



Revista Árvore

ISSN: 0100-6762

r.arvore@ufv.br

Universidade Federal de Viçosa
Brasil

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Revista Árvore, vol. 27, núm. 4, julho-agosto, 2003, pp. 427-434

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Viçosa, Brasil

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EXPERIMENTAL ALTERNATIVES FOR EVALUATION OF PROGENIES AND CLONES IN EUCALYPTUS BREEDING PROGRAMS¹

Elaine Aparecida de Souza², Isaias Olívio Geraldi³, Magno Antonio Patto Ramalho²e
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ABSTRACT - The feasibility of using augmented block designs and spatial analysis methods for early stage selection in eucalyptus breeding programs was tested. A total of 113 half-sib progenies of *Eucalyptus urophylla* and eight clones were evaluated in an 11 x 11 triple lattice experiment at two locations: Posto da Mata (Bahia, Brazil) and São Mateus (Minas Gerais, Brazil). Four checks were randomly allocated within each block. Plots consisted of 15 m long rows containing 6 plants spaced 3 m apart. The girth at breast height (cm/plant) was evaluated at 19 and 26 months of age. Variance analyses were performed according to the following methods: lattice design, randomized complete block design, augmented block design, Papadakis method, moving means method, and check plots. Comparisons among different methods were based on the magnitude of experimental errors and precision of the estimates of genetic and phenotypic parameters. General results indicated that augmented block design is useful to evaluate progenies and clones in early selection in eucalyptus breeding programs using moderate and low selection intensities. However, this design is not suitable for estimating genetic and phenotypic parameters due to its low precision. Check plots, nearest neighbour, Papadakis (1937), and moving means methods were efficient in removing the heterogeneity within blocks. These efficiencies were compared to that in lattice analysis for estimation of genetic and phenotypic parameters.

Key words: *Eucalyptus urophylla*, augmented block, lattice, randomized complete block, nearest neighbour, Papadakis, moving means, check plots, genetic and phenotypic parameters.

ALTERNATIVAS EXPERIMENTAIS NA AVALIAÇÃO DE PROGÊNIES E CLONES EM PROGRAMAS DE MELHORAMENTO DE EUCALIPTO

RESUMO - Visando estudar a viabilidade do emprego do delineamento em blocos aumentados e de métodos de análise espacial nas etapas iniciais dos programas de melhoramento de eucalipto, foram avaliados 121 tratamentos, sendo 113 progênies de meios-irmãos de *Eucalyptus urophylla* e oito clones, em dois locais: São Mateus, Estado do Espírito Santo, Brasil, e Posto da Mata, Estado da Bahia, Brasil. O delineamento utilizado foi um látice 11 x 11, com três repetições por local. Em cada bloco foram colocadas, aleatoriamente, quatro testemunhas. Cada parcela foi constituída por uma linha de seis plantas, no espaçamento 3 x 3 m. Os dados da circunferência à altura do peito (cm/planta), aos 19 e 26 meses de idade, foram submetidos à análise de variância, considerando as seguintes metodologias: látice, blocos aumentados, blocos ao acaso, método de Papadakis, método das médias móveis e testemunha intercalar. Realizou-se a comparação entre os diferentes métodos quanto à sua eficiência, a partir das seguintes estimativas: coeficiente de variação, herdabilidades e seus respectivos intervalos de confiança, correlação de Spearman e as metodologias de Fasoulas (1983) e Hamblin & Zimmerman (1986). Os resultados obtidos indicaram que o uso do delineamento em blocos aumentados é viável para seleção de progênies e clones nas etapas iniciais

¹ Recebido para publicação em 8.5.2001.

Aceito para publicação em 9.9.2003.

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dos programas de melhoramento genético de eucalipto, principalmente se for aplicada uma intensidade de seleção moderada; porém esse delineamento não se mostrou apropriado para a estimação de parâmetros genéticos e fenotípicos, devido à baixa precisão das estimativas. Os métodos de testemunha intercalar e análise de vizinhança, o Papadakis (1937) e o método das médias móveis foram eficientes no controle da heterogeneidade dos blocos, sendo esta eficiência similar àquela proporcionada pela análise em látice.

Palavras-chave: *Eucalyptus urophylla*, blocos aumentados, blocos ao acaso, látice, análise de vizinhança, método de Papadakis, médias móveis, testemunha intercalar e parâmetros genéticos e fenotípicos.

1. INTRODUCTION

Time is a limiting factor in eucalyptus breeding programs. However, a large number of progenies must be evaluated in the selection process. In this case, the most important restriction is the dimension of the experimental area. Because of the physical space occupied by an individual, there is restriction in the number of progenies and/or clones which can be evaluated in experiments with adequate replications.

Plant breeders have sought alternatives to solve these problems, such as using augmented block design (Federer, 1956, 1961a and 1961b), which allows to evaluate a large number of material with a manageable amount of work and experimental area. However, only a few published studies are available on the comparative evaluation of this design. Studies have been conducted with potato and common bean (Bearzoti, 1994; Souza, 1997). In the specific case of eucalyptus, information concerning this is scarce. Several methods of spatial analysis of experiments have been proposed. These have become available to plant breeders through the latest computing facilities. Among these are the Papadakis' (1937) and the moving means (Townley-Smith & Hurd, 1973) methods. Interest in this type of analyses has increased and new methods have been proposed (Bartlett, 1978; Wilkinson et al., 1983; Besag & Kempton, 1986; Magnussen, 1990; Samra et al., 1990; Vivaldi, 1990; Zimmerman & Harville, 1991; Loo-Dinkins, 1992; Ball et al., 1993; Brownie et al., 1993; Stroup et al., 1994; Helms et al., 1995; Anoshenko, 1994; Clarke & Baker et al., 1996). Nevertheless, information remains scarce on the relative efficiencies of these methods applied in breeding programs in Brazil.

This study was carried out to compare the efficiency of these methods in evaluating progenies and clones in eucalyptus breeding programs.

2. MATERIAL AND METHODS

The material used in this study consisted of 113 half-sib progenies of *Eucalyptus urophylla* and eight clones, evaluated in an 11 x 11 triple lattice design at two locations: Posto da Mata (17° 51' 48.65" S of latitude and 39° 46' 25.76" W of longitude in Bahia, Brazil) and São Mateus (18° 31' 05.58" S of latitude and 39° 47' 36.33" W of longitude in Espírito Santo, Brazil). A clone of *Eucalyptus grandis* one of *E. urophylla* as well as a seedlot of *E. grandis* and another of *E. alba*, were randomly allocated within each block as controls. Using these four checks as common treatment allowed to simulate a distinct experiment in augmented block design within each lattice replication. Plots consisted of 15 m rows, each one containing 6 plants planted 3 m apart. The girth at breast height was recorded in centimeters at ages 19 and 26 months. These data were processed through several methods of variance analysis to evaluate the efficiency of each process. Initially, the analyses were conducted with lattice and randomized complete block designs. Later, using information from each replication, three variance analyses were performed, by location, in the augmented block design. To compare regular treatments (progenies) of different blocks, an effective error was estimated by using the expression developed by Ferreira (cited by Barbosa, 1996):

$$MSE_e = \left[1 + \frac{1}{r+t-1} + \frac{r}{t(r+t-1)} + \frac{r \sum_{k=1}^b n_k^2}{t^2(r+t-1)} - \frac{2 \sum_{k=1}^b n_k^2}{t^2(r+t-1)} + \frac{b \sum_{k=1}^b n_k^2}{n^2(n+t-1)} \right] MSE$$

where

MSE_e = mean square of the effective error;

r = number of regular treatments (progenies);

t = number of common treatments (checks);

b = number of blocks;

n_k = number of treatments (regular + common) in the k block;

n = total number of plots, $n = \sum_{k=1}^b n_k$

MSE = mean square of the in block analysis' error.

Correction was also evaluated by means of the check plots method. Thus, an environmental index was obtained from the average performance of the four checks within each block. A covariance analysis was performed in randomized complete blocks using the environmental index as covariable.

Additionally, the data were analysed by the Papadakis' (1937) and moving average (Townley-Smith and Hurd, 1973) methods, using as adjustment the average performance of six neighbouring plots, three to the right and three to the left of the reference plot. For plots of boundary, the average of plots in front and behind of them was used.

Joint variance was carried out considering all the methods evaluated.

Comparison of these methods was made considering estimates of genetic and phenotypic parameters, such as coefficient of variation. The coefficient of variation (CV)

was estimated for expression: $CV(\%) = \frac{\sqrt{MSE_{Error}}}{\bar{x}} 100$,

where MSE_{Error} is error mean square obtained in analysis of variance in each method and \bar{x} is grand mean of experiment. Broad sense heritability on a progeny mean

basis was obtained for $h^2(\%) = \frac{MSP - MSE_{Error}}{MSP} 100$,

where MSP is progeny mean square estimated in analysis of variance in each method using only the 113 half sibs progenies. Confidence intervals of heritability were estimated by the expression presented by Knapp et al. (1985).

Additionally, Spearman's ranking correlation and Fasoulas' (1983) differentiation index (D) were estimated.

Fasoulas' (1983) differentiation index was obtained for expression $D = 200 \sum f / [n(n-1)]$, where f is the number of means that a given progeny exceeds significantly, after application of means test and n is the number of progenies. Also, coincidence index in the progenies that would be selected using the different methods was used by Hamblin & Zimmerman's (1986) expression

$$ES(\%) = \frac{A - C}{B - C} \cdot 100$$

where:

A = number of selected progenies with method considered as standard method;

B = number of selected progenies;

C = number of expected progenies by random coincidence; $C = b \cdot B$, where b is the intensity of selection used, and in this case $b = 0.10$.

3. RESULTS AND DISCUSSIONS

Error mean square and coefficients of variation (CV) estimates obtained at two locations and in the joint analysis of both ages are presented in Tables 1 and 3. Error mean square estimates were equivalent in both locations. The CV estimates varied from 10.40% in the augmented block design (BA3) in Posto da Mata to 34.59% in São Mateus. CV estimates obtained in São Mateus were higher than those in Posto da Mata and greater than those observed in eucalyptus experiments (Castro, 1992). This occurred because CV estimate is influenced by the average values (Pimentel Gomes, 1990). As mean squares of error were homogeneous between the two locations, the lower CV estimates obtained in Posto da Mata can be attributed to higher average at girth breast height in this location.

The efficiency of lattice compared with randomized complete block design was 45% and 24% at 19 and 26 months of age, respectively, in São Mateus, where CV estimate was high showing heterogeneity with blocks (Table 1). Eucalyptus seedlings of the same progeny coming from the nursery present dissimilarity in development. This fact contributes to higher heterogeneity within blocks. This difference disappears over time, explaining the reduction of efficiency of lattice at 26 months of age. No lattice efficiency was found in Posto da Mata showing homogeneity within blocks in this location.

Both nearest neighbour and the check plot methods resulted in a reasonable reduction of CV, around 25% and

21%, compared to the randomized complete block design, due to the heterogeneity within the blocks in São Mateus. The augmented block design produced a CV lower than that in the randomized complete block design and higher than that in the lattice design.

Heritability estimates were similar among the statistical methods in which replications were used because in these cases the values of the confidence interval limits were very close (Table 3). These heritability estimates do not agree with those obtained from the individual site variance analysis (Table 2) and confirm the observations made by Rosiele (1980) and Helms et al. (1995), which found that the difference between methods is evident in an isolated location, but it is not found in the joint variance analysis, due to genotypes x environments interaction. However, the results obtained in this study were similar to those reported by Pinto Júnior (1984) and Castro (1992). For the augmented block design there is a large discrepancy between the value of the h^2 estimate and the others, and no agreement was observed between the values of heritability estimates and their respective confidence intervals, compared to the estimates obtained by other methods.

Spearman's ranking correlation coefficients between family means were high, showing that there is not a great difference between the lattice design and other methods,

except for the augmented block design which was, in this case, 63% in São Mateus and 70% in Posto da Mata (Table 4 and 5).

Selection efficiency evaluated by the Hamblin & Zimmermann's expression (1986) was estimated considering different intensities of selection, 16% (selection of the 20 best progenies and 20 worst progenies), 33% (selection of the 40 best progenies) and 50% (selection of the 60 best progenies), respectively, based on the lattice analysis. There was a good agreement among the methods of variance analysis based on replication in both locations. However, for the augmented block design, the selection efficiency was lower, although it increased when the intensities of selection were moderate with $i=33\%$ and $i=50\%$ (Table 4 and 5). These results are in agreement with those obtained by Spearman's correlation, probably due to the fact that the averages in the augmented block design are estimated from only one replication, which affects the standard error of the progenies' means and, consequently, their classification. Therefore, the use of experiments with replications is necessary because the selection of the best progenies should be more strictly.

The differentiation index of the augmented block design was lower than those obtained by other methods (Table 4 and 5). From the 7260 pairs of possible contrasts

Table 1 – Error mean square (MSError) and coefficient of variation (CV%) estimates for all the statistical methods of girth at breast height (cm/plant) at ages 19 and 26 months in São Mateus and Posto da Mata

Quadro 1 – Estimativas do quadrado médio do erro (MSError) e coeficientes de variação (CV%) em todos os métodos estatísticos avaliados da circunferência à altura do peito (cm/planta) do eucalipto aos 19 e 26 meses em São Mateus e Posto da Mata

Designs ^{1/}	São Mateus				Posto da Mata			
	19 months		26 months		19 months		26 months	
	MSError	CV	MSError	CV	MSError	CV	MSError	CV
Lattice	7.939	26.60	13.510	16.88	8.650	11.96	14.248	11.83
BA1	11.698	25.87	16.936	18.09	15.893	14.48	24.923	15.27
BA2	13.303	34.59	31.824	27.16	9.051	10.88	18.924	13.13
BA3	6.391	25.79	18.181	21.29	8.239	10.40	13.869	11.37
RCB	11.536	32.06	16.741	18.80	8.659	11.97	14.248	11.83
Test	7.830	26.41	-	-	8.670	11.98	-	-
Papadakis	7.530	25.90	-	-	8.653	11.96	-	-
Moving average	7.229	25.38	-	-	8.689	11.99	-	-
Efficiency (%) ^{2/}	145.31		123.92		100.10		100.00	

^{1/} - Variance analysis: Lattice- in lattice design; BA1, BA2 e BA3- in augmented block design using data from replications 1, 2 and 3, respectively; RCB- in randomized complete block design.

- Covariance analysis in randomized complete block design: Test- employing the check plot as covariable; Papadakis- employing the Papadakis' (1937) method; Moving means- employing the moving mean as covariable.

^{2/} Efficiency of lattice compared with randomized complete block design.

among the 121 means, only 1.4% was significant in São Mateus, i.e., 104 contrasts, while the lattice design detected significant differences among 363 comparison pairs ($D=5.28\%$).

The general results obtained indicate that the augmented block design is a viable alternative to evaluate a greater number of genotypes in earlier stages of selection when the intensities of selection are moderate to low. For improving efficiency in this design, some other aspects should be considered such as the use of appropriate checks that represent the environmental variation of the segregating population in the study with, for example, the use of some of the genotypes being evaluated, as observed by Bearzoti (1994). On the other hand, the lower precision of the estimates of the genetic parameters obtained in this design do not rule out its usefulness in the stages for which it is recommended.

In the specific case of eucalyptus, this method can be very useful in clonal selection. Companies usually have a large number of clones to be evaluated, and obtaining

seedlings of all clones for evaluation in experiments with replications is very difficult. The augmented block design could be used and this would need less seedlings per clone because it does not require replication. Furthermore, the experimental area would be decreased allowing evaluation of a greater number of clones. At this stage early selection could be used contributing to a reduction in the selective cycle, already shown to be efficient in Brazil (Rezende et al., 1995; Marques Júnior et al., 1996). In this case, the selected clones would be evaluated precociously in experiments with replications for identification of the best ones and later introduced in the productive system.

The nearest neighbour methods (Papadakis and moving average) were shown to be efficient in removing environmental effects when heterogeneity within blocks was detected. In these cases, improvement in the experimental precision was almost always similar to the lattice design, based on genetic and phenotypic parameters estimates showing that the local control in rows and/or columns was as effective as the methods based on spatial analysis.

Table 2 – Heritability estimates and their respective confidence intervals for all the statistical methods of girth at breast height (cm/plant) at ages 19 and 26 months in São Mateus and Posto da Mata

Quadro 2 – Estimativas das herdabilidades e de seus respectivos intervalos de confiança em todos os métodos estatísticos avaliados da circunferência à altura do peito (cm/planta) do eucalipto aos 19 e 26 meses em São Mateus e Posto da Mata

Designs ^{1/}	São Mateus			Posto da Mata		
	h ² (%)	Lower	Upper	h ² (%)	Lower	Upper
19 months						
Lattice	42.37	20.83	58.73	43.15	21.91	59.29
BA1	25.62	-39.23	56.22	-28.96	-141.39	24.09
BA2	8.39	-71.47	46.08	13.60	-61.72	49.14
BA3	24.42	-41.47	55.51	1.27	-84.81	41.88
RCB	26.15	-0.60	46.79	43.38	22.87	59.21
Test	39.78	17.94	56.62	42.42	21.54	58.52
Papadakis	29.02	3.28	48.87	43.42	22.91	59.24
Moving average	33.05	8.77	51.77	43.11	22.48	59.02
26 months						
Lattice	52.06	34.54	65.38	52.50	35.14	65.69
BA1	19.63	-50.01	52.44	-0.20	-87.02	40.70
BA 2	-74.87	-226.37	-3.48	28.50	-33.45	57.69
BA3	-3.18	-92.57	38.95	15.65	-57.42	50.09
RCB	40.13	18.95	56.49	52.50	35.69	65.48

^{1/} - Variance analysis: Lattice- in lattice design; BA1, BA2 e BA3- in augmented block design using data from replications 1, 2 and 3, respectively; RCB- in randomized complete block design.

- Covariance analysis in randomized complete block design: Test- employing the check plot as covariable; Papadakis- employing the Papadakis' (1937) method; Moving means- employing the moving mean as covariable.

Table 3 – Summary of the joint variance analysis, heritability estimates and their respective confidence intervals for all the statistical methods of girth at breast height (cm/plant) at ages 19 and 26 months

Quadro 3 – Resumo das análises de variância conjuntas, estimativas das herdabilidades e de seus respectivos intervalos de confiança em todos os métodos estatísticos avaliados da circunferência à altura do peito (cm/planta) do eucalipto aos 19 e 26 meses

Designs ^{1/}	F (Prog.)	F (P x L)	CV (%)	Average	h ² (%)	Lower	Upper
19 months							
Lattice	2.02**	1.77**	16.37	17.59	46.73	26.82	61.85
BA	1.27 ^{NS}	1.02 ^{NS}	19.26	17.36	21.54	-46.86	53.82
RCB	1.83**	1.55**	18.07	17.59	39.98	18.24	56.76
Test	2.10**	1.62**	16.33	17.59	48.00	29.14	62.54
Papadakis	1.96**	1.54**	16.17	17.59	44.41	24.26	59.96
Moving average	1.97**	1.60**	16.04	17.59	44.68	24.62	60.15
26 months							
Lattice	2.39**	2.32**	13.88	26.83	52.63	35.32	65.79
BA	1.06 ^{NS}	0.83 ^{NS}	18.71	26.92	2.83	-81.36	42.50
RCB	2.24**	2.02**	14.67	26.83	48.44	30.20	62.53

^{1/} BA: joint analysis in the augmented block design employing the average progenies of BA2 in São Mateus and BA2 in Posto da Mata.

** F test significant at the level of 1 probability.

Table 4 – Spearman's correlation coefficients and coincidence index (%) between the means estimated in lattice design and other methods of variance analysis and Fasoulas' (1983) differentiation index (D%) of girth at breast height (cm/plant) at ages 19 and 26 months in São Mateus

Quadro 4 – Coeficientes de correlação de Spearman e índice de coincidência (%) entre as médias estimadas no látice e nos demais métodos de análise de variância e índice de diferenciação de Fasoulas'(1983) da circunferência à altura do peito (cm/planta) do eucalipto aos 19 e 26 meses em São Mateus

Designs ^{1/}	Spearman's correlation	Selected proportion (%)				D (%)
		16.5 ^{2/}	16.5 ^{3/}	33.0	49.6	
19 months						
Lattice	-	-	-	-	-	5.28
BA1	0.6568	31.25	43.75	51.85	53.33	1.06
BA2	0.6476	25.00	37.50	51.85	56.67	0.83
BA3	0.5790	37.50	31.25	29.63	50.00	2.30
RCB	0.8808	75.00	75.00	70.37	73.33	2.80
Test	0.9556	81.25	81.25	77.78	86.67	4.97
Papadakis	0.9281	81.25	75.00	77.78	80.00	3.10
Moving average	0.9168	81.25	68.75	77.78	80.00	3.57
26 months						
Lattice	-	-	-	-	-	4.21
BA1	0.6674	50.00	50.00	44.44	46.67	2.26
BA2	0.6149	25.00	31.25	44.44	46.67	0.29
BA3	0.6050	50.00	43.75	22.22	40.00	1.09
RCB	0.9354	75.00	68.75	74.07	80.00	3.14

^{1/} - Variance analysis: Lattice- in lattice design; BA1, BA2 e BA3- in augmented block design using data from replications 1, 2 and 3, respectively; RCB- in randomized complete block design.

- Covariance analysis in randomized complete block design: Test- employing the check plot as covariable; Papadakis- employing the PAPADAKIS'(1937) method; Moving means- employing the moving mean as covariable.

^{2/} and ^{3/} selection of 20 worst and best progenies, respectively.

Table 5 – Spearman's correlation coefficients and coincidence index (%) between the means estimated in the lattice design and other methods of variance analysis and Fasoulas' (1983) differentiation index (D%) of girth at breast height (cm/plant) at ages 19 and 26 months in Posto da Mata

Quadro 5 – Coeficientes de correlação de Spearman e índice de coincidência (%) entre as médias estimadas no látice e nos demais métodos de análise de variância e índice de diferenciação de Fasoulas' (1983) da circunferência à altura do peito (cm/planta) do eucalipto aos 19 e 26 meses em Posto da Mata

Designs ^{1/}	Spearman's correlation	Selected proportion (%)				D (%)
		16.5 ^{2/}	16.5 ^{3/}	33.0	49.6	
19 months						
Lattice	-	-	-	-	-	6.23
BA1	0.7512	56.25	37.50	59.26	63.33	1.27
BA2	0.7036	43.75	31.25	62.96	63.33	3.55
BA3	0.6395	50.00	37.50	48.14	46.67	3.11
RCB	0.9994	100.00	93.75	100.00	100.00	6.20
Test	0.9987	93.75	93.75	100.00	100.00	5.92
Papadakis	0.9964	93.75	87.50	100.00	96.67	6.22
Moving average	0.9988	93.75	87.50	100.00	100.00	6.18
26 months						
Lattice	-	-	-	-	-	9.48
BA1	0.7719	62.50	18.75	37.04	46.67	1.82
BA2	0.7457	56.25	50.00	62.96	63.33	3.94
BA3	0.6344	50.00	25.00	59.26	46.67	4.27
RCB	0.9999	100.00	100.00	77.78	80.00	9.42

^{1/} - Variance analysis: Lattice- in lattice design; BA1, BA2 e BA3- in augmented block design using data from replications 1, 2 and 3, respectively; RCB- in randomized complete block design.

- Covariance analysis in randomized complete block design: Test- employing the check plot as covariable; Papadakis- employing the Papadakis' (1937) method; Moving means- employing the moving mean as covariable.

^{2/} and ^{3/} selection of 20 worst and best progenies, respectively.

4. CONCLUSIONS

- Augmented block design is suitable in earlier stages of selection when intensities of selection are moderate to low. However, this design is not suitable for estimating genetic and phenotypic parameters due to low precision.

- Nearest neighbour, Papadakis (1937), moving means and check plot methods were efficient in removing heterogeneity within blocks. These efficiencies were compared to that in lattice analysis for estimation of genetic and phenotypic parameters.

5. ACKNOWLEDGEMENT

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