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L. L. B. Lanza · C. L. de Souza Jr.
L. M. M. Ottoboni · M. L. C. Vieira
A. P. de Souza

Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers

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Abstract To evaluate the genetic diversity of 18 maize inbred lines, and to determine the correlation between genetic distance and single-cross hybrid performance, we have used random amplified polymorphic DNA (RAPD), a PCR-based technique. Eight of these lines came from a Thai synthetic population (BR-105), and the others derived from a Brazilian composite population (BR-106). Thirty two different primers were used giving a total of 325 reproducible amplification products, 262 of them being polymorphic. Genetic divergence was determinated using Jaccard's similarity coefficient, and a final dendrogram was constructed using an unweighted pair-group method with arithmetical averages (UPGMA). Cluster analysis divided the samples into three distinct groups (GI, GII and GIII) that were confirmed by principal-coordinate analysis. The genetic distances (GD) were correlated with important agronomic traits for single-cross hybrids and heterosis. No correlation was found when group division was not considered, but significant correlations were detected between GI × GII and GI × GIII GDs with their respective single-cross hybrid grain-yield values. Three groups were identified; that is, the BR-106 population was divided in two different groups and the BR-105 population remained mostly as one group. The results indicated that RAPD can be used as a tool for determining the extent of genetic diversity among tropical maize inbred lines, for allocating genotypes into different groups, and also to aid in the choice of the superior crosses to be made among maize inbred lines, so reducing the number of crosses required under field evaluation.

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L. L. B. Lanza · L. M. M. Ottoboni · A. P. de Souza (✉)
Centro de Biologia Molecular e Engenharia Genética, Depto. de Genética e Evolução, IB, UNICAMP, CP 6109, CEP 13083-970, Campinas, SP, Brazil

C. L. de Souza Jr. · M. L. C. Vieira
Departamento de Genética ESALQ/USP, CP 83, CEP 13400-970, Piracicaba, SP, Brazil

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Introduction

Maize breeding programs are based on the development and selection of outstanding hybrids from inbred lines. Developing and selecting inbred lines for *per se* performance is quite easy, although time consuming. It is not possible to predict hybrid performance from inbred parent performance because of the high level of dominance for the grain-yield trait. Thus, hybrid performance is evaluated from extensive yield trials that are costly and time consuming. Moreover, the large number of possible hybrids produced from a relatively small number of inbred parents does not allow the evaluation of all hybrids (Smith 1986; Hallauer et al. 1988; Hallauer 1990; Bernardo 1994).

The use of genetic markers to assess the genetic divergence among pairs of inbred lines has been suggested as a means to overcome these drawbacks, allowing the prediction of single-cross hybrid performance. The use of isozymes (Heidrich-Sobrinho and Cordeiro 1975) and restriction fragment length polymorphism (RFLP) (Godshalk et al. 1990; Lee et al. 1990; Bernardo 1994; Dudley 1994) has been proposed to predict hybrid performance from the genetic divergence of inbred lines. In general, the correlations of the inbred lines and the grain yield of the hybrids are too low to be useful to predict hybrid performance. However, RFLP has proved useful for assigning maize inbreds to heterotic groups and for detecting relationships among them (Smith et al. 1990; Dudley et al. 1991; Melchinger et al. 1991; Bernardo 1993).

Random amplified polymorphic DNA, RAPD, is another class of genetic marker (Welsh and McClelland 1990; Williams et al. 1990), and its technical simplicity is its main advantage over RFLPs, although RAPDs are dominant and RFLPs are co-dominant markers

(Paran et al. 1991; Welsh and McClelland 1991). Thus, RAPD markers have also been proposed as an approach to assess genetic divergence among genotypes (Jain et al. 1994; Santos et al. 1994). Considerable efforts have been made in order to evaluate the effectiveness of a genetic marker system in comparison to others, by correlating its genetic similarity results (Messmer et al. 1991; Ragot and Hoisington 1993; Hahn et al. 1995). However, caution is necessary in comparing different systems due to: (1) the different nature of the information content; (2) different degrees of confidence for each method regarding genome saturation; (3) different base materials and their intrinsic polymorphisms; (4) awareness of the use of clustering and ordination methods to unveil a subgroup structure which might not necessarily obey the well-established patterns.

The tropical maize populations used in breeding programs differ from temperate maize populations. That is, tropical populations are usually composites, with greater variability than temperate synthetic materials. In this way, in contrast to temperate populations, it is difficult to allocate the tropical composites to well-defined heterotic groups based only in their divergent phenotypic characteristics. Due to the uniqueness of tropical maize germplasm, it would be very helpful to use molecular markers in their genetic evaluation.

In this report, two different populations have been used as base materials: BR-106, a Brazilian population, and BR-105, a Thai-derived population. These populations show a high degree of heterosis when intercrossed and have been allocated to distinct heterotic groups (Naspolini F° et al. 1981; Souza Jr. et al. 1993). The present paper reports the use of RAPD markers to: (1) evaluate the genetic divergence of maize inbred lines, (2) assign them to heterotic groups, and (3) correlate single-cross performance to the genetic divergence of the parental lines.

Materials and methods

Ten and eight S3-selected inbred lines from BR-106 and BR-105 maize populations, respectively, were used in this study. These lines were selected out of 400 based on testcrosses and on *per se* yielding performance; subsequently, they were selected for uniformity during three generations of inbreeding. BR-106 and BR-105 were released by the National Center of Maize and Sorghum Research (CNPMS, EMBRAPA), and both are early cycle with low plant height; BR-106 and BR-105 have yellow dent and orange flint kernels, respectively. BR-106 was derived by intercrossing three populations (Centralmex, Maya, and Dent Composite), followed by crossing with BR-108, an early cycle and low plant-height population, and then three generations of recombination. These populations have been included in the same heterotic group. BR-105 (Suwan-DMR) was introduced from Thailand and derived from an S_1 recurrent selection program, in which high selection intensity was practised; so, it is considered a synthetic. Usually, BR-105 has shown lower genetic variability than BR-106, and in crosses with BR-106 has a high level of heterosis. Therefore, these two populations have been allocated to distinct heterotic groups (Naspolini F° et al. 1981; Souza Jr. et al. 1993).

The inbred lines, BR-106 and BR-105, were crossed following a partial diallel mating design producing 80 interpopulation hybrids (Experiment 01). Twenty commercial hybrids were added for a total of 100 entries. Also, 100 single-cross intrapopulation hybrids were obtained by crossing ten inbred lines from the BR-105 population following a partial diallel mating design (Experiment 02). The inter- and intra-population hybrids were evaluated in two independent experiments (01 and 02) using a 10×10 lattice design, in three locations near Piracicaba, S.P. (Brazil) in 1994/1995, with two planting dates; thus, the hybrids were evaluated in six environments with two replications per environment. One-row plots were 40 m long, with 0.90 m between rows, and 0.20 m between plants within rows. At the same time, single-crosses produced from eight BR-106 inbred lines, following a complete diallel design, were evaluated under the same conditions as described above. Also, the 18 inbred lines were evaluated under the same conditions in a randomized, complete-block design. Data were recorded on plant and ear height and grain yield. Grain yield was recorded in kilograms per plot and transformed to ton/ha.

DNA extraction and amplification

Leaf tissue from each genotype was collected from 5-week-old plants. Equal quantities of leaf tissue from 16 plants of each line were bulked, lyophilized, ground with a mechanical mill, and stored at $-20°C$. Genomic DNA was isolated according to Hoisington et al. (1994). Approximately 300 mg of leaf tissue were homogenized in 9 ml of CTAB extraction buffer (100 mM Tris-HCl pH 7.5, 700 mM NaCl, 50 mM EDTA pH 8.0, 140 mM β-ME, 1% CTAB) and incubated at 65°C for 60 min. The samples were extracted twice with chloroform/isoamyl alcohol (24 : 1). After treatment with RNase, the DNA was precipitated with isopropanol, dissolved in TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA), and the concentration was determined. Samples were diluted to a final concentration of 2.5 ng/µl with a dilution buffer (10 mM Tris-HCl pH 8.0 and 0.2 mM EDTA) and stored at 4°C.

Amplifications were conducted with 10-mer primers from Operon Technologies Inc. (Alameda, Calif., USA). All amplification reactions were performed as reported by Williams et al. (1990), with minor modifications, using 25 ng of DNA. Controls were made by replacing DNA with water. Reaction mixtures (25 µl) contained 0.2 µM of primer, 2.0 units of *Taq* DNA polymerase (Gibco BRL, Gaithersburg, Md., USA), 2.5 µl of $10 \times$ supplied buffer, 0.1 mM of each dNTP, and 2.5 mM of $MgCl_2$. The amplifications were carried out in duplicate in a Perkin Elmer DNA Thermal Cycler. DNA denaturation was done at 94°C for 5 min., followed by a 45-cycle amplification (94°C, 1 min; 35°C, 1 min; 72°C, 2 min) and by a final extension step at 72°C for 7 min. Amplification products were separated by eletrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light.

Data analyses

Reproducible DNA bands, that is bands present in both repetitions of each sample, were scored for presence (1) or absence (0) of amplification products (the raw data is available on request). Pairwise comparisons of samples were used to estimate Jaccard's similarity coefficients (GS): $a/(n-d)$, where a = number of positive coincidences, n = total sample size, and d = number of negative coincidences (Jaccard 1908). Genetic distances (GD), between pairs of lines were estimated as $GD = 1 - GS$. Dendrograms were constructed using an unweighted pair-group method with arithmetical averages (UPGMA), and principal coordinate analysis (PCO, Gower 1966) was performed on inbred-lines allele frequencies (0 or 1). The first three principal coordinates were used to describe the dispersion of the 18 inbred lines according to their allele data. All

analyses were performed using the NTSYS-pc program, version 1.70 (Rohlf 1992).

Genetic distances between pairs of inbred lines were correlated with single-cross hybrid grain yield, plant height and ear height as well as with the mid-parent heterosis estimated for each trait. The mean values of single-crosses (SC) and the lines (L) averaged across environments were used to estimate heterosis as $h_{A,B} = SC_{(AB)} - 0.5(L_A + L_B)$. The GDs were first correlated with field data (experiment 01) from 80 interpopulation single-crosses (BR-105 × BR-106). Afterwards, the GDs and the respective crosses were separated according to the group association pattern observed in the dendrogram and the multivariate analysis. Two out of three groups included lines from BR-105 and BR-106 (GI and GII). The pairwise distances among these two groups (GI × GII) would include data from intra- (experiment 02) and inter- (experiment 01) population single-crosses. This way, intra- and inter-population single crosses were evaluated in two different experiments, with different associated intrinsic errors. As a consequence, line 01 (GI) from BR-105 and line 16 (GII) from BR-106 could not be included in the calculations since, as mentioned above, the data came from two different experiments. Only intra- or inter-population data were included in the correlation analyses, with respect to either one or the other set of experiments.

Results and discussion

Sixty eight 10-mer primers were pre-screened for the ability to detect polymorphism in three randomly chosen maize inbred lines (01, 11 and 17). The 32 primers that presented the highest degree of polymorphism (Table 1) were selected for use on the 18 maize inbred lines. These primers produced a total of 325 reproducible bands, from which 262 (80.6%) were polymorphic. An average of 11 bands per primer was obtained, ranging from 300 to 3000 bp (Fig. 1 A, B). Dendrogram stability is an important consideration in genetic diversity studies and, according to our results, the clustering pattern was maintained from 150 polymorphic bands up to 262 polymorphic bands. Thormann et al. (1994) reported that 327 RAPD bands were enough to produce a mean coefficient of variation of 10% for *Brassica*.

Genetic distances (GD) among pairs of inbred lines involving both the BR-105 and BR-106 populations ranged from 0.47 to 0.75, with an average of 0.63; from 0.29 to 0.77, with an average of 0.58 for lines from the BR-105 population; and from 0.11 to 0.77, with an average of 0.59 for lines from the BR-106 population (Tables 2 and 3). Even though, the GD averages of both populations were similar, GDs from BR-106 showed a wider range of variation than those from BR-105. These results were expected because BR-106 has a higher genetic variance than BR-105, as previously reported (Naspolini Fº et al. 1981; Souza Jr. et al. 1993).

Previous analysis of cross performance (Naspolini Fº et al. 1981) has allocated BR-105 and BR-106 into two different heterotic groups, but the dendrogram and principal coordinate analysis subdivided the inbred lines into three distinct groups (GI, GII, and GIII) (Figs. 2 and 3). These results disagree with the previously established group division based on the pedigree data, where only two groups were observed (Naspolini Fº et al. 1981). The disagreement between the data obtained by RAPD and pedigree could be attributed to the great variability of tropical maize populations. These tropical populations could be allocated to more than one heterotic group that can not be distinguished by phenotypic analyses alone. Hahn et al. (1995) reported that even though RAPD markers are useful for grouping inbred lines with different genetic backgrounds, RFLPs are better for determining the genetic relatedness between lines. Hahn et al. (1995) used temperate maize inbred lines that are derived from well-defined heterotic groups, which is not the case for the tropical maize lines used in the present work. Group I included three lines from BR-106 and one from BR-105; group II included seven lines from BR-105 and one from BR-106; group III included six lines from the BR-106 population. Average genetic distances among lines within these groups were 0.73, 0.52, and 0.46 for GI, GII, and GIII, respectively (Table 3). GI has the highest mean genetic distance due to line 01, the most divergent line of the group; however, the principal

Table 1 Primers used in the RAPD analysis of 18 S3 maize inbred lines

Operon primers	Sequence (5′ to 3′)	Total bands (no. polymorphic)[a]
OPB-03	CATCCCCCTG	13 (11)
OPB-04	GGACTGGAGT	11 (7)
OPB-05	TGCGCCCTTC	11 (5)
OPB-06	TGCTCTGCCC	9 (8)
OPB-07	GGTGACGCAG	7 (5)
OPB-08	GTCCACACGG	16 (14)
OPB-09	TGGGGGACTC	13 (12)
OPB-10	CTGCTGGGAC	15 (13)
OPB-11	GTAGACCCGT	15 (13)
OPB-12	CCTTGACGCA	12 (10)
OPB-13	TTCCCCCGCT	11 (9)
OPB-14	TCCGCTCTGG	7 (5)
OPB-15	GGAGGGTGTT	7 (3)
OPB-16	TTTGCCCGGA	9 (5)
OPB-17	AGGGAACGAG	19 (12)
OPB-18	CCACAGCAGT	14 (11)
OPC-03	GGGGGTCTTT	9 (5)
OPC-04	CCGCATCTAC	19 (15)
OPC-05	GATGACCGCC	4 (2)
OPC-07	GTCCCGACGA	11 (9)
OPC-09	CTCACCGTCC	9 (1)
OPC-13	AAGCCTCGTC	14 (10)
OPC-14	TGCGTGCTTG	14 (12)
OPC-16	CACACTCCAG	14 (11)
OPD-01	ACCGCGAAGG	5 (2)
OPD-02	GGACCCAACC	7 (5)
OPD-10	GGTCTACACC	7 (5)
OPD-11	AGCGCCATTG	10 (6)
OPD-19	CTGGGGACTT	11 (11)
OPD-20	ACCCGGTCAC	13 (8)
OPE-01	CCCAAGGTCC	9 (4)
OPE-08	TCACCACGGT	8 (5)

[a] Only reproducible bands were counted

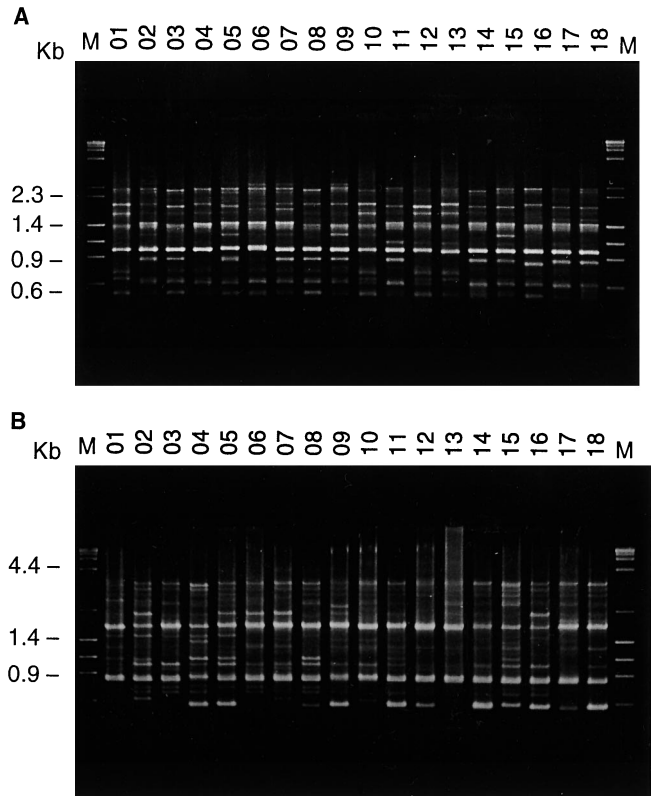

Fig. 1A–B RAPD profiles from 18 S3 maize inbred lines (1–18) with primers (**A**) OPB-18 and (**B**) OPC-07. *M* DNA molecular-weight standart ($\varphi \times Hae$III/λ *Hin*dIII)

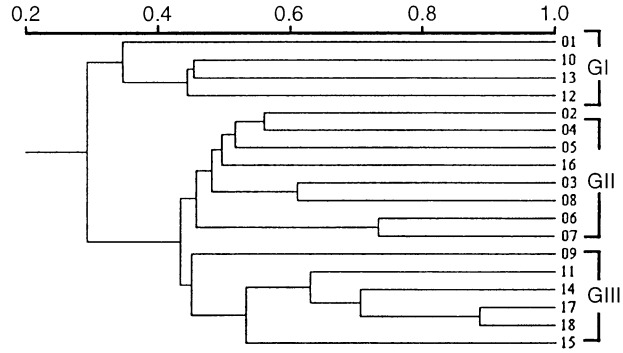

Fig. 2 Associations among 18 S3 maize genotypes revealed by UPGMA cluster analysis of the Jaccard genetic similarity (GS) coefficient calculated from 262 RAPD amplification products generated by 32 primers

coordinate analysis (Fig. 3), clearly showed that line 01 belongs to GI. The average genetic distances of GI × GII, GI × GIII, and GII × GIII inbred combinations were 0.75, 0.72, and 0.58, respectively (Table 3).

The correlation of GD values with single-cross grain-yield means (Table 2 and 4), considering the two previously identified heterotic groups, was very low, i.e., $r = 0.16$ (Fig. 4A). However, with the new groups established by RAPD analysis, the following correlations for grain yield were found for the GI × GII, GI × GIII, and GII × GIII combinations: $r = 0.70$, $r = 0.87$, and $r = 0.0$, respectively (Fig. 4B, C, D). These results could be explained, in part, by the higher values of GDs between lines from GI × GII and GI × GIII, than between lines from GII × GIII. These relationships are also depicted in the PCO (Fig. 3), based on GS values, where GII and GIII were closer to each other than GI is to GII or GI is to GIII. Principal coordinates (PCs) 1 and 2 accounted for 27.5% of the total variation while principal coordinate 3 (PC3) accounted for 8.5%. Although most of the variance was not explained, the three principal coordinates divided the inbred lines into three groups,

Table 2 Genetic distances (complement of Jaccard's estimator) for the 18 S3 maize inbred lines obtained from the 262 polymorphic bands

Inbred lines[a]	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
(01) 05-01.4B	0																	
(02) 05-05.2A	0.70	0																
(03) 05-17.1A	0.72	0.50	0															
(04) 05-18.6A	0.69	0.44	0.52	0														
(05) 05-19.1B	0.77	0.46	0.55	0.51	0													
(06) 05-23.2B	0.76	0.50	0.55	0.56	0.54	0												
(07) 05-33.5B	0.75	0.51	0.56	0.58	0.53	0.29	0											
(08) 05-34.2B	0.74	0.52	0.39	0.50	0.48	0.54	0.47	0										
(09) 06-03.5B	0.73	0.54	0.59	0.59	0.57	0.64	0.65	0.61	0									
(10) 06-06.3A	0.62	0.69	0.69	0.66	0.70	0.71	0.72	0.72	0.66	0								
(11) 06-08.1A	0.75	0.55	0.61	0.61	0.55	0.55	0.60	0.62	0.55	0.69	0							
(12) 06-08.2A	0.63	0.70	0.71	0.69	0.72	0.73	0.75	0.73	0.71	0.56	0.55	0						
(13) 06-14.4B	0.71	0.76	0.72	0.73	0.73	0.73	0.75	0.71	0.70	0.55	0.71	0.56	0					
(14) 06-24.7B	0.73	0.54	0.58	0.59	0.60	0.55	0.55	0.59	0.54	0.68	0.35	0.62	0.73	0				
(15) 06-28.1A	0.76	0.55	0.56	0.58	0.51	0.52	0.55	0.55	0.61	0.68	0.49	0.67	0.74	0.46	0			
(16) 06-29.7B	0.68	0.47	0.50	0.54	0.50	0.56	0.55	0.54	0.54	0.72	0.58	0.73	0.77	0.53	0.58	0		
(17) 06-37.5B	0.73	0.50	0.54	0.55	0.53	0.55	0.58	0.56	0.51	0.68	0.38	0.62	0.67	0.29	0.47	0.52	0	
(18) 06-44.1B	0.74	0.55	0.58	0.59	0.56	0.60	0.59	0.58	0.55	0.67	0.39	0.60	0.72	0.31	0.45	0.56	0.11	0

[a] Codes 1–8 were from BR-105/codes 9–18 were from BR-106

Table 3 Number of hybrids (N), mean, minimum, maximum, and standard deviation (SD) of genetic distances of individual line combinations calculated from RAPD data of 262 polymorphic bands for 18 S3 maize inbred lines

Group of line combinations	N	Mean	Min.	Max.	SD
BR-105 × BR-105	28	0.58	0.29	0.77	0.12
BR-106 × BR-106	45	0.59	0.11	0.77	0.14
BR-105 × BR-106	80	0.63	0.47	0.75	0.08
GI × GI	6	0.73	0.55	0.71	0.06
GII × GII	28	0.52	0.29	0.58	0.06
GIII × GIII	15	0.46	0.11	0.61	0.13
GI × GII	21	0.75	0.66	0.76	0.02
GI × GIII	17	0.72	0.60	0.74	0.04
GII × GIII	42	0.58	0.50	0.65	0.03

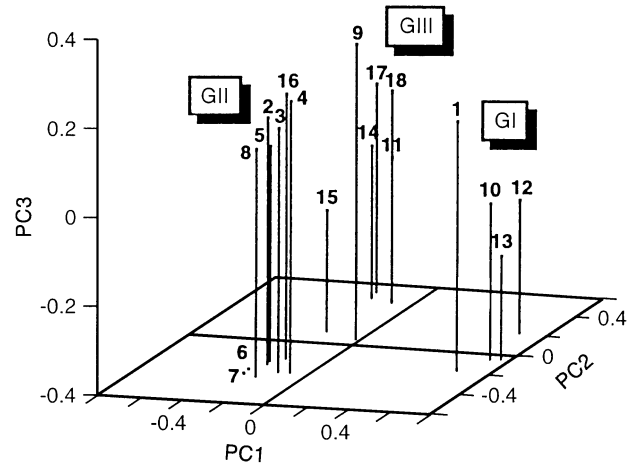

Fig. 3 Associations between 18 S3 maize inbreds revealed by principal coordinate analysis of Jaccard's similarity coefficients, calculated from 262 RAPD amplification products generated by 32 primers. The three group associations (*GI*, *GII* and *GIII*) are indicated

reflecting the same group-association observed in the final dendrogram (Fig. 2). Messmer et al. (1991) could not explain the total variance present in their data when using an ordination procedure to compare RFLP and allozyme measures.

Genetic distances among all inbred lines from the BR-105 and BR-106 populations had a low negative correlation with heterosis for grain yield ($r = -0.15$). Group division did not improve the heterosis correlation for GI × GII ($r = 0.13$) and GII × GIII ($r = -0.05$); however, heterosis for grain yield was more correlated with GD for the GI × GIII line combinations ($r = 0.63$). Plant and ear height were also examined, but exhibited poor association with the RAPD-based GDs either for the F_1 hybrids or heterosis for those traits (data not shown). This result was observed both when the previous heterotic group (Naspolini F° et al. 1981) or the RAPD-based GDs group division were considered. The GDs did not predict the best cross, but they could show that the previous heterotic division was unsuitable as GII (composed essentially of BR-105 lines) × GIII (exclusively BR-106 lines) GDs did not correlate with the performance of their crosses. However, GI × GII, or even the intrapopulation GI × GIII (BR-106) line combinations, showed expressive correlations among their GDs and the performance of their crosses. BR-106, as previously described, was derived from different populations and composites, and could be considered a broad-base composite with a higher extent of variability than the synthetic BR-105 population.

According to Lee (1995), significant challenges for predicting hybrid performance have remained because: (1) progeny derived from source populations must be tested in intergroup combinations; (2) a heterotic group may have important and perhaps unperceived genetic substructure, and (3) inbred lines of new uncharacterized or mixed origin may not fit into established groups. Our results reinforce the last statement and show that molecular-marker data can be used to unveil an unperceived substructure in a previous established heterotic pattern.

Currently, associations between hybrid performance and predicted heterozygosity have been stronger for crosses among lines of similar pedigrees (Frei et al. 1986; Lee et al. 1989; Smith et al. 1990), which could be related to the allelic states of "identical by descent" versus "identical in state" (Lee 1995). Also, the correlations between pedigree-based distances and molecular marker-based distances improve as more probes or marker loci are employed in the analysis (Moser and Lee 1994). On the other hand, the corresponding associations for intergroup crosses and lines of unknown pedigree have been too weak to be predictive (Godshalk et al. 1990; Melchinger et al. 1990; Boppenmaier et al. 1992; Dudley 1992). Bernardo (1994) suggested that single-cross yield could only be predicted effectively based on parental RFLP data and yields of related inbreds. Charcosset et al. (1991) proposed the need for linkage disequilibrium between the average degree of heterozygosity at marker loci and at QTLs for their association to occur. Bernardo (1992) identified the following conditions for the effective prediction of hybrid performance using molecular heterozygosity: (1) strong dominance effects, (2) the allele frequencies at individual loci in parental inbreds should be negatively correlated, (3) high trait heritability, (4) the narrow range variation of average parental allele frequencies, (5) 30–50% of QTLs (at least) have to be linked to molecular markers, and (6) not more than 20–30% of molecular markers have to be randomly dispersed or unlinked to QTLs. According to our results, only grain yield correlated consistently with RAPD-based genetic distances. Regarding individual plants, grain yield is a trait with a complex inheritance and low heritability; however, dealing with mean values, grain yield could be seen as a trait with high heritability and strong

Table 4 Single-cross hybrid grain yield and heterosis for inter- and intrapopulation crosses

Single crosses	Hybrid (ton/ha)	Heterosis (ton/ha)	Single crosses	Hybrid (ton/ha)	Heterosis (ton/ha)
Interpopulation (BR-106 × BR-105)			Interpopulation (BR-106 × BR-105)		
09 × 01	7.10	4.82	15 × 03	5.15	3.24
09 × 02	6.73	4.55	15 × 04	7.89	5.87
09 × 03	6.15	3.91	15 × 05	8.30	6.40
09 × 04	7.39	5.03	15 × 06	7.07	5.69
09 × 05	5.68	3.45	15 × 07	7.17	5.32
09 × 06	6.98	5.26	15 × 08	6.55	4.93
09 × 07	7.42	5.23	16 × 01	6.33	4.46
09 × 08	6.67	4.72	16 × 02	5.90	4.14
10 × 01	6.03	3.88	16 × 03	4.65	2.84
10 × 02	6.19	4.14	16 × 04	3.81	1.87
10 × 03	5.96	3.86	16 × 05	7.20	5.38
10 × 04	6.03	3.80	16 × 06	6.53	5.23
10 × 05	6.77	4.66	16 × 07	5.79	4.03
10 × 06	6.90	5.31	16 × 08	6.19	4.66
10 × 07	6.80	4.75	17 × 01	6.72	4.11
10 × 08	6.29	4.46	17 × 02	6.61	4.10
11 × 01	7.13	4.60	17 × 03	6.42	3.85
11 × 02	6.60	4.17	17 × 04	6.93	4.24
11 × 03	6.86	4.37	17 × 05	7.07	4.51
11 × 04	6.95	4.34	17 × 06	6.85	4.81
11 × 05	7.60	5.12	17 × 07	7.35	4.84
11 × 06	8.00	6.04	17 × 08	6.69	4.01
11 × 07	7.44	5.00	18 × 01	6.49	4.00
11 × 08	7.16	4.95	18 × 02	7.20	4.64
12 × 01	6.79	4.33	18 × 03	6.35	6.78
12 × 02	6.57	4.22	18 × 04	6.74	4.18
12 × 03	6.60	4.20	18 × 05	6.78	4.35
12 × 04	6.60	4.07	18 × 06	7.31	5.40
12 × 05	7.59	5.19	18 × 07	7.00	4.60
12 × 06	7.68	5.79	18 × 08	6.92	4.77
12 × 07	7.82	5.47			
12 × 08	6.88	4.76	Intrapopulation		
13 × 01	7.52	3.91	(BR-106 × BR-106)		
13 × 02	6.80	3.30	10 × 09	5.66	3.66
13 × 03	6.60	3.04	10 × 11	6.94	4.69
13 × 04	6.97	3.29	10 × 14	6.19	4.10
13 × 05	7.41	3.86	10 × 15	6.82	5.14
13 × 06	7.47	4.43	10 × 17	6.57	5.56
13 × 07	7.72	4.21	10 × 18	7.75	6.82
13 × 08	7.16	3.89	12 × 09	6.55	4.24
14 × 01	6.69	4.31	12 × 14	3.71	1.31
14 × 02	7.16	4.89	12 × 15	6.09	4.09
14 × 03	6.84	4.51	12 × 17	4.25	1.61
14 × 04	6.82	4.37	12 × 18	3.80	1.29
14 × 05	7.53	5.21	13 × 09	6.87	3.41
14 × 06	6.35	4.54	13 × 11	7.08	3.37
14 × 07	6.92	4.64	13 × 14	7.68	4.13
14 × 08	6.75	4.71	13 × 15	7.22	4.07
15 × 01	6.94	4.99	13 × 17	7.78	3.99
15 × 02	7.50	5.66	13 × 18	7.00	3.34

dominance effects, in agreement with Bernardo's predictions. Since we have found a positive correlation between RAPD-based genetic distances and F_1 grain yield, it can be expected that some of the RAPD markers are linked to QTLs; however, we can not determine the percentage of linked markers and linked QTLs. In order to determine whether the percentage of linked markers and QTLs correspond to Bernardo's last two conditions, more experiments have to be conducted.

Jain et al. (1994) have used RAPD markers to detect variation and the genetic relationships among 12 Indian and 11 exotic *Brassica juncea* genotypes. The authors could not find a consistent correlation between the genetic distances of RAPDs and single-cross performance for grain yield or heterosis**.** One of the explanations for this result might be that the group division in the dendrogram was not considered.

The results presented in this paper demonstrated direct and positive correlations between RAPD-based

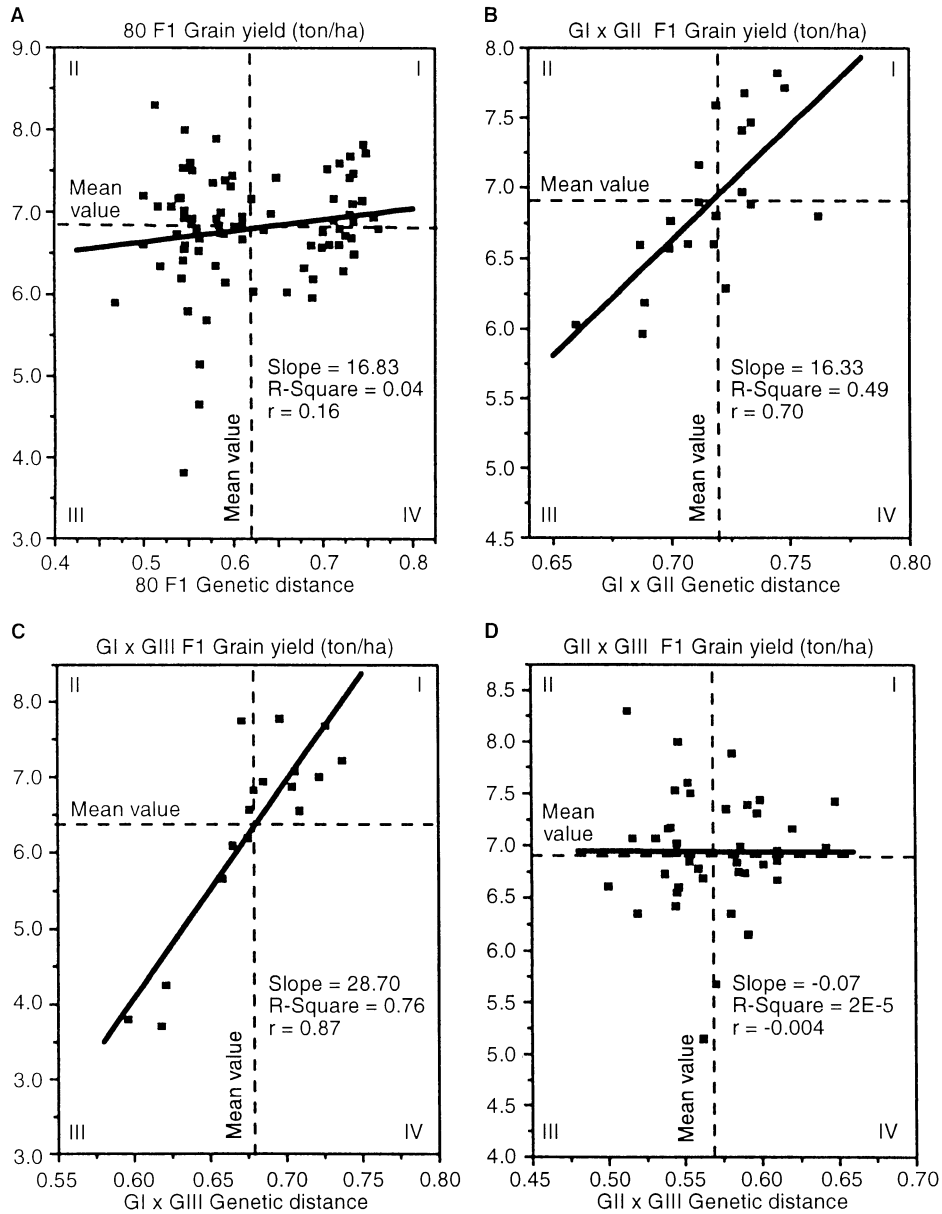

Fig. 4A–D Genetic distance (GD) versus F_1 performance for grain yield (ton/ha) in: (**A**) 80 interpopulation F_1s, (**B**) GI × GII, (**C**) GI × GIII and (**D**) GII × GIII. Quadrants are divided along mean values for the respective axes

genetic distances and maize single-cross hybrid grain yield, using inbred lines from tropical germplasm. To obtain these correlations, the dendrogram clustering pattern was considered and found to contain information that helped in establishing heterotic groups. In this way, the results showed that RAPD-based genetic distances could be used to establish consistent groups among maize inbred lines from tropical populations having a broad genetic base. In a traditional breeding program, thousands of crosses have to be done and F_1 grain yield has to be evaluated in experimental designs. According to our results, RAPD-based genetic distances could be used to help in the choice of the crosses to be made among tropical maize lines derived from a broad genetic base population, in this way reducing the number of single cross hybrids to be evaluated.

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