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Research Article

Combining ability of elite clones of *Eucalyptus grandis* and *Eucalyptus urophylla with Eucalyptus globulus*

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Abstract

In Brazil, eucalyptus breeding programs for cellulose production has used two species, *Eucalyptus grandis* and *Eucalyptus urophylla*. Nevertheless, it would be useful to introgress alleles from other species to improve wood quality and volume. The objective of this research was to evaluate the hybrid potential of elite clones of *E. grandis* and *E. urophylla* from the Aracruz Celulose Company S. A. with *Eucalyptus globulus* clones. To do so, six elite clones were crossed with ten *E. globulus* clones in a half-diallel mating design. The resulting hybrid combinations as well as the four check clones were evaluated in randomized complete block experiments with single plant plots and 40 replicates from September to October 2001 at three Brazilian sites, Aracruz and São Mateus in the Espírito Santo state and Caravelas in Bahia State. Two years later the circumference at breast height (CBH) and the wood density (WD) were measured. The means were submitted to diallel analysis according to the Griffing method (1956), adapted by Geraldi and Miranda Filho (1988). Although the number of clones involved was small, the crossings of elite clones of *E. grandis* and *E. urophylla* with clones of *E. globulus* were promising, especially for wood quality gains.

Key words: diallel, *Eucalyptus* breeding, hybrids, quantitative genetics, wood quality.

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Introduction

The success with improvement of eucalyptus in Brazil focusing on wood volume is undoubted but while the companies involved in the large-scale production of eucalyptus want the selection program for wood volume should continue they also want other factors to be included that could reduce specific wood consumption or the costs of the industrial process. To date, selection studies targeting the cellulose industry have concentrated on the species *Eucalyptus grandis* and *Eucalyptus urophylla* as well as their respective hybrids, although it would be very important to introgress alleles of other *Eucalyptus* species aimed at enhanced wood quality. One option is the species *Eucalyptus globulus* from temperate climates which although it probably has worse adaptation properties to tropical climates presents higher wood density and a lower lignin content than *E. grandis* and *E. urophylla*, allowing an increase in cellulose yield and reduced costs in regard to fiber extraction. The structural barrier is a major problem for producing F_1 hybrids with *E. globulus*, a species that has as much large flowers than *E. grandis* and *E. urophylla* (Potts and Dungey, 2004). In this case F_1 hybrid seeds can only be produced using *E. globulus* as the pollen parent (Gore *et al.*, 1990).

Since improvement is an accumulation of advantages (Rasmusson and Phillips, 1997), it is important that crossings are restricted to the elite clones of the species involved. One strategy is to use diallel crosses to evaluate the combining ability clones. In the case of the *Eucalyptus* species mentioned above, half-diallels would be used in which one group of parents of one species is crossed with that of another species (Geraldi and Miranda Filho, 1988). By means of diallel crossings it is possible to obtain information on the combining ability as well as draw conclusions on the type of genetic effect that prevails in the control of the traits.

Unfortunately, diallels have not been used very much in forest improvement. The present study was therefore undertaken with the objective of verifying the combining ability of clones of *E. grandis* and *E. urophylla* with *E. globulus* clones using half-diallel crossings.

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Material and Methods

The diallel was evaluated at three Brazilian sites of commercial eucalyptus stands of the Aracruz Celulose Company S. A., the sites being Aracruz (19°50' S, 40°12' W) and São Mateus (18°36' S, 40°01' W) in the state of Espírito Santos and Caravelas (17°47' S, 39°33' W) in the state of Bahia.

For the crossings, pollen of 10 superior *E. globulus* clones from Chile was sent to Aracruz Celulose S. A., this method allowing the characteristics of the clones to be preserved by the holder company. Six elite clones derived from *E. grandis, E. urophylla* and *Eucalyptus saligna* from Aracruz Celulose S. A. (Table 1) were used as mothers in partial diallel crossings with the *E. globulus* clones. Forty-seven hybrids of the 60 possible combinations were established.

The seeds obtained in the crossings were collected and grown in a greenhouse to obtain seedlings. Seedlings of the control clones were also produced. The experiments were carried out from September to October 2001, in a randomized blocks design with 40 replicates and one plant per plot and with a spacing between plants of 3m x 3m. The clones 1 to 4 were included as controls in both experiments. The management of the experiments was the same as for commercial stands.

Two years after the implantation of the experiments the following data were taken:

circumference at breast height (cm) and pilodyn penetration in the wood (mm). Based on the evaluation realized with the pilodyn the wood density was estimated in kg m^{-3} using the expression density = 615-11.R, where R is the pilodyn reading. For the establishment of this expression 142 two-year-old trees of different eucalypt species were previously evaluated with the pilodyn and the wood density thereafter determined in the laboratory. On these underlying evaluations the regression equation relating pilodyn reading and wood density was constructed.

Initially, the analysis of variance was realized per localization in each experiment and later the analysis of joint variance. Analyses of variance were realized using the *Procedure for General Linear Models* (PROC GLM) of the SAS® program (SAS, 2000). The means were grouped by the Scott Knott (1974) test using the Mapgen software.

Using the data means per localization and in the joint analysis, the estimates of the combining ability were obtained using the model of Griffing (1956), adapted to half-diallel crossings by Geraldi and Miranda Filho (1988). Detail of the diallel analysis can be found in Cruz *et al.* (2004). The model was the following:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + \bar{e}_{ij}$$

where Y_{ij} is the mean of the crossing involving the i^{th} parent of group 1 and the j^{th} parent of group 2; μ is the general mean of the diallel; g_i is the effect of the general combining ability of the i^{th} parent of group 1; g_j is the effect of the general combining ability of the j^{th} parent of group 2; s_{ij} is the effect of the specific combining ability and $\bar{e}_{ij}$ is the mean experimental error. Where

$$\hat{g}_i = \bar{Y}_{i\bullet} - \bar{Y}_{\bullet\bullet};\ \hat{g}_j = \bar{Y}_{\bullet j} - \bar{Y}_{\bullet\bullet} \text{ and } si_{ij} = Y_{ij} - \frac{Y_{i\bullet}}{q} - \frac{Y_{\bullet j}}{p} + \frac{Y_{\bullet\bullet}}{pq};$$

$\bar{Y}_{i\bullet}$ and $\bar{Y}_{\bullet j}$ is the mean of the crossing involving the parental of group 1 and the j^{th} parent of group 2; $\bar{Y}_{\bullet\bullet}$ is the general mean; p is the number the parental of group 1 and q numbers the parental of group 2.

Considering that not all combinations were obtained and that there were differences in the number of replicates, the diallel analyses were realized by the least square method using the *Procedure for Interactive Matrix Language* (PROC IML) of the SAS® program (SAS, 2000).

Table 1 - Relation of elite *Eucalyptus* clones used in the crossings with *Eucalyptus globulus*.

Elite clones	Origin
1	Hybrid with an *Eucalyptus grandis* mother, from Rio Claro, São Paulo (SP), Brazil
2	Hybrid with an *Eucalyptus saligna* mother, from Rio Claro, SP
3	Hybrid with an *Eucalyptus urophylla* mother, from Rio Claro, SP.
4	Hybrid with an *Eucalyptus grandis* mother, from Rio Claro, SP.
5	Hybrid of *Eucalyptus urophylla* from Flores-Indonésia.
6	Hybrid of *Eucalyptus grandis* from of Zimbabwe.

Results and Discussion

Despite the fact that a significant difference (p = 0.01) was detected between the stand sites the actual mean performance of the treatments varied little between sites. Considering the circumference at breast height, the difference between the highest mean obtained in São Mateus and the lowest in Aracruz was only 2.6 cm (8.5% of the general mean) while for wood density (WD), the amplitude of variation between the sites was 21.7 kg.m^{-3}, or about 5% of the general mean (Table 2).

The interactions between hybrids and controls x places were significant (p = 0.01), although the contribution of the interaction to the total variation was small for both traits. For example, for the circumference at breast height, the sum of squares (SQ) of the interaction was only 14.6% of the SQ of the treatments. Based on this data emphasis will be given to mean results that involve the three sites.

The circumference at breast height attained by the hybrids was 31.9% lower than that obtained by the controls but the opposite occurred for wood density. The hybrids presented a 5.5% superior WD mean to that obtained by the control clones (Table 2). An important factor to verify is

Table 2 - Means of hybrids and controls for circumference at breast height (CBH) and wood density (WD) obtained in the evaluation of diallel crossings of two-year-old *Eucalyptus globulus* and elite clones.

Trait		Sites			
		Aracruz	São Mateus	Caravelas	Mean
CBH (cm)	Hybrids mean	26.27	27.59	25.74	26.50
	Controls mean	36.54	40.15	40.05	38.92
	Mean	29.21	31.79	29.51	30.15
WD ($kg.m^{-3}$)	Hybrids mean	439.67	438.03	423.13	433.77
	Controls mean	423.67	417.68	392.64	411.33
	Mean	437.13	432.34	415.46	429.03

whether or not any hybrid presented better performance than the best control trees. For example, clone 4, the one most used by the company, presented superiority for circumference at breast height (Table 3) but when this clone was taken as reference none of the hybrids was placed in the same group as clone 4. This was the case because the species *E. globulus*, one of the parents, is less adapted to the conditions of southeastern Brazil than the clones of *E. grandis* and *E. urophylla*. Potts and Dungey (2004), show results where the hybrids were worse than parents, as happened with the circumference at breast height in this experiment.

For wood density all hybrids presented superior performance compared to the clones (Table 3). In the industrialization of wood for cellulose production, one of the factors that most affects the cost of the process is the lignin content (Do Cavaco, 2003). It is therefore strongly desirable to obtain clones that associate a higher wood weight per area with lower lignin content. Considering that one of the main advantages of *E. globulus* is the lower lignin content (Pereira *et al.*, 2000), the results of this study show that there is a possibility of associating this trait with greater circumference at breast height and greater wood density, which would result in lower costs for the extraction of cellulose fibers and higher wood weight. Still, the estimate of the phenotypic correlation between circumference at breast height and density was -0.75 ($p = 0.01$), indicating that it would initially be difficult to obtain hybrids that associate greater volume and wood density. In the literature there are few reports on this topic, although Raymond (2002) presents some results of estimates of the genetic correlation between basic wood density and tree diameter, with most values being close to zero although some estimates were negative. Paula *et al.* (2002) however obtained an estimate of 0.46 for the genetic correlation between wood volume and wood density.

An advantage of diallel crossings is the possibility not only of identifying good hybrid combinations but also drawing inferences on the combining ability of the parents involved (Cruz *et al.*, 2004). It should be emphasized, however, that although the number of clones used in our study was small the results of the analysis of variance of the diallel showed that the performance of the hybrids depended as much on the general combining ability (GCA) of

Table 3 - Means of the hybrids and clones used as controls for circumference at breast height (CBH) and wood density (WD) obtained in the evaluation of two-year-old trees from diallel crossings of *Eucalyptus globulus* clones (fathers) and elite clones (mothers), in the joint analysis.

Trait	M\F[2/]	1	2	3	4	5	6	7	8	9	10	*Per se*
CBH (cm)	1					24.03 D	32.08 C			25.55 D	30.35 C	37.76 B
	2	24.71 D[1/]	26.08 D	23.87 D	21.88 E	20.57 E	29.61 C	27.34 D	23.77 D	25.60 D	20.96 E	35.72 B
	3		20.73 E	26.86 D	23.04 E		32.98 C	24.32 D	22.87 E	23.03 E	31.32 C	38.00 B
	4		20.85 E	25.83 D	20.92 E	27.29 D	31.02 C	26.28 D	21.63 E	24.07 D	22.47 E	44.18 A
	5			29.55 C	30.31 C		34.59 B		30.58 C	31.98 C	34.67 B	
	6			28.40 C	21.13 E	28.49 C	33.44 C	28.90 C	20.70 E	33.87 C	24.11 D	
WD ($kg.m^{-3}$)	1					430.23 B	436.65 B			435.88 B	434.18 B	415.57 D
	2	436.92 B	434.08 B	439.86 B	435.84 B	435.41 B	439.18 B	433.98 B	433.39 B	439.59 B	445.35 A	414.93 D
	3		433.77 B	426.75 C	439.98 B		432.19 B	432.04 B	437.19 B	435.07 B	428.70 C	407.14 D
	4		440.40 B	436.69 B	444.61 A	433.84 B	442.20 A	441.69 A	444.88 A	438.52 B	455.56 A	407.66 D
	5			419.38 C	421.96 C		423.46 C		417.05 D	420.75 C	425.59 C	
	6			423.77 C	437.86 B	433.03 B	428.63 C	422.45 C	424.91 C	420.60 C	445.48 A	

[1/] For the same trait, treatments with the same letter belong to the same group ($p < 0.05$) by the test of Scott and Knott (1974); [2/] M - mother, F - father.

the parents of group 1 (GCA 1) and group 2 (GCA 2) as on the specific combining ability (SCA). The reason is that the F test was in all cases significant ($p < 0.01$) for these sources of variation (Table 4).

Although the differences were significant in all cases, the relative contribution of the GCA sum of squares was superior to the SCA. For the circumference at breast height the GCA explained 68% of the sum of squares among hybrids, while for the density the GCA explained 76%.

Initially these results allowed the conclusion that in the genetic control of these traits there is a predominance of additive effects, although dominance is also important. The occurrence of dominance in the expression of traits in the eucalypt crop has been mentioned in several papers (Rezende and Resende, 2000). For hybrid *E. urophylla* x *Eucalyptus pellita*, the relation between the variance of dominance and the additive variance for wood volume was higher (Bouvet and Vigneron, 1996). This shows that the variance of dominance contributes to the manifestation of the volume of trees. For the wood density, the results found in literature indicate that the variance of dominance is less important in the expression of this trait (Assis, 2000). These results agree with those obtained in our present study.

Comparing the participation of the GCA1 elite clones in relation to the GCA2 clones, the elite clones contributed less to the circumference at breast height and more to the wood density (Table 4). The contribution of the GCA of the elite clones was 43.0% for the circumference at breast height and 81% for the wood density. In the case of the circumference at breast height a greater variation of the GCA occurred between the clones of group 2, that is, in the group with the lowest frequency of favorable alleles for the trait under study. The opposite was observed for wood density (Table 4).

The specific combining ability (SCA) depends on the divergence in the parents and the occurrence of dominance (Vencovsky, 1987). Since the parents belonged to different gene pools and were from different origins, it can be inferred that the SCA was of lower magnitude due to the small contribution of dominance to the expression of the traits, especially for wood density.

For the circumference at breast height trait clone number 5 presented a positive GCA in the group 1 elite clones (Table 5). In group 2 (*E. globulus*), the clone with the highest positive GCA was number 6. The crossings that stood out for the SCA and in the means were 3 x 6, 5 x 6, 5 x 10, 6 x 6 and 6 x 9. For wood density the group 1 clones 2 and 4 presented positive GCA (Table 5). In group 2, clone 10 presented a GCA positive value. The crossings that stood out for the SCA estimate and the means were 2 x 10, 4 x 8, 4 x 10 and 6 x 10.

One of the main questions that arise is how the information of the diallel can be used by the breeders of a genus such as *Eucalyptus* that can be propagated sexually as well as asexually. In the first place the selection of the best trees for the establishment of new clones comes to mind. In the case of a species in which the information is obtained per tree, as in eucalypts, a great advantage of the diallel cross is that, while one identifies the crossing with highest mean, the estimate of the variability within the crossing can be obtained without additional efforts. This way one can identify the most promising segregating population for selection, *i.e.* one that associates high mean and variance values for the selected trait.

For tree selection the use of the combined selection as promoted by Resende (2002) and Bueno Filho and Vencovsky (2000) would be interesting, considering the performance of each tree as well as the performance of the hybrid combination to which it belongs. Another alternative would be the selection of trees based on the genotypic value, using the mixed model methodology based on the Best Linear Unbiased Prediction (BLUP) (Resende, 2002).

Once the information of the diallel is obtained, a good strategy would be that the breeder would repeat the best crossings to establish a large number of individual trees and be able to apply high selection intensity. As the amplitude of genetic variation between plants increases with the increase of the sample size (Steel *et al.*, 1997) when a large number of trees is evaluated it is possible to select ones with excellent performance within these populations and consequently achieve more success with selection.

Other information that can be extracted from the diallel is the identification of parents for the synthesis of a

Table 4 - Summary of the analyses of variance of the half-diallel, considering the mean of the three places for circumference at breast height (CBH) and wood density (WD) obtained in the evaluation of two-year-old trees from crossings of *Eucalyptus globulus* with elite clones.

		CBH (cm)			WD ($kg.m^{-3}$)		
SV[1]	DF	SS	MS	p	SS	MS	p
GCA1	5	6652.40	1330.48	0.00	53856.00	10771.20	0.00
GCA2	9	8661.87	962.43	0.00	12757.86	1417.54	0.00
SCA	30	7141.50	238.05	0.00	20432.10	681.07	0.00
Error	2227	123398.10	55.41		793658.30	356.38	

[1/] GCA1 - General combining ability for the clones of *Eucalyptus grandis* and *Eucalyptus urophylla* (elite clones); GCA2 - General combining ability for the clones of *Eucalyptus globulus*; SCA - Specific combining ability.

Table 5 - Estimate of the effects of the general combining ability of group 1 (GCA_i), general combining ability of group 2 (GCA_j) and specific combining ability (SCA_{ij}), for the circumference at breast height (CBH) and wood density (WD) obtained in the evaluation of two-year-old trees from diallel crossings of *E. globulus* clones (fathers) and elite clones (mothers) in the joint analysis.

Trait	M\F [2/]	1	2	3	4	5	6	7	8	9	10	CGC_i
CBH (cm)	1					-1.95	0.18			-1.12	1.75	-0.38
	2	!	3.15*	-0.52	0.17	-3.44*	-0.33	2.25*	1.16	0.89	-5.67*	-2.34*
	3		-4.16*	0.52	-0.62		1.08	-2.73*	-1.70	-3.64*	2.73*	-0.38
	4		-1.84[1/]	1.69	-0.54	3.52*	1.32	1.43	-0.73	-0.40	-3.92*	-2.58*
	5			-1.83	1.61		-2.34*		0.98	0.27	1.04	4.66*
	6			0.94	-3.65*	1.40	0.43	0.73	-4.98*	6.08*	-5.60*	0.73
	CGC_j	-0.95	-2.73*	-1.28*	-3.96*	-1.65*	4.28*	-0.57	-3.06*	-0.95	0.97	
WD ($kg.m^{-3}$)	M\F	1	2	3	4	5	6	7	8	9	10	CGC_i
	1					1.09	1.72			1.97	-3.32	1.20
	2	!	-1.49[1/]	5.06	-4.92	2.08	0.06	-2.10	-2.35	1.49	3.67	5.39*
	3		4.87	-1.37	5.89*		-0.25	2.64	8.13*	3.65	-6.31*	-1.29
	4		-0.48	-3.41	-1.46	-4.80	-2.22	0.30	3.84	-4.89	8.57*	10.69*
	5			1.47	-1.92		1.23		-1.80	-0.46	0.79	-11.50*
	6			-1.45	6.67	9.27*	-0.91	-4.05	-1.25	-7.92*	13.37*	-4.19*
	CGC_j	-1.13	-2.47	-3.25*	2.72	-4.72*	1.07	-1.97	-2.31	0.05	3.64*	

[1/]SCA_{ij}- internal part of the table; * - significant at 5% probability by the T test; ! - SCA_{ij} not estimated; [2/]M - mother, F- father.

compound targeting intrapopulational improvement, or of two compounds for interpopulational improvement. In the case of intrapopulational improvement, the choice could be directly realized in the parents with highest GCA values, or based on the best hybrid combinations, identifying the best trees for intermating.

Information from diallel crosses, especially in a situation such as in the present study, can further be used to identify testers for future evaluations of new introduced clones.

Finally, it is worth mentioning that the strategy of the use of pollen of elite clones of other companies for the realization of crossings with commercially planted clones is very useful since it allows the holder company of the clones to control the property rights of the genetic material and simultaneously allows the achievement of superior hybrids by and for companies that use the pollen. As highlighted by Rasmusson and Philips (1997), improvement is the accumulation of advantages. This way, all companies could share the headway made in genetics and consequently, the success of the genetic improvement in Brazil would certainly be amplified.

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