Diallel Crossing in *Pinus cembra*

III. Analysis of Genetic Variation at the Nursery Stage

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(Received 23rd February 1999)

Abstract

A 10 x 10 full diallel was made in a native population of stone pine *(Pinus cembra* L.) from high elevation, to provide information on the genetic variation and inheritance of important breeding traits. In October 1991, seeds were sown in individual polyethylene pots, in spruce humus. The families, including self-pollinated parents were arranged in a randomized complet block design with four replications and 12 seedlings per plot. Fifteen traits were measured during nursery testing. In addition, weight of 100 seeds and cotyledon number were assessed. Computer analysis of a balanced modified full diallel using the SAS program produced the results presented below. The most important result was that significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) differences occurred in all 17 traits for general and specific combining abilities as well for reciprocal effects. Maternal effects were significant in 13 traits, including diameter and total height. This suggests that the traits are controlled by nuclear (additive and non-additive) and extranuclear genes, and by nuclear x extranuclear gene interactions. Additive and non-additive genetic variances accounted for 25% and 27% of the total for height at age six and 14% and 22% for root collar diameter at the same age, respectively. These figures indicate that both variances were important for growth traits within the tested population. Parents were found with significant general combining effects for growth and other traits. Narrow-sense heritability estimates at family level ranged between 0.150 to 0.675 for cotyledon number and lamma shoots, respectively with 0.453 for total height at age six (H.6). By selecting the best 10 to 40 families, a genetic gain in H.6 of 10.6% to 5.5% could be achieved. An extra genetic gain could also be achieved by individual within family selection. Improvement of growth using both additive and non-additive gene effects should be possible.

Key words: Pinus cembra, diallel cross, additive variance, non-additive variance, combining ability, genetic correlation, heritability, genetic gain.

Introduction

The natural distribution area of stone pine is restricted to high elevations of the Alps and Carpathians (HOLZER, 1963; CRITCHFIELD and LITTLE, 1966). In the Alps, the species grows between 1200 m and 2500 m elevation (CONTINI and LAVARELLO, 1982) but the main zone is between 1500 m and 2000 m (HOLZER, 1975). In Romania, stone pine ranges between 1350 m and 1880 m elevation in the northern Carpathians (GUBESH, 1971) and between 1350 m and 1986 m in the southern Carpathians (BELDIE, 1941; TATARANU and COSTEA, 1952; OARCEA, 1966). However, the species was recently found at 2050 m elevation in the Retezat Mts in the southern Carpathians (BLADA, unpublished data).

Stone pine is important for: (1) reforestation of the subalpine zone to raise the timberline to its former limit, where it plays a leading role in slowing watersheds, stabilizing avalanche areas and reducing the effects of flash floods (HOLZER, 1972a, 1975); (2) creation of mixed spruce-larch-cembra stands at high elevations in order to increase windbreak resistance (BLADA, 1996); (3) its dense-brown-reddish wood is useful for handicrafts (CONTINI and LAVARELLO, 1982); (4) its high resistance to blister-rust caused by *Cronartium ribicola* FISCH. ex RABENH. (BINGHAM, 1972; HOLZER, 1975; HOFF et al., 1980; BLADA, 1987, 1994); (5) for landscaping purposes due to its conic-oval shape when grown as single trees (BLADA, 1996, 1997b).

Insofar as it can be determined very little breeding within stone pine has been reported; the main experiments have on cone and seed studies (ROHMEDER and ROHMEDER, 1955; NATHER, 1958; HOLZER, 1972b; BLADA and POPESCU, 1994), provenance testing (BLADA, 1997a), half-sib family testing (HOLZER, 1975; BLADA, 1996), full-sib family testing (BLADA, 1995, 1997b) and interspecific hybridization (BLADA, 1987, 1994).

A genetic improvement programme with stone pine has been launched in Romania (BLADA, 1990, 1995) with the following objectives: (1) phenotypic selection of parents in natural populations; (2) testing provenances and half-sib families; (3) intra and inter specific crosses; (4) full-sib families testing with estimation of main genetic parameters and selection of the best combiners and families; (5) seed orchards establishment with the best combiners for both mass seed production and as a base population for advanced- breeding generation. Five papers have been previously published as part of this programme (BLADA and POPESCU, 1994; BLADA, 1994, 1995, 1997a and b), and this paper is the sixth. The objective of the paper was to provide early information about genetic variation and inheritance of important traits useful in breeding stone pine.

Materials and Methods

Initial material and mating design

The 10 parent trees used in the crossing scheme *(Table 3)* were chosen from an old natural population at Gemenele located in the Retezat Mountains of Southern Carpathians, at about 1800 m elevation. Reproductive fertility was taken into consideration in parent selection in order to obtain the necessary number of flowers for pollination. The flowers were isolated in paper bags prior to local pollen dissemination. Fresh pollen was used for all crossings performed in July 1989. A full-diallel mating design according to GRIFFING's (1956) Method 1 was carried out.

Progeny test and experimental design

One hundred seedlots from the controlled crossings were collected in October 1990. Before sowing, the filled seeds were separated from empty seeds immersing each seedlot in 90% alcohol. This procedure ensured that only full seeds were sown; but, from a statistical standpoint, it may have skewed the data by decreasing the environmental error variance and increasing other components of phenotypic variance, such as SCA and reciprocal variances.

Two seeds per pot, i.e. 96 seeds per family, were sown in November 1990 in polyethylene pots (22 cm x 18 cm) in spruce humus. The second seedling was removed after germination.

Based on results of previous local experiments (BLADA, 1996) sowing stone pine seed during the autumn allowed the seed to develop embryos and to germinate the next spring eliminating the complicated, costly and risky 180 days of stratification recommended by KRIEBEL (1973). Since sowing was done during autumn, control measures for seed predation by mice in the nursery beds were necessary.

After sowing, the seeded pots were placed in nursery beds and arranged in a randomized complete block design. Each of the 100 families was represented by a 12 seedling plot in each of 4 blocks. The seedlings were kept in pots throughout the 6 years testing period.

Measurements

Fifteen traits were measured when the progenies were 2 to 6 years old. In addition, the weight of 100 seeds of each family was measured prior to sowing and cotyledon numbers were counted after seed germination *(Table 1)*. Lamma shoots were scored using a 5 point scale: 1 = no lamma shoots; 2 = only a small number of new needles present; 3 = slight flushing on leader and secondary branches; 4 = leader and/or branches with 1 cm to 2 cm growth; 5 = leader and/or branches with more than 2 cm of growth. Plot means comprised basic data for statistical analysis.

Statistical analysis

Although initially a full diallel mating design was used, the analysis was performed according to the modified full diallel mating design where the self pollinated parents were excluded; such analysis leads to unbiased estimates. However, the self pollinated parents were included in the material grown in the experiment so that comparison of croses with the selfs could be made in other analyses.

The mathematical model for analysis was a combination of HAYMAN (1954) and GRIFFING's (1956) models, such as:

$$x_{kij} = u + b_k + g_i + g_j + s_{ji} + m_i - m_j + r_{ij} + e_{kij} \qquad (1)$$

where x_{kij} = the mean performance in k-th block of the i-th parent mated to the j-th parent; u = the general mean; b_k = the effect of the k-th block; g_i and g_j = the general combining ability effects for the i-th and j-th parents, respectively; s_{ij} = the specific combining ability effect for the cross between the i-th and j-th parents so that $s_{ij} = s_{ji}$; m_i and m_j = the maternal effects of the i-th and j-th parents; r_{ij} = the difference caused by the direction of the cross between i-th and j-th parents, such that $r_{ij} = -r_{ji}$; e_{kij} = the random error.

Plot means of the 17 measured traits were analysed using the least – squares method by means of the computer DIALL programme prepared by SCHAFFER and USANIS (1969). The analysis of balanced modified full diallel according to GRIFFING's (1956) Method 3, was based upon the random model assuming that the parents were a random sample from a random mating population. This assumption makes possible estimates of the additive and non-additive genetic variance of the parent population.

The model of analysis of variance, expected mean squares and formulas for estimating the variance components are listed in *table 2*. Since data on individual seedlings was available, a separate analysis was performed in order to estimate the within plot variance (σ^2_w).

Standard errors (SE) of variance components were computed with the formula given by ANDERSON and BANCROFT (1952):

$$SE(\sigma^2_j) = \sqrt{\sum_i \frac{2a^2_i (MS_i)^2}{d.f_i + 2}} \qquad (2)$$

where : a_i are the coefficients of the inverse matrix of expected mean squares used to estimate the j-th variance component.

Table 1. – Measured traits.

Traits	Units	Symbol
100 seed weight	g	100 SW
Cotyledon number	No.	CN
Total height at ages 2, 3, 4, 5 and 6	cm	H.2, H.3, H.4, H.5, H.6
Annual height increment at ages 4, 5, and 6	cm	h.4, h.5, h.6
Diameter at root collar at ages 4, 5, and 6	mm	DRC.4, DRC.5, DRC.6
Number of buds arround the leader bud at age 6	No.	NBAL.6
Total number of branches at age 6	No.	TNB.6
Lamma shoots at ages 4 and 6	1...5	LS.4, LS.6

Table 2. – Analysis of variance of modified full diallel, random effects model, in a randomized block layout in one environment.

Source	Df	MS	E (MS)[1)]	F-test
Rep.	k-1	MS_{Rep}	$\sigma^2_w/n+\sigma^2_p + p(p-1)\sigma^2_{Rep}$	
GCA	p-1	MS_{GCA}	$\sigma^2_w/n+\sigma^2_p + 2k\,\sigma^2_{SCA} + 2k(p-2)\,\sigma^2_{GCA}$	
SCA	p(p-3)/2	MS_{SCA}	$\sigma^2_w/n+\sigma^2_p + 2k\,\sigma^2_{SCA}$	
Mat.	p-1	MS_{Mat}	$\sigma^2_w/n+\sigma^2_p + 2k\,\sigma^2_{Rec} + 2kp\sigma^2_{Mat}$	
Rec.	(p-1) (p-2) / 2	MS_{Rec}	$\sigma^2_w/n+\sigma^2_p + 2k\,\sigma^2_{Rec}$	
Error	$(k-1)(p^2-p-1)$	MS_E	$\sigma^2_w/n+\sigma^2_p$	
Within plot	kp(p-1) (n-1)	MS_W	σ^2_w	

1) $\sigma^2_w = MS_w$; $\sigma^2_e = MS_E$; σ^2_P = plot error = $\sigma^2_e - \sigma^2_w / n$; $\sigma^2_{Rec} = (MS_{Rec} - MS_E) / 2k$; $\sigma^2_{Mat} = (MS_{Mat} - MS_{Rec}) / 2kp$; $\sigma^2_{SCA} = (MS_{SCA} - MS_E) / 2k$; $\sigma^2_{GCA} = (MS_{GCA} - MS_{SCA}) / 2k(p-2)$; $(MS_{Rep} = (MS_{Rep} - MS_E) / p(p-1)$

p = parents; n = seedlings per plot; k = replications.

The component of variance σ^2_{GCA} was used to estimate the variance in general combining ability among all of the parents in this experiment and is used as an estimator of $1/4\sigma^2_A$. It is assumed that all epistatic components of genetic variance were insignificantly small. The component σ^2_{SCA}, the estimated variance in specific combining ability, is an estimator of $1/4\sigma^2_D$ (with the same assumptions). Therefore, an estimator of the additive genetic variance is $4\sigma^2_{GCA}$ and an estimate of the dominance genetic variance is $4\sigma^2_{SCA}$ (BECKER, 1984).

To estimate effectiveness of selection for early traits, three types of heritabilities were calculated.

The first heritability is the one commonly used for estimating the ratio of genetic (additive + non-additive) to total variance which is appropiate for estimating gain from selection among families when they are vegetatively propagated. This is broad-sense heritability and is estimated by:

$$h^2_1 = \frac{2\sigma^2_{GCA} + \sigma^2_{SCA}}{\sigma^2_{Ph1}} = \frac{2\sigma^2_{GCA} + \sigma^2_{SCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rec} + \sigma^2_e/k} \qquad (3)$$

where k = number of bloks.

Mass selection genetic gain (FALCONER, 1981) was estimated by:

$$\Delta G_1 = i_1 h^2_1 \sigma_{Ph1} \qquad (4)$$

where i_1 is the selection intensity taken from BECKER (1984); σ^2_{Ph1} is the phenotypic standard deviation which refers to family means.

The second heritability is appropiate for estimating gain from selection among half-sib families when they are sexually propagated. This is a narrow-sense heritability at familiy level, and is estimated by:

$$h^2_2 = \frac{2\sigma^2_{GCA}}{\sigma^2_{Ph1}} = \frac{2\sigma^2_{GCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rec} + \sigma^2_e/k} \qquad (5)$$

and gain from half-sib family selection is estimated by:

$$\Delta G_2 = i_1 h^2_2 \sigma_{Ph1} \qquad (6)$$

The third heritability is the one commonly used for estimating genetic gain from mass selection among randomly placed seedlings. This heritability was estimated by:

$$h^2_3 = \frac{4\sigma^2_{GCA}}{\sigma^2_{Ph2}} = \frac{4\sigma^2_{GCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rcc} + \sigma^2_p + \sigma^2_w} \qquad (7)$$

where: σ^2_w = within plot variance; σ^2_p = plot error = $\sigma^2_e - \sigma^2_w/n$; n = seedlings per plot.

The mass selection gain was estimated by:

$$\Delta G_3 = i_2 h^2_3 \sigma_{Ph2} \qquad (8)$$

where: i_2 = the selection differential for individual seedling selection; σ_{ph2} = phenotypic standard deviation and it refers to individual tree values.

If the parents of the best families are to be selected and intermated, i_1 should be doubled to give the expected genetic gain, such as:

$$\Delta G_4 = 2 i_1 h^2_2 \sigma_{Ph1} \qquad (9)$$

Results

Genetic variation

Performance comparisons between control-cross-pollinated (CP) – and control-self-pollinated (SP) progenies from the same parents are listed in *table 3.* Data on cross-pollination represent the mean female values over all males used in the mating design. These figures showed that throughout the life of the experiment, selfed families differed greatly in all but one (CN) trait from outcross families. Differences between the two categories of families were large. Thus 100 SW among the 10 parents in this diallel ranged from 16.6 g to 20.9 g for control-cross-pollinated and from 14.8 g to 18.4 g for control-self-pollinated families *(Table 3).* This variation caused significant maternal (extranuclear) gene effects, throughout the nursery period, in several traits, including growth ones. Means of the outcrossed families exceeded selfed ones by 52%, 59%, 39%, 89% and 43% in H.6, h.6, DRC.6, NBAL.6 and TNB.6, respectively. Therefore, it is clear that SP families performed significantly less well than CP ones. Inbreeding depression is expected in a naturally outcrossing species such as stone pine.

Variance components

Perhaps, the most prominent feature of this experiment was that diallel analysis of plot means from the 90 families showing significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) variation in general (GCA) and specific (SCA) combining abilities and in reciprocal effects for all traits except H.2. Significant or highly significant maternal effects also occurred in 13 out of 17 traits (*Table 4,* upper part). This suggests that the respective traits, including growth ones, were controlled by nuclear (additive and non-additive) and extranuclear genes and by nuclear x extranuclear gene interactions.

Variance component estimates, standard errors and dominance ratios are listed in *table 5.* The whole series of growth measurements indicate a progressive increase with age of the σ^2_{GCA} within σ^2_{Ph}. Thus, σ^2_{GCA} of the total height, increased from 2% at age two to 25% at age six. Also, σ^2_{GCA} of the root collar diameter increased from 8% at age four to 14% at age six. Similarly, σ^2_{SCA} of the total height growth ranged from 15% at age two to 27% at age six, whereas the σ^2_{SCA} of the root collar diameter was almost constant between age four and age six, i.e. 19% and 22%, respectively.

The diallel analysis indicated that GCA and SCA were important sources of variation. Dominance, σ^2_{SCA} exerted a greater influence on 10 out of 17 tested traits as evidenced by the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratios. However additive variance was higher in the remaining seven. The magnitude of the ratios, listed in *table 5* suggest that additive effects may be almost as important as non-additive ones in this very young stone pine population. Consequently, breeding strategy can employ both additive as well as non-additive variations, indicating that considerable progress under direct selection is possible.

Figure 1 shows a continuous increase of σ^2_{GCA}, σ^2_{SCA}, and σ^2_{Rec}, from H.2 to H.6, while contrast, σ^2_e displayed a more or less continuous decline in importance within the phenotypic

Table 3. – Performance comparisons between control-cross-pollinated (CP) and control-self-pollinated (SP) progenies from the parents.

Parents	100.SW (G)		CN (No.)		H.2 (cm)		H.3 (cm)		H.4 (cm)		H.5 (cm)		H.6 (cm)		h.4 (cm)		h.5 (cm)	
	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP
2	18.7	17.0	9.8	9.7	4.2	4.4	6.2	4.9	9.8	7.1	15.2	10.3	23.7	15.1	3.9	1.8	6.8	4.1
3	18.2	16.4	9.9	9.8	4.2	3.4	6.0	4.7	9.4	7.0	14.4	9.7	22.0	13.6	3.6	1.6	6.4	3.2
45	19.0	17.8	10.1	9.9	4.1	3.8	5.9	5.2	9.1	7.2	13.9	9.7	21.3	12.8	3.4	2.2	6.1	3.5
50	20.3	18.2	9.8	8.2	4.3	3.9	6.3	5.9	9.9	8.2	15.4	12.3	23.9	15.6	3.9	2.5	7.0	4.7
205	19.4	17.3	10.2	11.1	4.3	3.8	6.2	5.6	10.0	7.8	15.4	10.9	24.2	14.4	3.9	2.2	7.0	3.9
206	18.3	14.8	10.0	9.9	4.4	3.9	6.3	5.5	9.8	7.5	15.6	10.6	24.9	15.5	3.9	2.4	7.1	4.1
209	16.6	14.9	9.9	9.8	4.2	4.5	6.1	6.2	10.1	9.6	15.6	14.4	23.4	18.0	4.2	3.9	7.1	6.4
X	20.9	17.3	10.1	10.1	4.5	4.2	6.7	5.9	10.4	9.0	16.6	13.2	26.2	20.3	4.3	3.5	7.5	5.6
Y	19.7	18.4	9.9	10.1	4.3	4.1	6.3	5.8	3.7	7.7	15.2	11.4	23.8	17.2	3.9	2.6	6.8	4.5
Z	17.6	15.7	9.9	10.4	4.3	3.8	6.3	5.5	10.4	8.0	16.7	12.0	25.7	14.6	4.4	2.6	7.7	4.6
Mean	18.9	16.8	10.0	9.9	4.3	4.0	6.2	5.5	9.9	7.9	15.4	11.5	23.9	15.7	3.9	2.5	6.9	4.6
SUP[1]%	13		1		8		15		25		35		52		56		5.0	

Parents	h.6 (cm)		DRC.4 (mm)		DRC.5 (mm)		DRC.6 (mm)		NBAL.6 (No.)		TNB.6 (No.)		LS.4 (1....5)		LS.6 (1....5)	
	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP	CP	SP
2	10.5	6.4	4.9	3.3	7.3	4.9	10.5	7.1	3.8	2.3	4.4	2.6	3.4	3.8	2.4	3.5
3	9.8	5.7	4.9	3.2	7.2	4.6	10.6	6.6	3.5	1.4	3.9	1.5	3.4	3.8	2.2	2.7
45	9.1	4.7	4.8	3.9	6.9	5.0	10.3	7.6	3.4	2.0	4.3	2.9	3.3	3.7	2.4	3.4
50	10.7	6.2	4.9	4.0	7.1	5.5	10.5	7.0	3.1	1.3	4.2	3.0	3.7	4.3	2.8	3.9
205	10.2	5.0	5.1	3.9	7.5	5.3	11.1	8.3	3.8	2.2	4.4	3.6	4.1	4.8	2.8	4.4
206	11.2	6.8	4.9	3.6	7.3	5.0	10.5	7.2	3.6	1.6	4.0	2.5	3.0	2.6	2.2	2.7
209	10.1	7.1	4.9	4.6	7.2	6.8	10.4	8.6	3.7	2.6	4.9	5.2	3.8	4.2	2.7	3.7
X	11.7	9.2	5.4	4.9	8.0	6.9	11.5	9.4	3.5	2.2	4.8	3.9	2.9	3.2	2.2	2.0
Y	10.5	7.6	5.0	4.3	7.3	5.4	10.4	7.7	3.6	1.9	4.0	2.3	2.6	2.5	1.8	1.5
Z	11.0	6.9	4.9	3.6	7.3	5.0	10.5	6.8	3.9	1.9	4.1	2.8	4.0	4.9	2.7	4.2
Mean	10.5	6.6	5.0	3.9	7.3	5.4	10.6	7.6	3.6	1.9	4.3	3.0	3.4	3.8	2.4	3.2
SUP[1]%	59		28		35		39		89		43		-11		-25	

[1]) SUP(%) = superiority of CP relative to SP.

Table 4. – Analysis of variance of modified full diallel of the traits at different ages.

Source of variation	Df.	Mean squares								
		100.SW	CN	H.2	H.3	H.4	H.5	H.6	h.4	h.5
A. Full sib families										
Rep.	3	2.966	0.241	7.510	0.721	3.071	8.346	5.407	1.241	4.341
GCA	9	133.113°°°	1.391°	0.751	3.763°	12.865°°°	55.044°°°	182.958°°°	7.564°°°	17.522°°°
SCA	35	27.715°°°	0.479°	0.451°°°	1.303°°°	2.253°°°	6.440°°°	23.779°°°	0.991°°°	2.095°°°
Mat.	9	93.261°°°	2.250°°	1.038	2.971°°	6.809°°	22.411°	49.702°	3.522°°	6.467°
Rec.	36	23.026°°°	0.661°°°	0.580°°°	0.973°°°	2.214°°°	7.704°°°	21.540°°°	0.999°°°	2.289°°°
Error	266	0.410	0.267	0.146	0.190	0.617	1.964	1.885	0.356	0.771
Within plot	3960	–	1.259	0.695	0.971	1.912	6.815	16.167	1.035	2.775
B. Selfed families										
Rep.	3	1.290	0.330	0.630	0.150	0.190	2.030	1.100	0.040	0.830
Families	9	25.090°°°	0.810°°	0.440	0.840°°	2.840°°°	9.490°°°	20.280°°°	2.040°°°	3.760°°°
Error	27	0.620	0.200	0.210	0.220	0.320	0.730	3.920	0.150	0.380
Within plot										

Source of variation	Df	Mean squares							
		h.6	DRC.4	DRC.5	DRC.6	TNB.6	NBAL.6	LS.4	LS.6
A.Full sib families									
Rep.	3	2.880	1.163	3.422	1.865	3.542	0.459	1.576	0.434
GCA	9	43.882°°°	2.439°°	6.279°°°	12.153°°°	8.761°°°	4.750°°°	19.361°°°	8.886°°°
SCA	35	4.407°°°	0.697°°°	1.431°°°	2.425°°°	1.321°°°	0.609°°°	0.859°°°	0.641°°°
Mat.	9	8.487	2.009°	5.199°	6.572°	3.018°	0.723	1.023°	0.911
Rec.	36	4.187°°°	0.646°°°	1.417°°°	3.298°°°	1.039°°°	0.627°°°	0.457°	0.446°°°
Error	266	0.996	0.177	0.447	0.421	0.349	0.140	0.305	0.247
Within plot	3960	4.971	0.541	1.441	2.870	2.385	1.674	1.913	1.205
B. Selfed families									
Replications	3	0.34	0.160	0.320	1.170	1.020	0.110	0.290	0.290
Families	9	6.84°°°	1.190°°°	2.440°°°	3.180°	4.190°°°	0.660°°°	2.760°°°	3.640°°°
Error	27	1.22	0.150	0.210	1.020	0.230	0.130	0.300	0.630
Within plot									

Table 5. – Variance components (percents in brackets), standard errors and dominance ratios.

Components	Traits								
	100.SW	CN	H.2	H.3	H.4	H.5	H.6	h.4	h.5
σ^2_{GCA}	1.6514 (18) ±0.8951	0.0143 (4) ±0.0095	0.0047 (2) ±0.0052	0.0385 (8) ±0.0255	0.1663 (13) ±0.0863	0.7616 (18) ±0.3685	2.4941 (25) ±1.2255	0.1030 (16) ±0.0507	0.2417 (17) ±0.1173
σ^2_{SCA}	3.4211 (37) ±0.8079	0.0265 (7) ±0.0143	0.0381 (15) ±0.0132	0.1395 (28) ±0.0379	0.2049 (16) ±0.0660	0.5607 (13) ±0.1889	2.7433 (27) ±0.6934	0.0794 (12) ±0.0291	0.1659 (12) ±0.0616
σ^2_{Mat}	0.8806 (10) ±0.5032	0.0199 (5) ±0.0122	0.0057 (2) ±0.0057	0.0250 (5) ±0.0161	0.0576 (5) ±0.0370	0.1844 (5) ±0.1219	0.3530 (4) ±0.2729	0.0316 (5) ±0.0190	0.0524 (4) ±0.0352
σ^2_{Rec}	2.8364 (31) ±0.6625	0.0494 (13) ±0.0192	0.0543 (22) ±0.0167	0.0979 (20) ±0.0280	0.2003 (16) ±0.0641	0.7199 (17) ±0.2227	2.4650 (25) ±0.6201	0.0806 (12) ±0.0290	0.1904 (13) ±0.0664
σ^2_{e}	0.4098 (4) ±0.0354	0.2669 (71) ±0.0231	0.1461 (59) ±0.0126	0.1896 (39) ±0.0164	0.6174 (50) ±0.0533	1.9639 (47) ±0.1697	1.8849 (19) ±0.1628	0.3564 (55) ±0.0308	0.7708 (54) ±0.0666
σ^2_{Ph}	9.1993	0.3771	0.2489	0.4905	1.2465	4.1905	9.9403	0.6510	1.4212
σ^2_{W}	–	1.2595	0.6953	0.9708	1.9118	6.8154	16.1670	1.0349	2.7751
σ^2_{p}	–	0.1619	0.0882	0.1087	0.4581	1.3960	0.5378	0.2702	0.5395
$\sigma^2_{SCA} / \sigma^2_{GCA}$	2.1	1.9	8.1	3.6	1.2	0.7	1.1	0.8	0.7

Components	Traits							
	h.6	DRC.4	DRC.5	DRC.6	NBAL.6	TNB.6	LS.4	LS.6
σ^2_{GCA}	0.6185 (25) ±0.2936	0.0273 (8) ±0.0165	0.0760 (9) ±0.0423	0.1525 (14) ±0.0817	0.0649 (20) ±0.0318	0.1166 (17) ±0.0587	0.1099 (21) ±0.1094	0.1292 (28) ±0.0594
σ^2_{SCA}	0.4274 (17) ±0.1289	0.0651 (19) ±0.0204	0.1232 (15) ±0.0420	0.2511 (22) ±0.0708	0.0588 (18) ±0.0178	0.1218 (17) ±0.0387	0.0694 (14) ±0.0253	0.0497 (11) ±0.0189
σ^2_{Mat}	0.0539 (2) ±0.0470	0.0171 (5) ±0.0109	0.0474 (6) ±0.0281	0.0536 (5) ±0.0358	0.0012 (1) ±0.0043	0.0248 (4) ±0.0164	0.0071 (1) ±0.0056	0.0058 (1) ±0.0050
σ^2_{Rec}	0.4002 (16) ±0.1209	0.0588 (17) ±0.0187	0.1216 (15) ±0.0411	0.2354 (21) ±0.0663	0.0610 (18) ±0.0181	0.0866 (12) ±0.0301	0.0190 (4) ±0.0136	0.0249 (6) ±0.0131
σ^2_{e}	0.9960 (40) ±0.0860	0.1773 (51) ±0.0153	0.4473 (55) ±0.0386	0.4213 (38) ±0.0364	0.1404 (43) ±0.0121	0.3486 (50) ±0.0301	0.3053 (60) ±0.0264	0.2472 (54) ±0.0214
σ^2_{Ph}	2.4960	0.3456	0.8155	1.1137	0.3264	0.6984	0.5107	0.4565
σ^2_{W}	4.9710	0.5414	1.4408	2.8698	1.6743	2.3853	1.9131	1.2050
σ^2_{p}	0.5820	0.1322	0.3272	0.1822	0.0009	0.1498	0.1459	0.1468
$\sigma^2_{SCA} / \sigma^2_{GCA}$	0.7	2.4	1.6	1.6	0.9	1.1	0.6	0.4

variance; it was sharply reduced from 59% for H.2 to 19% for H.6. This decline supports the expectation that genetic estimates of the traits become more and more accurate with age. This assumption was also confirmed by the small standard errors of the variance component estimates *(Table 5)*.

Significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) maternal effects were found in 100.SW, CN, growth traits (H,h, and DRC), TNB.6 and LS.6 *(Table 4)*. This suggests that these traits were controlled by extranuclear genes. The largest maternal component of this study was 10% of the phenotypic variance (associated with 100.SW) whereas the smallest one of 1% was associated with NBAL.6 and LS.6. Maternal contribution of the growth traits did not exceed 6% of phenotypic variance. *Figure 1* shows that maternal variance (σ^2_{Mat}) curve exhibits a constant linear character. It requires a longer testing period in order to determine whether this variance maintains its significant participation in σ^2_{Ph}.

Reciprocal effects were highly significant ($p<0.001$) for all but one trait over the testing period *(Table 4)* suggesting nuclear x extranuclear gene interactions. Its contribution to the phenotypic variance was unexpectedly high. Its range of variation was between 16% and 25% for total height (H2 to H.6), 12% to 16% for annual height increment (h.4 to h.6) and 15% to 21% for diameter at root collar (DRC.4 to DRC.6) *(Table 5)*.

These results indicate that reciprocal effects are important in a breeding programme but material needs to be grown for a longer period to confirm this. The results provide support for using the diallel mating design for the estimation of GCA, SCA, maternal and reciprocal variances, even though it requires more effort and financial investment.

There was evidence that GCA, SCA, Mat. and Rec. variance components were associated with standard errors smaller than the estimates themselves, in all but two cases, thus making heritability estimates fairly reliable.

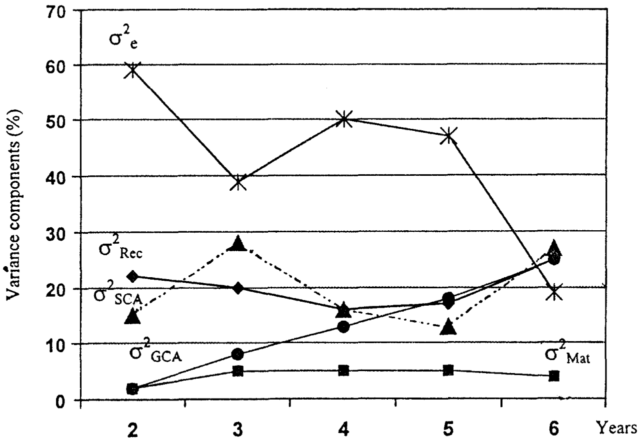

Fig. 1. – Changes in GCA, SCA, Mat and Rec and error variance for total height over 5 years.

Heritability

Estimates of the three types of heritabilities were listed in *table 6*. The greatest narrow-sense heritability on a family basis was 0.645 for LS.6 whereas the lowest one was 0.065 and was associated with H.2. This low heritability was expected since the contribution of σ^2_{GCA} to the σ^2_{Ph} was only 2% and its standard error was higher than the estimate itself *(Table 5)*.

Table 5 and *figure 2* present the change of variance components and heritabilities over time. Since the σ^2_{GCA} of total height progressively increased from age two (H.2) to age six (H.6), narrow-sense heritabilities at both family and individual level, increased accordingly, ranging from 0.065 to 0.453 and from 0.021 to 0.366, respectively *(Fig.2)*. In the same manner, the narrow-sense heritabilities of root collar diameter, for both family and individual level, increased from 0.228 to 0.321 and from 0.126 to 0.157 *(Table 6)*. It is obvious that (1) root-collar diameter heritabilities were lower than those for annual growth and total hight and (2) both family or individual heritabilities display the same progressive increase with age.

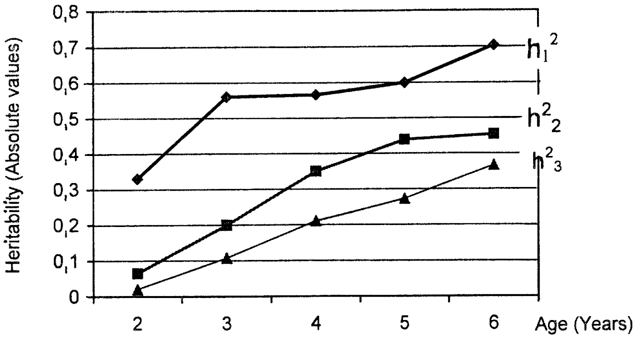

Fig. 2. – Heritability variation according to the seedling age, for total height.

As expected, broad-sense heritabilities at family level were greater than narrow-sense ones whereas individual narrow-sense heritabilities were the lowest. *Figure 2* displays an interesting parallel patern among the three heritability curves.

At present, narrow-sense heritabilities are of most use to tree breeders, but undoubtedly broad-sense ones will also be used as vegetative propagation methods and economical methods of producing specific crosses, such as supplemental mass pollination, become available.

In summary heritability estimates are high enough to ensure genetic progress in improving the main traits.

General combining ability

Parents used in this diallel were selected on the basis of fecundity only and therefore cannot be considered superior phenotypes but rather random samples used for assessment of genetic variation within the basic natural population.

General combinig ability effects (g.c.a.) calculated for each parent tree are listed in *table 7*. Both positive and negative significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) effects were found for most traits. Thus, parent X had the largest positive g.c.a effects for H.6 whereas parent Z was the second highest for the same trait. On the other hand, parents 3 and 45 had the largest negative values for H.6. Consequently, parents X and Z should be selected *(Fig.3)* for their high positive g.c.a effects and high breeding value in total height whereas parents 3 and 45, and some others should be rejected because of their high negative g.c.a. effects for the same trait. If two out of 10 randomly selected parent trees exhibited large positive g.c.a. effects for total height, then by extrapolation one may assume that 20% of trees within the basic natural population, could be selected as good combiners.

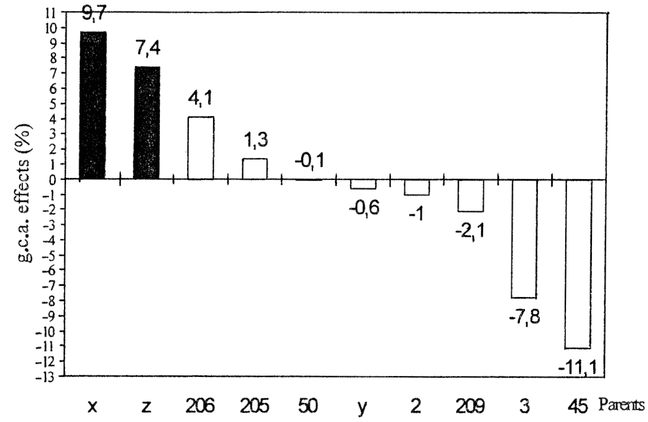

Fig. 3. – Adjusted general combining ability effects (%) for total height at age 6.

Table 6. – Estimates of phenotypic variances, phenotypic standard deviations and heritabilities.

Parameters	100.SW	CN	H.2	H.3	H.4	H.5	H.6	h.4	h.5
$\overline{X}$	18.6	10.0	4.3	6.2	9.9	15.4	23.9	3.9	6.9
σ^2_{Ph1}	10.5434	0.1911	0.1440	0.3868	0.9498	3.4792	11.0200	0.4867	1.0848
σ^2_{Ph2}	–	1.5458	0.8910	1.4189	3.1653	11.1916	27.2540	1.7027	3.4529
σ_{Ph1}	3.2471	0.4372	0.3795	0.6219	0.9746	1.8653	3.3196	0.6976	1.0415
σ_{Ph2}	–	1.2433	0.9439	1.1912	1.7791	3.3466	5.2205	1.3049	1.8582
$h^2_1=h^2_{bs}$	0.638	0.288	0.330	0.560	0.566	0.599	0.702	0.586	0.599
$h^2_2=h^2_{ns}$	0.313	0.150	0.065	0.199	0.350	0.438	0.453	0.423	0.446
$h^2_3=h^2_w$	–	0.037	0.021	0.108	0.210	0.272	0.366	0.242	0.280

Parameters	h.6	DRC.4	DRC.5	DRC.6	NBAL.6	TNB.6	LS.4	LS.6
$\overline{X}$	10.5	5.0	7.3	10.6	3.6	4.3	3.4	2.4
σ^2_{Ph1}	2.3676	0.2399	0.5560	0.9504	0.2859	0.5536	0.3916	0.4006
σ^2_{Ph2}	7.6720	0.8692	2.2122	3.8971	1.9260	3.0015	2.3740	1.6906
σ_{Ph1}	1.5387	0.4898	0.7455	0.9749	0.5347	0.7440	0.6258	0.6329
σ_{Ph2}	2.7698	0.9323	1.4874	1.9741	1.3878	1.7325	1.5408	1.3002
$h^2_1=h^2_{bs}$	0.703	0.499	0.495	0.585	0.660	0.641	0.738	0.769
$h^2_2=h^2_{ns}$	0.522	0.228	0.273	0.321	0.454	0.421	0.561	0.645
$h^2_3=h^2_w$	0.323	0.126	0.137	0.157	0.135	0.155	0.185	0.306

X = Trait mean

$\sigma^2_{Ph1} = 2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rec} + \sigma^2_P / k + \sigma^2_w / kn$; k = replications; n = seedlings / plot; $\sigma^2_{Ph2} = 2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{Mat} + \sigma^2_{Rec} + \sigma^2_P + \sigma^2_w$; $\sigma^2_P = \sigma^2_e - \sigma^2_w/n$; $h^2_1 = h^2_{bs} = (2\sigma^2_{GCA} + \sigma^2_{SCA}) / \sigma^2_{Ph1}$; $h^2_2 = h^2_{ns} = 2\sigma^2_{GCA} / \sigma^2_{Ph1}$; $h^2_3 = h^2_w = 4\sigma^2_{GCA} / \sigma^2_{Ph2}$; h^2_{bs}, h^2_{ns}, h^2_w = broad-sense, narrow-sense and within plot heritabilities, respectively.

Table 7. – Adjusted general combining ability effects.

Parent	Traits								
	100.SW	CN	H.2	H.3	H.4	H.5	H.6	h.4	h.5
2	-0,217	-0,154	-0,097	-0,012	-0,030	-0,179°	-0,236	-0.027	-0.111
3	-0,673°	-0,026	-0,074	-0.203	-0,469	-1,032	-1,869$^{\circ\circ\circ}$	-0.350	-0,512
45	0,127	0,107	-0,137	-0,340	-0,760°	-1,451°	-2,627$^{\circ\circ\circ}$	-0,552°	-0,863°
50	1,449$^{\circ\circ\circ}$	-0,185	0,031	0,110	0,027	0,053	-0,027	-0,033	0,035
205	0,552°	0,253	0,028	-0,005	0,098	0,048	0.317	0,005	0,020
206	-0,559°	0,045	0,078	0.046	-0,016	0,252	0.985	-0.059	0.189
209	-2,266$^{\circ\circ\circ}$	-0,020	-0,096	-0,153	0,212	0,164	-0,496	0,268	0.164
X	2,002$^{\circ\circ\circ}$	0,101	0,165	0,468$^{\circ\circ}$	0,559	1,086	2.328$^{\circ\circ\circ}$	0,401	0,549
Y	0,853$^{\circ\circ}$	-0,079	0,043	0,027	-0,119	-0,200	-0,143	-0,067	-0,191
Z	-1.308$^{\circ\circ\circ}$	-0,042	0,060	0,061	0.500	1,264	1.774$^{\circ\circ}$	0,415	0,724°

Parents	Traits							
	h.6	DRC.4	DRC.5	DRC.6	TNB.6	NBAL.6	LS.4	LS.6
2	0,004	-0,120	-0,058	-0,122	0,147	0,210	-0,073	-0,047
3	-0.697	-0,076	-0,080	-0,076	-0,355	-0.106	-0,005	-0.205
45	-1.358$^{\circ\circ\circ}$	-0.161	-0,392	-0,330	-0,006	-0.215	-0.153	-0,041
50	0,240	-0,048	-0.198	-0.161	-0,131	-0,498$^{\circ\circ}$	0.300	0,409°
205	-0,260	0,134	0,217	0,503	0,108	0.209	0,673$^{\circ\circ}$	0,398
206	0,760	-0,082	-0,031	-0,127	-0,292	0,050	-0,398	-0.194
209	-0.393	0.082	-0,133	-0.260	0,574°	0,091	0.374	0,311
X	1.192$^{\circ\circ}$	0,432°	0,655°	0,894$^{\circ\circ\circ}$	0,490°	-0,077	-0,513°	-0,280
Y	-0.017	0,046	0,009	-0,220	-0,342	-0,005	-0,812$^{\circ\circ\circ}$	-0,590$^{\circ\circ}$
Z	0.535	-0,045	0,009	-0.106	-0.188	0,344°	0,607$^{\circ\circ}$	0,242

Good combiners for total height, can be used for seed orchard establishment and for developing advanced generation breeding populations.

Correlations

Strong positive genetic correlations obtained *(Table 8)* between height and diameter traits imply that genetic gain in any of these traits even if selection was practiced on only one easy measurable trait, such as diameter. On the other hand, both low positive or negative correlations were found among all growth traits and any other trait from this experiment.

It is interesting to notice that 100.SW was moderately correlated only with root-collar diameter, i.e. DRC.4 to DRC.6.

These results need to be confirmed from long-term juvenille-adult correlations with additional traits of economic and ecologic values.

Selection and genetic gain

Selection is based upon the principle that genetic value of selected families or individuals will be better than the average value of individuals in the population as a whole (ZOBEL and TALBERT, 1984).

In this experiment, the selection can be made at family and individual level; individual selection refers to the best seedlings within families and best parents according to their g.c.a. effects.

The genetic gains that could be achived at both family and individual level are presented in *table 9* where, for instance, the gain for total height at age 6 (H.6) is, as follows:

– if the best 10, 20, 30 or 40 out of 90 tested full-sib families, were selected and generatively propagated, a genetic gain of 10.6%, 8.3%, 6.8% and 5.5%, respectively could be expected;

– if the best 5%, 10%, 15% or 20% individuals within the best families were selected, a genetic gain of 16.5%, 14.0%, 12.4% and 11.2%, respectively, could be achieved. At the same intensity of selection a lower gain in DRC.6 could be made, i.e. 6.0%, 5.1%, 4.5% and 4.1%, respectively;

– if the best 20 or 30 general combiners of 100 parents were selected and intermated and their progenies planted, a genetic gain in H.6 of 17.4% and 14.5%, respectively, could be obtained.

It is possible that all the above mentioned gains are underestimated because the parental selections were random with regard to any of the tested traits, including growth ones; for this reason the obtained gains relate to the initial (mother) population mean.

The gain at age six may be a good predictor of later results; however later age correlations, which are not available, will be more reliable for the final gain estimation.

The genetic gains, described above, can be multiplied for substantial returns in large planting programs.

Discussions

It should be noted that this was the first complete set of full-diallel families to be made in stone pine.

GCA and SCA variances

Results in this study indicate that σ^2_{SCA} was generally slightly greater than σ^2_{GCA} for total height, throughout the testing period. Conversely, σ^2_{GCA} consistently exceeded σ^2_{SCA} for annual increment in growth; this increment was more reliable than total height since the latter was stronghly influenced by transplanting and other early environmental effects. At the same time, σ^2_{GCA} of the total height progressively increased with age *(Fig.1)*, so that one may expect it will exceed σ^2_{SCA} in the coming years. But, at present, the magnitude of σ^2_{SCA} relative to σ^2_{GCA} for height, and some other traits, suggests that additive gene effects may be almost as important as non-additive gene action in very young stone pine progenies.

Maternal variance

The high variation in 100 SW among the 10 parents and their full-sib families resulted in significant maternal effects in most traits; including growth ones, suggesting extranuclear gene control. The maternal variance of total height (H.3 to H.6) was significant and the effects due to this source were constant over years, but relatively low throughout the testing period *(Fig. 1)*. However for annual height increment, which is the most reliable trait, the F-test significance at ages four to five disapeared at age six indicating non-durability of such effects. These results are in accordance with those reported, in other species, by several authors (HOUGH, 1952; SCHNELL, 1960; GREEN, 1971; BARNES and SCHWEPPENHAUSER, 1978; BRAMLETT et al., 1983). Significant extranuclear gene-effects were found for blister-rust resistance in nine year old, F_1 hybrids resulting from reciprocal crossing a *Pinus strobus* L. female with three *P.peuce* GRISEB. males (BLADA, 1992). The results are contradictory. Reliable information will only be obtained after a longer testing period. Until then, maternal effects should not be taken into consideration in the stone pine improvement programme.

Reciprocal variance

Compared to other reported results (MORGENSTERN, 1974; KRIEBEL et al., 1974; WYK, 1977; BARNES and SCHWEPPENHAUSER,1978; BARNES et al., 1992) reciprocal effects in the present experiment seem to be too large, particularly for growth traits. Hence, their contribution accounted for about the same percentage of the total variance as did g.c.a. or s.c.a. effects. These results indicate that such effects could be of major importance in a breeding program for young stone pine, but progenies should be grown for a longer period to confirm this observation.

Breeding strategy

Significant additive as well as non-additive variances were detected in growth traits, indicating that both variances could be used in stone pine improvement.

Reliable data on the amount of additive variance have already indicated the likely gains from programmes based on using best general combiners combined with clonal orchards. To date, only two good general combiners for total height were detected out of 10. But, the objective of the programme was to test more phenotypes and to select enough such combiners. If it is assumed that 20 or 30 parents selected out of 100 with good general combining ability will be used for establishing a seed orchard, using the improved seed in planting programmes could result in a genetic gain in height growth of 17.4% and 14.5%, respectively.

As earlier stated, non-additive variance can also be used. In an improvement programme to exploit non-additive variance most desirable parents would be the ones that had both high g.c.a. effects combined with other parents to consistently produce families with high s.c.a.. Their high g.c.a. would ensure a high expected full-sib family mean when the parents were crossed, and the high s.c.a. potential would provide the possibility of producing better than expected specific crosses. The progenies resulting from these crosses can be vegetatively propagated, providing there is the potential of (a) expanding the number of seedlings produced from a small number of full-sib seed

Table 8. – Genetic correlations between traits.

Traits	CN	H.2	H.3	H.4	H.5	H.6	h.4	h.5	h.6	DRC.4	DRC.5	DRC.6	TNB.6	NBAL.6	LS.4	LS.6
100.SW	0.02	0.42	0.60	–0.07	–0.05	0.14	–0.17	–0.14	0.26	0.65	0.41	0.53	–0.13	–0.72	–0.43	–0.24
CN		0.18	–0.03	–0.01	–0.02	0.03	–0.04	–0.05	–0.18	0.61	0.51	0.71	0.34	0.30	0.16	0.09
H.2			0.96	0.77	0.92	0.99	0.71	0.88	0.98	0.78	0.82	0.66	–0.43	–0.24	–0.26	–0.15
H.3				0.77	0.81	0.94	0.71	0.77	0.99	0.79	0.85	0.71	0.13	–0.16	–0.22	–0.06
H.4					0.99	0.94	0.99	0.99	0.84	0.50	0.65	0.48	0.37	0.38	0.29	0.34
H.5						0.97	0.96	0.99	0.88	0.47	0.61	0.42	0.22	0.37	0.22	0.26
H.6							0.88	0.96	0.95	0.61	0.74	0.56	0.13	0.32	–0.04	0.10
h.4								0.97	0.77	0.45	0.59	0.39	0.44	0.43	0.28	0.32
h.5									0.87	0.41	0.57	0.39	0.22	0.40	0.28	0.31
h.6										0.57	0.70	0.50	0.03	0.11	–0.18	0.09
DRC.4											0.95	0.95	0.32	–0.14	–0.21	–0.14
DRC.5												0.94	0.30	0.19	–0.16	– 0.16
DRC.6													0.38	0.03	0.02	0.04
TNB.6														0.05	0.22	0.40
NBAL.6															0.31	0.08
LS.4																0.95

Table 9. – Expected genetic gain (ΔG) for the main traits, if selected the best families (ΔG_1 ΔG_2), the best individuals within family (ΔG_3) and the bet g.c.a. combiners (ΔG_4).

Traits	$\Delta G_1 = i_1 h_1^2 \sigma_{ph1}$				$\Delta G_2 = i_1 h_2^2 \sigma_{ph1}$				$\Delta G_3 = i_2 h_3^2 \sigma_{ph2}$				$\Delta G_4 = 2 i_1 h_2^2 \sigma_{ph1}$		
	10	20	30	40	10	20	30	40	5%	10%	15%	20%	2 / 10	20 / 100	30 / 100
H.4	9.4	7.4	6.0	4.9	5.8	4.6	3.8	3.1	7.8	6.6	5.9	5.3	8.8	9.6	7.9
H.5	12.2	9.6	7.8	6.4	8.9	7.0	5.7	4.7	12.2	10.4	9.2	8.3	13.5	14.4	12.2
H.6	16.4	12.9	10.5	8.6	10.6	8.3	6.8	5.5	16.5	14.0	12.4	11.2	16.0	17.4	14.5
DRC.4	8.2	6.5	5.3	4.3	3.8	3.0	2.4	2.0	4.8	4.1	3.6	3.3	5.7	6.2	5.1
DRC.5	8.5	6.7	5.5	4.5	4.7	3.7	3.0	2.5	5.8	4.9	4.3	3.9	7.1	7.7	6.4
DRC.6	3.0	7.1	5.8	4.7	.0	3.9	3.2	2.6	6.0	5.1	4.5	4.1	7.5	8.2	6.8
TNB.6	18.6	14.7	12.0	9.8	12.2	9.6	7.9	6.4	12.9	11.0	9.7	8.7	18.5	10.1	8.4
LS.6	34.0	26.9	21.9	17.9	28.5	22.5	18.4	15.0	34.2	29.1	25.8	23.2	43.2	47.2	39.1

$\Delta G_1$and ΔG_2 = genetic gain if the best 10, 20, 30, 40 families are selected out of 90; ΔG_3 = gain from mas selection if the best 5%, 10%, 15%, 20% of individuals are selected within a family; ΔG_4 = gain if the best, 2 out of 10, 20 out 100 and 30 out of 100 parents, are selected.

(Armson et al., 1980); and (b) selecting superior trees, within full-sib families, for mass cutting propagation (Kleinschmit and Schmidt, 1977). In the same context, experiments with somaclonal embriogenesis techniques are underway in our laboratory (Palada, unpublished data). Further substantial gain can also be anticipated using biclonal orchards composed of parents with proven positive specific combining ability, or more importantly, the mass propagation by vegetative means of any good material derived from the exploitation of non-additive variation. Perhaps, the most practical method is the propagation of seedlings derived from bulked mixtures of full-sib families with proven s.c.a.

Conclusions

High variation was found in all traits, not only in cross pollinated families but also in selfed ones.

Selfed pollinated families performed worse than cross-pollinated ones for most traits.

General and specific combining ability variances of the growth traits were of practical significance; both of them can be used in an improvement programme for growth characteristics.

Reciprocal variance could also be taken into consideration for stone pine improvement.

Significant extranuclear gene effects were found in most traits but they are not reliable, yet.

High frequency of parents with a good general and specific combining ability for growth traits was detected within the parent population; they should be used for further crosses to get stocks for planting programmes and for advanced breeding populations.

Narrow-sense heritabilities at both family and individual level, progressively increased with age in all growth traits, indicating that the breeding programme could be successful.

Positive genetic correlations were found throughout the testing period suggesting that correlated responses for all growth traits will be obtained if selection is done on only one.

Selection can be done at family and individual within family level, as well as at individual parent level; therefore, three kinds of genetic gains can be expected.

Substantial genetic gain in growth and other traits could be achieved if the improved material – parents and families – will be used in planting programmes.

This test supports the adoption of a full diallel mating design in stone pine even if it requires more effort than a half-diallel one; this way it is possible to detect the maternal and reciprocal effects.

It is emphasized that all interpretations of the data were only up to the six year; to avoid erroneous decisions, observations should be confined and selections should be done later.

Acknowledgements

The author is grateful to Dr. I. Cabulea from Turda (Romania) Agricultural Research Station for his helpful comments on an early draft. The thorough comments of anonymous reviewer was invaluable in improving an earlier draft of this paper.

Literature

Anderson, R. L. and Bancroft, T. A.: Statistical theory in research. McGraw-Hill Book Co., New-York. 399 p. (1952). — Armson, K. A., Fung, M. and Bunting, W. R.: Operational rooting of black spruce cuttings. J.For. **78**, 341–343 (1980). — Barnes, R. D., Mullin, L. J. and Battle, G.: Genetic control of eight year traits in *Pinus patula.* Silvae Genetica **41**(6): 318–326 (1992). — Barnes, R. D. and Schweppenhauser, M. A.: *Pinus patula* progeny tests in Rhodesia, genetic control of nursery traits. Silvae Genetica **27**(5): 200–204 (1978). — Becker, W. A.: Manual of procedures in quantitative genetics. Fourth Edition. Acad. Enterp.Pullman, Washington. 190 p. (1984). — Beldie, A.: Observatii asupra vegetatiei lemnoase din Muntii Bucegi. Analele ICEF, Seria I, **6**: 39–43 (1941). — Bingham, R. T.: Taxonomy, crossability and relative hister rust resistance of 5 needle white pines. In: Bingham, R. T. et al. (Ed.): Biology of rust resistance in forest trees. USDA For. Serv., Misc. Publ., 1921, p. 271–280 (1972). — Blada, I.: Genetic resistance to *Cronartium ribicola* and height growth in some five needle pines and in some interspecific hybrids. PhD. Thesis, Academy of Agricultural and Forestry Sciences, 146 p. (1987). — Blada, I.: Breeding *Pinus cembra* by intra and inter-specific crosses. For. Res. Inst., Bucharest, Anual Report, No. 7 (Unpublished) (1990). — Blada, I.: Nuclear and extranuclear genetic effects in F_1 reciprocal hybrids between *Pinus strobus* and *P. peuce.* Silvae Genetica **41**(1): 34–38 (1992). — Blada, I.: Interspecific hybridisation of Swiss Stone Pine *(Pinus cembra* L.). Silvae Genetica **43**(1): 14–20 (1994). — Blada, I.: Diallel cross in *Pinus cembra:* I. Results of the three years old nursery test. IUFRO World Congress, 6 to 12 August 1995, Tampere, Finland (1995). — Blada, I.: Breeding of *Pinus cembra* and its nursery and planting operations. Anual Report No. 26, Forest Res. Inst., Bucharest. 5 p. (Unpublished) (1996). — Blada, I.: Stone pine *(Pinus cembra* L.) provenance experiment in Romania. I. Nursery stage at age 6. Silvae Genetica **46**(4): 197–200 (1997a). — Blada, I.: Diallel crossing in *Pinus cembra:* II. The nursery testing at age 6. pp 143–161. In: White, T. et al. (Eds.): Proceedings of the 24th Biennial Southern Forest Tree Improvement Conference, Orlando, Florida, U.S.A., June 9 to 12. 441 p. (1997 b). — Blada, I. and Popescu, N.: Variation in size and weight of cones and seeds in four natural populations of Carpathian stone pine. In: Schmidt, W. C. and Holtmeier, F. K., (Eds.): Proceedings International Workshop on Subalpine Stone pines and Their Environment. USDA, For. Serv., Intermountain Res. St. INT-GTR-309. pp. 154–158 (1994). — Bramlett, D. L., Deil, T. R. and Pepper, W. D.: Genetic and maternal influences on virginia pine seed germination. Silvae Genetica **32**(1–2):1–4 (1983). — Contini, L. and Lavarelo, Y.: Le pin cembro. INRA, Paris. 197 p. (1982). — Critchfield, W. B. and Little, E. L., Jr.: Geographic distribution of the pines of the world. Misc. Publ. 991. Washington, USDA For. Serv., 97 p. (1966). — Falconer, D. S.: Introduction to quantitative genetics. Longman and Co., New-York. 340 p. (1981). — Green, J. W.: Variation in *Eucalyptus obliqua.* L'Herit. New Phytol. **70**: 837–909 (1971). — Griffing, B.: Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. Sci. **9**: 463–493 (1956). — Gubesh, L.: Rãspândirea relictului glaciar zâmbrul *(Pinus cembra)* pe versantii sudici ai unor masive din Cãlimani. Ocrotirea Naturii **15**(2): 149–159 (1971). — Hayman, B. I.: The analysis of variance of diallel tables. Biometrics **10**: 235–244 (1954). — Hoff, R. J., Bingham, R. T. and McDonald, G. I.: Relative blister rust resistance of white pines. Eur. Jour. Forest. Path. **10**(5): 307–316 (1980). — Holzer, K.: *Pinus cembra* L. as a pioneer at timberline in the European Alps. FAO/ FORGEN 3/13 (1963). — Holzer, K.: Intrinsic Qualities and Growth Potential of *Pinus cembra* and *Pinus peuce* in Europe. In: Bingham, R.T. et al. (Eds.): Biology of Rust Ressistence in Forest Trees. USDA Misc. Publ. 1221, 99–110 (1972a). — Holzer, K.: Neue Wege in der Saatgutbeschaffung für Hochlagenaufforstungen durch Samenplantagen. Mitt. FBWA Wien, Heft 96, 39–49 (1972b). — Holzer, K.: Genetics of *Pinus cembra* L. Annales Forestales, 6/5, Zagreb, 158 p. (1975). — Hough, A. F.: Relationship of red pine seed source, seed weight and height growth in Kane plantation. N. E. Forest Exp. Station, Paper 50, Wash., USDA For. Serv. (1952). — Kleinschmit, J. and Schmidt, J.: Experiences with *Picea abies* cuttings propagation in Germany and problems connected with large scale application. Silvae Genetica **26**: 197–203 (1977). — Kriebel, H. B.: Method for germinating seed of five needle pines. IUFRO Instructions. 3 p. (1973). — Kriebel, H. B., Roberds, J. H. and Cox, R. V.: Genetic variation in vigor in a white pine incomplete diallel cross experiment at age 6. Proc. Eight Central States For. Tree Improv. Conf. (1974). — Morgenstern, E. K.: A diallel cross in black spruce, *Pinus mariana.* Silvae Genetica **23**(1–3): 67–70 (1974). — Nather, H.: Zur Keimung der Zirbensamen. Centralbl. f. Forstw. **75**(1): 61–70 (1958). — Oarcea, Z.: Contribuţii la cunoaşterea răspândirii şi vegetării pinului cembra în Retezat. Revista Pădurilor **9**: 495–497 (1966). — Rohmeder, E. and Rohmeder, M.: Untersuchungen über das Samentragen und Keimen der Zirbelkiefer *(Pinus cembra)* in den Bayrischen Alpen. Allg. Forstzeitschrift **10**: 83 (1955). — Schaffer, H. E. and Usanis, R. A.: General least squares analysis of diallel experiments. A computer programme – DIALL. Genetic Dept. Res. Rept. No. 1. North Carolina State University, Raleigh, N.C.. 61 pp. (1969). — Schnell, G. von: Die Abhängigkeit der Lebenskraft und der Pflanzengröße von der Keimschnelligkeit bei unterschiedlicher Korngröße, Allg. Forst und Jagdz. **131**(2): 34–37 (1960). — Tataranu, I. D. and Costea, C.: Un arbore de interes forestier: *Pinus cembra.* Revista Padurilor **11**: 3–14 (1952). — Wyk, G. von: Early growth results in a diallel progeny test of *Eucalyptus grandis.* II. A greenhouse study. Silvae Genetica **26**(1): 44–50 (1977). — Zobel, B. and Talbert, J.: Applied forest tree improvement. John Wiley and Sons, New-York. 505 p. (1984).