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Genetic Variability in a Breeding Population of *Eucalyptus urophylla* S.T. Blake

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Resumo

Os programas de melhoramento florestal têm sofrido grande pressão para apresentar resultados em vista do aumento da demanda de produtos e derivados. Neste sentido, a aplicação prática de marcadores moleculares nestes programas tem sido vantajosa. Este trabalho procura incorporar a utilização do marcador RAPD na avaliação da variabilidade genética em uma população-base de *Eucalyptus urophylla* com os objetivos de avaliar sua base genética e compor um banco de dados moleculares desta população composta por 61 indivíduos das procedências Flores e Timor e uma variedade comercial local. Esta população foi avaliada através de 70 locos RAPD polimórficos. Os resultados mostraram que a população-base apresenta uma ampla base genética com média de similaridade entre indivíduos de 0,3168. A variedade comercial apresentou a menor média de similaridade (0,2885). Cruzamentos baseados em distância genética são propostos.

Abstract

Tree breeding programs have been under tremendous pressure to show results since the demand for the derived products has greatly increased. Molecular markers have been used in breeding programs in an attempt to evaluate the genetic bases of populations involved in breeding programs, identify hybrids and parental lines, etc. The aims of this study were to evaluate genetic variability of a base population of *Eucalyptus urophylla* using RAPD, to assess the genetic base of populations and to construct a molecular data bank. The base population consisted of 61 individuals of Flores and Timor provenance and a local commercial variety. Seventy polymorphic loci were analyzed. The mean genetic similarity was 0.3168 for the base population and the commercial variety showed the lowest similarity (0.2885). Crosses based on genetic distance were proposed.

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Key words: RAPD, genetic variability, molecular markers, *Eucalyptus urophylla*, genetic similarity, breeding population, crosses.

Introduction

In Brazil, research on silviculture started at the beginning of the 20th century, with the main objective of supplying wood in order to reduce the deforestation of natural forests. The new silviculture was based on exotic species, mainly *Pinus* and *Eucalyptus* species (FERREIRA and SANTOS, 1997).

Among several *Eucalyptus* species growing throughout Brazil, *Eucalyptus urophylla* is very important because it is mainly used for hybridization with *Eucalyptus grandis*. Nowadays, commercial programs are based on clonal plantations, where hybrids between these species, known as "Eucalyptus urograndis", are those most extensively utilized. These hybrids show uniformity, productivity and fixation of economic traits.

The use of molecular markers in breeding programs has been very attractive for plants breeders. Since they are based on DNA, molecular markers allow rapid assays of genetic parameters. RAPD has been one of the most used molecular techniques over the last years, mainly because it is simple, inexpensive, of rapid execution and highly polymorphic. Although this technique provides a low content of genetic information per locus and low transferability of data between different laboratories, RAPD has been widely applied to the genus *Eucalyptus* for clone identification (LANGE et al., 1993), to construct linkage maps (GRATTAPAGLIA et al., 1995; GRATTAPAGLIA and SEDEROFF, 1994), to estimate outcrossing rate (GAIOTTO et al., 1997), for fingerprinting (CHEN and FILLIPIS, 1996), and to distinguish individuals (NESBITT et al., 1995).

Nevertheless, additional basic research as important as that carried out in the cited studies should be performed with the aim of monitoring and helping programs of *Eucalyptus* breeding. Such studies should include, for example, assessment and monitoring of genetic variability within base populations and the establishment of heterotic groups crossing based on genetic distance using molecular markers. Molecular markers can also be applied to evaluate redundancy and deficiency of collections and to provide data on the efficiency of the collection, main-

tenance, management and enhancement of a germplasm database (PHILLIPS et al., 1993; NEWBURRY and FORD-LOYD, 1993).

The objectives of the present study were to evaluate the genetic base of a *Eucalyptus urophylla* breeding population, to characterize individuals by molecular markers, and to provide information for crossing based on similarity data.

Material and Methods

Plant material: Sixty-one individuals of *E. urophylla* from the base population of the breeding program of the "Votorantim Celulose e Papel S.A. (VCP)" company were analyzed. The population comprised germplasm of Timor and Flores provenances from experimental areas and a group of individuals consisting of different provenances that represents the commercial variety obtained by the company after many breeding generations, defined here as "commercial variety" (*Table 1*).

Table 1. – Provenances, participation percent within the breeding population, NEI's diversity and mean similarity.

Provenance	Number of plants	% Within the population	H	S
Timor	10	16.4	0.2149	0.3228
Flores	25	41,0	0.2264	0.3308
Commercial Variety	26	42,6	0.2301	0.2885
TOTAL	61	100	0.2332	0.3168

Note: H = NEI's Diversity (1973); S = mean similiarity (JACCARD's index).

RAPD- The protocol used was based on that of WILLIAMS et al. (1990) adapted to *Eucalyptus grandis* by GRATTAPAGLIA and SEREDOFF (1994). Arbitrary primer screening was performed with 27 ten-base primers (Operon Technologies Inc., Alameda, California). For selection of polymorphic primers, the OPN kit and part of the OPX kit (OPX1- OPX7) were used. Only bands between 3054 and 506,517 bp according to the Ladder 1 Kb standard were considered.

Statistical Analysis- RAPD is a dominant marker scored considering band presence (1) and band absence (0). Only polymorphic loci were analyzed with the NTSYS v.2.02 (ROHLF, 1993), POPGENE v.1.2 (YANG and BOYLE, 1997), and TFPGA v.1.3 (MILLER, 1997) software.

The similarity matrices obtained with the NTSYS software and JACCARD's coefficient were used to assess genetic similarity among 61 plants studied.

The genetic variability of the breeding population was also analyzed in terms of heterozygosity (H_t) (NEI, 1978), genetic diversity (H) (NEI, 1972), number of migrants (Nm) and differentiation among groups (G_{st}) using the POPGENE v.1.2 software.

The TFPGA software was used to obtain WRIGHT's distance (1978) and the UPGMA cluster based on this coefficient.

Results and Discussion

Polymorphism- Twenty-seven primers were tested and 17 of them were used, yielding 128 polymorphic bands. Only 70 polymorphic bands were analyzed because of their better quality of amplification. The OPX7 primer was the most polymorphic one, presenting eight polymorphic markers.

Genetic analysis and guidance for crosses- The mean similarity among 61 plants, obtained by the similarity matrix, was 0.3168. That result indicates a broad genetic base within the breeding population assessed when compared with the values reported by KEIL and GRIFFIN (1994) for *E. grandis* clones (similarity of 0.5517), and by GAIOTTO & GRATTAPAGLIA (1997) in *E. urophylla* (similarity of 0.5 to 0.7).

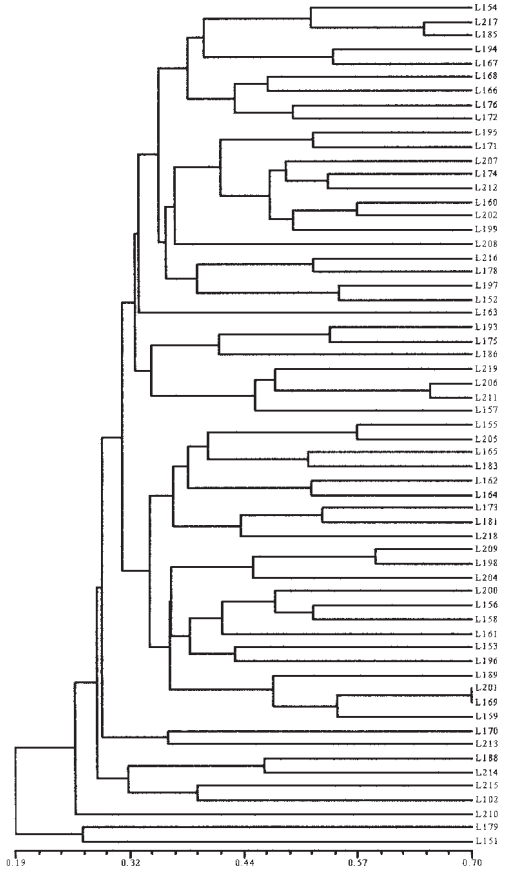

Figure 1. – UPGMA dendrogram of the plants from the base population based on RAPD data using JACCARD's genetic similarity matrix.

Among all the combinations made between individuals within the population, all of them based on the polymorphic loci, only 3 showed similarity higher than 0.6001 (*Figure 1*). The highest similarities were obtained between the following plants: L206 and L211 (0.6522), L185 and L217 (0.6471), and L201 and L169 (0.7000). Twenty-nine combinations showed similarity of 0.5001 to 0.6000, whereas 1,795 combinations showed similarity below 0.5000. In three combinations (L179 and L188, L179 and L168, and L179 and L212), the similarity between plants was 0%. BARIL et al. (1997) showed that it is possible to use genetic distance (or similarity) as an indicator to choose individuals to cross in *Eucalyptus* breeding programs. The use of genetic distance as a parameter to choose the best individuals for crosses is based on the ability to combine two divergent parents. By analogy, molecular data provide a large amount of information, being a powerful tool to choose the best cross. This combination would provide a superior hybrid. Thus, the plants found to have low similarity in the present study could eventually be used in crosses. On this basis, three pairs of plants with 0.000 similarity could be used to obtain full sibs for heterosis studies. Based on similarity matrix, more than 130 crosses were considered promising due to the low genetic similarity (0–20%) found between plants.

The evaluation of economical and silvicultural characteristics in conjunction with molecular data could be used to reduce the number of crosses between plants. The combination of

molecular data and silvicultural traits would decrease the amount of controlled pollinations and, consequently, the costs of breeding programs.

Variability within breeding population- A comparative analysis was performed for a better understanding of variability in Timor, Flores and in most of the commercial variety provenances that composed the studied breeding population (*Table 1*).

The value of H (0.2325) was close to data reported in the literature for natural populations of different *Eucalyptus* species. However, the coefficient of differentiation among groups (GST) was estimated to be 3.75%, a very low value when compared with other populations (HOUSE and BELL, 1994).

The uniformity among populations was also observed by NEI's (1972) genetic identity (*Table 2*). The genetic identity was higher between the Flores provenance and the commercial variety than between the Flores and Timor provenances or between the commercial variety and the Timor provenance. WRIGHT's genetic distance is shown in *Figure 2*. Thus, the result suggests that the germplasm of Flores is genetically more similar to the commercial variety than to the Timor provenance.

Table 2. – Result of analysis bei NEI's identity (1972).

	C.Variety	Flores	Timor
C.Variety	***	0.9887	0.9854
Flores	0.0114	***	0.9755
Timor	0.0147	0.0248	***

NEI's identity is given above the diagonal and genetic distance below the diagonal.

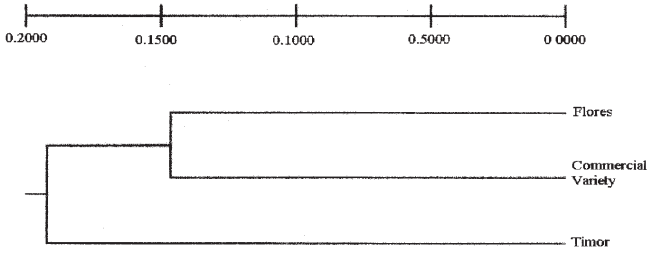

Figure 2. – UPGMA dendrogram of provenances based on RAPD data using WRIGHT's distance matrix.

Most of the germplasm of the breeding population came from Flores and Timor Islands (57.4% of the total). On those islands, the probability of bottlenecks is low and the resistance to genetic erosion could be strong (HOUSE and BELL, 1994), as shown by the number of migrants (Nm) found in this study. According to WRIGHT (1951), genetic drift could result in significant population differentiation if Nm<1, but not if Nm>1. The Nm observed was 12.847, which is considered higher than the values found in the literature, such as those reported by MORI and KAGEYAMA (2001) for two *Eucalyptus grandis* populations (3.628 and 6.107). The high Nm rate observed could be explained by the fact that part of the breeding population was set up using germplasm taken from the initial genetic improvement program developed by the VCP company. This implies generations of recombination in experimental areas of low intensity selection favoring random recombination among different individuals, reducing the rate of inbreeding and consequently the homozygosity. This process was also verified on the basis of the index of similarity shown by the commercial variety (0.2885), the Flores provenance (0.3308), and the Timor provenance (0.3228). For the commercial variety, generations of recombination in experimental areas could benefit genetic variation. The results also indicate that, despite the selection process, this material has been genetically variable within the breeding program.

Other authors have reported heterozygosity rates for isoenzymes and complete agreement has been found between gene diversity estimates derived from isoenzyme studies and from RAPD (ISABEL et al., 1995). Using isoenzyme markers, ARADHYA and PHILLIPS (1993a) obtained high heterozygosity (H = 0.495) for the Flores provenance, which was due to gene flow from other *Eucalyptus* species in surrounding areas.

Similarly, MARTINS-CORDER et al. (1996), also using isoenzyme markers, obtained 0.305 heterozygosity in a study on material of the same provenance (Flores). They also analyzed a population from Timor and found a mean heterozygosity of 0.283, a value close to that observed in the present study. According to MARTINS-CORDER (1994), the high heterozygosity found in the species is explained by natural hybridization with other species in the original region.

The heterozygosity observed in the present study demonstrated that the genetic base of the breeding population should be improved. Material from areas of high genetic variability could be introduced, as suggested by ARADHYA and PHILLIPS (1993b), increasing the ratio of Flores provenance. In spite of Flores provenance is 41% of breeding population, genes came from that wild provenance could be introduced.

Molecular information on diversity and genetic distance also helps to improve the genetic base in breeding programs. Molecular markers could be used to evaluate redundancy or deficiency in germplasm collections, thus increasing the amount of information about efficient assessment, gene maintenance, management and expansion of the germplasm database (PHILLIPS et al., 1993; NEWBERRY and FORD-LLOYD, 1993). Therefore, considering that the time spent on each selection cycle is important in tree breeding programs, molecular markers should be used to accelerate selection cycles.

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Direct and Indirect Measures of Stiffness and Strength Show High Heritability in a Wind-Pollinated Radiata Pine Progeny Test in New Zealand

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Abstract

Seventy-two first-generation open-pollinated (OP) families grown in a 12-year-old radiata pine progeny test were sampled to evaluate the effectiveness of several destructive and non-destructive measures of stiffness. The test was located in Kinleith forest, in the central North Island of New Zealand. Five to seven trees from each of 72 OP families were sampled for assessing various wood properties. Indirect (FAKOPP), non-destructive (clearwood sticks), destructive (HITMAN) and surrogate (density) traits were compared with each other. The measurements using FAKOPP were recorded on the standing trees; stiffness (MOE) and strength (MOR) were measured on clearwood sticks taken from standing trees; HITMAN on felled trees, and wood density (DEN) was also measured on each tree using discs. Diameter (DBH), branching cluster frequency (BR) and straightness (STR) were measured on all 32 replications of all 224 wind-pollinated families available in this trial.

The narrow-sense heritability estimates for HITMAN, MOE, MOR, FAKOPP and DEN were 0.47, 0.53, 0.54, 0.46 and 0.70, respectively. The genetic correlations for HITMAN: MOE, FAKOPP: MOE, DEN: MOE, MOR: DEN and clearwood MOE: MOR were 0.84, –0.69, 0.72, 0.88 and 0.98, respectively. The narrow-sense heritability estimate for DBH, STR and BR were 0.10, 0.17 and 0.06, respectively. Assuming clearwood MOE as the target trait for improving stiffness, HITMAN and DEN were found to be best indirect traits for selection. Predicted genetic gain from indirect selection of parents based on HITMAN and DEN was 80 and 78 percent, respectively, of that predicted from direct selection on MOE. There was some indication that density was a better predictor of strength than of stiffness.

Key words: Stiffness, strength, wood density, heritability, breeding strategy, *Pinus radiata*

Introduction

Wood stiffness, measured in terms of its modulus of elasticity (MOE), is the most important property of structural timber. Low stiffness is an important limitation of timber from radiata pine (*Pinus radiata* D. DON), particularly under short rotations favoured in the interests of reducing growing costs; such rotations result in a high proportion of juvenile wood and an associated reduction in wood quality (COWN, 1999; COWN *et al.*, 1999).

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