Estimating combining ability in popcorn lines using multivariate analysis

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Aiming to estimate the combining ability in tropical and temperate popcorn (Zea mays L. var. everta Sturt.) lines using multivariate analysis, ten popcorn lines were crossed in a complete diallel without reciprocals and the lines and hybrids were tested in two randomized complete block experiments with three replicates. Data were subjected to univariate and multivariate ANOVA, principal component analysis, and univariate and multivariate diallel analysis. For multivariate diallel analysis, variables were divided into group I (grain yield, mean weight of ears with grains, popping expansion, mean number of ears per plant, and final stand) and group II (days to silking, plant height, first ear height, and lodged or broken plants). The P2 line had positive values for agronomic traits related to yield and popping expansion for group I, whereas the P4 line had fewer days to silking and lodged or broken plants for group II. Regarding the hybrids, P2 × P7 exhibited favorable values for most of the analyzed variables and had potential for recommendation. The multivariate diallel analysis can be useful in popcorn genetic improvement programs, particularly when directed toward the best cross combinations, where the objective is to simultaneously obtain genetic gains in multiple traits.

Key words: Diallel analysis, genotype × environment interaction, principal components, Zea mays var. everta.

INTRODUCTION

Popcorn (Zea mays L. everta Sturt.) is a popular snack food among consumers in Brazil where its consumption has increased over the years, making popcorn economically attractive for farmers across the country (Mendes de Paula et al., 2010; Silva et al., 2011; Moterle et al., 2012). However, official data from Brazilian government institutions have revealed that popcorn production remains limited when compared to the crop’s potential market. The main limiting constraint is obtaining cultivars with multiple favorable agronomic traits (Arnhold et al., 2009; Moterle et al., 2012; Ribeiro et al., 2012; Silva et al., 2013). There are 48 registered cultivars in Brazil (MAPA, 2013), and most of them belong to popcorn industry represented by packing companies that keep the seed stock available only for a few partner producers.

Establishing national popcorn breeding programs aimed to provide varieties and hybrids with high agronomic potential and adapted to Brazilian conditions, diminishing dependence on foreign cultivars is a challenge (Moterle et al., 2012; Carvalho et al., 2013) especially due to the environmental diversity conditions, involving planting areas in tropical and temperate areas.

Obtaining hybrids with superior performance depends on choosing parents that possess a good genetic complementation of favorable agronomic traits (Hallauer and Miranda Filho, 1995). Among the methods that help breeders choose parents, diallel crosses have been widely used by plant breeders to obtain useful information, such as to estimate the general and specific combining ability from a set of parents; to classify heterotic groups; to estimate heterosis, and to develop hybrids (Melani and Carena, 2005; Cai et al., 2012). However, most data from diallel crosses experiments is analyzed by univariate techniques and, in general, traits evaluated individually may not be appropriate because correlations between different traits are not considered in that type of approach (Jung et al., 2007; Benin et al., 2009).

Using multivariate diallel analysis is an alternative because it is primarily used to combine various data obtained from the experimental unit, which facilitates the selection of promising parents and hybrids (Lin and Geng, 1986; Yan and Hunt, 2002; Ledo et al., 2003). Despite of the advantage in using the multivariate approach, few studies with diallel design have been published using this analytical technique (Ledo et al., 2003; de la Vega and Chapman, 2006; Jung et al., 2007; Benin et al., 2009;
Nascimento et al., 2010). For popcorn, this strategy may be an important choice, for instance, because two of the main traits of economic interest, popping expansion and yield, are negatively correlated (Dofing et al., 1991). Also, a set of other agronomic traits should be considered at the same time in popcorn research, such as the resistance to lodging, diseases, and pests (Zinsly and Machado, 1987).

Aiming to obtain new popcorn genotypes suitable for tropical and temperate areas, this study focused in estimating the general and specific combining abilities for two groups of traits using diallel crosses and multivariate analysis.

**MATERIALS AND METHODS**

Ten inbred lines derived from genotypes developed specifically for tropical and/or temperate region (Table 1) were used as parent lines in diallel crosses without reciprocals. The parents used are part of the group of popcorn lines from the Universidade Estadual do Norte Fluminense Darcy Ribeiro and Universidade Estadual de Maringá, Brazil, through a partnership between institutions.

The parents and hybrids were tested in two field experiments conducted in the municipalities of Campos dos Goytacazes (21º44'47" S, 41º18'24" W; 11 m a.s.l.) and Itaocara (21º39'12" S, 42º04'36" W; 60 m a.s.l.), Rio de Janeiro, Brazil. Treatments were arranged in a randomized complete block design with three replicates. Each plot consisted of two 5.0 m rows, spaced 0.9 m apart, and plants were spaced 0.2 m apart, with a usable area of 9.0 m².

The following traits were evaluated: mean plant height (PH, m) obtained from the soil level at the top of 10 competitive plants in each plot; first ear mean height (EH, m) expressed by soil level measurement at the first ear insertion in the same ten plants per plot; proportion of lodged and broken plants (LB); final stand (FS); mean area of 9.0 m².

The number of ears per plant (NE); number of days to silking (SILK); number of days to silking in the same ten plants per plot; proportion of lodged and broken plants (LB); final stand (FS); mean area of 9.0 m². The height (PH, m) obtained from the soil level at the top of 10 competitive plants in each plot; first ear mean height (EH, m) expressed by soil level measurement at the first ear insertion in the same ten plants per plot; proportion of lodged and broken plants (LB); final stand (FS); mean area of 9.0 m².

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**Table 1. Genealogy of the popcorn lines.**

| Inbred lines | Description of the population from which the inbred lines were obtained |
|--------------|-----------------------------------------------------------------------|
| L₁           | From the three-way hybrid ‘Zélia’, released by Pioneer Seeds; it consists of temperate and tropical inbred lines. |
| L₂           | From the composite with white grains ‘CMS-42’, released by Embrapa-Maize and Sorghum; it consists of tropical inbred lines. |
| L₃           | From the composite with white grains ‘CMS-42’, released by Embrapa-Maize and Sorghum; it consists of tropical inbred lines. |
| L₄           | From South American races in tropical regions. |
| L₅           | From the three-way hybrid ‘Zaeli’ which consists of temperate inbred lines. |
| L₆           | From South American races in temperate regions. |
| L₇           | From the modified one-way hybrid ‘IAC112’, adapted to tropical regions; it consists of inbred lines from the open pollinated variety ‘South American Mushroom’ crossed with inbred lines from the South American intervarietal hybrid ‘Guarani’ × ‘Amarela’. |

Data were analyzed considering ANOVA by environment, and the homogeneity of residual variances (or residual mean squares, RMSs) was subsequently tested using the ratio between the highest and lowest RMS. The model adopted for joint analysis was $Y_{ijk} = \mu + R/E_{jk} + G_i + E_j + GE_{ij} + \xi_{ijk}$, where $Y_{ijk}$ is the mean phenotypic value of the plot, $\mu$ is the mean, $R/E_{jk}$ is the effect of the $k^{th}$ replicate on the $j^{th}$ environment, $G_i$ is the fixed effect of the $i^{th}$ genotype, $E_j$ is the effect of the $j^{th}$ environment, $GE_{ij}$ is the effect of the interaction between the $i^{th}$ genotype and the $j^{th}$ environment, and $\xi_{ijk}$ is experimental error. Data were analyzed for homogeneity of variance (Bartlett’s test) and normality (Lilliefors). The partition of the complex interaction was performed for the traits in environments using the algorithm proposed by Cruz and Castoldi (1991).

For joint diallel analysis, model 2 proposed by Griffing (1956) was adopted, which considers parents and hybrids. Regarding multivariate diallel analysis, traits evaluated were divided into two groups. In the first group, the GY, EW, NE, PE, and FS were measured, and in the second group, SILK, PH, EH, and LB were measured. Subdividing variables into groups is considered to be an important strategy for popcorn genetic breeding. The first group in the present study included primary traits that are important for crop genetic improvement (including PE and GY), and the second group included traits that must be minimized such as earliness, plant height, and plant lodging or breaking to better integrate into crop succession schemes and as a result of greater susceptibility to lodging, diseases and pests compared with normal corn (Zinsly and Machado, 1987). The diallel analyses were performed using univariate analysis, considering the complete diallel method without reciprocals according to Griffing (1956) while estimating the effects of the general combining ability (GCA) and the specific combining ability (SCA).

Subsequently, principal components (PCs) were estimated based on the average standard of genotypes. After determining the number of PCs that involved a minimum 80% of the available range, scores for each PC and their relative importance were estimated. Next, we performed a diallel analysis of the component scores and obtained the effects of GCA and SCA for each component according to method described by Ledo et al. (2003). The
RESULTS AND DISCUSSION

Significant differences were observed (F test) for all of the traits, considering the genotype variation (lines + hybrids) and environment, which indicates variation among genotypes and environments tested (Table 2). Regarding the genotype × environment interaction (GxE), NE, PE, and FS were not significant (P > 0.05), which indicate the lack of differentiation in cultivars as a result of environmental variation. For the other traits, because there was significance for the GxE, we examined whether the type of interaction was simple or complex.

According to the algorithm proposed by Cruz and Castoldi (1991), simple interaction was predominant between all environments for all traits. These results indicate that the relative genotype positions remained unaltered within the set of environments evaluated, and thus, parents and hybrids can be jointly indicated between the environments.

The sum of squares of genotypes partition into the GCA and SCA sum of squares was significant for most traits, except for NE and SILK, regarding GCA (Table 2). These results indicate that additive and non-additive effects were involved in the genetic control of most traits. Moterle et al. (2012) evaluated eight temperate and tropical popcorn lines and found that PH and GY were significant for GCA and SCA, whereas AE was only significant for GCA, and PE did not exhibit variability resulting from additive and non-additive effects. Nevertheless, Freitas Júnior et al. (2006) also evaluated popcorn varieties but found significant additive and non-additive effects for GY and PE. For the GCA and SCA interaction with environment, there was no significance for NE, PE, and FS.

The multivariate diallel ANOVA of the model by Griffing (1956) revealed significant effects of the general combining ability on traits of the group I (GY, GW, NE, PE, and FS) and group II (SILK, PH, EH, and LB), which indicates that the parents have complementary genes and that traits can be considered altogether in diallel analysis (Ledo et al., 2003; Silva et al., 2008; Benin et al., 2009) (Table 3).

The first two components explained 86.78% (PC1: 66.79% and PC2: 19.99%) and 81.74% (PC1: 55.54% and PC2: 26.20%) of the total variation available among the means of diallel crossings for groups I and II, respectively, which indicates satisfactory interpretation of the variability manifested among genotypes (Table 2). Considering genotypes classified in group I, large and positive association was observed between principal component I (PC1) and GY, EH, NE, and PE, with values
of 0.95, 0.96, 0.87, and 0.85, respectively, but principal component 2 (PC2) had large and positive association only with PE (0.98). These results are consistent with other studies reporting the lack of an association or even a negative association between popping expansion and productivity-related traits (Dofing et al., 1991; Ziegler and Ashman, 1994).

For group II, large and positive association was registered for PC1 with PH (0.93) and EH (0.94), and a negative association with SILK (-0.66), which indicates that for the set of hybrid combinations evaluated, there are fewer chances of obtaining genetic progress in selecting plants that combine small plants and earliness (Table 2). The opportunity to obtain progenies with earlier maturation remains in identifying the most positive values of combining ability for PC1. However, progress in decreasing plant height is most likely not possible. Nevertheless, means for PH and EH were 2.03 m (2.69-1.89 m) and 1.20 m (1.74-0.90 m), respectively, and are considered suitable height values for crop mechanization, although PC1 was not significant for LB.

Regarding PC2 in group II, LB, and SILK had a positive correlation, whereas there was no association for the other traits, which indicates that progenies with higher negative combining ability values for PC2 should be selected to decrease plant height and the number of lodged and broken plants. Thus, positive and negative selection for CP1 and CP2, respectively, for group II is a good strategy for obtaining reduced SILK and LB in segregating populations of the diallel.

Choosing parents for forming segregating populations is crucial for successful breeding programs, in which the combining ability with the presence of complementary genes is largely responsible for success.

By estimates of the effects of GCA ($\hat{g}_i$), parents $P_2$, $P_3$, and $P_2$ expressed the most positive effects of $\hat{g}_i$ for PC1 of group I. For PC2 of group I, there were positive $\hat{g}_i$ values for parents $P_2$, $P_8$, $P_9$, and $P_{10}$ (Figure 1). Low GCA values indicate that the mean of hybrids in which line $i$ participates does not differ from the overall diallel mean. In contrast, high values –positive or negative– indicate that line $i$ is much better or worse than the other lines included in the diallel in relation to the mean of their hybrids (Sprague and Tatum, 1942). In the present study, only parent $P_2$ had positive association values for the two components; this outcome occurred as a result of the positive association of traits related to PC1 and popping expansion for PC2. For group II, the highest positive values for PC1 were found for parents $P_2$ and $P_4$, whereas the highest negative values for CP2 were found for $P_1$ and $P_4$. Thus, reducing days to silking and number of plants lodged or broken only could be associated with the $P_4$ line.

Group I: GY: grain yield (kg ha$^{-1}$), EW: mean weight of ears with grains (kg ha$^{-1}$), NE: mean number of ears per plant, PE: popping expansion (mL g$^{-1}$), FS: final stand.

Group II: SILK: number of days to silking, PH: mean plant height (m), EH: first ear mean height (m), LB: proportion of lodged and broken plants.

Figure 1. Estimates of the general combining ability (GCA) for 10 popcorn lines evaluated in two environments and two first principal components (PC1 and PC2).
The SCA effect is interpreted as a hybrid’s deviation compared with what would be expected based on the GCA of the hybrid’s parents. Thus, $s_{ij}$ values close to zero indicate that hybrids behave as expected based on GCA values, whereas high absolute $s_{ij}$ values indicate better or worse performance than expected (Sprague and Tatum, 1942). Our results indicated that the following hybrids had the highest positive $s_{ij}$ values for PC1 and PC2 of group I: $P_1 \times P_3$, $P_1 \times P_5$, $P_1 \times P_8$, $P_1 \times P_{10}$, $P_2 \times P_3$, $P_2 \times P_6$, $P_3 \times P_7$, $P_3 \times P_7$, $P_6 \times P_9$, and $P_7 \times P_8$ (Figure 2). However, only the $P_7 \times P_8$ had superior values of the GCA mean effect for CP1 and CP2, for traits related to yield and popping expansion. Thus, only the $P_3 \times P_4$ combination is promising because it has at least one superior parent for the mean effect of GCA compared with PC1 and PC2.

For group II, the hybrids $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_8$, $P_1 \times P_{10}$, $P_2 \times P_6$, $P_2 \times P_7$, $P_3 \times P_7$, $P_3 \times P_{10}$, $P_4 \times P_6$, $P_4 \times P_7$, and $P_7 \times P_8$ had the highest positive values for PC1. However, only the hybrids $P_2 \times P_7$, $P_1 \times P_4$, $P_2 \times P_6$, $P_2 \times P_9$, $P_2 \times P_{10}$, $P_4 \times P_6$, $P_4 \times P_7$, $P_5 \times P_{10}$, and $P_7 \times P_8$ are considered promising because they contain at least one parent with a high GCA estimate for PC1. However, only the hybrids $P_2 \times P_7$, $P_1 \times P_4$, $P_2 \times P_6$, $P_2 \times P_9$, $P_2 \times P_{10}$, $P_4 \times P_6$, $P_4 \times P_7$, and $P_7 \times P_8$ are promising with respect to reduced $s_{ij}$ values, whereas high absolute $s_{ij}$ values indicate that hybrids behave as expected based on GCA of the hybrid's parents. Thus, only $P_2 \times P_7$ combination is promising because it has at least one superior parent for the mean effect of GCA compared with PC1 and PC2.

**Figure 2.** Estimates of the specific combining ability (SCA) for 10 popcorn lines evaluated in two environments and two first principal components (PC1 and PC2).

**CONCLUSION**

The hybrids $P_2 \times P_7$ exhibited favorable values for most of the analyzed variables and had potential for recommendation. The multivariate diallel analysis can be useful in popcorn genetic improvement programs.

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