

# Effect of Different Progeny Test Strategies in the Performance of Eucalypt Clones

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## Abstract

The objective of the present study was to investigate whether the performance of an individual in clonal testing was affected by the location of the initial selection. The study also evaluated the effect of mass selection and BLUP (Best Linear Unbiased Prediction) on the performance of individuals in the progeny test and the clonal test. In the progeny test, 62 half-sib *E. grandis* families, 68 half-sib *E. urophylla* families and 62 full-sib families between *E. grandis* and *E. urophylla*, plus four checks were evaluated totaling 196 treatments. The experiments were carried out at three sites: Aracruz and São Mateus, in Espírito Santo State and Caravelas, Bahia State, Brazil. The 1000 best individuals were classified by mass selection and BLUP. In the clonal tests 257 clones were evaluated in two locations: Aracruz and Caravelas. In both the progeny test and the clonal test, two years after planting, the circumference at breast height and basic wood density were measured and the sum of standardized variables (*Z*) was estimated for simultaneous selection of both characteristics. The results indicated that the origin of the individual in a progeny test had a small effect on the performance of the clone when evaluated in other environments; the estimates of realized heritability and the linear regression coefficient were small in all the selection strategies. Thus, the correlation between the performance of trees selected in progeny tests and their respective clones in the clonal test was small.

*Key words:* Plant breeding, selection strategies, realized heritability, *Eucalyptus*.

## Introduction

At the beginning of the eucalyptus breeding programs, the breeding strategies were based on introductions of individuals of different origins and different species followed by the identification of those most adapted to the Brazilian environmental and mass selection for superior individuals and on half-sib family selection targeting improved seed production. In a second phase, priority was given to cloning rather than sexual methods of seedling production. Researchers began to select superior trees, especially in commercial plantations, most of

which, it seems, were natural hybrids involving *E. grandis* and *E. urophylla* (FERREIRA and SANTOS, 1997; REZENDE, 2001).

Significant progress was achieved when it was observed that, to obtain additional gains, superior individuals should be crossed to obtain new genotype combinations (GONÇALVES et al., 2001). Therefore companies with breeding programs intensified hybridization and conducted programs with half-sib and full-sib family selection. More elaborate statistical procedures began to be used to improve selection efficiency with the implementation of computational facilities. One such method is based on the use of mixed model analysis, especially the Best Linear Unbiased Prediction method (BLUP).

In eucalyptus cropping, some studies have compared the efficiency of some selection strategies (RESENDE, 2002; FREITAS et al., 2009; ROSADO et al., 2009). The superiority of combined selection and BLUP over mass selection and selection among and within half-sib progenies was theoretically demonstrated (RESENDE, 2002). In the field, all the selection strategies were effective in eucalypt breeding (FREITAS et al., 2009). However, no reports were found in the literature comparing the effectiveness of these strategies in future performance of the clones, i.e, the correlation between the performance of a selected individual in a progeny test and its performance in the clonal test. This information is essential for plant breeders to identify the best strategies for selection.

The objective of the present study was to verify whether the location of origin of the individual selected in the progeny test affected its performance in the clonal test. The study also evaluated the effectiveness of different individual selection strategies in the progeny test and its performance in the clonal test.

## Materials and Methods

### Progeny Test

The experiments were carried out in three areas of the company Fibria Celulose S. A., in Aracruz, ES, Brazil: 19° 50' lat S and 40° 12' long W, São Mateus, ES, Brazil: 18° 36' lat S and 40° 01' long W and Caravelas, BA, Brazil: 17° 47' lat S and 39° 33' long W.

In experiments set up between October and November 2001, 62 half-sib *E. grandis* families, 68 half-sib *E. urophylla* families and 62 full-sib families between *E. grandis* and *E. urophylla*, plus four checks were assessed, totaling 196 treatments. The experimental design was a 14 x 14 lattice design with 40 replications of single tree plots. The plants were spaced at 3 x 3 m and the usual management for commercial crops was used.

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Two years after planting, the data were obtained for circumference at breast height and pilodyn needle penetration in the wood (mm). Based on the pilodyn evaluation, the basic wood density ( $\text{kg}\cdot\text{m}^{-3}$ ) was estimated by the expression:  $\text{Density} = 615 - 11 \cdot \text{Pilodyn reading}$ . To establish this expression, 142 two-year-old trees of different eucalypt species were evaluated previously with the pilodyn and the wood density had been determined in the laboratory. The regression equation between the pilodyn reading and the basic wood density was constructed from these evaluations.

To obtain information on the two traits simultaneously, the data were standardized and the index of the sum of standardized variables (Z) was obtained (MENDES et al., 2009). Because the Z variable can assume both positive and negative values, a constant value of four was added to make all the values positive. In this case, the population mean of each trait, instead of zero assumed the constant value.

The progeny test data were analyzed by the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) procedure, using the SELEGEN computer program (RESENDE, 2007). Analyses were performed according to the following statistical model:  $y = X\beta + Wp + Z\gamma + \varepsilon$ , where  $y$ : vector of phenotypic data;  $\beta$ : vector of fixed effects of replication and blocks/replication added to the general mean;  $p$ : vector of fixed effects of progeny type (*E. urophylla* and *E. grandis* half-sib families and full-sib families between *E. grandis* x *E. urophylla*);  $\gamma$ : vector of genetic effects of individual trees, assumed to be random:  $\gamma \sim (\emptyset, G)$ ; with

$$G = \bigoplus_{k=1}^3 I_k \sigma_{gk}^2 (\emptyset, G);$$

$\bigoplus$  indicates the operation of direct matrix sum of matrices;  $I$  is the identity matrix and  $\sigma_{gk}^2$  the variance component associated with individual genetic effects. For half-sib progenies,  $\sigma_g^2$  is the individual additive genetic variance and for full-sib progenies  $\sigma_g^2$  is the individual genotypic variance);  $\varepsilon$ : vector of errors:  $\varepsilon \sim N$ . The capital letters represent the incidence matrices for these effects.

The coincidence index was estimated to assess the correlation between the performance of the trees selected by mass selection and BLUP, ignoring the random effects (HAMBLIN and ZIMMERMANN, 1986).

#### Clonal Test

The experiments were evaluated in two areas of the company Fibria Celulose S. A., in Aracruz, ES, Brazil: 19° 50' lat S and 40° 12' long W and Caravelas, BA, Brazil: 17° 47' lat S and 39° 33' long W.

To set up the clonal tests, 257 trees were cloned from the progeny test experiments described previously. Table 1 shows the origin of these clones. The experiments were set up between August and September 2007 in a randomized block design with 30 replications of single tree plots. The between plant spacing and experiment management were the same as in the progeny tests. The same traits as in the progeny tests were assessed when the trees were two years old.

The analysis was carried out using the least squares method (LSM) and restricted maximum likelihood / best

Table 1. – Number of clones in the clonal test from the *E. grandis* and *E. Urophylla* half sib families and *E. grandis* x *E. Urophylla* full sib families of progeny tests evaluated in Aracruz, Caravelas and São Mateus.

Type of families	Origin			Total
	Aracruz	Caravelas	S. Mateus	
<i>E. grandis</i>	6	4	9	19
<i>E. urophylla</i>	9	1	16	26
<i>E. grandis</i> x <i>E. urophylla</i>	65	51	96	212

linear unbiased prediction procedures (REML/BLUP). The analysis of variance by location and joint analysis of variance were performed using the *Procedure for General Linear Models* (PROC GLM) of the SAS® statistical software (SAS INSTITUTE, 2002).

For the REML/BLUP mixed model analysis, the SELEGEN computer program was used (RESENDE, 2007), which predicted the individual genotypic values. Analyses by location were performed according to the following statistical model:  $y = Wr + Zg + \varepsilon$ , where  $y$ : vector of phenotypic data;  $r$ : is the vector of fixed effects of the replication added to the general mean;  $g$ : is the vector of individual genotype effects, assumed to be random:  $g \sim N(0, G = I\sigma_g^2)$  ( $I$  denotes the identity matrix and  $\sigma_g^2$  the variance component associated with individual genetic effects) and  $\varepsilon$ : is the vector of errors:  $\varepsilon \sim N(0, R = I\sigma_e^2)$  ( $\sigma_e^2$ : environmental variance).

The joint analysis was performed according to the following statistical model:  $y = Xr + Zg + Wi + \varepsilon$ , where  $y$ : vector of phenotypic data;  $r$ : is the vector of fixed effects of location-replication combination added to the general mean;  $g$ : is the vector of individual genotype effects, assumed to be random:  $g \sim N(0, G = I\sigma_g^2)$  ( $I$  denotes the identity matrix and  $\sigma_g^2$ : the variance component associated with individual genetic effects);  $i$ : is the vector of the genotype x environment random effects:  $i \sim N(0, L = I\sigma_i^2)$  ( $\sigma_i^2$  genotype x environment variance) and  $\varepsilon$ : is the vector of errors:  $\varepsilon \sim N(0, R = I\sigma_e^2)$  ( $\sigma_e^2$ : environmental variance). The capital letters represent the incidence matrices for these purposes.

The realized heritability ( $h_R^2$ ) from mass and BLUP selection were estimated using different selection intensities: 5, 10, 15, 20 and 25%, according to the following estimator (adapted from FEHR, 1987):

$$h_R^2 = \frac{(Ms_j - Mo_j) / Mo_j}{(Ms_i - Mo_i) / Mo_i}$$

where:

$Mo_j$ : original mean of the progeny test population;  $Ms_j$ : mean of selected individuals in the progeny test;  $Mo_i$ : original mean of the clonal test;  $Ms_i$ : mean of the individuals in the clonal test, corresponding to the selected individuals in the progeny test.

As the progeny and clonal tests were conducted in different environments, the standardized heritability was also estimated in standard deviation units (Z) (FREY and HORNER, 1957) and then the heritability was estimated

by the parent/offspring regression, that is, the regression of the performance of the trees in the progeny test (x) and clonal test (y).

The genetic and phenotypic parameters were estimated from the expected mean square values according to the literature (BERNARDO, 2002).

## Results

### Progeny Test

Significant differences were found in the three traits considered among the progeny ( $P < 0.01$ ). Its decomposition showed variability among the *E. grandis* and *E. urophylla* half-sib progeny and *E. grandis* x *E. urophylla* full-sib progenies.

The coincidence between the trees classified by mass selection and BLUP, for Z, was 68.2% in Aracruz, 63.3% in São Mateus and 68.7% in Caravelas (Table 2).

Table 2. – Estimates of the coincidence index (CI, %) of the plants selected by mass selection and BLUP and selection gains (%) considering the two strategies, for the sum of Z (Z) in *Eucalyptus* progeny tests.

Locations	CI
Aracruz	68.2
São Mateus	63.2
Caravelas	68.7

Table 3. – Joint analysis of variance for circumference at breast height (CBH, cm), wood density (WD, kg m<sup>-3</sup>) and the sum of Z (Z), obtained in the evaluation of two-year-old clones of *E. grandis*, *E. urophylla* and *E. grandis* x *E. urophylla*.

Source of variation	DF	Mean square		
		CBH	WD	Z
Replications / locations	58	391.48**	69821.15**	0.28 n.s.
Locations (L)	1	29754.36**	328.41 n.s.	2.84 n.s.
Clones (C)	256	392.86**	17084.93**	29.76**
Origins (O)	2	232.32**	61001.66**	52.69**
AR <sup>1/</sup>	79	396.38**	17956.00**	39.47**
CA <sup>1/</sup>	55	345.12**	12829.00**	27.49**
SM <sup>1/</sup>	120	415.78**	17904.00**	24.21**
C x L	256	168.94**	1115.59**	8.52**
O x L	2	563.60**	3357.33**	23.77**
AR x L	79	166.86**	1061.54**	8.59**
CA x L	55	165.94**	968.79**	7.02**
SM x L	120	164.27**	1177.34**	8.88**
Erro	12897	14.10	339.17	1.15
Accuracy (%)		76	97	84
Mean clone		36.68	434.23	7.95
Mean AR		36.20	434.37	7.92
Mean CA		36.71	428.95	7.79
Mean SM		36.29	436.96	8.03

<sup>1/</sup> AR – selected clones in Aracruz, ES; CA – selected clones in Caravelas, BA e SM – selected clones in São Mateus, ES.

\*\* – Significant at 1% probability by F test; n.s – not significant.

### Clonal Test

The ten best clones were identified similarly in the comparison of the LSM and REML/BLUP analyses (data not shown). As the two procedures provided similar classification, the presentation of the results will refer to the LSM analysis.

Table 3 shows the analyses of variance of the 257 clones. The accuracy estimates indicated good experimental precision. For all traits, the estimated accuracy values were above 75%. Significant differences ( $P < 0.01$ ) occurred among locations for circumference at breast height (CBH) (Table 3). The highest estimates were obtained in Aracruz, where the average (37.9 cm) was 8% higher than that obtained in Caravelas (34.8 cm) (data not shown). Significant differences ( $P < 0.01$ ) were detected among the clones for all the traits. Taking origin as source of variation, significant differences were observed for the three traits ( $P < 0.01$ ) (Table 3). Considering Z, it was observed that clones from plants evaluated at São Mateus performed better but the difference was not significant. In principle, this result shows that the selection location of the tree did not significantly affect the clone performance in other conditions. The source of variation origin x location was significant ( $P < 0.01$ ). The same applied to the interactions involving clones of each origin x locations, indicating at first that the clone performance was not coincident at the two locations (Table 3). Estimates of genetic correlation ( $\hat{r}_g$ ) between the average clone performance of clones in the two locations highlights this observation. For example, in the case of Z, the ( $\hat{r}_g$ ) was only 0.58 (Table 4). Because there were significant interactions, results will be focused on the clone performance per site.

Estimates of broad sense heritability ( $h^2$ ) on the clone mean in Aracruz and Caravelas were above 93% and the confidence interval showed that they were different from zero at 95% probability (Table 4).

Table 5 shows the realized heritability ( $h^2_R$ ) estimates for Z, considering the two selections and different tree

Table 4. – Heritability estimates on clone mean ( $h^2_{mc}$ , %) in Aracruz, Caravelas and joint of two locations, genetic correlation ( $\hat{r}_g$ ) between the mean clone performance in two locations, for circumference at breast height (CBH, cm), wood density (WD, kg m<sup>-3</sup>) and sum Z (Z), obtained in the evaluation of *Eucalyptus* clones.

Parameters	CBH		
	Aracruz	Caravelas	Joint
$h^2_{mc}$	95.5 (94.7 – 96.2) <sup>1/</sup>	94.5 (93.6 – 95.4)	56.9 (44.8 – 66.4)
$\hat{r}_g$	-	-	0.42
Parameters	WD		
	Aracruz	Caravelas	Joint
$h^2_{mc}$	0.964 (95.9 – 96.9)	0.962 (95.5 – 96.9)	93.5 (91.6 – 94.8)
$\hat{r}_g$	-	-	0.91
Parameters	Z		
	Aracruz	Caravelas	Joint
$h^2_{mc}$	94.4 (93.4 – 95.2)	93.5 (92.4 – 94.6)	71.4 (63.3 – 77.6)
$\hat{r}_g$	-	-	0.58

<sup>1/</sup> Values in parentheses refer to the upper and lower limits of confidence intervals, at 5% probability.

**Table 5.** – Estimates of realized heritability at different selection intensities in a clonal test and linear regression coefficient (b) between the tree performance in progeny tests and *Eucalyptus* clonal tests for the sum of Z (Z), in Aracruz and Caravelas.

Locations	Mass selection					
	b	Realized heritability				
		5%	10%	15%	20%	25%
Aracruz	0.370**	0.002	0.069	0.181	0.234	0.301
Caravelas	0.302**	-0.090	0.061	0.161	0.180	0.248
BLUP						
Aracruz	0.301**	-0.030	-0.070	-0.060	0.024	0.095
Caravelas	0.245**	-0.119	-0.072	-0.069	0.090	0.190

\*\* Significant at 1% probability.

selection intensities in the progeny tests. All the ( $h^2_R$ ) estimates were of low magnitude in all the locations and increased with the increase in the number of clones selected, i.e, with decrease in selection intensity but even so, these values were very close to zero.

The linear regression coefficient (b) estimates were similar for Z in the two selections evaluated (Table 5). In two locations, the mass selection and BLUP estimates differed from zero, but although the estimates were significant, their magnitudes were low.

## Discussion

Normally, breeders handle several traits at the same time. The present study, for example, involved a tree growth trait and a wood quality trait. In this situation, simultaneous selection for traits is more efficient with a selection index. There are many selection indexes in the literature (BERNARDO, 2002). The index obtained by the sum of standardized variables (Z) is easy to estimate and interpret (MENDES et al., 2009). Furthermore, with standardized variables, the data can be compared in any situation. Therefore, discussions will be focused mainly on the sum of Z, which represents the breeder's objective.

The coincidence between the individuals selected by mass selection and BLUP was not high (Table 2). It could be inferred that mass selection was less efficient compared with BLUP. Although the same individual/progeny may not be identified by two or more selection strategies, individuals very similar in performance are identified.

In plant breeding, mixed model analysis by BLUP has frequently been used (RESENDE, 2002; NUNES et al., 2008; PIEPHO et al., 2008). The analysis by LSM and BLUP provided similar results when there was no data loss (BERNARDO, 2002). It is practically impossible to have balanced experiments where the plot consists of a single plant, mainly in respect to forest genetic breeding. The question is, at what level of imbalance would BLUP be most advisable. In the present study, 14% of the trees were lost in Aracruz and 11% in Caravelas and there was no advantage of BLUP over the LSM. Similar results were obtained by comparing eucalyptus clone classification by the mixed model and LSM procedures (SCARPINATI et al., 2009).

Currently, the use of the single plant plot has increased in forest genetic breeding experiments. This

strategy results in greater experimental precision due to higher number of replications (RESENDE, 2007). The estimated accuracy values were above 75%, indicating that the experimental accuracy was high to very high (RESENDE and DUARTE, 2007).

In experiments of this kind it is essential that genetic variation/variability occurs among clones. The clones assessed were derived from *E. grandis* and *E. urophylla* half-sib progenies and *E. grandis* x *E. urophylla* full-sib progenies. Thus, it was expected that they would differ for the three traits, as in fact occurred. Most clones were from *E. grandis* x *E. urophylla* full-sib progenies (Table 1). The superiority of the *E. grandis* x *E. urophylla* hybrids in meeting the demands of the pulp industry has been reported on several occasions due to their vigorous growth and good wood quality (BERTOLUCCI et al., 1995; ASSIS, 2000; REZENDE and RESENDE, 2000; VERRY, 2000; BOUVET and VIGNERON, 2009). Therefore, hybrids between these two species have been used commercially in several countries, including Brazil, South Africa, Colombia, Venezuela and the Congo (RESENDE, 2002).

One objective of the present study was to verify whether the origin of location of the selected individuals in a progeny test affected its performance when evaluated in other conditions. Although the origin as source of variation was significant ( $P < 0.01$ ), this effect was small (Table 3). In principle, these results showed that the progeny tests need not be conducted in a large number of environments. Unfortunately there are no reports in the literature, in the case of eucalyptus, on the influence of the progeny origin on clone performance in various environments.

One focus of the present study was to assess the selection efficiency of the progeny test in the clone performance in other environmental conditions. The study also assessed whether the selection strategy for the tree that will create the clone has different efficiency, considering the performance of the cloned individual. This efficiency can be compared by several procedures. One is the realized heritability or gain realized with the selection, i.e, the expected gain is estimated with selection of individuals in the progeny test and gain realized from the clone in the clonal test.

Another way to evaluate the selection efficiency is by the parent/offspring regression (HALLAUER et al., 2010). As the progeny and clonal tests were conducted in different environments (years and locations), the tree performance regression was estimated in the progeny test (x) and clonal test (y), using standardized data in standard deviation units (FREY and HORNER, 1957).

The heritability estimates for realized gain ( $h^2_R$ ) and the linear regression coefficient (b) were of low magnitude and provided similar interpretations, regardless of the selection method applied to the progeny test (Table 5). One hypothesis to explain the low heritability estimates obtained is the occurrence of the genotype x year interaction. Unfortunately, there are few reports of the occurrence of such interaction in eucalyptus cropping in Brazil. In the few studies found the genetic correlations between the performance of the tree in the progeny test and the clone derived from it were very low. In the aver-

age of different selection intensity, was only 27% (REIS et al., 2011). The authors comment that the low estimate of the genetic association between the elite trees and clone performance should be attributed to the interaction of genotypes x years.

Although the BLUP is the best predictor of the genotypic value, its efficiency in responding to selection in subsequent years was not different from mass selection, because it cannot predict the future effect of the genotype x year interaction. Thus, selection success always depends on the most accurate evaluation of the progenies and the use of alternatives to reduce the genotype x year effect. Thus, to reduce the effect of this interaction, the option would be to use a lower selection intensity on the trees in a progeny test, in order to increase the possibility of identifying clones with improved performance.

## Conclusions

The origin of the individual in the progeny test had a small effect on the clonal performance when evaluated in other locations.

Estimates of realized heritability and the linear regression coefficient in the two selection strategies used were small. That is, the correlation was low in the performance of selected trees in progeny tests and their respective clones in the clonal test. Reducing the selection intensity of the trees in progeny tests is an alternative to improve the efficiency of clonal test in the future.

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