Original Article

GENETIC DIVERGENCE AMONG MAIZE HYBRIDS IN CERRADO-PANTANAL ECOTONE

DIVERGÊNCIA GENÉTICA ENTRE HÍBRIDOS DE MILHO NO ECÓTONO CERRADO-PANTANAL

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ABSTRACT: In maize breeding programs can arise difficulties in relation to combination capacity studies for determination of heterotic groups, which are highly correlated with genetic divergence among the parents. The aim of this study was to estimate the genetic divergence measured for nine quantitative morphological traits in eleven single-cross hybrids of maize cultivated in the Cerrado-Pantanal ecotone. The experiment was conducted at Universidade Estadual de Mato Grosso do Sul – University Unit of Aquidauana. The experimental design was a randomized blocks with four replications. At harvest time, it were measured the following traits: plant height, ear insertion height, ear length, ear diameter, stem diameter, number of kernels per rows, number of rows per ear, weight of hundred grains and grain yield. In the application of hybrids cluster technique was adopted the Mahalanobis's generalized distance as dissimilarity measure, and for establishment of similar groups was applied the Tocher's method. The results indicated the existence of genetic variability among tested hybrids. The greatest genetic divergence was observed among the pairs MAXIMUS and XB6012, implying in heterotic gains. Crossings of lines extracted from hybrids 2B587HX and XB6012 with lines obtained from the other hybrids provide greater heterosis. The traits grain yield and ear insertion height were those who more and less contributed, respectively, for genetic divergence among hybrids.

KEYWORDS: Dissimilarity. Hybrid development. Genetic breeding. *Zea mays* L.

INTRODUCTION

Knowledge of the genetic diversity in available germplasms is fundamental for the optimal designing of breeding programs, the efficiency of which can be increased if superior crossings are pre-established. In the last five decades, a big number of maize (*Zea mays* L.) hybrids have been developed from genotypes with a restricted genetic base. This causes the risk of loss of genetic diversity and restricts the possibility of crossings among genetically divergent genotypes. Knowledge of the genetic relationships among breeding materials could help to prevent the great risk of increasing uniformity in the elite germplasm and could ensure long-term selection gains (OLIVEIRA et al. 2004).

The adequate choice of genotypes as sources of lines extraction can determine the success or economic return of a breeding program to develop maize hybrids. The trends are in order to use F2 populations originated from crossings of single hybrids, synthetic elite lineages and even commercial hybrids, as it the inappropriate choice of population will result in losses of time and resources destined for breeding (OLIVEIRA et al. 2004).

Front of numerous genitors frequently used in the maize breeding programs, hundreds of new lines are generated each year. Thus, the difficulty arises in relation to combination capacity studies for determination of heterotic groups, which are highly correlated with genetic divergence between the genitors. The determination of genetic dissimilarity among genitors contributes in the extraction of endogamic lines with considerable specific combination ability and thus allows exploiting the phenomenon of heterosis in hybrid crossings, increasing the chances to obtain segregating generations (PEJIC et al. 1998). These estimates are of great use in breeding programs and in the choice of genitors for mapping of genes.

In the genetic diversity study of a population, quantitative morphological traits are subjected to multivariate biometric techniques, allowing unify multiple information of a trait set (TORES et al., 2015). Given the above, the aim of this study was to estimate the genetic divergence measured for nine quantitative morphological traits in eleven single-cross hybrids of maize cultivated in the Cerrado-Pantanal ecotone.

MATERIAL AND METHODS

The experiment was installed at Universidade Estadual de Mato Grosso do Sul – Aquidauana Unit (UEMS/UUA), in the municipality of Aquidauana (MS), located in the Cerrado/Pantanal ecotone, comprising the coordinates 20º27'S and 55º40'W, with an average elevation of 170 m.

The soil was classified as Ultisol sandy loam texture, with the following chemical features in the layer 0 – 0.20 m: pH (H_2O) = 6.2; Al exchangeable ($cmol_c$ dm^{-3}) = 0.0; Ca+Mg ($cmol_c$ dm^{-3}) = 4.31; P (mg dm^{-3}) = 41.3; K ($cmol_c$ dm^{-3}) = 0.2; Organic matter (g dm^{-3}) = 19.7; V (%) = 45.0; m (%) = 0.0; Sum of bases ($cmol_c$ dm^{-3}) = 2.3; cation exchange capacity (or CEC) ($cmol_c$ dm^{-3}) = 5.1. The climate according to the Köppen classification is Aw with humid and hot summers and dry winters (TEODORO et al, 2015). The cumulative rainfall during the experiment 450 mm and maximum and minimum temperatures of 91.4 and 66.2 º F, respectively.

The experimental design was a randomized block with four replications. The area was split into four blocks with a total of seventy-four plots, each with 15.75 m² (3.15 x 5.0 m) and two meters spacing between blocks. The treatments consisted of eleven single-cross hybrids of maize (Table 1).

Table 1. Features of eleven single-cross hybrids of maize grown in Aquidauana, MS, Brazil, 2012.

Commercial Name	Business	Cycle
AG 9010	Agroceres	Very early
FÓRMULA TL	Syngenta	Very early
MAXIMUS	Syngenta	Early
P30F53	Pioneer	Early
P3340	Pioneer	Early
STATUS TL	Syngenta	Early
XB 6010	Semeali	Very early
XB 6012	Semeali	Early
2B587	Dow Agrosciences	Early
30A30	Agromen	Hyper early
30A37	Agromen	Very early

In preparation of the experimental area was used glyphosate herbicide for desiccation, in doses of 1 kg ha^{-1}. After drying and complete death of the plants, the furrows were opened using a seeder, performing manually the seeding under no-tillage system, on 2012-02, ten days after desiccation, which were distributed six seeds per meter in the rows, spaced 0.45 m. With about fifteen days after emergence of the plants were carried thinning keeping four plants per linear meter for establishment of 88,889 plants ha^{-1}.

The fertilization at sowing time consisted of 300 kg ha^{-1} formulation 4-20-20. In topdressing nitrogen was used urea as a source of N, applying 100 kg ha^{-1} on the surface when the plants had five to eight fully expanded leaves. It was made the control of fall armyworm (*Spodoptera frugiperda* Smith) 30 days after sowing using Triflumuron insecticide at a rate of 75 mL ha^{-1}. For control of pre-emergence weeds, it was used 1,125 g ha^{-1} of active ingredient atrazine and subsequently weeding.

At harvest time, when the grain had about 18% humidity, measurements were made of plant height and ear insertion height, being carried out with a ruler graduated in five plants per plot. In each plot it were randomly harvested five ears, which it were numbered according to the plants evaluated for determining ear diameter, ear length, stem diameter, number of kernels per rows and number of rows per ear.

The harvesting and threshing of maize ears were performed manually in three central rows of five meters length, according to the cycle of each hybrid. We determined the weight of hundred grains by manual counting, weighing and correction of moisture to 13%. We estimated the grain yield by extrapolation of production harvested in the area useful for one hectare, correcting for a 13% wet basis.

In the application of clustering technique of hybrids, we adopted the Mahalanobis's generalized distance (1930) as dissimilarity measure, considering the degree of dependence among the nine traits evaluated. In relation to the establishment of similar groups was applied the hierarchical agglomerative optimization method proposed by Tocher (RAO, 1952), whose calculations were equally grounded in Mahalanobis's generalized distance (D²) and Unweighted Pair Group Method

with Arithmetic Mean (UPGMA), proposed by Sneath and Sokal (1973). We applied the criterion of Singh (1981) to quantify the contribution of each trait in the dissimilarity among hybrids. All analyzes were carried using the statistical software SAS (SAS INSTITUTE, 1999).

RESULTS AND DISCUSSION

There was genetic variability among corn hybrids for all traits, confirming the results obtained in other studies conducted in this region (TORRES et al., 2013; TEODORO et al, 2014a, b) The dissimilarity measures estimated from the Mahalanobis's distance matrix (D²), based on nine quantitative traits are delineated in Table 2. The magnitude of the observed distances (0.03<D²<4.20) evidences the existence of large genetic divergence among the hybrids, allowing the choice of contrasting genitors for the extraction of endogamic lines. Simon et al. (2012) obtained similar results, which evaluating nineteen single-cross hybrids of maize in the Brazilian Cerrado found high genetic variability.

Table 2. Estimates of genetic dissimilarity of eleven maize hybrids from the Mahalanobis's distance matrix (D²) based on nine quantitative traits.

Hybrid	30A37HX	AG9010	MAXIMUS	P30F53	FÓRMULA	P3340	XB6010	STATUS	30A30HX	2B587HX
XB6012	2.19	3.31	4.20	2.17	1.77	2.11	2.16	2.46	1.48	4.06
30A37HX		1.12	1.75	2.03	1.65	1.72	2.14	1.37	1.01	1.90
AG9010			0.87	1.88	2.80	2.44	2.18	3.06	1.89	2.60
MAXIMUS				2.81	3.24	2.73	2.97	3.28	1.58	1.87
P30F53					0.80	0.52	0.03	3.12	2.06	1.98
FÓRMULA						0.19	0.79	1.83	1.16	1.60
P3340							0.53	2.27	1.16	1.30
XB6010								3.20	2.13	2.00
STATUS									1.38	1.76
30A30HX										1.96

Cluster analysis aims to gather the genitors (or any other type of sample unit) through classification criterion in several groups, so that there is homogeneity within the group and heterogeneity between groups. This way, in a dendrogram, large level change indicates the union of heterogeneous hybrids. Using 60% similarity as a criterion for defining groups based on the UPGMA method from nine quantitative traits evaluated, it were formed five groups (Figure 1). Four hybrids are containing in the group I (P30F53, XB6010, FÓRMULA and P3340), one in the group II (2B587HX), three in the group III (30A37HX, 30A30HX and STATUS), one in the group IV (XB6012) and two in the group V (AG9010 and MAXIMUS).
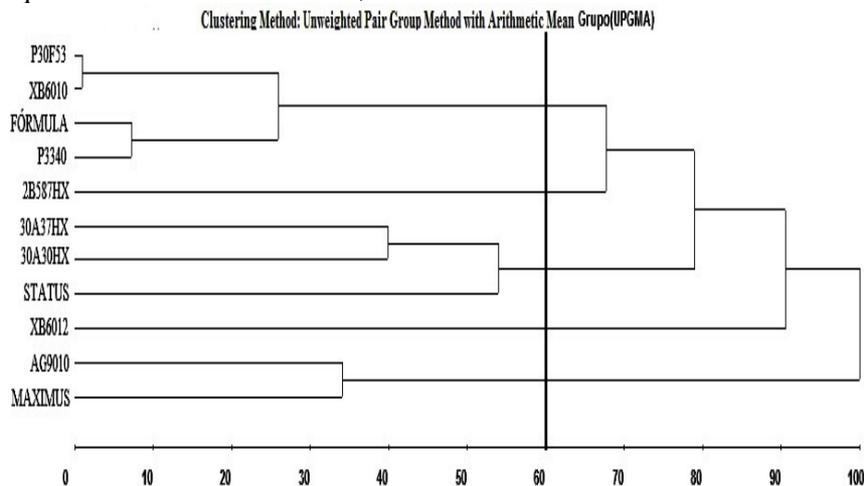

Figure 1. Illustrative dendrodram of dissimilarity pattern established by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for eleven maize hybrids through nine morphological traits.

Cluster analysis by Tocher's optimization method provided the formation of five distinct groups of hybrids (Table 3). Tocher's optimization method (RAO, 1952) allows establishing mutually exclusive clusters of objects according to an objective function that adopts the criterion of optimization, which minimizes the average distance intra-cluster and maximizes the average distance inter-cluster. The dissimilarity among hybrids established by UPGMA method based on the arithmetical mean of dissimilarity measures is a tool that associated with Tocher's optimization method provides greater safety in discriminating different genotypes to genetic divergence. Both cluster methods similarly have hybrids allocated in groups with greater genetic similarity, a fact that has also been observed by other authors (SMITH et al. 1997; OLIVEIRA et al., 2010; ROTILI et al. 2012; SIMON et al. 2012; PREISIGKE et al. 2013 and GOMES JÚNIOR et al. 2014).

Table 3. Cluster analysis of maize hybrids by Tocher's method, based on the dissimilarity expressed by Mahalanobis's generalized distance.

Groups	Hybrids
I	P30F53, XB6010, P3340 and FÓRMULA
II	AG9010, MAXIMUS and 30A37
III	STATUS and 30A30HX
IV	2B587HX
V	XB6012

The most divergent hybrids according to Mahalanobis's distance (D^2=4.20) were XB6012 and MAXIMUS, which were also allocated into different groups (groups V and II) by Tocher's method. These results suggest the possibility of lineages extracted from hybrids of these groups had distinct genetic divergence and consequently heterosis in hybrid combinations. Another point that reinforces this hypothesis is that these hybrids constituted the groups with the highest amplitude between them (3:23) also obtained by Tocher's optimization method (Table 4).

Table 4. Intergroup estimated average distances by Tocher's optimization method, involving eleven maize hybrids.

Groups	II	III	IV	V
I	2.38	2.12	1.72	2.05
II		2.03	2.12	4.06
III			1.86	1.97
IV				3.23

Groups formed by just one individual suggest that these individuals are more divergent compared to the other. This facilitates the projection of breeding works, finding distinct genotypes for future crosses. In this sense, there is interest in obtaining distinct populations in maize breeding program, increasing the chances of crossings. Therefore, the extraction of lines of hybrids 2B587HX and XB6012 for crosses with lines belonging to other genotypes may result contrasting genitors and, concomitantly, heterotic gains.

In order to reduce efforts and perform the most efficient and economical breeding program is possible to select some of the hybrids that were considered less divergent as is the case of P30F53 and XB6010, which showed D^2=0.03, in addition to belonging to the same group formed by UPGMA and Tocher's optimization methods.

The Singh's method (1981), based on the Mahalanobis's D^2, considers of lesser importance characteristics that express less variability. It is suggested, therefore let it be discarded in this case, the traits number of rows per ear, plant height, ear insertion height and ear length (Table 5), that showed minor contribution to the divergence.

Table 5. Relative contribution of the traits evaluated to genetic divergence by Singh's method (1981), based on Mahalanobis's generalized distance (D^2).

Trait	Percentage value
Ear lenght	1.56
Ear diameter	7.10
Number of rows per ear	0.46

Number of kernels per rows	4.98
Stem diameter	9.75
Ear insertion height	0.11
Plant height	13.68
Weight of hundred grains	18.33
Yield grains	44.12

The traits that most contributed to the genetic divergence among the hybrids were grain yield and weight of hundred grains, corroborating the results obtained by Rotili et al. (2012). According to Hallauer et al. (1988), the great interest in evaluating the relative importance of the traits refers to possibility of discarding of the features that contribute little to the discrimination of the evaluated material, reducing workforce, time and cost spent by experimentation. Moreover, the identification of traits that most contributed to the dissimilarity justifies its use in evaluation of genetic divergence among maize hybrids.

CONCLUSIONS

There is genetic variability among tested hybrids. The higher genetic divergence was observed among MAXIMUS and XB6012, implying in heterotic gains.

Crossings of lines extracted from hybrids 2B587HX and XB6012 with lines extracted from other hybrids provide higher heterosis.

The traits grain yield and ear height were that most and least contributed, respectively, to the genetic divergence among hybrids.

RESUMO: Em programas de melhoramento em milho podem surgir dificuldades quanto a estudos de capacidade de combinação para determinação de grupos heteróticos, que estão altamente correlacionados com a divergência genética entre os genitores. O objetivo deste trabalho foi estimar a divergência genética mensurada para nove descritores morfológicos quantitativos em onze híbridos simples de milho cultivado no ecótono Cerrado-Pantanal. O experimento foi conduzido na área experimental da Universidade Estadual de Mato Grosso do Sul, Aquidauana (MS). O delineamento experimental utilizado foi o de blocos casualizados com quatro repetições. No momento da colheita, foram mensurados os seguintes descritores: altura de planta, altura de inserção da primeira espiga, diâmetro da espiga, diâmetro do colmo, número de grãos por fileira, número de fileiras por espiga, massa de cem grãos e rendimento de grãos. Na aplicação da técnica de agrupamento de híbridos foi adotada a distância generalizada de Mahalanobis como medida de dissimilaridade, e para o estabelecimento de grupos similares foi aplicado o método de Tocher. Os resultados indicaram a existência de variabilidade genética entre os híbridos testados. A maior divergência genética foi observada entre os pares MAXIMUS e XB6012, implicando em ganho heterótico. Cruzamentos de linhagens extraídas dos híbridos 2B587HX e XB6012 com linhagens obtidas a partir dos demais híbridos proporcionaram maior heterose. Os descritores rendimento de grãos e altura de inserção da espiga foram os que mais e menos contribuíram, respectivamente, para a divergência genética entre os híbridos.

PALAVRAS-CHAVE: Dissimilaridade. Desenvolvimento de híbridos. Melhoramento genético. *Zea mays* L.

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