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Selection efficiency in *Brachiaria* hybrids using *a posteriori* blocking

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ABSTRACT - *This paper compares the efficiency of Brachiaria hybrid clone selection by either traditional analysis or a posteriori blocking, to adequately consider the effect of flower competition in the border rows of the experimental plots (border effect). Results demonstrated that a posteriori blocking improved selection and the reliability of the genotypic evaluation. Of the ten best clones. four did not coincide in the two approaches. The ranking was altered as well, which demonstrated that the indication of the five best clones. selected to proceed to pasture trials based on the traditional evaluation, was misleading. This paper confirms the usefulness of a posteriori blocking. Furthermore, the results revealed lhe need to impose more effective competition on plants in the border rows to avoid erroneous selection when conducting evaluations for agronomic performance in Brachiaria trials.*

Key words: competition; forage breeding; genetic parameters; REML-BLUP; repeatability.

INTRODUCfION

Grasses of the *Brachiaria* genus, especially *B. brizantha* and *B. decumbens,* currently account for about 49% of all forage seed produced in Brazil and represent 85% of the seed sold in the Cerrado region (Valle et aI. 2004a). In Central Brazil basically two cultivars of two species (cv. Basilisk of *B. decumbens* and cv. Marandu of *B. brizantha)* are planted on an estimated area of some 100 million hectares.

In Brazil, new cultivars are commonly selected from the natural variation in germplasm collections introduced from their original habitats, mainly the Afriean savannas. This procedure has been successful for several *Brachiaria* species, for which selection of natural genotypes and their use has been facilitated by apomixis, that is, assexual reproduction through seeds (Valle et aI. 2004b).

Several apomictic genotypes available in the germplasm banks have desirable agronomic characteristics and are adequate for the most varied production conditions throughout BraziI. AlI cultivars available today have limitations that can be improved through breeding (Miles et aI. 2004). Amplification of genetie variability in the breeding of predominantly apomictic grasses inevitably implies the use of hybridization. Since 1988 the ongoing *Brachiaria* breeding program ofEmbrapa (Valle et aI. 1993, 1999) has generally used crosses of artificially tetraploidized *B. ruziziensis* as the source of sexuality, pollinated by apomictic *B. decumbens* or *B. brizantha.* These crosses produce interspecific hybrids with desirable traits of interest for the breeding program and the cultivar development process (Valle et aI. 2000), which are

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experimentally evaluated as potential new cultivars in trials as described in this paper.

Initial stages of evaluation and selection in *Brachiaria,* either with accessions or hybrids, involve a number of genotypes. Due to the limited quantity of avai!able seed or vegetative tillers and size of the experimental area needed, linear plots (5 m) and few replications (2 or 3) are generally used. Such experiments tend to be biased due to spatial variation or fertility leveis, for example, and inter genotypic competition, which could result in an erroneous identification of elite genotypes. In these experiments, inter genotypic competition could affect the prediction of the genotypic value of the clones and reduce genetic gain. Recently, differences were detected in the selection of sugar cane genotypes, between the traditional analysis method and the *a posteriori* blocking, the latter being recommended when environmental variation (fertility) and intra-plot competition are identified.

The so-called *a posteriori* blocking technique is a useful approach, which takes spatial variation and the effect of competition into account (Federer 1998, Gilmour 2000). For this purpose, a new block arrangement is considered, which involves a practical evaluation of the experiments. Visible border effects, for example, differential patches in the plots or natural ferti!ity gradients, among other aspects, are taken into consideration. Effects of differential phenotypic expression, determined by the competition in the border rows of an experiment, may be due to the absence or to the use of inefficient borders.

This paper compares the efficiency of selection and estimation of genetic parameters in *Brachiaria* hybrid clones, using the traditional and the *a posteriori* blocking analysis to accommodate the competition associated to the border effect in the experimento

MATERIALAND METHODS

In the experiment 49 clones were evaluated, of which 26 were interspecific hybrids between *B. ruziziensis* x *B. brizantha,* 7 were hybrids between *B. ruziziensis* x *B. decumbens,* 14 were second-generation hybrids from a cross between a sexual hybrid *(B. ruziziensis* x *B. brizantha)* and other *B. brizantha* accessions and two were checks, *B. brizantha* cv. Marandu (M) and *B. decumbens* cv. Basilisk. (D)

The experiment was carried out at Embrapa Gado de Corte, in Campo Grande, MS, (lat 20°28' S, long 55°40' W, 530 m asl, soi! type 'Alic Latosol A', clayey texture)

(Mothci et aI. 1979). According to Kõppen the climate type is Aw, humid tropic, with a rainy summer and dry winter season.

The plots were established in a 7 x 7 lattice design, with two replications and seven plants per plot, ofwhich the five central plants were considered. The experimental design with the treatment distribution in the replications is presented in Table 1.

Two months before planting, the soil pH was corrected with 2.5 t ha' of dolomitic lime. Fertilization consisted ofNPK 05-20-20 (500 kg ha"), and additional N (100 kg ha"). Maintenance fertilization was applied in the second year (100 kg ha" ofN and K_2O, and 50 kg ha'! ofP$_2$0$_{S)}$'

The plots were cut 10 times but the first cut was not considered for analysis. Cuts 3, 4 and 10, on 07/30/ 02,09/27/02 and 07/01/03, respectively, were dry season cuts and the others, cut on 10/29/02, 12/02/02,01/07/03, 02/11/03,03/18/03 and 04/04/04, were evaluations ofthe rainy season. The total dry matter production (TDMP) and leaf dry matter production (LDMP) in kilograms per plot (kg plor") in the dry and rainy seasons were compared as four independent variables.

Analysis of experimental data

For the traditional analysis of the lattice experiment, the following statistical model was applied: univariate model for clones, considering heritability and repeatability simultaneously - Model70 ofthe SELEGEN REML-BLUP program (Resende 2002b).

$$Y = Xf + Zg + Wb + Tp + e,$$

where:

y, f, g, b, p, and e: are data; fixed effects (combination replication-evaluation); individual genotypic effects (random); permanent block within replication effects (random); permanent environmental effect in plots (random) and random error vectors, respectively.

X, Z, W and T: are matrices of incidence for f, g, b, p, respectively.

The distribution and structure of means and variances were given by:

$$y|b,V \sim N(Xb, V)$$
$$g|\sigma_g^2 \sim N(0, I\sigma_g^2)$$
$$b|\sigma_b^2 \sim N(0, I\sigma_b^2)$$
$$p|\sigma_p^2 \sim N(0, I\sigma_p^2)$$
$$e|\sigma_e^2 \sim N(0, I\sigma_e^2)$$

Table 1. Experimental design of the data evaluation of *Brachiaria* hybrids. Campo Grande, MS

BLOCK		REPLICATION		I				BLOCK		REPLICATION		11			
B7	M	44	45	46	47	48	49	B14	7	14	21	28	35	D	49
B6	36	37	38	39	40	41	D	B13	6	13	20	*zt*	34	41	48
B5	*ng*	30	31	32	33	34	35	B12	5	12	19	26	33	40	47
B4	22	23	24	25	26	*ZI*	28	B11	4	11	18	25	32	39	46
B3	15	16	17	18	19	20	21	BI0	3	10	17	2A	31	38	45
B2	8	9	10	11	12	13	14	B9	2	9	16	23	30	37	44
Bl	1	2	3	4	5	6	7	B8	1	8	15	22	*ng*	36	M

M: *B. brizantha* cv Marandu; D: *B. decumbens* cv Basilisk.

The covariances among alI random effects in the model were considered nonexistent.

Thus:

$$E\begin{vmatrix} y \\ g \\ b \\ P \\ e \end{vmatrix} = \begin{vmatrix} Xb \\ 0 \\ 0 \\ 0 \\ 0 \end{vmatrix} \quad \text{and} \quad Var\begin{vmatrix} y \\ g \\ b \\ P \\ e \end{vmatrix} = \begin{vmatrix} V & Z & G & W & B & T & P & R \\ GZ' & G & 0 & 0 & 0 \\ BW' & 0 & B & 0 & 0 \\ PT' & 0 & 0 & P & 0 \\ R & O & 0 & O & R \end{vmatrix}$$

, where:

$G = /a2_g$

$B = /\sim$

$P = / a2_p$

$R = /a2_e$

$V = Z/ a2_g Z + W/ \sim hW' + T'/ a2_p T + / a2_e$
$= ZGZ' + WBW' + TPT'R$

The distributions and structures of means and variances; mixed model equations and iterative estimators of variance components by REML via algorithm EM (Expectation maximization) are given by Resende (2002a).

The following parameters were estimated:

$h_{\cdot}^{\cdot} = \dfrac{CJZ}{CJ_g^z + CJ_h^1 + CJ_p^z + CJ_{\cdot}^1}$: individual broad-sense

heritability (determination coefficient of genotypic effects) within replication for a given measurement; where:

$0''$: : genotypic variance among clones;

$0'' \sim$: permanent variance among blocks;

$0''2_p$: variance ofthe perrnanent environmental effects in plots;

$0''2_e$: residual variance.

$h \sim = \dfrac{CJ1}{CJ_g + CJ_{\cdot}^1} \sim + g$:adjusted individual heritability;

$h \sim = \dfrac{1}{CJZ_g + \bullet \dots}$ adjusted clone average heritability,

where m is the number ofmeasurements and ris the number ofreplications;

$r = \dfrac{CJ2_g + CJ1_b + CJ1}{CJ1_g \div CJ_b \div CJ2_p \div CJZ_e}$ individual repeatability in the replication.

The estimates of adjusted heritability were used to compare models. A more direct means of comparison is the calculation of selection accuracy for two alternative analysis models, based on the assumption that the true genetic and phenotypic parameters are those of the most complete model. These parameters (matrices G and V of the complete model) are used to compute the accuracy by the two models, both the simple (traditional analysis) as well as the more complete one *(a posterior i* blocking analysis). This approach considers the alteration in alI components of variance simultaneously when the analysis model is changed.

The variance of the prediction error in genotypic values (PEV) by the traditional model (t), considering *a posteriori* blocking (b) as the true model, was calculated by the following equation:

PEVt/b=Var(g/-gb)=Ct,v;lvbv;' Ct-Ct'V;1Cb-Cb'V;Ct+Gb, where V and G were defined above and C = ZG. PEV by the *a posteriori* blocking model was calculated by: PEVb/b=Var(gh-gb)=Gb-Cb'V-/'Cb With PEV for each genotype, the accuracy was calculated using Ac=[I-PEV/cri] 112, where Cfi is the genotypic variance estimated by the *a posteriori* blocking model.

Mixed model equations (BLUP procedure) were used to predict the genotypic values of hybrids for the evaluated traits.

Before the joint analysis of all cuts for a given season - dry or rainy - separate analyses were run for each cut to evaluate the heterogeneity of variances

among cuts. The data were corrected by the following expression, according to Resende (2004):

$$J'an := \left(S''1\sim \right), \text{ where:}$$

y: original data vector for the specific variable.

Sgi: genetic standard deviation in cut i;

Sg: average of genetic standard deviations of ali cuts in dry or rainy seasons;

S: average of phenotypic standard deviations of ali cuts in the dry or rainy seasons;

Sjl: phenotypic standard deviation in cut i;

The idea of applying the ratio of the square roots ofheritability in environment i (h.) and ofthe heritability means in ali environments (h$_m$) is an attempt to consider both the heterogeneity in genetic as well as in residual variance, as implied in the heritability estimates. In other words, the method takes the heterogeneity in heritabilities into account.

If one considers that the predictor BLUP, applied in the analysis of ali measurements (environments) simultaneously, balances the data by an average heritability valid for ali measurements, the final weighted data in e~ch environment (measurement) are given by (h/hm)·h m=h/h$_m$.

This calculation depends simultaneously on the heritability in the target environment for selection (in this case, the overall mean environment of all evaluations) and on the reliability ofthe data in each environment, given by the function (hi) ofthe heritability in each environment. The smaller the heritability in a certain environment, the lower the weight attributed to information from this environment. This, in practice, is coherent and desirable.

Considering the lack of competition along the borders ofthe experiment and the effect on the genotypes growing there (Table 1), a post-blocking was performed so that these hybrids were placed in new blocks and the old blocks were maintained but modified to accommodate the removal ofthe border hybrids.

This rearrangement resulted in additional four blocks, adding up to 18 blocks altogether. The first extra group included genotypes 1,8, 15,22,29,36, and M; the second: 7,14,21,28,35, D and49, inreplication I; thethirdincluded 1,2,3,4, 5, 6 and 7; and the fourth, M, 44, 45, 46, 47,48 and 49, in replication 11.

With this new setup, parameters were estimated using the same statistical modeI. This constitutes the analysis based on *a posteriort* blocking. Alternatively,

a model with two fixed effects was adopted: one considering the border plots and the other considering the remaining plots, but maintaining the original block designo The genotypic values were predicted for each trait in the rainy and the dry seasons, and the hybrids were ranked in decreasing order to facilitate selection.

The leaf dry matter production in the dry and rainy seasons were considered in an additive selection index, with economic weights defined as a function of the proportion of production in the two periods (greater production in the rainy season) and the agronomic importance of each (greater importance of this production in the dry season). Thus, these two traits were assigned equal weights.

For ali statistical analysis the software package for genetics and statistics SELEGEN - REML/BLUP was used (Resende 2002b). Model70, described above, was used to estimate the components of variance and prediction of genotypic values in the univariate model of clones in lattice, considering heritability and repeàtability simultaneously. Model 101 - additive selection index, as proposed by Resende (2002a) - was used to estimate the selection indices for gain in a genotype group formed by several traits.

RESULTS AND DISCUSSION

Table 2 displays the estimates of the variance components and genetic parameters for the evaluated traits in the dry and rainy seasons in hybrid clones of *Brachiaria,* considering the traditional method of lattice analysis as well as the *a posteriori* blocking method.

The altered block composition to circumvent the border effect in this experiment resulted in an increase in genotypic variance and also in adjusted heritability for the evaluated traits. This was true both in the dry and rainy seasons, with exception ofleaf dry matter in the dry season, where results were identical by either approach.

The adjusted heritability refers to a heritability free ofall adjusted random environmental effects in the model; in this case, the denominator consists of the genotypic and residual variances only. The adjusted heritability allows for comparisons of alternative models of analysis since it is a function ofthe residual variance particular to the adjustment of each model (the smaller the residual variance the better the model) and also includes the amount of genetic variance recovered by the analysis modeI. Such heritability is free of fluctuation in the remaining

Table 2. Estimates of genetic and phenotypic parameters for total dry matter production (TDMP) and leaf dry matter production (LDMP), in kg plor', evaluated in *Brachiaria* spp genotypes, in the dry and rainy seasons, by the traditional method (TRAD) and by *a posteriori* blocking (BLOC), in Campo Grande, MS

Parameters	TDMP-TRAD	TDMP-BLOC	LDMP-TRAD	LDMP-BLOC
		Dry Season		
V_g	0.1315	0.1594	0.0628	0.0627
V.	1.3242	1.3269	0.3806	0.3810
V_b	0.0020	0.4086	0.0007	0.1574
V_j	0.5265	0.0895	0.1940	0.0328
h^2_{8J}	0.09	0.11	0.14	0.14
h^2_{mcaj}	0.37	0.42	0.50	0.50
AC_2	0.500	0.581	0.540	0.630
AC_1	0.499	0.572	0.537	0.619
Ac,	0.497	0.572	0.535	0.620
r	0.33	0.33	0.40	0.40
		Rainy Season		
$V,$	0.1408	0.1542	0.0919	0.1113
V.	1.5300	1.5301	0.4290	0.4289
$V,$	0.0036	0.2441	0.0011	0.0875
$V,$	0.4345	0.1832	0.1913	0.0879
h^2_{8J}	0.08	0.09	0.18	0.21
h^2_{rncaj}	0.52	0.54	0.72	0.76
AC_2	0.559	0.613	0.682	0.734
AC_1	0.558	0.605	0.681	0.724
Ac_o	0.557	0.605	0.680	0.727
r	0.27	0.28	0.40	0.40

Genotypic variance among treatments (V), residual variance (V_e). variance among blocks in the lattice (V_b), permanent environmental variance (VI')' adjusted individual heritability (h;), adjusted average clone heritability (~caj)' selective accuracy of clones that appear twice, once or never as border, respectively (Ac_2, AC_1 and Ac_O) and individual repeatability (r).

components of variance relative to environmental effects, for it is proportional only to the error or to the residual random variance not adjusted in the model. The adjusted heritability is associated to the shrinkage factor for the genotypic effects in the mixed model equations,

since Â, = $\underline{1}$ ~: , even for models with several random
    haj

effects besides the error. Therefore, the adjusted heritability expresses the reliability of the adjusted phenotypic values for alI fixed effects and remaining random effects of the model, as indicators of the true genotypic effects. The best model is the one with the most reliable adjusted phenotypic values for alI remaining effects ofthe model.

Similar results of efficiency were observed in the adjusted selection accuracy (Table 2). As a consequence, the selection efficiency in *a posteriori* blocking over the traditional analysis was 1.10 and 1.17 for the total and leaf dry matter production in the rainy season, respectively

and 1.22 for total dry matter production in the dry season. The superiority of *aposteriori* blocking over the traditional analysis for these three traits ranged from 10 to 22% in terms of adjusted heritability.

The major difference between the estimates by the two analyses are observed for the environmental variance between blocks and the permanent environmental variance. By the traditional approach, unlike by *aposteriori* blocking, the lattice block effect was quite small for alI traits. The opposite result was observed for the permanent environmental effect. In other words, *aposteriori* blocking redistributed the permanent environmental effect in the genotypic variance and among blocks. This result demonstrates that traditional blocking was not efficient (low variation among blocks). On the other hand, post-blocking was effective (high variation among blocks detected, due to better growth of the hybrids along the borders ofthe experiment), indicating that the border effect was eliminated when the new blocks were formed and that genotypic effects were predicted free ofthe differential or

reduced competition effects that are imposed on the plots allocated along the borders. Furthermore, by using *a posteriori* blocking the selection was more precise and the genotypic evaluation more reliable.

The benefit of the re-distribution of variability, when adopting the post-blocking model could also be detected by the selection accuracy (Table 2) associated to the two analysis models, assuming that the correct parameters are the ones provided by the most complete model *(a posteriori* blocking). By this model the accuracy was higher than by the traditional model for all traits studied.

The adjustment for the competition effect affected the ranking of the best clones rather strongly (Table 3). Of the best five candidates for new cultivars, there was no alteration in the two first individuals selected when only the dry season yield variables were taken into account. From that point onwards however the rank and genotypes were significantly altered. Three of the best individuals for total dry matter production by the a posteriori approach would not be selected by the traditional method, nor the six best individuals for leaf dry matter production.

Four of the ten best individuals selected by *a posteriori* blocking for total dry matter production in the rainy season, when 70-80% of the annual yield of grasses is produced (Jank et al. 2005), did not appear among the ten best by the traditional method. The order was also significantly altered for leaf dry matter production.

In the rainy season, out of the 10 best genotypes for total dry matter production, the traditional method selected nine which were in the border whereas the a posteriori blocking identified only five of these. The number of border

clones was therefore reduced by half. This had been expected since out of a total of 98 plots, 28 were border plots, which is practically one third of all plots. Thus, according to the probability or mathematical expectations, between 3 and 4 of the best 10 genotypes should really be in the border. The number 5 instead of 4 could be due to random deviations from the mathematical expectation. Furthermore, the simple effect of reducing the number of selected clones due to the position in the border clearly justifies the use of *a posteriori* blocking.

Based on the alternative model with fixed effects (one considering the border plots and the other the remaining plots), the ten best genotypes for total dry matter production, in decreasing order were clones 28, 2, 48, 24, D, 9, 20, 14, 30, and 26~This ranking is almost identical to the one established by *a posteriori* blocking, since only one of the selected genotypes was exchanged. The ranking order was also practically identical. The newly selected genotype was clone 26, which was not in the border, like 38, selected by *a posteriori* blocking. This approach did therefore not reduce the number of selected genotypes along the border of the experiment. Similar results were obtained for the other traits. Therefore, the results obtained by *a posteriori* blocking were considered for all practical aspects of discussion.

When leaf dry matter production in the rainy and the dry seasons are considered as different characters for the selection index, one verifies that out of the ten best individuals, four did not coincide in the two approaches (Table 3). The ranking was also altered to such a degree that the five best genotypes indicated to proceed to pasture

Table 3. *Brachiaria* hybrids ranked in decreasing order of their genotypic values (in brackets) for total dry matter production (TDMP) and leaf dry matter production (LDMP), in kg plor', in the rainy and dry seasons, analyzed by the traditional approach (Trad) and *a posterion* blocking (Bloc), as well as the rank based on an additive selection index

| Rainy Season | | | | Dry Season | | | | Index | |
| TDMP | | LDMP | | TDMP | | LDMP | | | |
Trad	Bloc	Trad	Bloc	Trad	Bloc	Trad	Bloc	Trad	Bloc
28(4.11)	2(4.10)	2 (2.76)	2(2.87)	D(4.1O)	D(4.25)	D(2.22)	D(2.23)	1	3
D(4.04)	28(4.06)	7(2.76)	48 (2.76)	3 (3.87)	3 (3.88)	3(2.21)	3 (2.19)	7	30
2(4.01)	48(4.02)	49 (2.71)	24(2.68)	M(3.86)	36(3.85)	1 (2.19)	36(2.18)	49	38
48 (3.99)	24(4.00)	1(2.69)	46(2.65)	14(3.84)	14(3.79)	M (2.15)	11 (2.11)	3	2
14(3.97)	D(3.96)	48(2.69)	28(2.64)	49(3.80)	M(3.78)	7 (2.14)	30(2.10)	2	11
49(3.95)	14(3.86)	28(2.65)	38(2.64)	1 (3.79)	11 (3.74)	36(2.10)	M(2.07)	M	48
M(3.89)	20(3.85)	46(2.61)	49(2.63)	36(3.77)	37 (3.70)	49(2.10)	1 (2.04)	14	1
1(3.87)	9(3.84)	21 (2.60)	7 (2.63)	5 (3.75)	38 (3.68)	14(2.09)	14(2.03)	21	14
24(3.87)	26(3.83)	14(2.57)	26(2.60)	21 (3.73)	5 (3.67)	5 (2.07)	38 (2.02)	28	21
46(3.83)	38(3.79)	24(2.56)	21 (2.60)	6(3.71)	30(3.66)	21 (2.07)	39(2.01)	D	49

trials by the traditional evaluation, would be seriously mistaken for not taking the border effect into account. Of the tive best, only two genotypes were identical by both methods. Furthermore, the tive best clones by the traditional approach all grew along the plot borders. By *a posteriori* blocking only two were found in the border, as expected, based on the probability cited above.

In fact the first ten individuais classitied by the index in the traditional approach all grew along the plot borders, i.e., with no competition. By using *a posteriori* blocking, three out of the ten selected grew on the border, among them the 2nd, 3rd and 5th in the ranking.

The results revealed the usefulness and reliability of a posteriori blocking, but also the need to plan more effective borders to impose competition on plants in the border rows, and thus avoid erroneous selection when conducting evaluations for agronomic performance in *Brachiaria* trials.

Eficiência na seleção de híbridos de *Brachiaria* via uso da blocagern *a posteriori*

RESUMO - **O** *presente trabalho teve como objetivo comparar a eficiência da seleção envolvendo clones híbridos de* Brachiaria, *por meio da análise tradicional e da blocagem* a posteriori, *visando considerar o efeito da competição associada ao efeito de borda do experimento. Os resultados revelaram que a blocagem* a posteriori *conduziu a uma seleção mais correta e a uma maior confiabilidade da avaliação genotípica. Verificou-se que, dos dez melhores clones selecionados quatro não coincidem nas duas abordagens de análise. A ordem também se altera, de forma que a seleção dos cinco melhores para ensaios de pastejo, conforme a análise tradicional, conduziria a sérios erros. Além da utilidade da técnica da blocagem* a posteriori, *os resultados revelam a necessidade do uso de bordaduras mais efetivas no planejamento da experimentação para fins de seleção em* Brachiaria.

Palavras chave: competição, melhoramento de forrageiras, parâmetros genéticos, REML-BLUP, repetibilidade.

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