.1814	3.1151	0.5464
$\acute{o}_{Ph1} = ?\acute{o}_{Ph1}^2$		4.3750	2.1849	1.4770	1.7650	0.7392
GCV (%)		22.0	25.8	14.6	26.3	20.3

Table 2. ANOVA, variance components ($\acute{o}^2$) (percents in brackets), standard errors (SE) and genetic coefficient of variation (GCV) for 136 open-pollinated stone pine families.

Family[1]	H.6	h.6	RCD.6	TNB.6	TNBAL.6
1	35.0	16.3	15.6	13.0	5.6
2	34.6	15.3	13.8	11.5	5.3
3	31.8	14.8	13.6	10.7	5.1
4	30.6	13.6	13.0	10.1	4.9
5	30.4	13.6	12.7	10.0	4.9
6	28.1	13.4	12.5	10.0	4.9
7	27.6	11.7	12.4	9.7	4.8
8	27.3	11.4	12.3	9.6	4.7
9	25.7	11.1	12.2	9.6	4.7
10	25.2	11.0	12.2	9.4	4.6
X_1	29.6	13.2	13.0	10.4	4.9
127	14.2	5.5	8.0	4.3	2.5
128	14.1	5.5	8.0	4.3	2.4
129	14.0	5.4	7.9	4.3	2.4
130	13.9	5.3	7.9	4.3	2.4
131	13.7	5.1	7.9	4.2	2.3
132	13.7	4.8	7.8	4.2	2.3
133	13.5	4.6	7.6	4.1	2.3
134	13.1	4.4	7.6	4.0	2.2
135	12.7	4.2	7.2	3.8	1.9
136	11.5	4.1	7.2	3.5	1.8
X_2	13.4	4.9	7.7	4.1	2.2
X	19.6	8.2	9.8	6.6	3.5
D1(%)	120.7	170.6	69.3	152.6	120.3
D2(%)	51.3	61.8	32.9	56.5	43.3

Table 3. Ranking of the 10 best and the 10 poorest stone pine families based on nursery performance

Legend:

Dl = differences (%) between mean of the best (X_1) and the poorest (X2) group of the 10 families;

D2 = differences (%) between mean of the best group (Xi) and the test mean (X);

[1] The best and the poorest families were not the same for every trait.

collar diameter and 88 % for total number of branches. Therefore, a significant contribution of genetic variance was noted not only for total height but for the other traits as well.

The data suggest that additive genetic control is high in all traits. Rather than relying on a constructed F-test, Snyder & Namkoong (1978) recommended that the magnitude of a variance component be compared with its standard error. The variance component is deemed to be important if it is estimated to have a standard error less than half the magnitude of the component. In this experiment, the variance components for all traits had standard errors about seven times lower than the estimated components themselves (Table 2, line 5). This indicates that the genetic variances and heritabilities were reliable and that a selective breeding program using additive variation will be effective in improving any tested trait.

In summary, because the amount of variation was considerable an improvement program with stone pine would be practical.

Genetic and phenotypic correlations

Highly significant (p<0.001) phenotypic correlations were found among all but two traits (Table 4). Genetic correlations among growth characteristics, i.e. total and annual height growth and root collar diameter were high or very high ranging between 0.804 and 0.969. These correlations suggest that selection for one trait should lead to strong positive indirect responses in the others. Both total height and diameter at root collar were moderately and highly associated with the total number of branches, with genetic correlations ranging from 0.571 to 0.713. Phenotypic correlations for the same traits were highly significant (p<0.001) and positive. Selection for total height growth or growth in root collar diameter should lead to an indirect increase in the total number of branches, but this is a negative feature of trees because an increased number of branches means lower wood quality. Therefore, reduction in the incidence of the total number of branches in the next generation is likely to be achieved most readily by selection against this trait. Consequently, the breeder should act towards breaking this undesirable positive correlation and to select in favour of fast-growing trees with a small number of branches. Similar genetic correlations have been reported for the full-sib family test (Blada 1999).

Heritability

Table 5 presents a summary of the heritability estimates and their 95% confidence intervals for the open-pollinated families. All estimates of heritability in this study may be somewhat upwardly biased because the experiment was restricted to a single nursery where family-site interactions were not accounted for. Since the additive genetic variance of all analysed traits was high, family narrow-sense heritabilities were also high, ranging between 0.870 and 0.968. The heritability estimate for root collar diameter was 0.938 and was about the same magnitude as the corresponding estimates for growth in height. Similarly, high estimates were obtained for annual height growth, total number of branches and total number of buds around the leader bud. High heritability has also been

observed in the *P. cembra* full-sib progeny test carried out in the same nursery (Blada 1999). All estimates calculated here fell within the 95% confidence interval suggesting their reliability.

Traits	h.6	RDC.6	TNB.6	TNBAL.6
H.6	0.969 / 0.937***	0.881 / 0.830***	0.571 / 0.559***	0.703 / 0.622***
h.6		0.804 / 0.783***	0.422 / 0.432***	0.660 / 0.638***
RCD.6			0.713 / 0.674***	0.715 / 0.622***
TNB.6				0.354 / 0.333***

Table 4. Genetic correlations (upper line) and phenotypic correlations (lower line) (Df = 134). *** p < 0.001

Traits	hf^2(CI)	AG (%) when selecting best 30,35 40 and 45 families out of 136 tested			
		30	35	40	45
H.6	0.968 (0.960 - 0.975)	28.8	26.8	25.1	23.4
h.6	0.941 (0.922 - 0.951)	33.4	31.1	29.1	27.2
RCD.6	0.938 (0.922 - 0.951)	18.8	17.6	16.4	15.3
TNB.6	0.966 (0.958 - 0.973)	34.4	32.1	30.0	28.0
TNBAL.6	0.870 (0.838 - **0.898)**	25.2	23.5	22.0	20.5

Table 5. Family narrow-sense heritability estimates (h f)with 95 % confidence interval (CI) and expected genetic gain (AG) at family level.

In conclusion, all analysed traits were under strong genetic control and thus, appeared quite amenable to genetic selection.

Expected genetic gain

Table 5 presents estimates of gain, as a percentage of the nursery test mean, which might be expected in growth and other traits after one generation of selection. If the best 30, 35,

40 or 45 of the 136 families were selected and used in a planting program, genetic gains in total height growth and diameter at root collar of 28.8%, 26.8%, 25.1% and 23.4% and 18.8%, 17.6%, 16.4% and 15.3%, respectively could be expected. Similar genetic progress can be made in the other traits tested. This genetic gain could result in substantial returns if large planting programs are developed. These results suggest that growth improvement through family selection in slow-growing stone pine is possible. Increased use of stone pine may lead to a situation where fast-growing trees become commercially valuable.

Implications for breeding

As stated earlier, stone pine is a very slow-growing species. However, according to our previous report (Blada 1999) and to the nursery test results presented in this study, growth traits of stone pine are under strong genetic control. Consequently, selection on the basis of progeny performance in the nursery test could provide substantial improvement in diameter, height and total number of branches. Improving height and diameter growth in stone pine is the main objective to be achieved. Therefore, action will concentrate on the production of improved seed for operational planting, based on the results acquired from this six-year nursery test of 136 half-sib progenies. This population was divided into two equal parts. One part is already planted in field trails to be used for estimation of genetic variation including juvenile-mature genetic correlations. The other half of the population will be used for seed orchard development according to Zobel & Talbert (1984) recommendations.

After field testing the breeding strategy will be improved according to the new estimated genetic parameters. The objective is to incorporate the early testing procedures into an operational improvement program.

Two types of production seed orchards are planned:

(i) A clonal seed orchard will be developed using as ortets the best 45 female trees; selection was based on the height and diameter performances of their open-pollinated families (Figure 1). The commercial Fl seedlings will be planted in regions to which they are adapted, e.g., on sites relatively similar to those of the wild female parents. Taking into account our previous observations (Blada 1990, unpublished data), this clonal seed orchard will have the first seed crop about seven years after grafting.

(ii) A seedling seed orchard will be established by planting the fastest growing seedlings selected from the best 45 families out of the 136 tested, i.e., the best offspring of the trees planted in the clonal seed orchards. This is a first generation seed orchard. To maintain a large genetic base, 80 seedlings of each family will be planted at 3 x 3 m, requiring an area of 3.2 ha. The first commercial F2 crop is expected to be available about 20 years after planting.

By planting improved material from the two types of seed orchards, significant genetic gains should be obtained. In estimating these gains, one can use the procedures indicated by Namkoong et al. (1966); and Shelbourne (1992).

Fig.1 - Regression between total height growth and root collar diameter
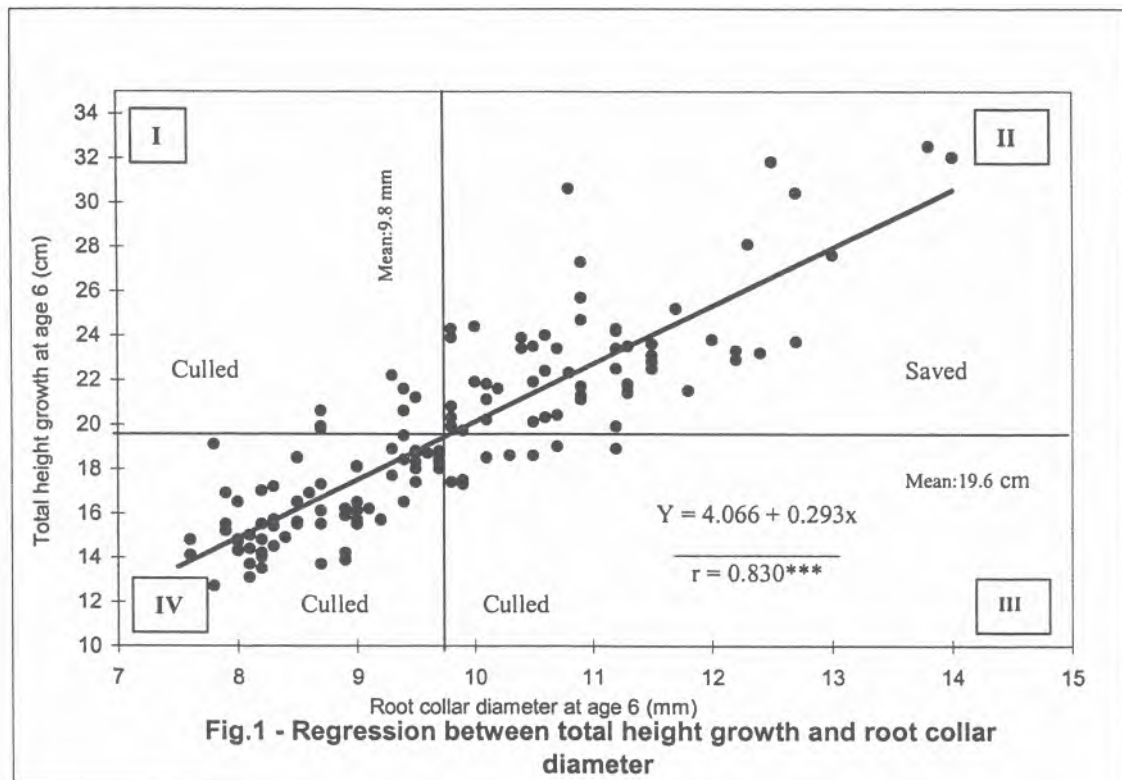
If the best 45 tested progenies are used directly in operational planting, a genetic gain in total height of 23.4% could be expected (Table 5, row 1).

The provenance test (Blada 1997a) demonstrated that the improvement of growth in height and diameter by provenance selection is also possible even if the species is a very slow growing one.

It should be noted that our decision to utilize early selection to develop production seed orchards after only six years of testing was encouraged by similar work reported by others. For example, Lambeth et al. (1983) suggested that most selection in loblolly pine *(Pinus taeda* L.) is currently carried out between ages five and ten years. Also, Lowe & Van Buijtenen (1989); and Bridgwater & McKeand (1997) were in favour of early selection.

CONCLUSIONS

Although stone pine is a very slow-growing species, high genetic variation among half-sib families in growth traits and total number of branches was found.

The additive genetic variation detected in this breeding population can be incorporated into an operational program or as a base for an advanced breeding population.

Genetic and phenotypic correlations suggest that correlated responses for growth traits and total number of branches should be obtained through indirect selection.

The high variability of the material and comparatively high heritability estimates showed that consistent genetic gain in growth and total number of branches is possible.

Results of this experiment indicated that early height growth could be used as an early testing trait for stone pine; consequently, early evaluation trials will permit making crosses among the best parent trees after only a few years of testing.

By using results of this early testing, corroborated with previous results, an operational improvement program for stone pine was developed.

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