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Inbreeding depression in *Eucalyptus* clones

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ABSTRACT - *Most* Eucalyptus *plantations are developed from clones. The inbreeding depression estimate allows plant breeders to plan their breeding programs more accurately. To obtain information concerning inbreeding depression, 10 clones were inbred. The clonal (F_1) and the inbred generation (F_2) were evaluated in two contiguous experiments carried out in Aracruz, Espirito Santo, Brazil. A randomized complete block design with one-tree plots and 40 replicates was applied in both experiments. The experiments were installed in August 2001 and evaluated two years later. The inbreeding depression varied among the clones and was in average 17.5% for the circumference at breast height and 4.0% for the wood basic density. In most cases it was possible to select F_2 generation trees with a better performance than their (F_1) parents, indicating this strategy as promising for breeders to select superior plants.*

Key words: quantitative genetics, breeding, circumference at breast height, wood basic density.

INTRODUCTION

Eucalyptus is considered an allogamous tree with a natural selfing rate varying from 10% to 30% (Eldridge et al. 1993). Selfing therefore leads to inbreeding depression during the tree development, expressed in a higher mortality and less vegetative growth in the nursery and field stages.

The achievement of estimates of inbreeding depression allows inferences on the genetic population structure and the type of predominant gene action in the genetic trait control. There are few reports on eucalyptus of these estimates and most of them involve populations of species that are not used in Brazil (Hardner and Potts 1995, Griffin and Cotterril 1988).

Given the tendency of companies to use only superior clones in their plantations, it would be interesting to know the inbreeding depression of these clones and to know if it differs among them. Besides, it would be important to verify the viability of proceeding to the selection of superior trees within the segregant populations derived from these clones. When there is no overdominance in the genetic control of these traits, it is possible to select recombinant individuals with a better performance than the parent clone amongst the clone-derived segregant populations. Unfortunately, no information on this possibility was found in literature.

In this setting, we estimated the inbreeding depression for some traits of economical importance in eucalypt to verify if

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the inbreeding depression differs among the clones and to propose a selection strategy using inbreeding.

MATERIAL AND METHODS

Two experiments were conducted to estimate the inbreeding depression in eucalypt in the Aracruz Celulose S.A. Company, in Aracruz, state of Espirito Santo, Brazil. The geographical coordinates of the plantation site were 19º 50' 19" lat S and 40º 12' 43" long W. Ten commercial eucalypt clones of the company were used for the study. The trees of each clone were artificially selfed to obtain F_2 generations.

With the seedlings obtained by cloning the 10 clones and with seeds obtained from the selfings, two experiments were conducted in contiguous areas. One experiment evaluated the clones (F_1 generation) and the other F_2 generation. The experimental design was of randomized complete blocks with one-tree plots and 40 replicates in a plant spacing of 3 x 3 m.

The experiments were set up in August 2001 using the procedure normally adopted by the company: 80 cm deep furrows were opened and phosphate was simultaneously applied at the bottom. The plant spots were fertilized with 100 g of N:P:K (6:30:6). Later, the plantlets were inserted and irrigated to guarantee the survival.

Two years after the installation of the experiments the following data were collected:

- circumference at breast height (CBH in cm);
- penetration of the pilodyn pin into the wood (mm).

Based on the penetration of the pilodyn pin into the wood the basic wood density was estimated as follows:

- basic wood density (BWD in kg m^{-3}), being BWD = 615 - 11 * pilodyn reading. In a previous stage, various two-year-old eucalypt trees of different species had been evaluated for this purpose using pilodyn; the wood density was thereafter determined in the laboratory. Based on these evaluations a regression equation was constructed between the pilodyn reading and the basic wood density.

The data obtained for the CBH and BWD were, initially, subjected to analysis of variance per experiment and later to the joint variance analysis, now involving both generations. The analyses were done by software SAS (SAS Institute 1993), using the Procedure for General Linear Models – Proc GLM. The Scott-Knott (1974) test was performed with the treatment means to verify which treatments belonged to the same group.

The following estimates were also obtained by the means:

Inbreeding depression (ID)

$ID(\%) = \frac{\bar{F}_{1_i} - \bar{F}_{2_i}}{\bar{F}_{1_i}} x100$, where $\bar{F}_{1_i}$ is the mean of clone i and $\bar{F}_{2_i}$ the mean of generation F_2 of clone i.

Mean components (*m+a* and *d*)

Considering that generation F_2 is an equilibrium population whose mean for the segregant population derived from clone i is obtained by: $\bar{F}_{2_i} = m + a + d$ (Vencovsky 1987), the mean of generation F_1 will be: $\bar{F}_{1_i} = m + a + 2d$ (Lima et al. 2000). Thus, the estimate of *m+a* can be obtained by $2\bar{F}_{2_i} - \bar{F}_{1_i}$ and *d* by $\bar{F}_{1_i} - \bar{F}_{2_i}$, where *m+a* is the contribution of the loci in homozygosis to the mean of each clone and *d* is the contribution of the loci in heterozygosis to the mean of each clone. To obtain the estimates of *m+a* and *d* in percentage, the obtained values were divided by the mean of the respective generation F_2 and multiplied by 100.

Broad-sense heritability estimate ($h^2_{a_i}$)

Considering that the variance among plants of a clone ($\sigma^2_{C_i}$) is wholly environmental ($\sigma^2_{C_i} = \sigma^2_E$) and that the phenotypic variance among plants of generation F_2 ($\sigma^2_{F_i}$) from the referred clone has genetic ($\sigma^2_{G_i}$) and environmental variance (σ^2_E), that $\sigma^2_{G_i} = \sigma^2_{F_i} - \sigma^2_{C_i}$. Thus, the estimator of the heritability is: $h^2_{a_i} = \sigma^2_{G_i} / (\sigma^2_{G_i} + \sigma^2_E)$, where σ^2_E is the mean environmental variance among trees of all clones evaluated and $\sigma^2_{G_i} + \sigma^2_E$ and variance among generation F_{2_i} plants.

Expected gain with selection (GS)

$GS(\%) = \frac{h^2_{a_i} \, x \, ds_i}{mc_i} x100$, where ds is the differential of selection (ds = $ms_i - mc_i$) where ms_i is the mean of the four best trees in generation F_2 of clone i and mc_i is the value equivalent to the mean obtained in generation F_1 from clone i, obtained in the experiment close to that of generation F_2.

RESULTS AND DISCUSSION

Any individual in a tree population of sexual reproduction is product of the union of two gametes. When doubled, these gametes correspond to two parent lines. Thus, any plant in this population is in fact a single-cross hybrid. A vegetatively propagated tree of this population represents a clone. Consequently, any clone is equal to F_1 generation of a single-cross hybrid. Analogously, trees from a selfed clone represent F_2 generation of the referred hybrid. For this reason, when referring to clone propagation F_1 generation will be considered and F_2 generation when referring to plants originated by selfing in this paper.

In the evaluation of F_1 generation, the estimate of the coefficient of variation (CV) was of small magnitude for the basic wood density (BWD) and of greater magnitude for the circumference at breast height (CBH) (Table 1). As mentioned above, the variation among trees of a same clone in F_2 generation is of genetic and environmental nature, due to segregation and recombination of the genes. As expected, under this condition the experimental precision was lower (data not shown).

Significant difference among the clones was detected in both generations (Table 2). The most outstanding clones for the CBH were 3, 4, 5, 7, and 8 (Table 2). For the BWD, clones 2, 4 and 5 presented the best performance (Table 2). These results evidenced the possibility of identifying clones, as those with the numbers 4 and 5, which associate the phenotypes of interest for the company with the evaluated traits.

Main objective of this study was the estimation of the inbreeding depression (ID). The existence of generation effects in the analyses of variance (Table 1) indicated that there was inbreeding depression. Mean results of F_1 and F_2 generations show that in the mean of the 10 clones, the depression was 17.5% for CBH and 4.0% for BWD (Table 2). The estimate of the inbreeding depression obtained for the CBH is compatible with the estimates reported in literature under other conditions. Reports of inbreeding depression in two-year-old *E. grandis* of 40.0% were observed for the CBH and in three-year-old trees of 26.0% for the same trait in relation to the progenies obtained in the cross of *E. grandis* x *E. urophylla* (Campinhos et al. 1998). In 19-month-old *E. globulus* an inbreeding depression of 21.0% was estimated for the diameter at breast height (Hardner and Potts 1995). These authors stated that the depression slightly increased with age; at 43 months it was 24.0% for the diameter at breast height. In 45-month-old *E. regnans* the estimate for the inbreeding depression was 11.0% for the diameter at breast height (Griffin and Cotterril 1988).

For the occurrence of inbreeding depression dominance and/or epistasis must be present in the trait control, besides the occurrence of loci in heterozygosis. No reports were found on the occurrence of epistasis in the genus *Eucalyptus*. On the other hand, the presence of dominance has been detected for the wood

Table 1. Summary of the joint analyses of variance for circumference at breast height (CBH) and basic wood density (BWD) obtained in the evaluation of F_1 and F_2 generations of two-year-old elite clones

Sources of variation	df	CBH cm		BWD kg m^{-3}	
		MS	P	MS	P
Replications/generation	78	35.27	0.00	389.46	0.00
Generations (G)	1	3184.57	0.00	46399.4	0.00
Treatments (T)	9	271.96	0.00	16839.9	0.00
G x T	9	112.76	0.00	2819.67	0.00
Pooled Error	567	16.68		247.03	
CV (%)		16.71		3.70	

Table 2. Estimate of the percent inbreeding depression (ID) of some two-year-old elite clones for circumference at breast height (CBH) and basic wood density (BWD)

Treatments	CBH cm			BWDkg m^{-3}			Survival %	
	Generation			Generation			Generation	
	F_1	F_2	ID	F_1	F_2	ID	F_1	F_2
1	25.36 B	22.42 B	11.59	430.37 C	407.13 C	5.40	92.50	82.50
2	26.09 B	20.24 C	22.41	445.70 A	423.24 B	5.04	95.00	85.00
3	27.80 A	21.01 C	24.42	387.39 E	379.34 D	2.08	95.00	57.50
4	28.17 A	20.88 C	25.90	444.23 A	441.13 A	0.70	97.50	67.50
5	26.55 A	23.34 B	12.07	448.39 A	427.29 B	4.71	95.00	87.50
6	25.24 B	22.03 B	12.69	436.45 B	414.33 B	5.07	92.50	72.50
7	27.55 A	19.46 C	29.35	440.95 B	401.52 C	8.94	90.00	55.00
8	28.30 A	28.88 A	0.00	427.83 C	394.59 C	7.77	95.00	97.50
9	23.45 C	20.20 C	13.84	430.00 C	430.29 B	0.00	90.00	62.50
10	23.90 C	18.55 C	22.39	423.94 D	423.14 B	0.19	92.50	60.00
Mean	26.24	21.70	17.47	431.52	414.20	3.99	93.50	72.75

In each column, treatments with the same letter are allocated to the same group by the Scott-Knott test ($P < 0.05$)

volume (Bouvet and Vigneron 1996, Rezende and Resende 2000). Dominance was however was not observed for the basic wood density (Assis 2000).

Another objective of the study was to verify if the inbreeding depression differs among the clones. The existence of a significant interaction treatments x generations (P<0.01) shows that the inbreeding depression was not the same among the clones (Table 1). Considering, for example, the CBH, the inbreeding depression was more pronounced for the clones 3, 4 and 7 while it was zero for clone 8 (Table 2). Basically, these results show that clones 3, 4 and 7 have a greater proportion of loci in heterozygosis for this trait in relation to clone 8. For the BWD, the inbreeding depression was most expressive for clones 7 and 8.

Table 3. Estimates of the percentual contribution of the loci in homozygosis ($m+a$) and in heterozygosis (d) for the mean of some two-year-old elite clones for circumference at breast height (CBH) and basic wood density (BWD)

Treatments	CBH		BWD	
	cm		kg m^{-3}	
	m+a	***d***	***m+a***	***d***
	%	%	%	%
1	86.89	13.11	94.29	5.71
2	71.11	28.89	94.69	5.31
3	67.68	32.32	97.88	2.12
4	65.04	34.96	99.30	0.70
5	86.27	13.73	95.06	4.94
6	85.46	14.54	94.66	5.34
7	58.46	41.54	90.18	9.82
8	100.00	0.00	91.58	8.42
9	83.93	16.07	100.00	0.00
10	71.15	28.85	99.81	0.19
Mean	79.09	20.91	95.82	4.18

It had been expected that the inbreeding depression in eucalyptus were greater. One of the reasons that this was not the case is that since the rate of natural selfing varies from 10.0% to 30.0% in this genus (Eldridge et al. 1993), part of the lethal alleles is naturally eliminated in each generation, reducing the inbreeding depression stepwise. Another reason is that during the establishment of the seedlings, there is mortality of the plantlets that express deleterious alleles. This mortality also occurs in the field during the development of the trees. This would lead to an underestimation of the inbreeding depression estimates.

Field mortality was observed in this study. It was verified that one of the main effects of inbreeding is plant mortality (Table 2). In some cases, as for clones 3, 4, 7, 9, and 10, the mortality was higher than 32.0%. This indicates that these clones have a high genetic load, which is expressed when selfed. This result is similar to that obtained by Hardner and Potts (1995) in 43-month-old *E. globulus.*

The results obtained in relation to inbreeding depression can be confirmed by means of the estimates of the contribution of the loci in homozygosis ($m+a$) and in heterozygosis (d) to the performance of the different clones (Table 3). In general, it was verified that the contribution of the loci in homozygosis was considerably more expressive than that of the loci in heterozygosis, especially for the BWD. Besides, clone 7 once again evidenced to have a greater proportion of loci in heterozygosis.

Comparisons of the estimates of $m+a$ and d with other allogamous plants are difficult, principally due to the difference in the considered traits. However, taking for example maize and the grain yield trait as reference, it is verified that the contribution of d is around 70.0% and of $m+a$ around 30.0% (Ramalho et al. 2003). There are however other traits in maize, whose results are quite similar to those obtained for the CBH in eucalyptus - for example the plant and ear height, where the estimate of the contribution of d is around 30.0% (Hallauer and Miranda Filho 1988).

Another question that arises is if it would be possible to obtain individuals with a superior performance to that of F_1 generation within a segregant population (F_2). This fact is expected when the trait is controlled by genes whose predominant allelic interaction is additive or of partial dominance. To answer this question the expected gain with selection within F_2 generation, in relation to the mean of F_1 generation of each clone was estimated (Table 4). For this estimate the mean variance among the trees of each clone in F_1 generation was considered as environmental variance. Even though relatively small populations had been used in F_2 generation (around 40 trees) there would be the possibility of obtaining new clones superior to the parent clone in almost all cases. In some cases, the estimated gain was

Table 4. Estimates of the broad-sense heritability (h_a^2) within the F_2 populations and of the expected gain with selection (GS) in relation to the mean of the parent clone, for circumference at breast height (CBH) and basic wood density (BWD) at two years of age

Treatments	(h_a^2)		GS (%)	
	CBH	**BWD**	**CBH**	**BWD**
1	0.52	0.69	12.82	1.81
2	0.58	0.55	6.97	0.70
3	0.52	0.86	1.79	6.57
4	0.60	0.72	1.71	5.39
5	0.44	0.62	6.53	0.79
6	0.51	0.65	10.60	0.78
7	0.55	0.78	0.00	0.00
8	0.63	0.78	21.65	1.28
9	0.70	0.84	20.15	7.39
10	0.51	0.81	5.09	7.60

quite expressive, as for example for the populations of clones 1, 6, 8, and 9 for the CBH. In the case of the density, the estimated gains were more expressive for populations 3, 4, 9, and 10. It is important to underline that clone 4 is the most planted by the company and that it would be possible to obtain, within a segregant population of this clone, trees with a greater BWD which would be very important for the cellulose industry.

Evidently, these estimates cannot be considered very trustworthy as there are no replications of the trees evaluated in each F_2. It would be very important that these were corroborated, multiplying the selected individuals and proceeding with the comparison of their performances compared to the parents. If these results were confirmed, it would be evidenced that in the genetic trait control the mean degree of dominance is lower than 1 and moreover, that this could be a strategy of obtaining new superior clones. The best clones of the company should therefore be selfed. In this case, an adequate strategy would be to harvest seeds in the center of the commercial stands, which would allow the achievement of a large number of seeds selfed with little work.

This way it would be possible to evaluate a large number of inbred trees in each population. Those that manifest lethal alleles could be eliminated in advance in the nursery. Besides, as there is the possibility of obtaining a large number of descendents a high selection intensity could be applied.

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Depressão endogâmica em clones comerciais de eucalipto

RESUMO - *Na cultura do eucalipto, a maioria dos plantios é realizada com clones. A estimativa da depressão por endogamia desses clones permite aos melhoristas planejarem seus programas de melhoramento mais eficientemente. Visando obter informações a esse respeito, as gerações clonal (F_1) e autofecundada (F_2) de 10 clones foram avaliadas em dois experimentos contíguos, conduzidos no município de Aracruz, ES, Brasil. O delineamento utilizado foi o de blocos casualizados, com 40 repetições e parcela de uma planta. Os experimentos foram instalados em agosto de 2001 e avaliados dois anos depois. A depressão por endogamia variou entre clones, sendo, em média, de 17,5% para a circunferência à altura do peito e de 4,0% para a densidade básica da madeira. Para a maioria dos clones foi possível selecionar plantas na geração F_2 com desempenho superior clone parental (F_1), evidenciando que esta pode vir a ser uma boa alternativa para os melhoristas procederem à seleção de plantas superiores.*

Palavras-chave: genética quantitativa, melhoramento, circunferência à altura do peito, densidade básica da madeira.

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