CBAB
CROP BREEDING AND
APPLIED BIOTECHNOLOGY

Realized heritability in the selection of *Eucalyptus* spp. trees through progeny test

Raoni Ribeiro Guedes Fonseca[1], Flávia Maria Avelar Gonçalves[2], Leonardo Novaes Rosse[3], Magno Antonio Patto Ramalho[2], Adriano Teodoro Bruzi[4], and Cristiane Aparecida Fioravante Reis[2]

Abstract - *Full-sib progenies were evaluated with the objective of comparing the performance of selected individuals in progenies tests with their clones in clonal tests. The experiments were carried out in randomized complete block design with five replications, five plants per plot, spaced at 3 m x 3 m, and four controls. The wood volume of each tree was determined after four years of growth. The 173 selected trees in the progeny test were used in the clonal tests in two different locations. The same experimental design was used with 20 replications, one tree plot, spaced at 3.5 m x 2.15 m with a border, and 13 controls (commercial clones). The growth traits of each tree were determined after two years of planting. Genetic parameters were estimated using general linear models. Realized heritability was greater than 80% indicating that the selection of trees in the progeny test was efficient.*

Key words: full-sib progenies, breeding, clones selection, genotypes by locations interaction, selection gain.

INTRODUCTION

The successful culture of *Eucalyptus* for production of cellulose and paper in Brazil has derived mainly from the careful selection of clones and competent technical management, and has contributed to an increase in wood production from 20 m³ ha⁻¹ per year in 1960 to 40 m³ ha⁻¹ per year in 1998 accompanied by a concomitant increase in cellulose yield (Vencovsky and Ramalho 2006). In commercial plantations it is common elite trees are selected and cloned with the aim of increasing productivity. Since such trees that originate from natural hybridization exhibit large genetic variability, these are expected to be exploited by breeders for further selection (Zobel and Talbert 1984).

However, nowadays additional genetic enhancement of such trees is difficult from population resampling, and other methods are needed in order to increase the chances of generating novel superior genotypic combinations (Gonçalves et al. 2001). The technique of artificial hybridization allows the combination of desired characteristics (Chaperon 1987, Assis 2000, Assis and Máfia 2007). This method involves the controlled crossing of individuals and the generation of various progenies that may be subsequently evaluated in field experiments. Such experiments, which are referred to as progeny tests, represent a substantial expense for the wood industry and demand considerable knowledge and skill of the breeders.

[1] Universidade de Rio Verde, Fazenda Fontes do Saber, C. P. 104, 75.901-970, Rio Verde, GO, Brazil.
[2] Universidade Federal de Lavras, Departamento de Biologia, Campus Universitário, s/n, C. P. 3037, 37.200-000, Lavras, MG, Brazil.
[3] Veracel S.A., BA-275, km 24, C. P. 21, 45.820-970, Eunápolis, BA, Brazil.
[4] Souza Cruz S.A., Avenida General Plínio Tourinho, Bom Jesus, 83.880-000, Rio Negro, PR, Brazil.

In progeny tests, the selection of individuals for cloning has been performed exclusively on the basis of the phenotypes and such an approach has certainly resulted in improved productivity (Marcó and White 2002). However, with the computing technology advances, the mixed model methodology has been introduced into *Eucalyptus* breeding programs in order to improve the selection procedure (Resende 2002). As part of this procedure, *Eucalyptus* elite trees are identified by Best Linear Unbiased Prediction (BLUP) and subsequently evaluated through clonal tests in which a large number of clones are examined in order to maximize the chances of identifying trees that exhibit genetic merit. In this context, it is important to determine if there is good correspondence between the performance of trees selected in the progeny tests and their clones selected in the clonal tests. The knowledge of such correlation is very important in the development of new breeding strategies. Little information is available in literature concerning this aspect. The aim of the present study was to compare the performance of *Eucalyptus* trees selected in progeny tests with the performance of the clones derived there from.

MATERIAL AND METHODS

The present work was based on data provided by Veracel Celulose S.A. company located in Eunápolis, Bahia, Brazil (16º 22'39" lat S, 39º 34'49" long W and 189 m alt asl). The investigation was conducted in two phases, namely, a progeny test involving the evaluation of full-sib progenies and clonal tests involving the evaluation of the selected trees in the progeny tests.

Progeny tests

Full-sib progenies, obtained from the controlled hybridization of six *Eucalyptus* species (Table 1), were used in experiments conducted in the different locations 1, 2 and 3 (Table 2). The experiments were allocated in a randomized complete block design with five replications, five plants per plot, spaced at 3 m x 3 m, and four controls (commercial clones). The experiments were implanted in August 2000, and the wood volume (m³) of each tree was determined after four years of growth.

Statistical analyses were performed using the procedure for general linear models from software SAS (SAS Institute Inc 2000) and Selegen (Resende 2007). The ranking of the trees was established by considering genetic deviations by the mixed models methodology

Table 1. Progenies derived from controlled crossing of *Eucalyptus* species

Crosses	Progeny number
E. urophylla x *E. brassiana*	6
E. urophylla x *E. globulus*	16
E. urophylla x *E. grandis*	107
E. urophylla x *E. pellita*	5
E. urophylla x *E. tereticornis*	1
E. urophylla x *E. urophylla*	25
E. grandis x *E. brassiana*	1
E. grandis x *E. globulus*	13
E. grandis x *E. grandis*	3
E. grandis x *E. pellita*	1
E. grandis x *E. urophylla*	18
Total	196

*196 progenies were evaluated by locations, being 132 of then common to all three locations.

Table 2. Locations of experiments

Locations	Latitude (South)	Longitude (West)	Altitude (m)
1	16° 47'25,09"	39° 18'23,23"	73
2	16° 05'40,59"	39° 19'10,13"	117
3	16° 20'20,70"	39° 35'35,61"	189
4	16° 18'07,36"	39° 20'25,84"	122
5	16° 41'38,41"	39° 23'29,40"	102

(BLUP). The individual and joint analyses involved all of the 132 progenies common to the three locations and four controls were carried out using the procedure for general linear models from SAS (SAS Institute Inc 2000) and according to the following statistical model: $Y_{ijwl} = m + p_i + l_w + b_{j(w)} + (pl)_{iw} + e_{ij(w)} + d_{l(ijw)}$ in which: Y_{ijwl}: observation of progenie i, in the replication j, in the location w; m: mean effect of the trait; p_i random effect of progenie $i = 1, 2, 3, ..., 132$; a_w: random effect of location $w = 1, 2, 3$; $b_{j(w)}$: random effect of replication j, within of the location w; $(pl)_{iw}$: random effect of interaction between progeny i and location w; $e_{ij(w)}$: random effect error of each plot, within of the location w, $e_{(ij)} \cap N(0, ^2)$. $d_{ij(k)}$: random effect plant k within of the plot ij. The genetic variances between progenies (σ_p^2) by location and joint analysis, the variances of the interaction progenies x locations (σ_{pl}^2) and the error associated with these estimations, were estimated (Ramalho et al. 2005).

Clonal tests

The 173 trees selected in the progeny test were used in the clonal tests, which were implanted in 2005 and conducted in locations 4 and 5 (Table 2). The experiments

were carried out in randomized complete block design with 20 replications, single tree plot, spaced at 3.5 m x 2.15 m with a border, and 13 controls (commercial clones). The wood volume (m³) of each tree was determined after two years of growth.

Statistical analyses were performed using the procedure for general linear models from SAS (SAS Institute Inc 2000). The individual analyses were realized and subsequently the joint analysis involved all of the clones common to the two locations. The joint analyses were realized according to the following statistical model: $Y_{ijw} = m + c_i + l_w + (cl)_{iw} + b_{j(w)} + e_{(ijw)}$, in which: Y_{ijw} observation of clone i, in repetition j; in locations w; m: mean effect for the trait; c_i: randon effect of clone i ($i = 1, 2, 3,..., n$); l_w random effect of location $w = 1, 2$; $b_{j(w)}$: random effect of repetition j, within of the location w; $(cl)_{iw}$: random effect of interaction between clone i and location w; $e_{(ijw)}$ random effect of error, $e_{(ij)} \cap N(0, ^2)$.

Genetic and phenotypic parameters were estimated from the expected mean square values obtained in the analyses of variance. The genetic variances between clones (σ_c^2) by location and joint analysis, the variances of the interaction clones x locations (σ_d^2) and the errors associated with these estimates were established using the methodology described by Ramalho et al. (2005). The heritability was estimated by the expression, $\frac{\sigma_c^2}{\sigma_F^2} \times 100$, in which σ_F^2 is the phenotypic variance between the mean of clones by location and multi-locations. The errors of the heritability estimate were obtained by Knapp et al. (1985).

Since the progeny and clonal tests were carried out in different years and locations, the results obtained were adjusted in order to allow direct comparisons between the two tests. For this purpose, relative mean volumes were calculated from the volumes of the trees selected in the progeny and clonal tests in comparison with the volumes of the control clone '361' cultured under identical conditions. Similar procedures were used for each of the replications.

On the basis of data obtained employing a selection intensity of 10%, the following parameters were estimated: (i) the selection gain (expressed as a percentage) in relation to the mean values of the controls $GS\% = \frac{(Ms_j - Mo_j)}{Mo_j} \times 100$; and (ii) the realized heritability (Fehr 1987) as estimated according to the estimator $h_r^2 = \frac{(Ms_j - Mo_j)/Mo_j}{(Ms_i - Mo_i)/Mo_i}$, where Mo_i is the mean of all trees selected in the progeny test, Ms_i is the mean of the trees in the progeny test leading to the best 10% clones, Mo_j is the mean of the clonal test, and Ms_j is the mean of the 10% best clones in the clonal test.

Results and Discussion

Progeny tests

The analysis of variance for wood volume revealed significant F-test values ($P \leq 0.01$) for the sources of variation associated with locations, treatment and the interaction between treatment x locations. Decomposition of the treatment effects revealed that there were significant differences between progenies, controls and the contrast progeny vs controls, indicating that the mean values of the controls were different from those of the progenies (Table 3).

Table 3. Variance joint analyses and estimation of the genetic parameters obtained from the individual and joint analyses carried out in the progeny tests for years old trees for wood volume (m³)

Sources of variation	df	Mean squares
Replications/locations	2	0.044**
Locations (L)	12	1.261**
Treatments (T)	135	0.134**
Progenies(P)	131	0.112**
Controls(C)	3	0.042**
P vs C	1	3.186**
T x L	270	0.010**
P x L	262	0.010**
C x L	6	0.015
(P vs C) x L	2	0.058**
Error	1945	0.008
Within	8859	0.007
Mean		0.165
CV (%)		54.207
R² (%)		25.349
$\hat{\sigma}_{p1}^2$ [a]		$80^+ (\pm 9)$ [b]
$\hat{\sigma}_{p2}^2$ [c]		$51^+ (\pm 0)$ [b]
$\hat{\sigma}_{p3}^2$ [d]		$49^+ (\pm 6)$ [b]
$\hat{\sigma}_{p}^2$ [e]		$49^+ (\pm 0)$ [b]
$\hat{\sigma}_{pl}^2$ [f]		$2^+ (\pm 0)$ [b]
$\hat{\sigma}_{pl}^2/\hat{\sigma}_{p}^2(\%)$		4.081^+
r_{12} [g]		0.809^+
r_{13} [g]		0.782^+
r_{23} [g]		0.782^+

** Significant at the 1% probability by F test; + Volume (m³/plant) x 10^{-5}; [a] Genetic variance between the progenies in location 1; [b] Errors associated with the estimates $\hat{\sigma}_{p1}^2$, $\hat{\sigma}_{p2}^2$, $\hat{\sigma}_{p3}^2$, and $\hat{\sigma}_{pl}^2$; [c] Genetic variance between the progenies in location 2; [d] Genetic variance between the progenies in location 3; [e] Genetic variance between the progenies in the joint analysis; [f] Variance of interaction between progenies x locations; [g] Correlation coefficients of the average performance of progenies in paired locations (1 x 2, 1 x 3 and 2 x 3).

The variation among progenies in all of the locations can be clearly observed in Figure 1. When considering all locations, the amplitude of variation is equivalent to 0.515 m³, i. e. 3.12-fold greater than the general mean of the progenies. Of the 10.713 trees evaluated in the experiments, 3184 exhibited volumes larger than one standard deviation above the mean value of the controls, thus demonstrating the high potential of the population for selection.
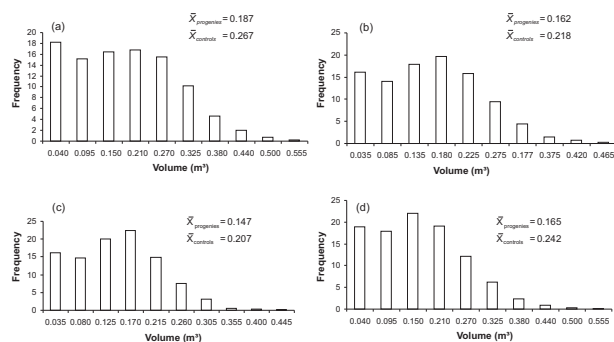

Figure 1. Progeny tests: distribution of progenies according to volume (m³ plant⁻¹) in (**a**) location 1, (**b**) location 2, (**c**) location 3, (**d**) all three locations.

Estimates of the genetic variance among progenies were significantly different from zero, suggesting the existence of variation for wood volume. The corresponding component to the interaction progenies x locations $(\hat{\sigma}^2_{pl})$ was, however significant, small (i.e. $\hat{\sigma}^2_{pl} < \hat{\sigma}^2_{p}$). Estimates of the correlations between the average performances of the progenies in the locations considered, two by two, ranged from 0.78 to 0.81 (Table 3). From these results we can infer that the interaction progenies x locations was of small magnitude, and has a major contribution to the simple part, as suggested by Vencovsky and Barriga (1992). The existence of an interaction progenies x locations in the culture of *Eucalyptus* in Brazil has been described previously (Pereira et al. 1997, Nunes et al. 2002). However, as confirmed in the present study, the participation of this component of interaction to the total variation is small. Pereira et al. (1997) for example, reported a value of $\hat{\sigma}^2_{pl}/\hat{\sigma}^2_{p} < 17\%$ following studies involving the selection of *Eucalyptus camaldulensis*. In principle, these results favor the selection of individual trees since they are selected in one location and clones will be evaluated and, subsequently, recommended for cultivation in locations that may not necessarily be the same as that employed in the selection process.

Clonal tests

The joint analysis demonstrated that there were significant differences ($P \leq 0.01$) between the clones and between the locations (Figure 2). No significant differences were detected, however, in the interaction between clones x locations indicating that the clones performed similarly in both locations (Table 4). Estimates of the genetic variance in each location and in the locations average were all significantly different from zero. It is noteworthy that the heritability estimates for selection in the mean of the clones was high on location and also in joint analysis, indicating favorable selection conditions (Table 4). The heritability estimates obtained were higher than those reported previously for *Eucalyptus* spp. (Volker et al. 1990, Paula et al. 2002, Pádua et al. 2004).
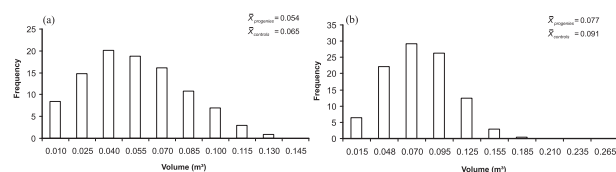

Figure 2. Clonal tests: distribution of clones according to volume (m³ plant⁻¹) in (**a**) location 4, (**b**) location 5.

Table 4. Variance joint analyses and estimation of the genetic parameters obtained from the individual and joint analyses carried out in the clone test for two year old trees

Source of variation	df	Mean Squares
Replications/locations	38	0.004**
Locations (L)	1	0.304**
Clones (C)	175	0.029**
C x L	175	0.001
Error	9696	0.001
Mean		0.071
CV(%)		33.370
R²(%)		52.710
$\hat{\sigma}^2_{c4}$ [a]		7⁺ ($\pm$ 0) [b]
$\hat{\sigma}^2_{c5}$ [c]		11⁺ ($\pm$ 1) [b]
$\hat{\sigma}^2_{c}$ [d]		8⁺ ($\pm$ 0) [b]
$\hat{\sigma}^2_{cl}$ [e]		2⁺ ($\pm$ 0) [b]
$\hat{\sigma}^2_{F4}$ [g]		7⁺
$\hat{\sigma}^2_{F5}$ [g]		11⁺
$\hat{\sigma}^2_{F}$ [h]		8⁺
$\hat{h}^2_{4}(\%)$ [i]		96.159⁺ (95 - 97) [k]
$\hat{h}^2_{5}(\%)$ [j]		96.954⁺ (96 – 97.5) [k]
$\hat{h}^2(\%)$ [l]		97.712⁺ (96 - 98) [k]
r_{45} [m]		0.959⁺

** Significant at the 1% probability by F test; ⁺ Volume (m³/plant) x 10⁻⁵; [a] Genetic variance between clones in location 4; [b] Errors associated with the estimates $\hat{\sigma}^2_{c4}$, $\hat{\sigma}^2_{c5}$, $\hat{\sigma}^2_{c}$ and $\hat{\sigma}^2_{cl}$; [c] Genetic variance between clones in location 5; [d] Genetic variance between clones in the joint analyses; [e] Variance of interaction between controls x locations; [f] Phenotypic variance between clones in location 4; [g] Phenotypic variance between clones in location 5; [h] Phenotypic variance between clones in the joint analyses; [i] Heritability in location 4; [j] Limits (range) associated with estimates; [k] Heritability in location 5; [l] Heritability in the joint analyses; [m] Genetic correlation coefficient between locations 4 and 5.

The estimate of the gain with selection between *Eucalyptus* clones, with reference to clone 361, was high (34%). Garcia and Nogueira (2005) estimated gains of 22% with the selection, with reference to the mean of the clones. It is important to emphasise that, in the present study, the estimate of gain was based only on the gain of individuals selected in the progeny test ($n = 173$), and that a higher value could have been achieved if all individuals ($n = 10713$) had been considered.

Since the progeny and clonal tests were conducted in different years and locations, it was necessary to adjust the data by reference to the values determined for the commercial clone 361, which was common to all experiments. The relative mean volume of the 17 trees selected ($M_{Si} = 1.99$) was 1.48-times higher than the mean of the 173 trees ($M_{oi} = 1.421$). On the other hand, the mean of the clones derived ($M_{sj} = 1.479$) from such trees was 1.34-fold larger than the overall mean volume ($M_{oj} = 1.103$) (Figure 3). In this condition, the realized heritability (h^2), was 0.84. The high estimate obtained suggests that the conditions employed were favorable for breeding since the selection of trees in the progeny test was efficient in identifying the best clones. Realized heritability estimates for *Eucalyptus* are not available in literature and hence comparisons with other reports are not possible.

Figure 3. Distribution of individuals selected in the progeny test (**a**) and clone test (**b**) according to volume (relative to that of clone 361). (Mo_i is the mean value of the original population (individuals selected in the progeny test), Ms_i is the mean value of the individuals selected in the original population (Mo_i), Mo_j is the mean value of the clonal test, and Ms_j is the mean value of the individuals of population Mo_j corresponding to the individuals selected in population Mo_i).

Herdabilidade realizada na seleção de árvores de *Eucalyptus* spp. por meio de teste de progênie

Resumo - *Progênies de irmãos germanos foram avaliadas com o objetivo de comparar o desempenho de árvores selecionadas em testes de progênies com seus respectivos clones nos teste clonais. Os experimentos foram implantados em blocos completos casualizados com cinco repetições e cinco plantas por parcela, no espaçamento de 3 x 3 m e quatro testemunhas. O volume de madeira de cada árvore foi avaliado no segundo e quatro anos. As 173 árvores selecionadas no teste de progênies foram avaliadas em testes clonais em dois diferentes locais. O mesmo delineamento experimental foi utilizado, porém com 20 repetições e parcelas de uma planta, no espaçamento de 3,5 x 2,15 m e 13 clones comerciais como testemunhas. Os caracteres de crescimento de cada árvore foram determinados no segundo ano de campo. Parâmetros genéticos foram estimados usando modelos lineares generalizados. A herdabilidade realizada foi superior a 80%, indicando que a seleção de árvores no teste de progênies foi eficiente.*

Palavras-chave: progênies de irmãos germanos, melhoramento, seleção clonal, interação genótipos x ambientes, ganho de seleção.

REFERENCES

Assis TF (2000) Production and use of Eucalyptus hybrids for industrial purposes. In Dundgey HS, Dieters MJ and Nikles DG (eds.) **Hybrid breeding and genetics of forest trees: proceedings of the QFRI/CRC-SPF symposium**. Department of Primary Industries, Brisbane/QLD, p. 63-74.

Assis TF and Máfia RG (2007) Hibridação e clonagem. In A Borém (Ed) **Biotecnologia Florestal**. UFV, Viçosa, p.93-121.

Chaperon H (1987) Vegetative propagation of *Eucalyptus*. In **Anales del symposio sobre silvicultuira y melhoramiento genético de especies forestales**. Vol 1, Centro de Investigaciones e Experiencias Forestales, Buenos Aires, p. 215-223.

Fehr WR (1987) **Principles of cultivar development**. Macmillan Publishing, New York, 487p.

Garcia CH and Nogueira MCS (2005) The application of the REML/BLUP methodology for eucalypt clones selection. **Scientia Forestalis 68**: 107-112.
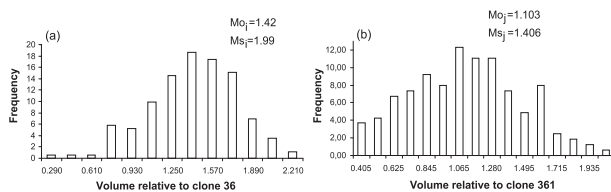
Gonçalves FMA, Rezende GDSP, Bertolucci FLG and Ramalho MAP (2001) Progresso genético por meio da seleção de clones de eucalipto em plantios comerciais. **Revista Árvore 25**: 295-301.

Knapp SJ, Stroup WW and Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. **Crop Science 25**: 192-194.

Marcó M and White TL (2002) Genetic parameter estimates and genetic gains for *Eucalyptus grandis* and *E. dunnii* in Argentina. **Forest Genetics 9**: 205-215.

Nunes GHS, Rezende GDSP, Ramalho MAP and Santos JB (2002) Implicações da interação genótipos x ambientes na seleção de clones de eucalipto. **Revista Cerne 8**: 49-58.

Pádua FA, Trugilho PF, Rosado SCS, Lima JT, Cardoso Júnior AA and Oliveira AC (2004) Estimate of the genetic parameters of growth stresses in Eucalyptus clones. **Scientia Forestalis 66**: 152-160.

Paula RC, Pires IE, Borges RCG and Cruz CD (2002) Prediction of genetic gains in forest improvement. **Pesquisa Agropecuária Brasileira 37**: 159-165.

Pereira AB, Marques Júnior OG, Ramalho MAP and Althoff P (1997) Eficiência da seleção precoce em famílias de meios-irmãos de *Eucalyptus camaldulensis* Dehnh., avaliadas na região noroeste do Estado de Minas Gerais. **Revista Cerne 3**: 25-32.

Ramalho MAP, Ferreira DF and Oliveira AC (2005) **Experimentação em genética e melhoramento de plantas**. Editora UFLA, Lavras, 326p.

Resende MDV (2002) **Genética biométrica e estatística no melhoramento de plantas perenes**. Embrapa Informação Tecnológica, Brasília, 975p.

Resende MDV (2007) **Software Selegen – REML/BLUP: sistema estatístico e seleção genética computadorizada via modelos mistos**. Embrapa Florestas, Colombo, 359p.

SAS INSTITUTE INC. SAS/STAT User' Guide, version 8, ed. Cary: NC, 1999. 3384p.

Vencovsky R and Barriga P (1992) **Genética biométrica no fitomelhoramento**. Revista Brasileira de Genética, Ribeirão Preto, 496p.

Vencovsky R and Ramalho MAP (2006) Contribuições do melhoramento genético no Brasil. In Paterniani E (ed) **Ciência, agricultura e sociedade**. EMBRAPA, Brasília, p. 41-74.

Volker PW, Dean CA, Tibbits WN and Ravenwood IC (1990) Genetic parameters and gains expected from selection in *Eucalyptus globulus* in Tasmania. **Silvae Genetica 39**: 18-28.

Zobel BJ and Talbert, J (1984) **Applied forest tree improvement**. John Wiley and Sons, New York, 511p.