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GENETIC DISTANCE BASED ON SSR AND GRAIN YIELD OF INTER AND INTRAPOPULATIONAL MAIZE SINGLE CROSS HYBRIDS

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ABSTRACT - The objective of this work was to correlate the genetic distances between the progenitors obtained by microsatellite markers with the grain yield of inter and intrapopulational maize single cross hybrids. Three S_0 populations derived from commercial single cross hybrids were used to obtain 163 hybrids (110 interpopulational and 53 intrapopulational). The two best hybrids and two worst hybrids of each the inter- and intrapopulational crosses were selected and their progenitors maintained through self-pollination of the second ear of each S_0 plant, genotyped with 47 SSRs. The Modified Roger's Distance (MRD) between each pair of S_1 inbred lines, the number of alleles and the polymorphic information content (PIC) of each primer were estimated. The genetic distances between progenitors were correlated with the grain yield of the inter- and intrapopulational hybrids. The number of obtained alleles was 186, with a mean of 3.96 alleles. The PIC varied from 0.49 to 0.80, with a mean of 0.65. The mean genetic distance between all S_1 inbred lines was 0.75, varying from 0.40 to 0.89, indicating the existence of variability between the S_1 inbred lines. The correlation between MRD and grain yield was high and significant for the interpopulational crosses ($r = 0.84$, $P \leq 0.01$) and low and not significant ($r = 0.18$, $P \geq 0.05$) for intrapopulational crosses.

KEY WORDS: *Zea mays*; Heterosis; SSR; Correlation; Bootstrap.

INTRODUCTION

Knowledge on genetic distances between genotypes is very helpful for allowing an improvement of the sampling efficiency and use of germplasm. Breeders can make use of such information when taking decisions, as for example on which progenitors are to be chosen to obtain hybrid combinations that will maximize the expression of heterosis (Cheres *et al.,* 2000). The genetic distance between inbred lines, obtained through molecular markers, is being considered a feasible alternative for predictions of hybrid performance and heterosis in crosses (Melchinger, 1999).

Based on the hypothesis of association between heterosis and the frequency of heterozygous loci affecting the traits, Hallauer *et al.* (1988) suggested the prediction of heterosis based on molecular markers. Likewise, Árcade *et al.* (1996) considered the participation of heterozygosity in the phenomenon of heterosis and suggested a predictive potential for heterosis based on the quantification of genetic distance between the progenitors.

Several studies with DNA markers have been realized with the objective of predicting the performance of hybrids by genotyping of the progenitors (Lanza *et al.,* 1997; Ajmone-Marsan *et al.,* 1998; Drinic *et al.,* 2002; Barbosa *et al.,* 2003; Reif *et al.,* 2003a; Xu *et al.,* 2004). Results found in literature are generally somewhat inconsistent regarding the efficiency of prediction of hybrid performance by molecular markers, since correlations of intermediate magnitude between the performances of the hybrids and the genetic distances between their progenitors (Ajmone-Marsan *et al.*, 1998) as well as low correlations have been found (Boopenmaier *et al.*, 1992; Dhillon *et al.*, 1993; Melchinger, 1999).

Microsatellite markers or SSR are sees as the most promising for studies on genetic diversity and hybrid prediction, since they present Mendelian inheritance and codominant behavior, in other words, they allow the identification of homozygous and heterozygous genotypes in the population. In addi-

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tion, there is already a large set of microsatellites available for maize; many of them were identified as associated to the QTLs for grain yield (SIBOV *et al.*, 2003).

The objective of this work was to correlate genetic distances obtained from microsatellite markers among the progenitors and the grain yield of inter and intrapopulational maize single cross hybrids.

MATERIAL AND METHODS

Three S_0 populations from the commercial single cross hybrids, P30F45, Dow657 and DKB333B were used. The populations were codified as population A (P30F45), population B (Dow657) and population C (DKB333B). Inter and intrapopulational crosses were made to obtain the hybrids, starting from the S_0 populations. The second ear of each progenitor S_0 was selfed, to obtain the S_1 generation that was genotyped with SSR markers.

The inter and intrapopulational hybrids were evaluated using commercial hybrids and their S_0 populations as checks, in two environments in southeastern of Brazil in a lattice design (13 x 13) with two replicates. 163 hybrids were evaluated, 110 interpopulational (34 AB, 48 AC and 28 BC) and 53 intrapopulational (14 A, 17 B and 19 C). The data were submitted to individual and combined analyses of variance.

The selection of parental inbred lines for genotyping with microsatellites was done considering the means for grain yield of the inter- and intrapopulational hybrids. The two best hybrids and two worst hybrids of each the inter- and intrapopulations crosses were selected, resulting in 24 hybrids. 48 S_1 inbred lines were identified and selected for the analyses (Table 1).

The DNA was isolated from a bulk of one-week-old leaf tissue from 20 plants of each S_1 inbred line, using the method described by SAGHAI-MAROOF *et al.* (1984).

Forty-seven SSR markers uniformly distributed throughout the genome were analyzed for each S_1 inbred line. Information regarding map position and sequence repeat for each of the SSR primer use can be found in Table 2. From these SSR primers, eleven are associated with QTLs for grain yield and other agronomic traits identified in tropical maize (SIBOV *et al.*, 2003).

The SSR amplification reactions were carried out in 0.2 mL tubes using Thermocyler Mastercycler Gradient (Eppendorf). The PCR amplification consisted of: denaturation for 1 min at 95°C, followed by two cycles of 1 min at 95°C, 1 min at 65°C and 5 min at 72°C. The annealing temperature was then reduced by 1°C every two cycles until a final temperature of 55°C was reached. The last cycle was repeated 30 times and was terminated with continuous cycle at 4°C. The 10.65 µL reaction mix consisted of 1 ng μL^{-1} template DNA, 1X reaction buffer supplied by the manufacturer (Promega), 1.5 mM $MgCl_2$ (Promega), 0.2 mM each dNTP (Invitrogen), 2 units of *Taq* DNA polymerase (Promega) and 0.3 mM of each primer. After amplification, 4 µL of loading-dye (30% glycerol, 0.25% bromophenol blue and 0.25% xyleno cyanol) was added to each amplification reaction, which was separated by electrophoresis in a horizontal gel system (Hoefer SE 600 Gel Casters) using 0.5X TBE (Tris/Borato 0.045 M and EDTA 0.001 M pH 8.3) on a 3% ultrapure agarose gel (GibcoBRL); a molecular weight standard (100 pb) was used. After electrophoresis, the gels were stained with ethidium bromide (0.5 µG/mL) and photographed under UV light. The gels ran at 140 V for 2 hours.

TABLE 1 - *Code of the S_1 inbred lines genotyped with 47 SSR markers.*

Code	Inbred lines
1 a 8	Inbred lines of interpopulational crosses AB
9 a 16	Inbred lines of interpopulational crosses AC
17 a 24	Inbred lines of interpopulational crosses BC
25 a 32	Inbred lines of intrapopulational crosses A
33 a 40	Inbred lines of intrapopulational crosses B
41 a 48	Inbred lines of intrapopulational crosses C

We calculated the Modified Roger's Distance (MRD) between all of the S_1 inbred lines (GOODMAN and STUBER, 1983) as:

$$MRD_{(IJ)} = \left[\sum_{k=1}^{I} (p_{ik} - p_{jk})^2\right]^{-1/2} \Big/ 2n$$

Were, p_{ik} e p_{jk} are the allele frequencies of the *j*th allele at the *i*th marker in the two S_1 inbred lines under consideration, is the number of markers, and *I* refers to the number of alleles at the *i*th marker. The estimate of allelic frequencies was obtained through the TFPGA software, version 1.3 (MILLE, 1997).

According with REIF *et al.* (2005) the MRD is especially suitable in studies based on allelic informative marker data for examining (i) the prediction of heterosis with genetic dissimilarities or (ii) the establishment of heterotic groups.

For each SSR locus the average number of alleles per locus and polymorphic information content (PIC) were calculated. PIC, a measure of the allelic diversity at a locus, was estimated for each of the polymorphic SSR loci detected in the present study using the following equation:

$$PIC = 1 - \sum_{i=1}^{t} p_i^2 - 2 \sum_{i=j+1}^{t} \sum_{j=1}^{t-1} p_1^2 p_j^2$$

where p_i and p_j are the frequencies of the *ith* and *j*th alleles at a locus with *t* alleles in a population, respectively (BOTSTEIN *et al.*, 1980).

Cluster analysis was performed on the shared allele distance matrix using the unweighted pair-group method with arithmetic average (UPGMA) as implemented in NTSYS-pc v. 2.1 (ROHLF, 2000). The cophenetic correlation coefficient was calculated, and Mantel's test (MANTEL, 1967) was performed to check the goodness of fit of a cluster analysis for the matrix on which it was based.

The bootstrap method was utilized to verify if the number of SSR loci needed to precisely determine the genetic distance estimates between the S_1 inbred lines. For each pair of S_1 inbred lines, the MRD was estimated using re-sampling from different sizes (2, 4, 6, 42, 44, 46 SSRs), each repeated 10,000 times with the statistical software GQMol (CRUZ and SHUSTER, 2004). The software estimates correlations of values from the original distance matrix with other matrices, obtained considering the re-

TABLE 2 - *SSR markers, map localization (Bin), sequence repeat, number of alleles and polymorphic information content (PIC).*

SSR locus	Bin	Repeat	Alleles	PIC	SSR locus	Bin	Repeat	Alleles	PIC
BNLG1484	1.03	AG	4	0.67	UMC1014	6.04	GA	4	0.63
BNLG1016	1.04	AG	7	0.80	UMC1857	6.04	TAA	4	0.59
BNLG2238	1.04	AG	3	0.49	UMC1653	6.07	GAAA	6	0.78
UMC1297	1.05	GA	3	0.58	UMC1409	7.01	GCTC	3	0.66
BNLG615	1.07	*	3	0.55	BNLG434	7.03	*	4	0.68
UMC1845	2.03	AG	6	0.77	BNLG1666	7.04	AG	3	0.65
BNLG166	2.04	*	4	0.61	DUPSSR13	7.04	CA	4	0.56
BNLG2077	2.07	AG	4	0.73	UMC1154	7.05	AC	3	0.59
UMC1230	2.09	TAA	3	0.60	BNLG1176	8.05	AG	4	0.69
UMC1394	3.01	AT	4	0.58	BNLG1607	8.06	AG	3	0.64
BNLG1951	3.06	AG	5	0.72	BNLG240	8.06	*	4	0.66
BNLG2241	3.06	AG	4	0.71	UMC1069	8.08	GGAGA	3	0.61
BNLG1318	4.01	AG	4	0.62	UMC1638	8.09	CTCCGG	4	0.60
UMC1943	4.02	*	3	0.55	BNLG1724	9.01	AG	4	0.65
BNLG1755	4.05	AG	3	0.61	BNLG2122	9.01	AG	6	0.81
UMC1088	4.05	CT	5	0.69	UMC1893	9.02	AGC	4	0.64
BNLG1621	4.06	AG	3	0.66	UMC1804	9.07	AG	5	0.75
BNLG589	4.10	*	4	0.59	UMC1380	10.00	CTG	3	0.56
BNLG1006	5.00	AG	3	0.59	UMC1318	10.01	GTC	6	0.81
BNLG105	5.02	*	3	0.58	UMC1432	10.02	AG	3	0.58
UMC1221	5.04	CT	4	0.68	BNLG2336	10.04	AG	3	0.49
UMC1792	5.08	CGG	4	0.58	BNLG1250	10.05	AG	3	0.65
BNLG386	5.09	*	5	0.58	Total			186	
BNLG1600	6.00	AG	3	0.70	Mean			3.96	0.65
BNLG1371	6.02	*	6	0.60					

* Not information

samplings from different sample sizes. The software also calculates another parameter: a value of stress (S) that indicates an adjustment between the original matrix, considering all of the 48 SSRs and re-sampling matrices (KRUSKAL, 1964).

Spearman correlation coefficients were calculated for genetic distances between the S_1 inbred lines and grain yield from inter and intrapopulation hybrids (STEEL and TORRIE, 1980). The analysis was performed with the statistical software SAS version 6.03 (SAS INSTITUTE, 1998).

RESULTS AND DISCUSSION

The number of obtained alleles was 186, with a mean of 3.96 alleles per primer. The highest number of alleles was identified in primer BNLG1016. Of the 47 primers, 19 amplified three alleles, 18 four alleles, 4 amplified 5 alleles, 5 amplified 6 alleles and 1 primer amplified 7 alleles. The mean number of alleles per SSR locus is similar to that obtained in other studies dealing with maize. MENKIR *et al.* (2004) used 38 lines and 33 SSRs and found 5.33 alleles per locus; LU and BERNARDO (2001), with 40 lines and 83 SSRs, found 4.9 alleles per locus and LE CLERC *et al.* (2005), with 133 cultivars and 51 SSRs found 3.9 alleles per locus.

The PIC varied from 0.49 for primer BNLG2238 to 0.80 for primer BNLG1016, with a mean of 0.65. The PIC values are in agreement with those VAZ PATTO *et al.* (2004) observed who genotyped 104 maize inbred lines with 15 SSR markers. According to the authors, the PIC was between 0.33 and 0.89, with a mean of 0.56. XIA *et al.* (2004), when genotyping 155 maize inbred lines with 79 SSR markers found a PIC mean of 0.60.

The correlation between the number of alleles and the PIC was high ($r = 0.82$, $P \leq 0.001$). This result is in line with that of VAZ PATTO *et al.* (2004) who observed a correlation value of 0.85.

The mean, minimum and maximum genetic distances between the eight S_1 inbred lines used in each group of crosses are shown in Table 3. The

TABLE 3 - *Number of inbred lines (N), mean, minimum, maximum, and standard deviation (SD) of genetic distances calculated from 47 SSRs for 48 maize S_1 inbred lines.*

Crosses	N	Mean	Min.	Max.	SD
A X B	8	0.71	0.47	0.81	0.12
A X C	8	0.66	0.47	0.86	0.09
B X C	8	0.74	0.47	0.86	0.10
A X A	8	0.43	0.34	0.76	0.12
B X B	8	0.71	0.40	0.85	0.09
C X C	8	0.62	0.34	0.76	0.11
Among all*	48	0.75	0.40	0.89	0.09

* inter + intrapopulational crosses.

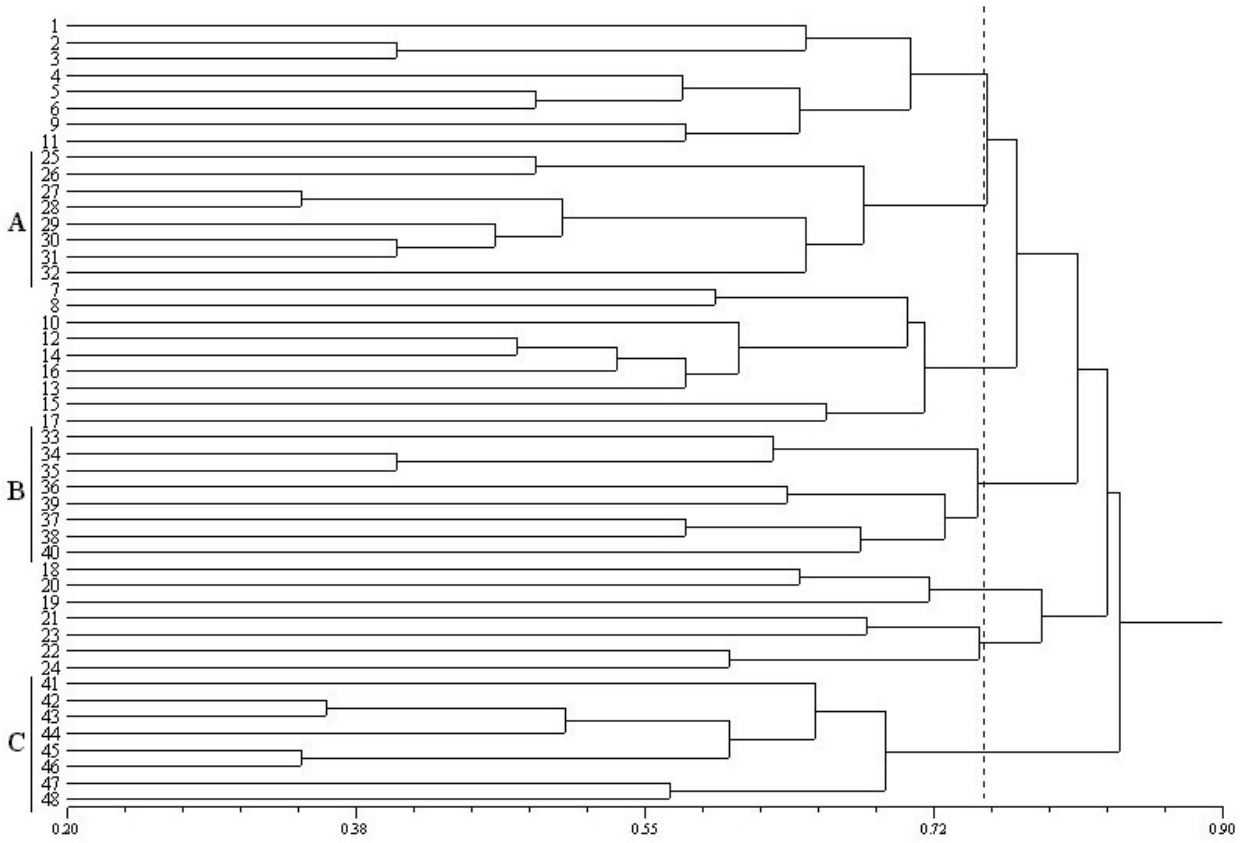

FIGURE 1 - Association among the 48 S_1 inbred lines generated by UPGMA cluster analysis of genetic distance calculated from 47 SSR markers. (A, B e C) S_1 inbred lines of the intrapopulation crosses A, B and C.

mean genetic distance between all S_1 inbred lines was 0.75, varying from 0.40 to 0.89, indicating the existence of great genetic variability between the S_1 inbred lines. The mean genetic distance in the interpopulational crosses was 0.70 and 0.59 in the intrapopulational crosses. The amplitude of variation for the genetic distance was greater in the intrapopulational crosses (0.42).

The dendrogram obtained by the UPGMA of the genetic distances based on SSRs can be found in Fig. 1. Cluster analysis showed a good fit to the matrix on which it was based (cophenetic correlation coefficient [r] = 0.82, P < 0.0001, 10,000 permutations). VAZ PATTO *et al.* (2004) consider a correlation value above 0.56 as ideal.

For the determination of the groups in the den-

TABLE 4 - *Spearman correlation coefficient of genetic distance with grain yield of inter and intrapopulational crosses.*

Crosses	N	Grain yield
Interpopulational	12	0.84 **
Intrapopulational	12	0.18 ns
Inter + intrapopulational	24	0.55 **

** significant at $P = 0.01$. ns not significant.

drogram the mean genetic distance (0.75) between all S_1 inbred lines was established as cut off point (Fig. 1, dotted line). In the dendrogram, one notes that the S_1 inbred lines associated to each intrapopulational crosses clustering together (intrapopulation A, B and C). Based on the presumption that these inbred lines were derived from three different commercial hybrids and considering the distinct heterotic groups, microsatellites were effective at allocating these inbred lines in heterotic groups in this study.

BARBOSA *et al.* (2003), which genotyped 18 S_3 inbred lines with AFLP and SSR markers (eight derived from population BR-105 and ten from BR-106) observed that the 18 inbred lines grouped in two quite distinct groups. According to the authors, previous studies had already allocated the two populations in different heterotic groups, leading to the conclusion that both marker types were effective at allocating inbred lines in their respective heterotic groups. According with REIF *et al.* (2003a), SSRs are a valuable complementation to field data for the identification of heterotic groups in maize.

Bootstrap analyses indicated that 25 SSRs were necessary for a precise estimation of the genetic distance between the 48 S_1 inbred lines in this study (Fig. 2). The correlation between the original matrix (47 SSRs) and the matrix of re-sampling (25 SSRs) was 0.88, with a stress value (S) of 0.049. Each re-sampling was repeated 10,000 times. According to KRUSKAL (1964), a stress value below 0.05 indicates excellent precision.

PEJIC *et al.* (1998) stated that 20-30 SSRs were sufficient to estimate the genetic distances between 33 maize inbred lines with precision. BARBOSA *et al.* (2003) determined that 29 of the 68 SSR markers would be needed for a precise estimation of the genetic distance between 18 maize inbred lines. In our study, the genetic distance between the 48 S_1 lines was estimated with 47 SSRs, allowing the conclusion that there was good genotyping, mainly because the microsatellites were chosen aiming to achieve a representative physical cover of the maize genome (Table 2).

Temperate maize populations used to development of inbred lines have a narrower genetic base than tropical maize populations, once they are generally synthetics derived from crosses of few inbred lines (BARBOSA *et al.,* 2003). There are few studies on tropical germplasm trying to correlate the hybrid performance with the genetic distance between their progenitors, making comparisons among results difficult. For LANZA *et al.* (1997) tropical maize germplasm presents a broad genetic base since these genotypes were obtained by intercrossing of different populations. In this case, there is no allocation of these populations in well-defined heterotic groups as in the case of temperate germplasm.

The correlation between the genetic distance of the progenitors (GD) and grain yield of the hybrids was high and significant for the interpopulational crosses ($r = 0.84$, $P \leq 0.01$) and low and not significant in intrapopulational crosses ($r = 0.18$, $P \geq 0.05$) in our study. The correlation between GD and grain yield was 0.55 ($P \leq 0.01$), considering the inter- and intrapopulational crosses jointly (Table 4). Several studies with maize have indicated the occurrence of correlations between the genetic distance of the progenitors and hybrid performance, although with different magnitudes (LEE *et al.,* 1989; SMITH *et al.,* 1990; AJMONE MARSAN *et al.,* 1998; MELCHINGER *et al.,* 1999; BENCHIMOL *et al.,* 2000; BARBOSA *et al.,* 2003). Results of the present study are in line with those found by REIF *et al.* (2003b) who genotyped seven maize populations with 85 SSR markers. The correlation between the genetic distance and grain yield was high in the intergroup crosses ($r = 0.63$). According to the authors this high correlation could be related to additive and dominant effects together

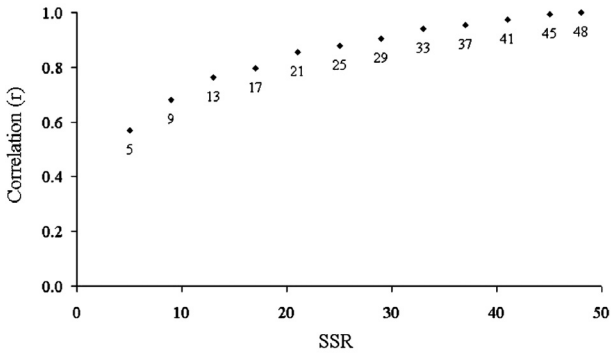

FIGURE 2 - Analysis of bootstrap for a precise estimate of the genetic distance between the 48 S_1 inbred lines.

with high values of specific combining ability between these populations.

DRINIC *et al.* (2002) genotyped 12 inbred lines from different origins with 21 SSR markers. The inbred lines were crossed in a diallell without reciprocal crosses both within each set and among sets. The authors observed that were no better correlation between grain yield and genetic distance in the intragroup crosses compared to the intergroup crosses. In some cases, the correlation was higher in the intergroup crosses.

In a review article, MELCHINGER (1999) pointed out that only intragroup crosses present a high correlation between the genetic distance (GD) and midparent heterosis (MPH), but not intergroup crosses. For intragroup crosses, the correlation (GD, MPH) is generally positive too. This can be explained by (i) hidden relatedness between some parents considered as being unrelated based on their pedigree records and (ii) presence of the same linkage phase between QTL and marker loci in the maternal and parental gametic arrays of intragroup hybrids, which results in a positive covariance between GD and MPH. In the intergroup crosses, the maternal and parental gametic array may differ in the linkage phase for many QTL marker pair; as a consequence, positive and negative terms cancel each other in their net contribution to the covariance (GD, MPH), resulting in a low or zero correlation.

Results of the present study show that the correlation between the genetic distance of the progenitors and the grain yield of the hybrids can be high in intergroup crosses as well. One possible explanation for the results is associated to a greater mean genetic distance between the inbred lines in interpopulational crosses (0.70) than in the intrapopulational (0.58) contributed to a smaller value of coancestry coefficient [*f*.] (MALÉCOT, 1948) in the interpopulational crosses. Accordingly, for crosses there exists a tight association between GD and grain yield because both measures are a linear function of *f*., hence, they increase with decreasing *f*.

Since the inbred lines were grouped in three distinct heterotic groups, according to their origin, the SSR markers can be useful to select divergent inbred lines from among these heterotic groups to obtain hybrids with a superior performance. This evidences that the three hybrids used to generate the populations form different heterotic groups. One should take into consideration that the inbred lines used to obtain each commercial hybrid should also be from different heterotic groups.

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