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QUANTIFYING THE GENETIC DIVERGENCE AMONG MAIZE HYBRIDS USING WARD-MLM STRATEGY

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ABSTRACT – The aim of this study was to estimate the genetic divergence of twelve maize hybrids grown in Cerrado-Pantanal ecotone, based on nine agronomic traits and using the Ward-MLM procedure. The experiment was installed at Universidade Estadual de Mato Grosso do Sul, Aquidauana Unit, in a randomized blocks design with four replications. The following traits were measured: plant height, ear insertion height, ear diameter, ear length, stem diameter, number of kernels per rows, number of rows per ear, weight of hundred grains and grain yield. The significance of the nine traits was analyzed by F test at 5% probability. Data were simultaneously analyzed using the Ward-MLM to compose the hybrids groups through the cluster. There was genetic variability among the maize hybrids assessed. Hybrids with high heterotic effect can be obtained by crossing individuals of the group II with group III.

Key words: dissimilarity, hybrid development, genetic breeding, *Zea mays* L.

QUANTIFICAÇÃO DA DIVERGÊNCIA GENÉTICA ENTRE HÍBRIDOS DE MILHO USANDO O PROCEDIMENTO WARD-MLM

RESUMO – O objetivo deste trabalho foi estimar a divergência genética usando o procedimento Ward-MLM em 20 híbridos de milho cultivados no ecótono Cerrado-Pantanal baseada em nove caracteres agronômicos. O experimento foi instalado na Universidade Estadual de Mato Grosso do Sul, Unidade de Aquidauana Unit, em um delineamento de blocos casualizados com quatro repetições. Mensuraram-se os seguintes caracteres: altura de plantas; altura de inserção da primeira espiga; diâmetro da espiga; diâmetro do colmo; número de grãos por fileira; número de fileiras por espiga; peso de 100 grãos; e produtividade. A significância dos nove caracteres foi analisada pelo teste F a 5% de probabilidade. Os dados foram analisados simultaneamente usando o procedimento Ward-MLM para compor os grupos de híbridos por meio da análise de agrupamento. Existe variabilidade genética entre os híbridos de milho avaliados. Híbridos com alto efeito heterótico podem ser obtidos do cruzamento ente indivíduos contidos no grupo I com os do grupo II.

Palavras-chave: dissimilaridade, desenvolvimento de híbridos, melhoramento genético, *Zea mays* L.

Knowing the genetic divergence in available germplasms is fundamental for the optimal designing of breeding programs, and its efficiency can be increased if superior crossings are pre-established (Rigon et al., 2015). At 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 divergence and restricts the possibility of crossing between genetically divergent genotypes. Knowledge of the genetic relationships among breeding materials can help to prevent the great risk of increasing uniformity in the elite germplasm, and can also ensure long-term selection gains (Oliveira et al. 2004).

The adequate choice of genotypes as sources of lines extraction can to determine the success or economic return of a breeding program to develop maize hybrids. The trends are in order to use F2 populations with genetic variability or double hybrids with yield potential originated from crosses of single hybrids, synthetic elite lineages and even commercial hybrids, as it the inappropriate choice of population will result in loss of time and resources destined for breeding (Smith et al. 1997; Gutierrez et al. 2003; Franco et al., 2005; Ortiz et al, 2008; Oliveira et al., 2010; Rotili et al. 2012; Simon et al. 2012; Gomes Júnior et al. 2014).

Front to numerous genitors frequently used in the maize breeding programs, hundreds of new lineages 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 heterosis phenomenon in hybrid crosses, increasing the chances to obtain segregating generations. These estimates are of great use in breeding programs and also in the choice of genitors for mapping of genes (Rigon et al., 2015).

In a population genetic divergence study, quantitative morphological descriptors are subjected to multivariate biometric techniques, allowing unifying multiple information of a trait set. The modified location model (MLM), proposed by Franco et al. (1998), is a new strategy to quantify the variability using quantitative and qualitative traits simultaneously. MLM has two stages. In the first, Ward clustering method (Ward, 1963) defines the groups using the Gower dissimilarity matrix (Gower, 1971). In the second stage, the vector average of the quantitative trait is estimated by MLM procedure, for each subpopulation, regardless of the qualitative traits values.

In this context, the aim of this study was to estimate the genetic divergence with Ward-MLM procedure in twelve maize hybrids cultivated in the Cerrado-Pantanal ecotone based on nine agronomic traits.

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. The region climate, according to the classification described by Köppen-Geiger, is Aw (Savanna Tropical), with cumulative rainfall over the experiment of 450 mm and maximum and minimum temperatures of 33 and 19ºC, respectively.

Experimental design used was randomized blocks with four replications. The area was divided into four blocks with a total of seventy-four plots, each with 15.75 m² (3.15 x 5.0 m), spaced two meters spacing between blocks. The treatments consisted of 20 maize hybrids (Table 1), which it has shown satisfactory agronomic performance in the study region (Torres et al., 2013; Teodoro et al., 2014).

In the preparation of the experimental area, it was performed application of glyphosate herbicide for desiccation. After drying and complete death of the plants, the furrows were opened using a seeder. Seeding was done manually under no-tillage, 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 plants emergence, we carried thinning keeping four plants per linear meter for establishment of 88,889 plants ha^{-1}.

Fertilization at sowing time consisted of 300 kg ha^{-1}, at formulation 4-20-20. In topdressing, urea was used as nitrogen source, applying 100 kg ha^{-1} on the surface when the plants showed five to eight fully

TABLE 1. Commercial Name, business and genetic class of 20 maize hybrids gowing in Aquidauana, MS, 2012.

Commercial Name	Business	Genetic class	Cycle
AG 9010	Agroceres	Híbrido Simples	Very early
FÓRMULA TL	Syngenta	Híbrido Simples	Very early
MAXIMUS	Syngenta	Híbrido Simples	Early
P30F53	Pioneer	Híbrido Simples	Early
P3340	Pioneer	Híbrido Simples	Early
STATUS TL	Syngenta	Híbrido Simples	Early
XB 6010	Semeali	Híbrido Simples	Very early
XB 6012	Semeali	Híbrido Simples	Early
XB 7253	Semeali	Híbrido Triplo	Early
2B433	Dow Agrosciences	Híbrido Triplo	Very early
2B512	Dow Agrosciences	Híbrido Triplo	Early
2B587	Dow Agrosciences	Híbrido Simples	Early
2B604	Dow Agrosciences	Híbrido Simples Modificado	Early
2B655	Dow Agrosciences	Híbrido Triplo	Early
20A55	Agromen	Híbrido Triplo	Early
20A78	Agromen	Híbrido Triplo	Early
30A30	Agromen	Híbrido Simples	Hyper early
30A37	Agromen	Híbrido Simples	Very early
30A91	Agromen	Híbrido Simples Modificado	Early
30A95	Agromen	Híbrido Triplo	Early

expanded leaves. The control of *Spodoptera frugiperda* was made at 30 days after sowing using Triflumuron insecticide at 75 mL ha^{-1}. For control of pre-emergence weeds, we used 1,125 g ha^{-1} of active ingredient atrazine.

At harvest, we measured in five plants of each plot the agronomic traits plant height (PH) and ear insertion height (EIH), being carried out with a ruler graduated in five plants per plot. In each plot were randomly harvested five sheaves, which were numbered according to the plants evaluated by determining ear diameter (ED), ear length (EL), stem diameter (SD), number of kernels per rows (NKR) and number of rows per ear (NRE).

Harvest and threshing of maize ears were performed manually in three central rows of five meters length, according to the cycle of each hybrid. The weight of hundred grains (WHG) was determined by manual counting, weighing and correction of moisture to 13%. Grain yield (YIE) was estimated by extrapolation of harvested production in 1 ha, correcting by plant stand and for 13% wet basis.

The significance of the nine agronomic traits was analyzed by F test (ANOVA), at 5% probability. Data were analyzed simultaneously, using Ward-MLM for composing the hybrids groups through the cluster. For the Ward cluster method, the distance matrix was provided by Gower's algorithm (Gower, 1971). The definition of the ideal number of groups was performed according to the pseudo F and pseudo t² criteria. Difference among groups, correlation between the variables and canonical variable (CAN) were graphically evaluated. The distance for the traits distribution proposed by Franco et al. (1998) was used for determining the dissimilarity among the formed groups. All analyzes were carried using the SAS statistical software (SAS INSTITUTE, 1999).

Results and Discussion

There was significant differences ($p<0.01$) between genotypes for all evaluated traits, allowing to infer about the existence of genetic variability in the population (Table 2). Coefficient of variation ranged from 3.79% (ED) and 15.21% (PH), revealing high experimental precision. It is important to mention that the hybrids evaluated obtained satisfactory agronomic performance and the yield grain average obtained is above the average of the state and Mato Grosso do Sul and Brazil, which is around 5.0 Mg ha^{-1} (Conab, 2015). Similar results were reported in other studies with maize hybrids growing in this region (Torres et al., 2013; Teodoro et al., 2014).

By the likelihood function procedure (pseudo F and pseudo t²), the ideal number of groups was equal to three. Gonçalves et al. (2009) and Barbé et al. (2010) reported that the of likelihood function analysis can to define more precise criteria in the formation of groups, resulting in the determining the less subjective groups. Barbé et al. (2010), Cabral et al. (2010) and Pessana et al. (2011) observed results of similar magnitude.

Group I was composed by 12 maize hybrids (2B655HX, 30A37HX, 2B433HX, 30A95HX, Maximus, P30F53, Fórmula TL, 2B604HX, Status TL 2B587HX, 30A91HX and 20A55HX) and was characterized by having the lower means for the traits SD, EIH, PH, WHG e YIE (Table 3). Group II was formed by five hybrids (XB6012, 2B5124, 20A78HX, AG 9010 and 30A30HX), which obtained intermediate values for all evaluated traits. Group III was formed by three hybrids (P3340, XB6010 and XB7253), which stand out by obtaining the higher means for SD, EIH, PH, WHG and YIE.

TABLE 2. Summary of the analysis of variance for the traits evaluated in 20 maize hybrids grown in ecotone Cerrado/Pantanal.

SV	DF	EL	ED	NRE	NKR	SD	EIH	PH	WHG	YIE
Blocks	3	0.20	0.04	1.05	0.20	0.02	117.18	999.84	15.51	392,842.05
Hybrids	19	4.15*	0.14*	9.83*	18.64*	0.11*	336.34*	2,242.32*	113.58*	10,181,913.20*
Residue	57	1.04	0.03	1.46	4.80	0.02	67.79	941.67	6.41	1,224,690.65
Mean	---	14.43	4.69	15.96	28.87	2.00	102.95	201.73	33.88	7,686.31
CV (%)	---	7.07	3.79	7.57	7.59	6.77	8.00	15.21	7.47	14.40

*Significant at 1% probability by F test; SV: sources of variation; CV: coefficient of variation; DF: degrees of freedom; EL: ear length; ED: ear diameter; NRE: number of rows per ear; NKR: number of kernels per rows; SD: stem diameter; EIH: ear insertion height; PH: plant height; WHG: weight of hundred grains; YIE: yield grain.

It can be observed in Table 4 that the groups I and II were the most similar between them. Such groups, by presenting the same pattern of similarity, are not recommended for use in breeding programs by hybridization, so that genetic variability is not restricted, in order to derail the gains to be obtained by selection. His occurs because genetically related parents tend to share many genes or alleles in common, and when two of these parents are crossed, there is little stimulus, attributed low level of allelic heterozygosity at the crossing (Cruz et al., 2014).

The longest distance was found between the groups II and III. This high divergence, at first, allows recommending the crossing between these pairs, in order to maximize heterosis in progenies and increase the possibility of segregating in advanced generations due to different numbers of loci in which the dominance effects are evident (Cruz et al., 2014). In addition, to consider the grain yield extremely important trait for selecting superior hybrids in breeding programs, individuals from group II and III presented the highest means. Thus, it may be possible to generate hybrids with high heterotic effect due to different numbers of loci in which the dominance effects are evident.

In the analysis based on the canonical variables (CAN), we found that the first two variables accounted for 100% of the variance. Thus, the two-dimensional representation is the most suitable for representing the data set. The Ward-MLM procedure was employed to quantify the genetic variability in research on maize (Gutierrez et al. 2003; Franco et al., 2005; Ortiz et al, 2008), turnip-feed (Padilha et al, 2005), tomato (Goncalves et al., 2009), common bean (Cabral et al., 2010), chili/pepper (Sudre et al., 2010) and banana (Pestana et al., 2011). These authors observed that the first two CAN explained the variability between the groups higher than 80% and the two-dimensional graph was suitable for viewing the relationship between the groups.

By the graphical analysis of the first two CAN, we observed a move away from group III from other groups formed by Ward-MLM procedure and its greater distancing with the hybrids from the group II (Figure 1). These results reinforce the hypothesis of obtaining hybrids with high grain yield by crossing between individuals from group II and III.

TABLE 3. Groups, number of hybrids per group, hybrids and mean of each trait evaluated in 20 maize hybrids grown in ecotone Cerrado/Pantanal.

Group	Number of hybrids	Hybrids	Trait	Mean	Standard deviation
1	12	2B655HX, 30A37HX, 2B433HX, 30A95HX, Maximus, P30F53, Fórmula TL, 2B604HX, Status TL 2B587HX, 30A91HX and 20A55HX	EL	14.81	0.70
			ED	4.81	0.13
			NRE	16.83	1.59
			NKR	28.67	1.78
			SD	1.97	0.09
			EIH	99.33	6.27
			PH	194.08	10.41
			WHG	30.92	4.48
			YIE	6980.80	1873.21
II	5	XB6012, 2B5124, 20A78HX, AG 9010 and 30A30HX	EL	12.62	1.39
			ED	4.62	0.16
			NRE	14.40	0.94
			NKR	26.96	4.28
			SD	2.16	0.40
			EIH	112.14	12.15
			PH	203.44	24.97
			WHG	39.20	1.52
			YIE	8346.46	1352.29
III	3	P3340, XB6010 and XB7253	EL	16.00	0.40
			ED	4.40	0.26
			NRE	12.93	0.92
			NKR	32.00	0.35
			SD	2.30	0.20
			EIH	119.13	19.32
			PH	262.17	20.56
			WHG	42.60	3.10
			YIE	9731.50	1476.07

EL: ear length; ED: ear diameter; NRE: number of rows per ear; NKR: number of kernels per rows; SD: stem diameter; EIH: ear insertion height; PH: plant height; WHG: weight of hundred grains; YIE: yield grain.

TABLE 4. Distance between groups formed by the Ward-MLM procedure proposed by Franco et al. (1998).

Group	**I**	**II**	**III**
I	0	64.87	72.59
II		0	135.96
III			0

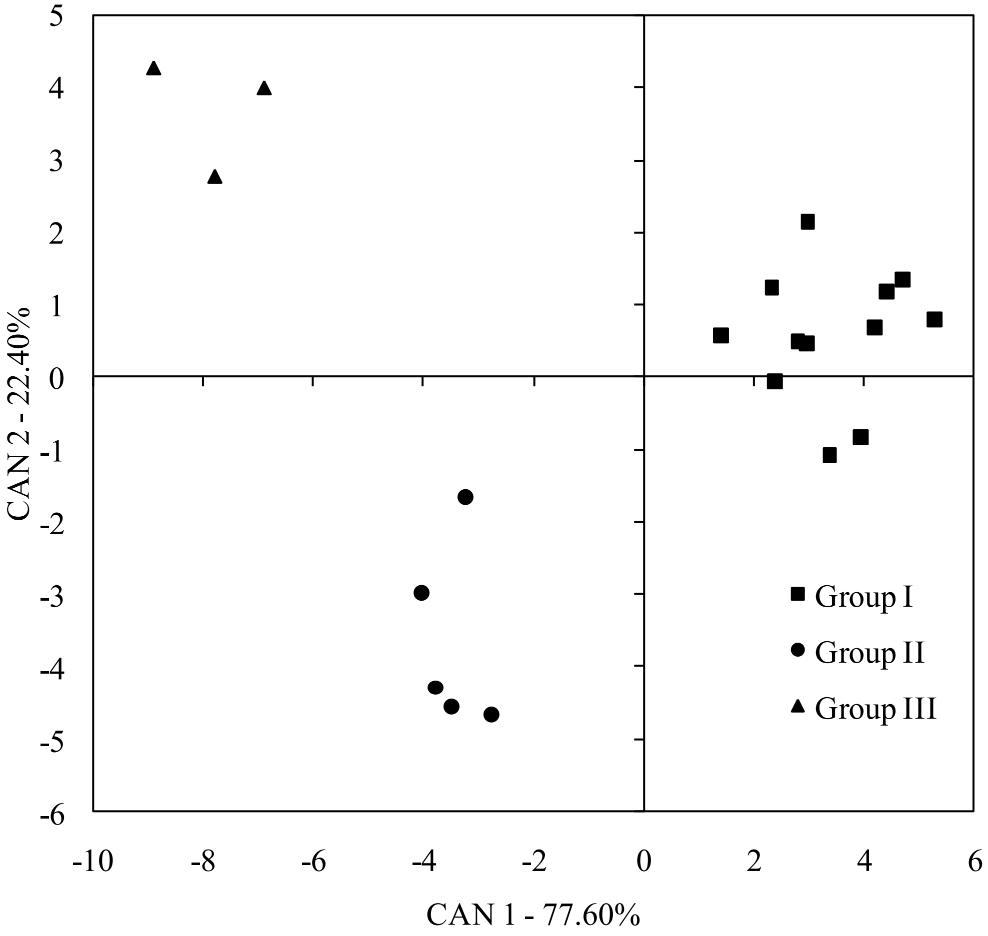

FIGURE 1. Graph of the first two canonical variables (CAN 1 and CAN 2) for the three groups of maize hybrids formed by Ward-MLM procedure.

Conclusions

There is genetic variability of the evaluated traits in hybrids adapted to ecotone Cerrado/Pantanal region.

Ward-MLM statistical procedure is a useful tool for detecting genetic divergence and group hybrids, using agronomic traits.

F2 populations with high genetic variability or double hybrids with high heterotic effect may be obtained bye crossing of individuals from group II with group III.

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