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Crosses recommendation method for obtaining *Eucalyptus* spp. hybrids assisted by molecular markers

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ABSTRACT - *The genetic and morphological diversity among parents selected in progeny tests of* Eucalyptus grandis *and* E. urophylla *was used as a criterion to choose the best crosses for a circulant partial diallel design. In a first stage, 127 parents were selected from 503 clone trees (70* E. urophylla *and 57* E. grandis *trees) considering two traits: the Mean Genetic Distance (MGD) values obtained by RAPD markers and Annual Mean Increment (AMI) values. In a second stage, these parents were analyzed with other RAPD markers and grouped by the Tocher method. These groups were used to prepare a diallel set, involving the two most divergent groups of parents of the species. Other silvicultural data were used to calculate the average Euclidean distances, which also emphasizes the great variability existing among and within populations. Correlations between the genetic distances obtained by RAPD markers and the average Euclidean distances were negative or very low.*

Key words: Genetic diversity, forest tree improvement, RAPD markers, selection.

INTRODUCTION

The Brazilian forest industry has a great potential for *Eucalyptus* production given the vast capability and technology available for developing clonal seedlings. Clonal plantations form highly uniform forests which facilitate silvicultural practices (Ratnieks and Assis 1993).

The selection of genotypes for clonal plantations depends on the progeny selection and best choice of crosses to prioritize specific hybrid combinations. Besides assuring the superiority of the segregant population (Cruz and Regazzi 1994), hybridization has been a useful tool in eucalypt breeding programs considering the different species and origins. However, choosing parents that provide a more favorable heterosis is still a serious challenge. A primary strategy for predicting the best hybrid combinations is the use of progeny tests associated with the genetic diversity among the parents.

Diallel crosses as proposed by Griffing (1956), Gardner and Eberhart (1966) and Kempthorne and Curnow (1961) are among the leading methodologies for analyzing parental diversity and estimating the best combinations

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for progeny tests. Other options include multivariate techniques such as principal components, canonical variables, dissimilarity, and hierarquical clustering methods that facilitate the identification of genetically divergent groups for a given set of traits (Cruz and Regazzi 1994).

Besides genetic tests based on the analysis of variance and decomposition of the different sources of variation, the analysis of the improved population using molecular markers has been adopted for establishing divergent clusters in a population (Rocha et al. 2002, Gaioto et al. 1997, Lanza et al. 1997, Barbosa-Neto et al. 1996). RAPD markers (Welsh and McClelland 1990, Williams et al. 1990) stand out as the most frequently used molecular markers for genetic diversity analyses of *Eucalyptus* and have been used for genetic map construction (Grattapaglia and Sederoff 1994, Byrne et al. 1997), clonal identification, association among parental molecular genetic distances, and prediction of the F1 generation performance (Grattapaglia et al. 1992, Sale et al. 1996).

Studies on the relationship between genetic dissimilarity measures and heterosis have also produced conflicting results (Dias et al. 2004). While the efficiency of hybrid combination selection based on molecular diversity analyses has been satisfactory and recommended, many other studies have not confirmed these results (Brustin and Charcosset 1997, Marsan et al. 1998, Dudley et al. 1991, Charcosset et al. 1991, Lanza et al. 1997).

Among the most widespread molecular marker techniques (RFLP, RAPD, AFLP, and microsatellites), RAPD is the most commonly used for forest species (Williams et al. 1990, Welsh and Mcclelland 1990) due to its rapidity and easiness of implementation (Rocha et al. 2002). The RAPD uses arbitrary primers for random amplification of genomic loci. Due its large genome sampling capacity, the RAPD genetic distances favor the selection of more divergent genotypes for negative or positive associated crosses. This methodology also allows the evaluation of genetic divergence without the need for field tests, which would cause a six to seven-year wait to complete one generation of *Eucalyptus* species. The low informative content per loci and reproducibility problems are the main limitations of the RAPD technique.

Objectives of the present study were to analyze the genetic diversity of a clone tree group selected from *Eucalyptus urophylla* and *Eucalyptus grandis* populations using RAPD molecular markers and morphological traits, to compare quantitative and molecular distances and also recommend crosses for a circulating partial diallel design.

MATERIAL AND METHODS

Genetic material

Five hundred and three *E. grandis* and *E. urophylla* clone trees from different origins were selected in five progeny tests (189, 192, 290, 291 and 391) with variable ages, on the Farm Itabaiana, Açailândia, state of Maranhão (lat 5º 05' S, long 47º 39' W, 260 m asl). The temperature varied from 26.4 to 24.5 ºC (annual average 25.4 ºC) and the average rainfall was 1473 mm.

DNA extraction

Healthy leaves were collected from the 503 clone trees, ice-stored or dehydrated in a ventilated oven at 42 ºC for 24 hours, were sent to the Laboratory of Molecular Genetics and Microorganisms/BIOAGRO - UFV, and stored at -10 ºC. The total DNA was extracted using the Doyle and Doyle (1990) method, with the following modifications: extraction buffer with 1% insoluble PVP and 0.4% β-mercaptoethanol, and DNA precipitation with isopropanol and 2.5 M ammonium acetate.

Amplification of DNA fragments

The 503 clone trees were previously analyzed with Operon primers OPF03, OPF05, OPF07, OPF19, OPE06, and OPE08. Of the 503 trees, 127 genotypes were selected based on their genetic diversity. Secondly the 127 were analyzed using the Operon primers OPF 01, OPF 02, OPF 09, OPF 12, OPF 14, OPF 16, OPE 01, OPE 03, OPE 14, OPE 15, and OPE 16. The amplification reactions were performed according to Williams et al. (1990). Amplified products were analyzed by electrophoresis in 1.5% agarose gel and stained with ethidium bromide (0.2 mg mL^{-1}). The bands were visualized and photographed on an ultraviolet transilluminator, using an Eagle Eye Video System (Stratagene).

Molecular data analysis

RAPD bands were scored considering presence (1) or absence (0) of a determined DNA fragment for the different samples. The genetic distance values were calculated using the Jaccard coefficient - Genes software (Cruz 1997). The genetic distance estimates were also decomposed into Average Genetic Distance (AGD) and Specific Genetic Distance (SGD) as follows:

$$SGD = d_{ij(RAPD)} \text{ and } AGD = \frac{\sum_{i=1}^{n} d_{i.}}{n},$$

where "n" is the total genotype number.

Quantitative data

Total height (Ht), diameter at breast height (DBH) and Annual Medium Increment (AMI) were measured in the experimental field. AMI was obtained as follows:

AMI = (Vol.1111)/6

where AMI is expressed in m^3 $(ha.year)^{-1}$ and volume was obtained for each experiment, giving a variable volume equation.

Analysis of variance and genetic parameter estimates

Data from three field experiments, 291, 192 and 290, were used in the analysis of variance**.** The treatments were distributed in randomized blocks, with five plants per family and five blocks. The dissimilarity values for quantitative traits DBH, Ht and AMI were obtained by the Average Euclidean Distance.

Cluster analysis

The Tocher Method was used to identify the most divergent and closest genotypes of each species (Cruz and Regazzi 1994). Based on the dissimilarity matrix for quantitative traits and molecular markers, a cluster analysis of the 127 parents was carried out using the UPGMA method (Unweighted Pair Group Method Arithmetic Average).

Correlation and coincidence analysis

The correlation analysis based on Pearson coefficient and coincidence analysis for genetic distance values by RAPD and Euclidean distance was performed with Genes software.

Analysis procedure

The AGD and EDG estimates were used to select 127 among the 503 clone trees evaluated. For the 127 clone trees selected, 11 other RAPD primers were used to study diversity and cross recommendation.

RESULTS AND DISCUSSION

Breeding recommendation based on molecular analyses

In a first stage selection, the RAPD molecular analysis with 26 polymorphic DNA fragments was used to obtain the genetic distance values for the 503 clone trees. In this study, the Average Genetic Distance values (AGD) of each individual were considered for the selection. One hundred and twenty-seven clone trees (70 *E. urophylla* and 57 *E. grandis* parents) were selected based on the AGDs generated from RAPD markers and AMI values, reflecting annual variations in the development of a new population with fewer genotypes. The 127 selected clone trees were those with increased AMI and high AGD values.

In a second stage, the DNA of the 127 clone trees was amplified using 11 other primers for a total of 74 polymorphic DNA fragments. The number of amplified DNA fragments varied from 4 to 9, with an average of 6.72 DNA fragments per primer. While some primers produced highly polymorphic amplification patterns others showed low polymorphism (data not shown). Polymorphism data for these DNA fragments were used to generate a genetic distance matrix and perform cluster analyses. A dendrogram was developed for each species, although their interpretations are difficult due to the high number of parents included in the analysis.

The simultaneous evaluation of many genotypes impairs the acquisition of a larger number of polymorphic fragments for the genetic diversity estimates. Dias et al. (2004), Ferreira et al. (2004) and Picoli et al. (2004) used the bootstrap methodology to estimate dissimilarity matrices with different numbers of markers. They suggested the use of 284 to 377 markers to obtain correlation values above 90% among the matrices estimated with fewer markers and a parametric matrix. The reduced number of markers used in this work, 26 and 74, result, respectively, in correlation values: of 0.38 and 0.67 and stress: of 0.41 and 0.12, considering the samplings accomplished by Ferreira et al. (2004). These results indicated that the number of 26 markers used in the first stage of this work results in low precision of the clustering, complicating especially, the separation of the genetically less divergent groups. However, considering that in the following stage of evaluation, only genotypes of the most divergent groups were chosen the associate error tends to be smaller.

According to the second analysis of both species (*E. grandis* and *E. urophylla*) using 74 DNA markers, the smallest genetic distance between trees was 10.9%, between parents G97 (Mount Spurigion) and G98 (Woodum Kangaroo River - WWSA). The largest genetic distance was 82.6%, between parents U63 (*E. urophylla* var. *plantyphylla*) and U109 (Anhembí - Island of Flores).

The smallest distance for E. grandis was still 10.9% between parents G97 and G98, and the largest 70.8%, between G47 (Woodum Kangaroo River - WWSA) and G74 (Aracruz - ES - originally from Mount Speac). The largest distance for *E. urophylla* remained at 82.6%, between U63 and U109, and the smallest 12.5%, between U31 (Aradetung - Flores) and U36 (Lewotobi - Flores).

At this step, the Tocher method was used to the separation and classification of the more similar genotypes. The Tocher method allows the obtaining of

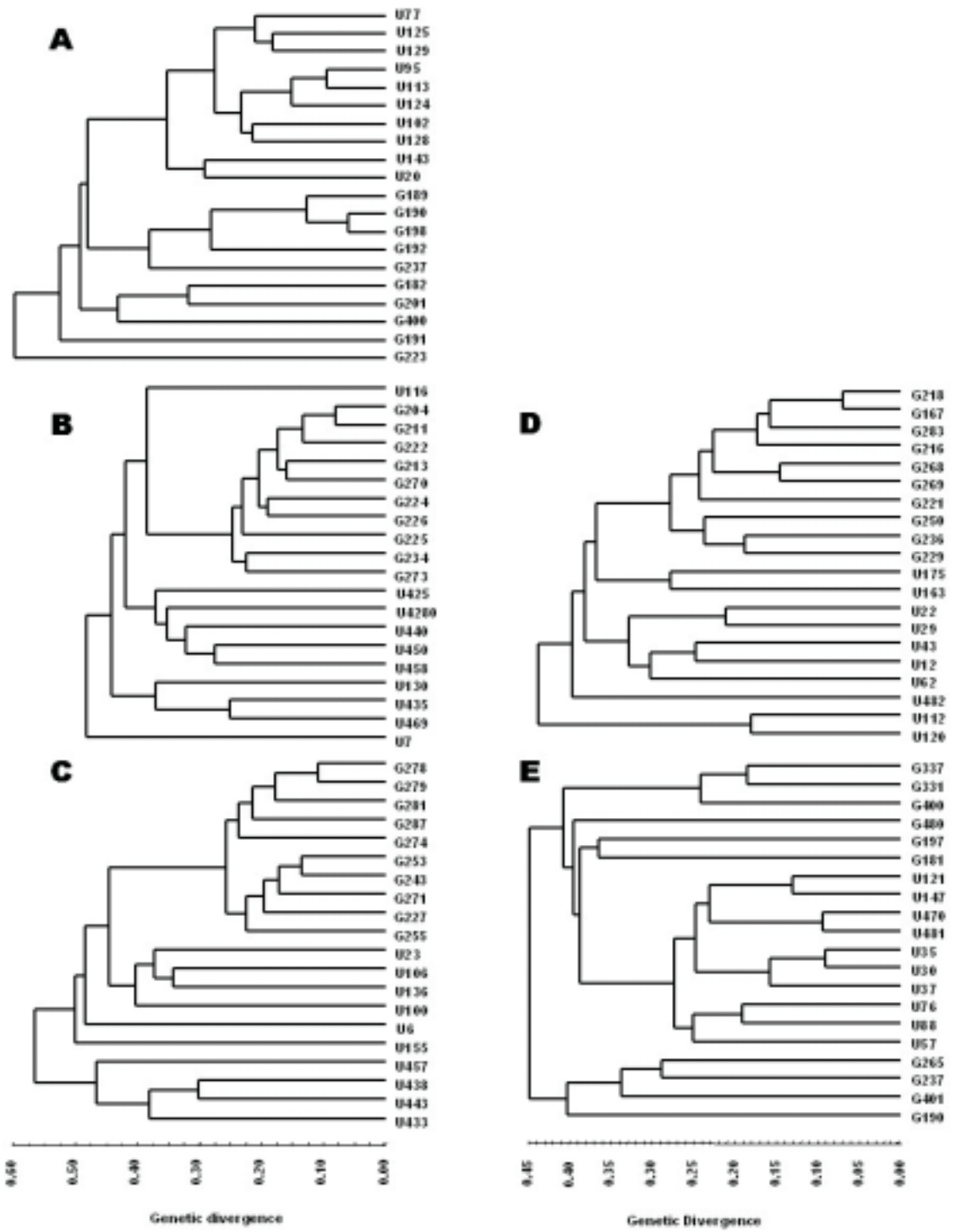

Figura 1. Dendrogram generated by the UPGMA method using RAPD marker data, for selected *E. grandis* and *E. urophylla* hybrids of the diallel prepared with parents: (A) GR1 x UR7; (B) GR2 x UR6; (C) GR5 x UR1; (D) GR3 x UR5 (Table 1)

exclusive groups that shows homogeneity inside and heterogeneity among groups, of easiness of interpretation. The Tocher cluster analysis allowed the identification of seven *E. urophylla* and six *E. grandis* groups of genotypes that should be used as parents considering their genetic divergence (data not shown).

Therefore crosses between the most heterogeneous groups are recommended. Figure 1 shows the dendrogram for selected *E. grandis* and *E. urophylla* genotypes selected to compose the diallel designs. Were recommended the breeding of the more divergent genotypes in five partial circulating diallel designs: grG1 x grU7, grG2 x grU6, grG3 x grU5, grG5 x grU1 and grG4 x grU2 (Table 1).

Genetic diversity based on quantitative traits

ANOVA was used to analyze the silvicultural trait data from three field experiments (290, 291 and 192). The progeny effect was significant at 1% of probability by the F test, for both the DBH and Ht traits in all experiments. The existence of significant statistical differences between progenies indicates the existence of variability in the base population. Were also observed, that the residual variance component ($\hat{\sigma}^2$) for DBH trait for the tree experiments was negative (Table 2). This indicates a competitive effect between progenies. In this case the use of the intra-class correlation coefficient is recommended as a correction factor of the variance estimate component ($\hat{\sigma}_d^2$) (Xavier et al. 1996).

In comparison with experiment 290, experiment 192 and 291 (Table 2), showed higher mean heritability values at the family level. The high heritability values indicated that the phenotypic value is a good predictor of the genetic value for the tree experiments, allowing gain by selection for the next generation.

The parental biometric data (DBH, Ht and AMI) selected in experiments 290, 189, 192, and the combined experiments 391 and 291 (same age) were used to obtain the average Euclidean distance function, generating a dissimilarity matrix used for cluster analyses by UPGMA (data not shown).

Considering the genotypes of the experiments 391 and 291 the smallest distance was 0.35 between parents U468 and U38 (both from Teixeira de Freitas/BA - origin: Ilegele - Flores), and the largest distance was 12.86 between parents U102 (Teixeira de Freitas - origin: Ilegele - Flores) and U62 (Ilimandiri - Flores). In experiment 189 the smallest distance was 0.58 between G400 and G234 (Belo Oriente), and the largest was 12.29 between G256 (Itabira/FRDSA - Atherton) and G213 (Belo Oriente). In the experiment 192 the smallest distance was 0.59 between U136 (Anhembí T10B71 – islands near Timor) and U473 (origin not identified), and the largest distance was 10.58 between U438 (Anhembí T8i70 - islands near Timor) and U23 (Anhembí T10B71 - islands near Timor). In the experiment 290, the smallest distance was 2.27 between U57 (Anhembí - SP T8c51 – Timor) and U6 (Lassance, ex-Anhembi – Flores), and the largest distance was 10.16 between U6 and U109 (Linhares with origin in other islands near Timor).

The small values between different origins are usually not expected in some pairs of genetic distance. The random sampling, number of markers and evaluated genotypes may produce results of this type.

Correlation between molecular and quantitative diversity

The correlation analysis using the Pearson coefficient was performed using the genetic distances based on molecular markers and Euclidean distances (Table 3). Except for experiment 290, positive correlations were found. The smallest correlation coefficient (-0.11) was found for the parents of experiment 290, and the largest correlation coefficient (0.21) for the parents of experiments 291 and 231. These values are relatively low, considering the positive correlation values from 0.024 (experiment 189) to 0.21 (experiment 290).

The low correlation between the molecular analysis and quantitative analysis can be explained by the low correlation between the analyzed DNA fragments and the measured phenotypic traits. The DNA regions amplified by RAPD markers are distributed along the genome with no evidence of directed amplification for specific DNA sequences.

The coincidence analysis for the molecular and Euclidean distance values resulted in the largest percentages of coincidence for experiments 391 and 291 (42.68% for the lowest and 35.36% for the highest sampled values, respectively). The lowest percentages occurred in experiment 192, which was 12.5% for the lowest and highest values (Table 3).

Due to the random nature of RAPD markers and the genome-wide sampling, the possibility of high correlation values between molecular and quantitative distances is small. Backmann (1992) showed that phenotypic differences are not necessarily correlated with the genetic mechanisms related with genetic divergence, since the morphological divergence is not necessarily a function of genetic differences; different gene pools can be manipulated to generate similar phenotypes (Róldan-Ruiz et al. 2001). The lack of correlation between genetic

Table 1. *Eucalyptus* parents recommended for the circulating partial diallel, based on Tocher cluster analysis of 127 genotypes and parental groups recommended for the circulating partial diallel

Group	Code	Provenance	Origin	Group	Code	Provenance	Origin	Group	Code	Provenance	Origin	Group	Code	Provenance	Origin
GR 1	G278	Mount Spurgion	Mount Spurgion	GR 5	G337	Without Provenance	Without Origin	UR 3	U113	Ilegele Flores	Ilegele Flores	UR 7	U457	Ilegele Flores	Ilegele Flores
GR 1	G279	Woodum Kang. River	Without Origin	GR 5	G480	Mount Spurgion	Mount Spurgion	UR 3	U124	T. Freitas	Ilimandiri - Flores	UR 7	U438	Anhembí T8i70	Outras Ilhas
GR 1	G281	Windsor Tableland	Windsor Tableland	GR 5	G197	Windsor Tableland	Windsor Tableland	UR 3	U20	T. Freitas	Ilegele Flores	UR 7	U443	Ilegele Flores	Ilegele Flores
GR 1	G287	Aracruz - ES	Gadgarra - QLD	GR 5	G181	Aracruz - ES	Cairns Dan	UR 3	U125	T. Freitas	Ilimandiri - Flores	UR 7	U433	Without provenance	Without origin
GR 1	G274	Windsor Tableland	Windsor Tableland	GR 5	G265	Aracruz - ES	Herberton SF	UR 3	U77	Anhembí T10B71	Outras Ilhas	UR 7	U23	Anhembí T10B71	Outras Ilhas
GR 1	G253	Itabira - FRDSA	Atherton - QLD	GR 5	G331	Without Provenance	Without Origin	UR 3	U143	T. Freitas	Ilimandiri - Flores	UR 7	U6	Lassance ex Anhembi	Flores
GR 1	G243	Divinolandia	Without Origin	GR 5	G401	Without Provenance	Without Origin	UR 3	U129	Lewotobi Flores	Lewotobi Flores	UR 7	U106	T. Freitas	Ilimandiri-Flores
GR 1	G255	Aracruz - ES	Mount Speac	GR 5	G237	Aracruz - ES	Cairns Dan	UR 3	U128	T. Freitas	Ilegele Flores	UR 7	U136	Anhembí T10B71	Outras Ilhas
GR 1	G271	Windsor Tableland	Windsor Tableland	GR 5	G400	Belo Oriente - Cenibra	lote 06/87	UR 3	U102	T. Freitas	Ilegele Flores	UR 7	U100	Resende IPEF	Timor
GR 1	G227	Aracruz - ES	Herberton SF	GR 5	G190	Aracruz - ES	Mount Speac	UR 3	U95	T. Freitas	Ilimandiri - Flores	UR 7	U155	Anhembí -SP T8i70	Flores
GR 2	G256	Itabira - FRDSA	Atherton - QLD	GR 6	G237*	Aracruz - ES	Cairns Dan	UR 4	U135	T. Freitas	Ilimandiri - Flores				
GR 2	G273	Itabira - FRDSA	Atherton - QLD	GR 6	G400*	Belo Oriente - Cenibra	lote 06/87	UR 4	U109	Linhares - ES FRDSA	Outras Ilhas				
GR 2	G234	Belo Oriente - Cenibra	lote 06/87	GR 6	G190*	Aracruz - ES	Mount Speac	UR 4	U105	Ilegele Flores	Ilegele Flores				
GR 2	G222	Aracruz - ES	Cairns Dan	GR 6	G198	Woodum Kang. River	Without Origin	UR 4	U21	T. Freitas	Ilimandiri - Flores				
GR 2	G204	Itabira - FRDSA	Atherton - QLD	GR 6	G189	São Mateus - ES	Atherton - QLD	UR 4	U15	Ilimandiri - Flores	Ilimandiri - Flores				
GR 2	G224	Aracruz - ES	Cairns Dan	GR 6	G192	Aracruz - ES	Cairns Dan	UR 4	U118	T. Freitas	Ilimandiri - Flores				
GR 2	G213	Belo Oriente - Cenibra	lote 06/87	GR 6	G201	Without Provenance	Without Origin	UR 4	U16	Egon II Flores	Egon II Flores				
GR 2	G226	Aracruz - ES	Mount Speac	GR 6	G182	Aracruz - ES	Cairns Dan	UR 4	U137	Lewotobi Flores	Lewotobi Flores				
GR 2	G211	São Mateus - ES	Atherton - QLD	GR 6	G191	Aracruz - ES	Cairns Dan	UR 4	U468	T. Freitas	Ilegele Flores				
GR 2	G270	Woodum Kang. River	Without Origin	GR 6	G223	Aracruz - ES	Without Origin	UR 4	U96	Brotas-SP IPEF	Timor				
GR 3	G218	Aracruz - ES	Without Origin	UR 1	U121	T. Freitas	Ilimandiri - Flores	UR 5	U482	T. Freitas	Ilegele Flores				
GR 3	G167	Mount Lewis A	Mount Lewis A	UR 1	U147	Aradetung Flores	Aradetung Flores	UR 5	U22	T. Freitas	Ilimandiri - Flores				
GR 3	G283	Aracruz - ES	Mount Speac	UR 1	U470	Aradetung Flores	Aradetung Flores	UR 5	U112	T. Freitas	Egon Flores				
GR 3	G216	São Mateus - ES	Atherton - QLD	UR 1	U481	T. Freitas	Ilegele Flores	UR 5	U43	Ilimandiri - Flores	Ilimandiri - Flores				
GR 3	G268	Mount Lewis A	Mount Lewis A	UR 1	U35	Aradetung Flores	Aradetung Flores	UR 5	U120	Aradetung Flores	Aradetung Flores				
GR 3	G269	Aracruz - ES	Mount Speac	UR 1	U76	Anhembí -SP T8g71	Timor	UR 5	U12	T. Freitas	Egon Flores				
GR 3	G221	Aracruz - ES	Without Origin	UR 1	U57	Anhembí -SP T8c51	Timor	UR 5	U175	Itamarandiba-MG	Timor				
GR 3	G250	Belo Oriente - Cenibra	lote 06/88	UR 1	U88	Ilegele Flores	Ilegele Flores	UR 5	U62	T. Freitas	Ilimandiri - Flores				
GR 3	G236	Aracruz - ES	Mount Speac	UR 1	U37	T. Freitas	Ilegele Flores	UR 5	U29	T. Freitas	Ilimandiri - Flores				
GR 3	G229	Itabira - FRDSA	Atherton - QLD	UR 1	U30	T. Freitas	Ilimandiri - Flores	UR 5	U163	Anhembí T10B71	Outras Ilhas				
GR 4	G244	São Mateus - ES	Atherton - QLD	UR 2	U45	T. Freitas	Ilegele Flores	UR 6	U116	Anhembí -SP T10b71	Timor				
GR 4	G272	Aracruz - ES	Gadgarra - QLD	UR 2	U473	Anhembí T10B71	Outras Ilhas	UR 6	U425	Without provenance	Without origin				
GR 4	G176	Woodum Kang. River	Without Origin	UR 2	U46	T. Freitas	Ilegele Flores	UR 6	U130	Lewotobi Flores	Lewotobi Flores				
GR 4	G248	Aracruz - ES	Without Origin	UR 2	U24	T. Freitas	Ilimandiri - Flores	UR 6	U428	Without provenance	Without origin				
GR 4	G185	Belo Oriente - Cenibra	lote 06/88	UR 2	U485	Ilegele Flores	Ilegele Flores	UR 6	U469	Lewotobi Flores	Lewotobi Flores				
GR 4	G206	Aracruz - ES	Mount Speac	UR 2	U38	T. Freitas	Ilegele Flores	UR 6	U450	T. Freitas	Ilegele Flores				
GR 4	G262	Aracruz - ES	Dimbulah - QLD	UR 2	U162	T. Freitas	Ilegele Flores	UR 6	U458	T. Freitas	Ilegele Flores				
GR 4	G293	Without Provenance	Without Origin	UR 2	U26	T. Freitas	Ilegele Flores	UR 6	U440	Ilimandiri - Flores	Ilimandiri - Flores				
GR 4	G261	Without Provenance	Without Origin	UR 2	U141	Anhembí T8h69	Outras Ilhas	UR 6	U435	Without provenance	Without origin				
GR 4	G398	Without Provenance	Without Origin	UR 2	U81	T. Freitas	Ilimandiri - Flores	UR 6	U7	Ilegele Flores	Ilegele Flores				

Tabela 2. Parameter estimates and components of variance for three analyzed experiments (192, 290 and 291). Mean among family heritability (h_m), mean within family heritability (h_d), individual heritability within the block (h_b) and within the experiment (h_e), coefficient of variation among plots (CV_1), coefficient of variation within plots (CV_4), block variance ($\hat{\sigma}^2_b$), genetic variance among families ($\hat{\sigma}^2_g$), genetic variance within families ($\hat{\sigma}^2_{gd}$), phenotypic variance within families ($\hat{\sigma}^2_d$) and residual variance ($\hat{\sigma}$)

	Experiment 192		Experiment 291		Experiment 290	
Genetic parameters	**DBH**	**Ht**	**DBH**	**Ht**	**DBH**	**Ht**
h^2 (Family average)	0.7979	0.8010	0.8009	0.7841	0.5770	0.6623
h^2 (within Family)	0.1909	0.2465	0.1706	0.2305	0.0499	0.1020
h^2 (individual in block)	0.2406	0.2936	0.2205	0.2740	0.0664	0.1281
h^2 (individual in expt.)	0.2391	0.2887	0.2164	0.2545	0.0654	0.1245
CV exp. (CV_1)	9.4530	9.1221	8.5037	8.8361	8.1732	7.4177
CV gen. (CV_4)	10.2887	10.0235	9.3402	9.2233	5.8460	6.3612
CV_4/CV_2	-	2.5637	-	2.2655	-	1.9220
$\hat{\sigma}^2_b$	0.0437	0.1812	0.1522	0.9737	0.1661	0.4361
$\hat{\sigma}^2_g$	0.4382	0.7983	0.4497	0.8698	0.1936	0.4743
$\hat{\sigma}^2_{gd}$	1.3146	2.3949	1.3491	2.6095	0.5808	1.4228
$\hat{\sigma}^2_d$	6.8852	9.7148	7.9084	11.3198	11.6305	13.9458
$\hat{\sigma}$	-0.0378	0.3644	-0.1998	0.5085	-0.1569	0.3852

Tabela 3.Pearson correlation and coincidence analysis between quantitative and molecular distances for parents selected in experiments 189, 192, 290, 291, and 391

	Experiment 189	Experiment 290	Experiment 192	Experiments 391 and 291
Variance (Q)	6.729	3.81	6.273	7.613
Variance (M)	0.009	0.0069	0.014	0.0056
COV(E,M)	0.058	-0.018	0.061	0.044
$r_{pearson}$	0.024**	-0.116	0.2058**	0.213**
Nr of date pairs	465	21	28	276
Coincidence analysis				
Superior values	48	1	1	35
Superior values (%)	34.53	16.67	12.5	42.68
Inferior values	36	1	1	29
Inferior values (%)	25.90	16.67	12.5	35.37
Size sample	139	6	8	82

diversity indices of quantitative and molecular nature is shown in many studies and can have numerous causes (Reed and Frankham 2001). It must however be emphasized that molecular tools are used to complement the classical evaluation methods of genetic variation and that different methods can reveal different variation patterns of genetic diversity.

In this case, each species makes up a heterotic group for itself. However, the close relationship between E. grandis and E. urophylla decreases the hybrid gain for closer crosses. Crosses directed by molecular markers may allow new combinations for the various genetic compositions of each pair of parents. Another advantage is that fewer crosses need to be evaluated.

CONCLUSIONS

The large amount of data generated by the analyses of the 503 genotypes made the interpretation of cluster analysis by hierarchical models unfeasible. In this study, the combination of the genetic distances obtained by RAPD and AMI to assist the selection of the best and most divergent parents was satisfactory. The analysis of diversity using quantitative data from the average Euclidean distance estimates and cluster analysis showed the existence of high variability among and within families and provenances. Low correlation was found between the genetic distances calculated using RAPD markers and average Euclidean distances.

Método para recomendação de cruzamentos visando a obtenção de híbridos de *eucalyptus* spp. assistido por marcadores moleculares

RESUMO - *A diversidade entre genitores selecionados em testes de progênies de* Eucalyptus grandis *e* E. urophylla *foi avaliada para identificação dos melhores cruzamentos utilizando dialelo parcial circulante. Para a análise da diversidade foram utilizados marcadores moleculares RAPD e distância Euclidiana média calculada a partir dos dados silviculturais: de diâmetro a altura do peito (DAP), altura total (Ht) e incremento médio anual (IMA). As análises por RAPD foram feitas em duas etapas, na primeira, 127 genitores foram selecionados de 503 árvores. Posteriormente os 127 genitores foram agrupados pela técnica de Tocher e utilizados na montagem de dialelos parciais circulantes, envolvendo dois grupos de genitores mais divergentes entre as duas espécies. Foram observados altos valores de diversidade genética entre e dentro de procedência e baixos valores de correlação entre as distâncias genéticas obtidas por marcadores RAPD e distância Euclidiana média.*

Palavras-chave: Diversidade genética, melhoramento florestal, marcadores RAPD, seleção.

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