

# Potential of maize single-cross hybrids for extraction of inbred lines using the mean components and mixed models with microsatellite marker information

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**ABSTRACT.** The present study examined the importance of mean ( $m+a'$  and  $d$ ) components in the performance of single-cross hybrids for the formation of new populations and determined the contribution of the mixed model (best linear unbiased predictor of random effects, BLUP) method associated with molecular markers for the choice of crosses to obtain interpopulation hybrids. Ten single-cross commercial hybrids of different companies were used for this purpose, producing all possible double-cross hybrids through a complete diallel. The hybrids were evaluated in 15 locations in the agricultural year 2005/2006, using randomized complete block design with three repetitions. In three of these locations, estimates of  $m+a'$  and  $d$  were obtained. DNA was extracted from the single-cross hybrids and 20 SSR primers were used, nine of which were linked to QTL for yield. There was no correlation between  $m+a'$  of the single-cross hybrids with general combining ability ( $r = -0.15$ ) inferring that populations with lines with high means do not always produce good hybrids. Also, it was observed that the correlation between the genetic distances with specific combining ability varied from 0.31 to 0.80 in the inter-group hybrids, while in the intra-group hybrids these estimates were low and non-significant. The heritability value obtained by BLUP

was high and greater than that obtained by ordinary least squares ( $h^2 = 0.95$  and  $0.86$ ), confirming the greater selection accuracy by the BLUP method. There were no differences between the accuracy values obtained with microsatellite information and without this information, inferring that there was no advantage of progenitor information on balanced data. It can be concluded that the estimate  $m+a'$  should not be used as a deciding parameter about the potential for extracting lines from a given population. The heritability and accuracy values obtained by BLUP allow the inference that it is possible to predict success in the choice of progenitors to obtain interpopulation hybrids.

**Key words:**  $m+a'$ ; Specific combining ability; BLUP; Molecular markers; Interpopulation hybrids

## INTRODUCTION

The choice of the base population to obtain elite lines in breeding programs is of utmost importance. A question that has arisen lately is about the type of germplasm to use for this purpose.

Populations of narrow genetic basis, originating from maize single-cross hybrids, have been considered as preferential germplasms in breeding programs compared to open pollination varieties, since the latter are little improved. Single-cross hybrids have the advantage of being previously tested in several environments, thus associating high yield with a large number of loci with fixed favorable alleles. Besides the single-cross hybrids, also populations from synthetic elite lines, related or not, have been used, as well as populations derived from back crossings as alternatives for extracting new lines (Hallauer and Miranda Filho, 1988; Hallauer, 1990; Troyer, 1994).

Beginning with a single-cross hybrid diallel, the plant breeder can obtain information to aid in the choice of which hybrids to use to form a new population. Estimates of specific combining ability (SCA) between single-cross hybrids serve as parameters to predict the average performance of its lines after crossings. That is, hybrids with greater SCA would be potential populations for extracting lines, in such a way that, lines originating from these hybrid pairs, on average, would have high SCA, better exploiting the hybrid vigor.

Most studies published on plant breeding experiments have considered the effects of both SCA and general combining abilities (GCA) as fixed. This supposition impedes the breeder from making inferences about SCA as a parameter for choosing the parents since a fixed effect cannot be extrapolated to the progenies. André (1999), states that, by definition, breeding values, such as SCA and GCA, are random and assuming them as such, one can predict them using methods that combine the best linear unbiased estimate of fixed effects (BLUE) and the best linear unbiased predictor of random effects (BLUP). Lately, several studies have pointed to this same idea (Henderson, 1984; Bernardo, 1996; Resende, 2002; Piepho et al., 2008). According to André (1999), using BLUE to estimate fixed effects and BLUP for predicting SCA and GCA, in general, is less biased than the ordinary least squares (OLS) method.

The use of molecular markers for predicting the performance of maize hybrids has been proposed by several authors (Reif et al., 2003; Amorim et al., 2006). However, until now, the

results are not consistent (Guimarães et al., 2007). For this reason, the use of molecular marker information associated with the mixed model method has been suggested with advantages in predicting genetic values, such as SCA and GCA (Bernardo, 1996; André, 1999). Thus, micro-satellite markers could be used since they have uncountable advantages (Reif et al., 2005).

Other parameters, such as  $m+a'$  and  $d$ , have been proposed for decision-making on the choice of potential hybrids to use in forming populations for the extraction of lines (Lima et al., 2000; Bison et al., 2003). The estimate  $m+a'$  represents the loci with fixed alleles, i.e., the average of all lines in  $S_{\infty}$ . According to Troyer (2006), the percentage of yield of hybrids due to heterosis decreases with time due to increase in locus fixation with favorable alleles in the lines ( $m+a'$ ), which means that an increase in the participation of the average of parental lines in hybrid yield occurs, in relation to heterosis *per se*. Therefore, the choice of hybrids with greater proportion of fixed favorable loci should be considered a priority.

Another component of the average that should be taken into account is  $d$ , representing the deviation of heterozygotes in relation to the means. This component is useful in indicating the potential genetic variability, since selecting vigorous lines assumes the existence of dispersion or genetic variation among them. However, the use of this parameter as a variability measure has not found agreement among researchers (Abreu, 1997; Lima et al., 2000).

Both estimates of  $m+a'$  and  $d$ , as well as estimates of SCA should be taken into account for selecting populations. However, emphasis has been given to the estimate of  $m+a'$  as a deciding factor (Lima et al., 2000; Bison et al., 2003).

The objective of the present study was to determine the importance of mean components ( $m+a'$  and  $d$ ) in the choice of maize single-cross hybrids as potential parents and the contribution of the BLUP associated with molecular markers in the choice of parents with greater SCA for the production of interpopulation hybrids.

## MATERIAL AND METHODS

Ten commercial single-cross hybrids, from different companies, were used (Table 1). A complete diallel was conducted with these hybrids, obtaining 45 double hybrids, which were evaluated simultaneously with their progenitors, in different environments, in a total of 55 treatments. The treatments were evaluated in 15 locations in the States of Minas Gerais, Bahia and Goiás (Table 2).

**Table 1.** Description of the ten commercial single-cross hybrids used as progenitors.

| Code | Hybrid  | Company         | Kernel texture | Cycle |
|------|---------|-----------------|----------------|-------|
| 1    | DKB199  | Monsanto        | Semi-dent      | SMP   |
| 2    | 30F90   | Pioneer         | Flint          | SMP   |
| 3    | A2555   | Nidera Sementes | Flint          | SMP   |
| 4    | DKB333B | Monsanto        | Semi-dent      | SMP   |
| 5    | DOW657  | Dow AgroScience | Semi-dent      | P     |
| 6    | AG8060  | Monsanto        | Semi-dent      | P     |
| 7    | 30F87   | Pioneer         | Flint          | SMP   |
| 8    | DOW8420 | Dow AgroScience | Flint          | P     |
| 9    | 30K75   | Pioneer         | Semi-dent      | SMP   |
| 10   | AG7000  | Monsanto        | Semi-dent      | SMP   |

P = early cycle; SMP = semi-early cycle.

**Table 2.** Characteristics of the environments used for the evaluation of the simple and double hybrids.

| Environment | Municipality          | Latitude | Longitude | Altitude (m) |
|-------------|-----------------------|----------|-----------|--------------|
| 1           | Lavras, MG            | 21°13'S  | 44°58'W   | 910          |
| 2           | Guarda-Mor, MG        | 17°34'S  | 47°08'W   | 1010         |
| 3           | Barreiras, BA         | 12°08'S  | 45°00'W   | 452          |
| 4           | Jussara, GO           | 23°35'S  | 52°28'W   | 250          |
| 5           | Lavras, MG            | 21°12'S  | 44°58'W   | 951          |
| 6           | São Gotardo, MG       | 19°18'S  | 46°03'W   | 1058         |
| 7           | Ijaci, MG             | 21°09'S  | 44°56'W   | 859          |
| 8           | Ijaci, MG*            | 21°09'S  | 44°56'W   | 859          |
| 9           | Lavras, MG            | 21°13'S  | 45°03'W   | 918          |
| 10          | Ribeirão Vermelho, MG | 21°10'S  | 45°04'W   | 884          |
| 11          | Candeias, MG          | 20°46'S  | 45°19'W   | 967          |
| 12          | Paracatu, MG          | 17°13'S  | 46°39'W   | 580          |
| 13          | Carrancas, MG         | 21°24'S  | 44°38'W   | 1005         |
| 14          | Itutinga, MG          | 21°23'S  | 44°46'W   | 958          |
| 15          | Ingai, MG             | 21°22'S  | 44°45'W   | 980          |

\*Planted during the dry season.

The experimental design was randomized complete blocks, and the variable analyzed was unhusked corn ear yield (kg/ha) corrected for 13% moisture.

Diallel analyses were carried out using method IV, proposed by Griffing (1956). Estimates of SCA and GCA were obtained using mixed model methodology (MMM), thus obtaining BLUEs and BLUPs.

With MMM, the estimates of fixed effects and the prediction of combination abilities were obtained in a manner similar to that presented by Bernardo (1995), proposed for partial diallel crossings.

The considered linear model was given by:

$$y = X\beta + Z_1g + Z_2s + e \quad (\text{Equation 1})$$

where  $y$  is the observation vector;  $\beta$  is the vector of fixed effects (general means, and locations);  $g$  is the vector of GCA effects;  $s$  is the vector of SCA effects;  $e$  is the residue vector, and  $X$ ,  $Z_1$  and  $Z_2$  are the incidence matrices of effects  $\beta$ ,  $g$  and  $s$ , respectively.

The joint solution for fixed and random effects was obtained by the equation system, according to Henderson (1984):

$$\begin{bmatrix} \beta^0 \\ \hat{g} \\ \hat{s} \end{bmatrix} = \begin{bmatrix} X'X & X'Z_1 & X'Z_2 \\ Z_1'X & Z_1Z_1 + A_1^{-1}\gamma_1 & Z_1'Z_2 \\ Z_2'X & Z_2'Z_1 & Z_2'Z_2 + A_2^{-1}\gamma_2 \end{bmatrix}^{-1} \begin{bmatrix} X'y \\ Z_1'y \\ Z_2'y \end{bmatrix} \quad (\text{Equation 2})$$

$$\gamma_1 = \frac{\sigma_e^2}{\sigma_{GCA}^2} \quad \gamma_2 = \frac{\sigma_e^2}{\sigma_{SCA}^2}$$

where  $A_j$  is the additive genetic similarity matrix between the hybrids, where the elements in the diagonal were considered equal to one unit. The elements out of the diagonal were completed using the expression  $s_{ij} = 1 - d_{ij}$ , where  $d_{ij}$  is Roger's modified distance (RMD) value.  $A_2$  is the genetic

dominance matrix between the crossings, where the elements out of the diagonal equal to the values defined as the dominance coefficient, as suggested by Henderson (1984) and Van Vleck (1993).

According to Reif et al. (2005), RMD has low residual sum of squares for the genetic distances in comparison to Roger's distance, which is linearly correlated with the coancestry coefficient (Malecot, 1948). For this reason, this study proposed the use of RMD to analyze the parenthood relationship between the hybrids; moreover, this dissimilarity expression is indicated for microsatellite markers and heterosis prediction (Reif et al., 2005). Similar methodology was applied by Bauer et al. (2006).

Estimates of residual variance, additive variance and dominance components were obtained with the restricted maximum likelihood (REML) method, using an iteration process based on the algorithm EM, or expectancy maximization. In this case BLUPs were obtained, since the parametric values of the variables are substituted by their estimates (Henderson, 1984). Also, initial values of variance components were considered as those obtained from the random method by OLS. All analyses were performed using the SAS® PROC IML program. The program to calculate BLUPs was adapted from Reis et al. (2005).

Heritability ( $h^2$ ) was estimated based on the means of the hybrids, using the expression:

$$\hat{h}^2 = \frac{\frac{1}{4}\sigma_A^2 + \frac{1}{4}\sigma_D^2}{\frac{1}{4}\sigma_A^2 + \frac{1}{4}\sigma_D^2 + \frac{\sigma_E^2}{nk}} \quad (\text{Equation 3})$$

where  $n$  and  $k$  correspond to the number of environments and repetitions per environment, respectively.

The predicted breeding values for each hybrid were obtained according to Resende (2002). The accuracy of the methods OLS and MMM was tested using the square root of the heritabilities ( $\hat{h}^2$ ) and a regression between the predicted genetic values by BLUP and their respective phenotypic values.

To estimate the mean components, self pollination was done with the single-cross hybrids. The hybrids  $F_1$  and their respective  $F_2$  generations were evaluated in only three locations. The experiments also were carried out as randomized complete blocks, and the variable evaluated was unhusked corn ear yield. The estimates of mean components  $m+a'$  and  $d$  were obtained using a procedure similar to that presented by Vencovsky (1987), consisting of obtaining the estimates of  $m+a'$  using the contrast  $2F_2 - F_1$ . The values of  $m+a'$  and  $d$  of the three environments were compared only for the data of the three environments where the data were obtained.

Twenty seeds of each single-cross hybrid were sown in 128-cell trays. The trays were placed in a greenhouse, and after the second leaf pair had emerged, DNA was extracted according to Saghai-Marouf et al. (1984). Extracted DNA was quantified in a fluorimeter and diluted to a concentration of 10 ng/mL.

Subsequently, the materials were genotyped with 20 microsatellite primers, 9 of them linked to kernel yield (Amorim et al., 2006). The reactions of simple sequence repeats were prepared according to Balestre et al. (2008).

A binary matrix was constructed from the simple sequence repeat results, and subsequently, the binary data were converted to allele frequencies. The parents' genetic distances were calculated using RMD (Wright, 1978) using the NTSYS-PC 2.1 program (Rohlf, 2000).

Genotype grouping was established by the Tocher's method (Rao, 1952), from the genetic distance data using the GENES 2007.0.0 software (Cruz, 2001). This optimization

method consists of partitioning the genotypes in non-empty and mutually exclusive subgroups through the maximization or minimization of a pre-established similarity measure (Cruz, 2001). Also, the most and least divergent crossings were obtained using the GENES 2007.0.0 software (Cruz, 2001).

The correlation study was performed using Pearson's coefficient ( $r$ ) standardized by Mantel's test (1967). The correlation between the values of mean components with GCA of single-cross hybrids in the three evaluated locations was analyzed.

The data obtained with molecular markers were correlated with SCA of all hybrids in the 15 locations. Also, the genetic distances were correlated with SCA of the intra- and inter-group hybrids, obtained by the Tocher's grouping method.

## RESULTS

In the joint analysis of the three locations where estimates of  $m+a'$  and  $d$  were obtained, the contribution of  $d$  for the unhusked corn ear yield was 68.3% in the single-cross hybrids, on average, which means that there was an endogamy depression of about 34.2% for the character. In contrast, the contribution of loci with additive genetic effects ( $m+a'$ ) was 31.7%, a result similar to that observed in previous studies (Lima, 2000; Souza Sobrinho, 2001). The values of  $m+a'$  varied from 11.24 to 51.56% (Table 3).

**Table 3.** Estimate of  $m+a'$  in percentage and absolute values, general combining ability (GCA) and  $d$ , and the correlation values between the parameters  $m+a'$  and  $d$  with GCA and weight of unhusked ears (WUE) of the single-cross hybrids evaluated in 3 environments, in the agricultural year 2005/2006.

| Hybrid   | $m+a'$              | D                   | GCA                 | % $m+a'$            |
|----------|---------------------|---------------------|---------------------|---------------------|
| 1        | 3.799 <sup>ns</sup> | 7.643**             | -0.165              | 33.2                |
| 2        | 2.771 <sup>ns</sup> | 8.076**             | 0.308               | 20.55               |
| 3        | 3.818 <sup>ns</sup> | 5.161 <sup>ns</sup> | -0.447              | 42.52               |
| 4        | 2.971 <sup>ns</sup> | 7.472**             | -0.539              | 35.88               |
| 5        | 3.990 <sup>ns</sup> | 5.758 <sup>ns</sup> | -0.087              | 40.93               |
| 6        | 3.666 <sup>ns</sup> | 8.553**             | 0.506               | 30.00               |
| 7        | 2.336 <sup>ns</sup> | 8.504**             | 0.225               | 21.25               |
| 8        | 3.325 <sup>ns</sup> | 7.108**             | 0.366               | 31.87               |
| 9        | 1.188 <sup>ns</sup> | 9.378**             | -0.06               | 11.24               |
| 10       | 5.657 <sup>ns</sup> | 5.315 <sup>ns</sup> | -0.133              | 51.56               |
| Variable | $m+a'$              | D                   | GCA                 | WUE                 |
| $m+a'$   | -                   | -                   | -0.15 <sup>ns</sup> | -0.18 <sup>ns</sup> |
| D        | -                   | -                   | 0.47 <sup>ns</sup>  | 0.33**              |

\*\*Significant at 1 and 5% by the  $t$ -test. ns = non-significant.

There was no significant correlation between the estimates of  $m+a'$  and the average of the single-cross hybrids in the three locations (Table 3). In contrast, there was an average and significant correlation between the heterozygote deviation ( $d$ ) and the means of the single-cross hybrids, highlighting that their yield was more affected by heterosis. It is possible to observe in the same table that the loci of the hybrids in heterozygosis with directional dominance ( $d$ ) showed an average correlation, although non-significant, with the effects of GCA ( $r = 0.47$ ); in contrast, there was no significant correlation between GCA and  $m+a'$  ( $r = -0.15$ ).

The average genetic distance of the 10 single-cross hybrids was 0.84. This relatively high distance is due to the use of hybrids of 4 different companies, i.e., different sources.

The single-cross hybrids were placed into five distinct heterotic groups using the Tocher's grouping method (Table 4). Group 1 was formed by two genotypes from the same company (Monsanto) and one genotype of Pioneer, while group 4 was formed only by hybrids of the company Pioneer. The other groups were mixed, consisting of hybrids of the companies Monsanto, Dow Agroscience and Nidera Seeds. The inter-group hybrids from group 1 were the most divergent, with an average distance 0.87.

**Table 4.** Grouping of the 10 single-cross hybrids by Tocher's optimization method, average distance between intra- and inter-groups and partition of the most and least divergent crossings of each hybrid.

| Group formation |                     |                     |              |
|-----------------|---------------------|---------------------|--------------|
| Groups          | Genotypes           |                     |              |
| 1               | 1 (Monsanto)        | 2 (Pioneer)         | 4 (Monsanto) |
| 2               | 3 (Nidera Sementes) | 5 (Dow Agroscience) |              |
| 3               | 6 (Monsanto)        | 8 (Dow Agroscience) |              |
| 4               | 7 (Pioneer)         | 9 (Pioneer)         |              |
| 5               | 10 (Monsanto)       |                     |              |

  

| Inter- and intra-group distance |                  |        |                  |
|---------------------------------|------------------|--------|------------------|
| Groups                          | Average distance | Groups | Average distance |
| 1                               | 0.72             | 2 x 5  | 0.88             |
| 1 x 2                           | 0.88             | 3      | 0.71             |
| 1 x 3                           | 0.91             | 3 x 4  | 0.81             |
| 1 x 4                           | 0.86             | 3 x 5  | 0.77             |
| 1 x 5                           | 0.80             | 4      | 0.84             |
| 2                               | 0.84             | 4 x 5  | 0.71             |
| 2 x 3                           | 0.85             |        |                  |
| 2 x 4                           | 0.85             |        |                  |

  

| Crossing | Most divergent pairs |        |        | Crossing | Least divergent pairs |        |      |
|----------|----------------------|--------|--------|----------|-----------------------|--------|------|
|          | RMD                  | SCA    | WUE    |          | RMD                   | SCA    | WUE  |
| 1 x 6    | 0.95                 | 0.506  | 9036   | 1 x 4    | 0.6519                | -0.877 | 6678 |
| 2 x 8    | 0.92                 | -0.078 | 8217   | 2 x 4    | 0.7246                | 0.454  | 8875 |
| 3 x 9    | 0.92                 | -0.243 | 7783   | 3 x 5    | 0.8367                | -0.970 | 6531 |
| 4 x 9    | 0.95                 | 0.326  | 8497   | 4 x 10   | 0.7583                | -0.696 | 7004 |
| 5 x 1    | 0.89                 | 0.001  | 7929   | 5 x 9    | 0.7071                | 0.027  | 7956 |
| 6 x 4    | 0.89                 | 0.138  | 8388   | 6 x 8    | 0.7071                | 0.024  | 8272 |
| 7 x 3    | 0.91                 | 0.002  | 8007   | 7 x 1    | 0.7746                | -0.126 | 7958 |
| 8 x 1    | 0.94                 | 0.326  | 8655   | 8 x 10   | 0.7416                | 0.114  | 8157 |
| 9 x 4    | 0.95                 | 0.326  | 8497   | 9 x 6    | 0.7416                | 0.055  | 8320 |
| 10 x 3   | 0.91                 | 0.071  | 8060   | 10 x 7   | 0.6708                | -0.026 | 8101 |
| Average  | 0.923                | 138.5  | 8306.9 |          | 0.731                 | -202.1 | 7785 |

RMD = Roger's modified distance; SCA = specific combining ability; WUE = weight of unhusked ear (kg/ha).

BLUPs of SCA and GCA are presented in Table 5. There was an effect of GCA on the averages of the hybrids. This means that the parents contributed differently to the crossings in which they took part, i.e., the additive effects had a significant effect on the crossings.



**Table 5.** Average of unhusked corn ear yield (WUE) in kg/ha, genetic distance (GD) and BLUPs of the general combining ability (GCA) and specific combining ability (SCA) of maize hybrids evaluated in 15 environments in the agricultural year 2005/2006.

| Hybrids | WUE (kg/ha) | GCA    |      |  |
|---------|-------------|--------|------|--|
| 1       | 9013        | -0.028 |      |  |
| 2       | 9054        | 0.286  |      |  |
| 3       | 7923        | -0.083 |      |  |
| 4       | 9187        | -0.141 |      |  |
| 5       | 7475        | -0.147 |      |  |
| 6       | 9656        | 0.264  |      |  |
| 7       | 8870        | 0.025  |      |  |
| 8       | 8187        | -0.004 |      |  |
| 9       | 8225        | 0.006  |      |  |
| 10      | 8839        | -0.057 |      |  |
| Hybrids | WUE (kg/ha) | SCA    | GD   |  |
| 1 x 2   | 8973        | 0.455  | 0.77 |  |
| 1 x 3   | 8243        | 0.245  | 0.87 |  |
| 1 x 4   | 6678        | -0.877 | 0.65 |  |
| 1 x 5   | 7929        | 0.001  | 0.89 |  |
| 1 x 6   | 9036        | 0.506  | 0.95 |  |
| 1 x 7   | 7958        | -0.126 | 0.77 |  |
| 1 x 8   | 8655        | 0.326  | 0.94 |  |
| 1 x 9   | 8264        | 0.242  | 0.92 |  |
| 1 x 10  | 6635        | -0.889 | 0.76 |  |
| 2 x 3   | 8341        | 0.085  | 0.87 |  |
| 2 x 4   | 8875        | 0.454  | 0.72 |  |
| 2 x 5   | 7971        | -0.192 | 0.87 |  |
| 2 x 6   | 8618        | 0.078  | 0.88 |  |
| 2 x 7   | 8738        | 0.277  | 0.85 |  |
| 2 x 8   | 8217        | -0.078 | 0.92 |  |
| 2 x 9   | 8008        | -0.203 | 0.82 |  |
| 2 x 10  | 8597        | 0.212  | 0.85 |  |
| 3 x 4   | 7790        | -0.080 | 0.87 |  |
| 3 x 5   | 6531        | -0.970 | 0.84 |  |
| 3 x 6   | 7907        | -0.197 | 0.84 |  |
| 3 x 7   | 8007        | 0.002  | 0.91 |  |
| 3 x 8   | 8242        | 0.113  | 0.88 |  |
| 3 x 9   | 7783        | -0.243 | 0.92 |  |
| 3 x 10  | 8060        | 0.071  | 0.91 |  |
| 4 x 5   | 7971        | 0.078  | 0.89 |  |
| 4 x 6   | 8388        | 0.138  | 0.89 |  |
| 4 x 7   | 7163        | -0.488 | 0.84 |  |
| 4 x 8   | 7718        | -0.138 | 0.87 |  |
| 4 x 9   | 8497        | 0.326  | 0.95 |  |
| 4 x 10  | 7004        | -0.696 | 0.76 |  |
| 5 x 6   | 8397        | 0.191  | 0.82 |  |
| 5 x 7   | 8137        | 0.199  | 0.89 |  |
| 5 x 8   | 7723        | -0.092 | 0.88 |  |
| 5 x 9   | 7956        | 0.027  | 0.71 |  |
| 5 x 10  | 8177        | 0.196  | 0.85 |  |
| 6 x 7   | 9344        | 0.732  | 0.84 |  |
| 6 x 8   | 8272        | 0.024  | 0.71 |  |
| 6 x 9   | 8320        | 0.055  | 0.74 |  |
| 6 x 10  | 8868        | 0.481  | 0.81 |  |
| 7 x 8   | 7538        | -0.354 | 0.85 |  |
| 7 x 9   | 8019        | -0.081 | 0.84 |  |
| 7 x 10  | 8101        | -0.026 | 0.67 |  |
| 8 x 9   | 7885        | -0.116 | 0.82 |  |
| 8 x 10  | 8157        | 0.114  | 0.74 |  |
| 9 x 10  | 8004        | 0.033  | 0.74 |  |



In relation to SCA estimates, these varied from -0.889 to 0.732 t/ha in the hybrids 1 x 10 and 6 x 7, demonstrating that there was a great influence of non-additive effects in the hybrid combinations and that the parents showed great divergence in these loci, thus yielding negative and positive values for SCA (Table 5).

The correlation of the genetic distances between the inter-group hybrids with SCA was small and significant ( $r = 0.31$ ) for the 15 environments. In contrast, there was no association between intra-group hybrids and their genetic distances ( $r = 0.03$ ). Similar results have been reported in previous studies (Reif et al., 2003; Amorim et al., 2006). Moreover, when more divergent inter-group hybrids were used (groups 1 vs 2, 1 vs 3, 1 vs 4, and 1 vs 5) the correlation between RMD and the specific ability was high and significant ( $r = 0.80$ ) (Table 6).

**Table 6.** Correlation between Roger's modified distance (RMD) and BLUPs of the specific combining ability (SCA) of double hybrids evaluated in the harvest 2005/2006.

| Variables                       | SCA <sup>1</sup>    |
|---------------------------------|---------------------|
| Yield                           | 0.98**              |
| RMD inter-groups                | 0.31 <sup>+</sup>   |
| RMD intra-groups                | 0.03 <sup>ns</sup>  |
| RMD most divergent inter-groups | 0.80**/++           |
| RMD inter-groups 1 and 2        | 0.57**/++           |
| RMD 10 most divergent           | 0.66* <sup>+</sup>  |
| RMD 10 least divergent          | -0.24 <sup>ns</sup> |

\*\*\*/Significant at 1 and 5%, respectively, by the *t*-test. ++/Significant at 1 and 5%, respectively, by Mantel's test with 5000 simulations. ns = non-significant.

The partition of the most divergent groups totaled 21 crossings, i.e., 47.8% of the double hybrids evaluated. However, it was observed that the inter-group hybrids from group 2 (groups 1 vs 2, 2 vs 3, 2 vs 4, and 2 vs 5) also were quite divergent and, when added to the hybrids from group 1, the correlation between RMD and SCA was 0.57 (Table 6). In this case, 68.9% of the double hybrids and 79% of the inter-group double hybrids were evaluated; demonstrating that, in most of the crosses with high divergence degree there is a high and significant correlation between RMD and SCA.

Another result that deserves attention is the partition of the most and the least divergent crosses (Table 4). In this case, for the most divergent crossing of each parent, there was a strong association between RMD and SCA ( $r = 0.66$ ), while there was no significant correlation between the variables for the most similar pairs (Table 6).

Griffing's additive/dominant genetic model in association with the BLUP methodology was highly effective in predicting genotypic values, since regression adjustment between phenotypic values observed in the 15 environments and the predicted genetic values was practically perfect ( $R^2 = 0.97$ ). Heritability estimate obtained by BLUP was high, as well as its accuracy, demonstrating the efficacy of the method in predicting genotypic values with greater confidence than with OLS (Table 7).

There were differences of estimates of variance components whether or not using the information of microsatellite markers in the parenthood matrix (Table 7). However, there was no difference among accuracy estimates. Thus, it can be inferred that, for balanced data, there is no difference in BLUP accuracy with the inclusion of molecular markers.

**Table 7.** Variance components, heritability estimates ( $\hat{h}^2$ ), selective accuracy ( $a_c$ ) obtained through mixed methods (eBLUPs) and ordinary least squares (OLS) and regression adjustment ( $R^2$ ) between the predicted genotypic values and phenotypic values of the diallel of 10 single-cross hybrids in the harvest 2005/2006.

| Methods                | Parameters       |                  |                        |
|------------------------|------------------|------------------|------------------------|
|                        | $\hat{h}^2$      | $a_c$            | ( $R^2$ ) <sup>#</sup> |
| BLUPs + RMD            | 0.947            | 0.973            | 0.97                   |
| BLUPs + I <sup>2</sup> | 0.938            | 0.968            | 0.97                   |
| OLS                    | 0.858            | 0.927            | -                      |
| Variance components    | $\sigma^2_{GCA}$ | $\sigma^2_{SCA}$ | $\sigma^2_e$           |
| BLUPs + RMD            | 0.086            | 0.257            | 0.844                  |
| BLUPs + I <sup>2</sup> | 0.048            | 0.227            | 0.817                  |
| OLS                    | 0.068            | 0.286            | 1.289                  |

RMD = Roger's modified distance; I<sup>2</sup> = identity of parenthood matrix.

<sup>#</sup>Predicted breeding values using the expression  $y_{ij} = \mu + g_i + g_j + s_{ij}$ .

## DISCUSSION

The breeder has to consider several key parameters, as has been mentioned, during the decision-making process of which populations should be used to extract lines.

According to Lima et al. (2000) and Viana (2007), the populations from single-cross hybrids with a greater proportion of fixed favorable alleles would be considered for potential extraction of inbred lines. However, as seen in Table 3, there was no significant correlation between  $m+a'$  and  $d$  with GCA in the three environments. In contrast, there was a trend of heterozygous loci explaining the estimates of GCA, indicating that the general combining abilities of the single-cross hybrids used in this study could have been more affected by the heterozygous loci than by the fixed ones.

The expression of GCA can be written as  $CGC = (p_i - p_j)[a + (1-2t)d]$ , where the first part of the expression refers to parent divergence and the second one to the effect of allele substitution. In this study, on average,  $m+a'$  had little contribution to the hybrid means (31.7%). In contrast, 68.3% of the hybrid means was due to the heterozygous loci, which also contributed to GCA. Thus, if there is little difference in the allele frequency of the additive effect loci ( $a$ ), these could have almost nil contribution to the effects of GCA.

Therefore, the hybrids that could be considered for potential extraction of lines (3, 5 and 10) did not show high GCA values for the three environments in which they were evaluated; quite on the contrary, they had negative estimates for this parameter (Table 3). Also, it can be noted that these hybrids did not show the highest estimates of SCA, not completely exploiting the hybrid vigor (Table 5).

Therefore, it can be inferred that the estimate  $m+a'$  should not be used alone as a parameter for choosing populations, as suggested by some studies (Lima et al., 2000; Bison et al., 2003), since the breeder can be at risk of choosing the process with highly productive inbred lines that do not yield promising hybrids. This is due to the weak association between performance of the lines *per se* and of their respective hybrids, i.e., the small correlation between the means of the progenitors and that of the hybrid in question (Troyer, 1994). The importance of fixed favorable alleles in high frequency in a maize population is evident; how-

ever, when the populations to be used for the extraction of lines originate from single-cross hybrids, and provided the objective of the breeder is to obtain interpopulation hybrids, which is common in this type of germplasm, this parameter becomes secondary. In this case, hybrids with greater SCA should be preferred due to the high correlation with performance of the crossings (Table 6).

It should be noted that the estimates of  $m+a'$  were obtained in some of the environments, which does not allow one to make inferences about the results obtained in all fifteen environments. However, in most studies found in the literature, these estimates were obtained in three locations, which allows this study to retain the information as a parameter for selecting potential populations despite its evaluation in only three of the fifteen environments (Lima et al., 2000; Bison et al., 2003; Viana, 2007).

The results obtained with microsatellite markers demonstrate that they can be used as a tool for selecting populations to effectively exploit heterosis. In this experiment, there was a high and significant correlation between SCA and the genetic distance of the inter-group hybrids with high divergence degree (Table 6). These data are in agreement with previous studies, when high correlations between SCA and RMD were observed for inter-group hybrids and low correlations in intra-group hybrids (Drinic et al., 2002; Reif et al., 2003; Amorim et al., 2006).

High divergence values were observed in the inter-group hybrids in this experiment (0.86) in contrast with intra-group values (0.79); moreover, there was great variability of SCA for the inter-group hybrids, thus, concurring with the increase in the correlation of RMD with SCA. Also, Tocher's optimization method can be considered as promising for allocation of hybrids in distinct heterotic groups, especially for making the interpretation of the results easier and for providing the breeder with greater ease in direct crossings of inter-group hybrids.

The most diverging pairs of hybrids resulted, on average, in greater estimates of SCA in relation to the least divergent pairs (Table 4). Moreover, they showed a high correlation between their genetic distance and SCA (Table 6), thus demonstrating that, crossing the most divergent pairs, on average, coincides with greater estimates of SCA, which is an alternative to direct crossings.

The components of residual variance and additive and dominant variances obtained in this study using the BLUP and OLS methods were very similar; however, it can be observed that BLUP yielded greater reduction of residual variance and a small decrease in the variance of dominance, but with a small increase in the additive variance. This sharp decrease in residual variance and small change in the other ones concurs with the increase in heritability estimate and accuracy estimate (Table 7). This result confirms the properties of least variance and prediction error of BLUP in relation to OLS even when the data are balanced, as has been observed in other studies (Panter and Allen, 1995a).

The heritability estimate obtained in this study was higher than that obtained by Bernardo (1994, 1995) in partial diallel:  $\hat{h}_2 = 0.81$  and 0.88. Thus, it is possible to predict success in the selection of progenitors to obtain interpopulation hybrids.

Greater accuracy was also obtained by the method BLUP in relation to the OLS method, giving the breeder greater confidence in the selection of potential progenitors for the production of interpopulation hybrids (Table 7). According to André (1999), BLUPs of SCA have smaller average error and bias than the SCA obtained by OLS; therefore, SCA predictions with BLUP are better than those with OLS.

In this study, crossings 6 x 7 and 1 x 6 gave the best double hybrids (Table 5) and, consequently, the highest SCA (0.732 and 0/506 t/ha). Since these progenitors (1, 6 and 7) have

high averages and due to the high heritability shown, it can be inferred that the lines originating from these pairs of progenitors when crossed will have, on average, these same values of SCA, allowing the selection of hybrids superior to their parents.

Due to the high correlation between the predicted genetic values with the phenotypic values and high accuracy, it can be inferred that BLUP showed smaller prediction standard error, a finding also reported by Panter and Allen (1995a). These authors also observed that when using parenthood information, there is a small improvement in the estimate values (Panter and Allen, 1995b). From the observations made in this study, there was a difference in the estimates of variance components between BLUP and the microsatellite information or without the latter (Table 7). However, there were no differences in the estimates of accuracy and heritability, with a slight advantage for the model with parenthood information with microsatellite data, inferring that in conditions of balanced data there is no advantage in using parenthood information. Despite this fact, microsatellite markers can be used as an alternative for parenthood information in the prediction of untested hybrids, especially for better prediction of heterosis, as observed by the correlations between SCA and RMD.

It can be concluded that the estimate of  $m+a'$  should not be used as a parameter to make inferences about the potential of a population for extraction of lines. The combining abilities of the populations should be evaluated previously. Microsatellite markers associated with the techniques used in this study were useful tools in decision-making about which crossing should be made to maximize heterosis, besides obviously determining the populations in groups that effectively exploit the SCA.

The BLUP method was more effective than OLS in selection accuracy and heritability estimate, inferring that, even with balanced data, this method should be preferred for predicting SCA values for the selection of parents to produce interpopulation hybrids. The use of microsatellite markers did not alter selection accuracy, but it could be a useful tool in association with BLUP for predicting untested hybrids.

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