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Evaluating genetic relationships between tropical maize inbred lines by means of AFLP profiling

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Diversity among tropical maize inbred lines that compose breeding programs, is not well known. The lack of this information has made the arrangement of heterotic groups to be used for breeding purposes difficult. Methods of molecular analysis have been used as efficient alternatives for evaluating genetic diversity, aiming at heterotic group arrangement and acquisition of new hybrids. In this study, AFLP (amplified fragment length polymorphism) was used to investigate the genetic relationships among 96 tropical maize inbred lines from two different origins. The polymorphism level among the genotypes and the possibility of their allocation in heterotic groups were evaluated. Besides, correlations among genetic diversity and flowering time were analyzed. Nine primer combinations were used to obtain AFLP markers, producing 638 bands, 569 of which were polymorphic. Genetic similarities (GS), determined by Jaccard's similarity coefficient, varied from 0.345 to 0.891, with an average of 0.543. The dendrogram based on the GS and on the UPGMA cluster method did not separate the inbred lines in well-defined groups. Aiming at separating the lines into more accurate groups, Tocher's optimization procedure was carried out, 17 groups being identified. Association between flowering time and germplasm pools was detected. AFLP showed itself to be a robust assay, revealing a great power of detection of genetic variability in the tropical germplasm, and also demonstrated to be very useful for guiding breeding programs.

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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 crosses are pre-established. In the last five decades, a big number of maize lines has been developed from genotypes with a restricted genetic base. This causes the risk of loss of genetic diversity and restricts the possibility of crosses 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 also ensure long-term selection gains.

Different methodologies are available to investigate genetic diversity. Analyses based on morphologic and biochemical traits as well as on pedigree data have been used for this purpose for a long time and they have been showing distinct degrees of confidence. Morphologic characteristics are very limited, mainly because of environmental influence and, therefore, they do not always express genetic relationships. Besides, these traits reveal differences that are not comprehensible in terms of genetic distances (Smith and Smith 1989).

Biochemical data obtained by isozymes significantly overcome these problems, since proteins portray the genetic base with more fidelity. Nevertheless, the reduced coverage of the genome, due to few available and polymorphic loci, constitutes a striking factor for the generalized application of this technique (smith 1988).

Although there are several methods to study the diversity, none of them showed themselves to be as efficient as the molecular markers. Data obtained by molecular techniques overcome most limitations that exist in the other kinds of analysis. Characteristics such as (1) an almost unlimited number of markers, (2) absence of environmental influence, (3) a great number of polymorphic loci, (4) access to contribution of both parents and (5) possibility of comparing genotypes,

based on the DNA, make this type of marker very powerful for genetic diversity estimates.

In maize, restriction fragment length polymorphisms (RFLPs) have long been used for this purpose (Melchinger et al. 1990, 1992; Messmer et al. 1992; Gerdes and Tracy 1994). The greatest advantage of RFLP for maize analysis is the large number of polymorphic loci found in breeding materials (Messmer et al. 1992). Studies with elite lines from the U.S. Corn Belt and also with some European maize inbred lines showed that RFLPs are suitable to (1) define heterotic groups, (2) assign inbred lines to such groups, (3) reveal genetic relationships among lines and, (4) identify diverse germplasm sources. However, RFLPs show several drawbacks, which stimulated the development of alternative marker systems based on the polymerase chain reaction (PCR) such as the AFLPs – amplified fragment length polymorphisms (Zabeau and Vos 1993; Vos et al. 1995).

AFLPs, genomic fragments detected after selective PCR amplification, in addition to being highly reproducible, have the generation of multiple bands in a single assay as a principal advantage. The use of AFLP to estimate genetic diversity was demonstrated at first in 58 maize inbred lines (Smith et al. 1993, 1994), followed by other studies in rice (Mackill et al. 1996), soybean (Powell et al. 1996), barley (Russell et al. 1997), sugarcane (Besse et al. 1998; Lima et al. 2002), coconut (Perera et al. 1998; Teulat et al. 2000), cotton (Abdalla et al. 2001) and other species. In maize, AFLP markers were also used in the investigation of (1) correlations between genetic distance and heterose for profit (Ajmone-Marsan et al. 1998), (2) genetic variability among dent lines in the U.S. (Pejic et al. 1998), (3) diversity among selected lines in temperate climates (Chitto et al. 2000) and (4) relationships among precocious European maize lines (Lübberstedt et al. 2000). However, until this moment, no study with this marker has been carried out for tropical material.

Not much is known about genetic diversity among tropical maize inbred lines that compose breeding programs. Knowledge about genetic diversity in tropical material would allow more adequate choices of parents possible, optimizing the use of the genetic potential in hybrid programs. The objectives of this study were to use AFLP markers to genetically identify 96 tropical maize inbred lines, allocate them into heterotic groups and relate the information obtained with the flowering time of the lines.

MATERIAL AND METHODS

Plant material

A total of 96 tropical maize inbred lines, from the Agronomic Institute of Campinas (IAC) Genebank, Brazil, were analyzed, including 45 historical Brazilian inbred lines and 51 recent inbred lines derived from populations introduced from the CIMMYT. Identification, origin and information about the flowering time of these genotypes are described in Table 1. The abbreviations "AL", "IA", "IP", "PM", "SLP" and "VER" refer to Brazilian inbreds and the abbreviation "L" refers to CIMMYT-derived inbreds. All inbred lines were led to homozygosity by successive self-fertilizations.

Thirty seeds of each inbred line were planted in the field, at the Experimental Center in Campinas. Young leaves from at least 15 plants, from 6 to 8 weeks, were collected, freeze-dried (72 h, -60°C, 05 to 10 Hg microns), grounded to powder using a mechanical mill (Ciclotec – 1093 Sample Mill, Tecator) and stored in a -20°C freezer.

DNA extraction and quantification

Genomic DNA of the leaves was extracted following the method described by Hoisington et al. (1994). A total of 300 mg of leaves, freeze-dried and grounded to power, was used together with CTAB extraction buffer (100 mM Tris-pH 7.5, 700 mM NaCl, 50 mM EDTA pH 8.0), followed by two successive extractions with chloroform/isoamilic alcohol (24:1). The DNA of each sample was still submitted to a final extraction with phenol/chloroform and lunged with TE buffer (10 mM Tris pH 8.0; 1 mM EDTA pH 8.0). The DNA obtained was examined regarding its quality and concentration in 0.8% agarose gels, using increased concentrations of phage λ as a pattern.

AFLP analysis

AFLP analysis profiles were performed as described by Vos et al. (1995), using the "AFLP Analysis Kit" (Life Technologies-GIBCO BRL, Gathersburg, MD, USA), following the patterns of the manufacturer. The genomic DNA (400 ng) of each inbred line was isolated and digested simultaneously at 37°C, for two hours, by the *Eco*RI and *Mse*I enzymes. The resulting restriction fragments were linked in 24 µl of a specific adapter solution (*Eco*RI/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl pH 7.5, 10 mM Mg-acetate, 50 mM K-acetate) and 1 µl of DNA ligase and then diluted ten times in a TE buffer. Next, 5 µl of diluted DNA were amplified in a PTC^{TM}-100 termocyclator (Programmable Thermal Controller/MJ Research, Inc.) for 20 cycles (94°C for 30 s, 56°C for 60 s, 72°C for 60 s),

Table 1. *Identification of the 96 tropical maize inbred lines used in the AFLP-based genetic similarity assessment.*

Inbred line	Selected from:	Flowering time (days)	Origin	Inbred line	Selected from:	Flowering time (days)	Origin
AL124	CATETO	71	IAC-Brazil	L101	Pool27	63	CIMMYT-Mexico
AL218	CATETO	65	IAC-Brazil	L105	Pop.26	57	CIMMYT-Mexico
AL491	CATETO	72	IAC-Brazil	L110	Pop.24	60	CIMMYT-Mexico
AL516	CATETO	66	IAC-Brazil	L111	Pop.26	57	CIMMYT-Mexico
AL519	CATETO	65	IAC-Brazil	L112	Pop.26	65	CIMMYT-Mexico
AL526	CATETO	62	IAC-Brazil	L114	Pop.26	60	CIMMYT-Mexico
AL535	CATETO	64	IAC-Brazil	L116	Pop.27	57	CIMMYT-Mexico
AL583	CATETO	69	IAC-Brazil	L117	Pop.24	67	CIMMYT-Mexico
AL604	CATETO	65	IAC-Brazil	L118	Pop.27	65	CIMMYT-Mexico
AL614	CATETO	66	IAC-Brazil	L120	Pop.28	57	CIMMYT-Mexico
AL628	CATETO	65	IAC-Brazil	L121	Pop.27	61	CIMMYT-Mexico
AL673	CATETO	69	IAC-Brazil	L123	Pop.27	66	CIMMYT-Mexico
AL745	CATETO	65	IAC-Brazil	L126	Pop.27	60	CIMMYT-Mexico
AL758	CATETO	72	IAC-Brazil	L128	Pop.24	55	CIMMYT-Mexico
AL761	CATETO	77	IAC-Brazil	L130	ACROSS7543	60	CIMMYT-Mexico
IA278	CATETO	62	IAC-Brazil	L131	ACROSS7543	64	CIMMYT-Mexico
IA606	CATETO	64	IAC-Brazil	L132	Pool23	66	CIMMYT-Mexico
IA2938	CATETO	68	IAC-Brazil	L134	Pop.24	51	CIMMYT-Mexico
IA3040	CATETO	68	IAC-Brazil	L137	Pop.36	65	CIMMYT-Mexico
IACB	Pop.TX303	69	IAC-Brazil	L155	Pop.25	61	CIMMYT-Mexico
IP48	CATETO	70	IAC-Brazil	L156	Pop.36	62	CIMMYT-Mexico
IP301	CATETO	70	IAC-Brazil	L157	Pop.27	61	CIMMYT-Mexico
IP330	CATETO	61	IAC-Brazil	L158	Pop.27	54	CIMMYT-Mexico
IP365	CATETO	70	IAC-Brazil	L160	Pop.28	50	CIMMYT-Mexico
IP398	CATETO	71	IAC-Brazil	L161	Pop.26	53	CIMMYT-Mexico
IP661	CATETO	70	IAC-Brazil	L162	Pop.26	53	CIMMYT-Mexico
IP701	TUXPEÑO	61	IAC-Brazil	L163	Pop.26	51	CIMMYT-Mexico
IP3644	CATETO	65	IAC-Brazil	L164	Pop.27	62	CIMMYT-Mexico
IP3668	CATETO	66	IAC-Brazil	L165	Pop.27	63	CIMMYT-Mexico
IP3854	CATETO	63	IAC-Brazil	L166	Pop.28	51	CIMMYT-Mexico
IP3855	CATETO	67	IAC-Brazil	L167	Pop.36	50	CIMMYT-Mexico
IP3999	CATETO	67	IAC-Brazil	L168	Pop.24	62	CIMMYT-Mexico
IP4022	CATETO	64	IAC-Brazil	L169	Pop.26	62	CIMMYT-Mexico
L1	MJ268	54	CIMMYT-Mexico	L170	Pop.27	51	CIMMYT-Mexico
L2	MJ274	60	CIMMYT-Mexico	L171	Pop.28	64	CIMMYT-Mexico
L3	Pop.24	60	CIMMYT-Mexico	L172	Pop.28	60	CIMMYT-Mexico
L4	Pop.24	60	CIMMYT-Mexico	PM129	TUXPEÑO	63	IAC-Brazil
L5	Pop.26	61	CIMMYT-Mexico	PM219	TUXPEÑO	65	IAC-Brazil
L6	Pop.26	54	CIMMYT-Mexico	PM308	TUXPEÑO	65	IAC-Brazil
L8	Pop.28	61	CIMMYT-Mexico	PM421	TUXPEÑO	62	IAC-Brazil
L9	Pop.36	59	CIMMYT-Mexico	PM518	TUXPEÑO	69	IAC-Brazil
L10	Pop.36	52	CIMMYT-Mexico	PM624	TUXPEÑO	69	IAC-Brazil
L11	Pop.27	57	CIMMYT-Mexico	PM684	TUXPEÑO	64	IAC-Brazil
L12	Pop.27	66	CIMMYT-Mexico	PM888	TUXPEÑO	70	IAC-Brazil
L13	Pop.26	52	CIMMYT-Mexico	PM2837	TUXPEÑO	67	IAC-Brazil
L14	Pop.27	61	CIMMYT-Mexico	SLP103	TUXPEÑO	61	IAC-Brazil
L15	Pop.27	62	CIMMYT-Mexico	SLP365	TUXPEÑO	65	IAC-Brazil
L100	Pool27	62	CIMMYT-Mexico	VER266	TUXPEÑO	69	IAC-Brazil

using primers carrying one selective nucleotide. Products of pre-amplification were diluted fifty times in a TE buffer and were used as a template for selective amplification with two primers carrying three selective nucleotides: *Eco*RI 5′end-labeled with $\gamma[^{33}P]$-ATP (4000 Ci mmol^{-1}) and T4 polynucleotide kinase and *Mse*I without labeling. The reaction was amplified in the PTC^{TM}-100 termocyclator, using the following cycles: 94°C for 30 s, 65°C (− 0.7°C/cycle) for 30 s and 72°C for 60 s during 12 cycles, until the optimal annealing temperature of 56°C was reached, resulting in a total of 23 cycles which were necessary for complete amplification. The nine primer combinations used in the amplification are similar of those described by Vos et al. (1995) (Table 2).

Twenty µl of formamide buffer (98% formamide, 10 mM EDTA, 0.025% xylene cyanol w/v, 0.025% bromophenol blue w/v) were added to the selective

Table 2. *Number of polymorphic AFLP bands observed using 9 AFLP primer combinations.*

Primer combination	Number of bands	Polymorphic bands	Polymorphism rate(%)
E+AAC/M+CTC	83	74	89.15%
E+AAG/M+CTG	98	86	87.75%
E+AAG/M+CTC	109	98	89.91%
E+AAG/M+CAC	48	42	87.50%
E+AAC/M+CAT	68	61	89.70%
E+ACA/M+CAT	58	53	91.37%
E+ACA/M+CTG	59	50	84.74%
E+AAC/M+CAG	53	52	98.11%
E+AAC/M+CTT	62	53	84.12%
Totals	638	569	89.15%

amplified product, 3.5 µl of this mixture for each inbred line was applied in a 6% denaturing polyacrylamide gel and submitted to electrophoresis (Sequi-Gen® GT-Nucleic Acid-Electrophoresis Cell/BIO RAD Apparatus of electrophoreses), for 4 h in 75 W. Detection of AFLPs was made after transference to a paper filter (Whatman 3MM), covered with PVC film and vaccum dried (Gel Dryer Model 583, Hydrotech™ Vaccum Pump/BIO RAD) and exposed to a hypersensitive autoradiograph film (MP Hyperfilm™/ Amersham Life Science, UK) for fifteen days.

Polymorphism levels and genetic similarity estimation

Manual scoring of the autoradiographies was performed using a binary system, considering presence (1) or absence (0) of bands in each combination of genotypes. PICs – polymorphism information content – for the polymorphic loci were calculated using the PIC-AFLP routine developed in SAS software (SAS Institute 1999), planning to evaluate the discriminatory capacity of the AFLP markers and the importance of the alleles in the analysis of genetic diversity.

The matrix raw data was used to calculate genetic similarities among the maize inbred lines. Estimates of genetic similarity (GS) among all genotypes were calculated according to Jaccard's similarity coefficient (Jaccard 1908): $Gs_{ij} = a/(a + b + c)$, where Gs_{ij} corresponds to the genetic similarity between lines i and j, a is the number of polymorphic bands present in both individuals, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

Cluster analysis based on the similarity matrix was carried out using the unweighted pair-group method with arithmetic averages (UPGMA), as suggested by Sneath and Sokal (1973). The cophenetic coefficient between the similarity matrix and the cophenetic values matrix was also calculated. All preliminary clustering procedures were performed using the NTSYSpc software, version 2.1 (Rohlf 1997).

Tocher's optimization procedure

Complementary cluster analysis was carried out to obtain heterotic groups using Tocher's optimization procedure using the Genes Software (Cruz 2001). Diversity among inbred lines can be better visualized by the classification of an original group into several groups, according to some rule of similarity or dissimilarity. Analyses were performed using the dissimilarity matrix, and the average distances among the different groups obtained by Tocher's procedure were used to draw a dendrogram showing the relationships among groups.

Bootstrap analysis

A routine was developed for bootstrap analysis using SAS software version 8.2 (SAS Institute 1999) to verify if the number of polymorphisms detected by AFLP was enough to supply precise estimates of GS. Polymorphic markers were submitted to 500 bootstrap resamplings, as recommended by Tivang et al. (1994), simulating different number of markers (29, 74, 119, 164, 209, 254, 299, 344, 389, 434, 479, 524 and 569, the total number of markers obtained). Hence, 500 genetic similarity estimates for each pair of genotypes combination were obtained. The average, the variance and the coefficient of variation were estimated across the bootstraps samples for each number of markers. An exponential function was fitted to evaluate the effect of sample size in the accuracy of the GS estimates.

RESULTS AND DISCUSSION

Levels of polymorphism

Nine primer combinations were selected from a previous study (Barbosa et al. 2003), where 20 out of the 64 possible combinations were used. The importance of appropriate selection of primer combi-

nations, which show greater levels of polymorphism for the whole genome, has been described in investigations of diversity analysis (Qi and Lindhout 1997; Lima et al. 2002) as well as in mapping studies using AFLP profiles (Castiglioni et al. 1999). Thus, the 9 combinations, which displayed the higher level of polymorphism among the genotypes, were chosen.

Analysis of the 96 tropical maize inbred lines, with the primer combinations selected, identified 638 AFLP fragments, 569 of which (89.18%) were polymorphic. These 569 markers were then used to estimate the genetic similarity between the genotypes. The number of polymorphic fragments detected for each pair of primers, ranged from 42 (EAAG/MCAC) to 98 (EAAG/MCTC), with an average of 63 polymorphic fragments per combination. Based on the percentage of polymorphic bands, levels of polymorphism were calculated and they varied from 84.12% (EAAC/MCTT) to 98.11% (EAAC/MCAG). The selected primers, the number of polymorphic bands and the rate of polymorphism among lines are listed in Table 2.

PIC has been used in marker comparison studies concerning the analysis of polymorphism levels (Hongtrakul et al. 1997; Lübberstedt et al. 2000; Manifesto et al. 2001; Garcia et al. 2004). In our work, a great number of markers presented PIC values between 0.8 and 1.0, but the average was 0.68. The level of polymorphism observed in this study agrees with previous studies, which determined the great amount of polymorphism detected in maize by molecular markers (Dudley et al. 1991; Ajmone-Marsan et al. 1995; Smith et al. 1997). Despite the applicability of the different types of markers in detecting polymorphism, AFLP efficiency has been shown to be higher than the efficiency of other markers, due to its capacity of revealing a great number of bands per reaction (Ajmone-Marsan et al. 1995; Pejic et al. 1998; Lübberstedt et al. 2000, Garcia et al. 2004). It should be considered, however, that this level of polymorphism is not only due to the technique used but also to the considerably high diversity among the genotypes analyzed.

Evaluation of number of markers

To verify if the number of loci used was sufficient to obtain genetic distances with good precision, King et al. (1993), Halldén et al. (1994) and Tivang et al. (1994) employed the bootstrap-analysis method. With the same purpose, this analysis was also used for our data, and, as expected, it was shown that the accuracy of the GS estimates increased according to the growth of the number of polymorphic loci analyzed. Based on graphical analyses (data not shown), it was observed that the mean coefficient of variation (CV) decreased with an increase in the number of AFLP markers used. A mean CV of 5.43% was obtained using 569 polymorphic markers. An average CV around 10% has been recommended as a suitable value, and using this CV, accepted in literature, about 168 markers would have been sufficient to obtain this level of precision. Nevertheless, Garcia et al. (2004) proposed the use of a mean CV of 5%, due to the need for more precision. Even working with this lower margin, it is possible to conclude that the number of markers used is adequate.

Genetic similarities and cluster analysis

Jaccard's similarities were calculated using presence/absence of bands in the autoradiographies, where only the bands with good resolution and constancy in the gels were considered. Jaccard's coefficient has been preferred in plant breeding and evolution studies with dominant markers, due to its good comparison capacity in analyzing genotypes of the same species, when more genetic similarities are expected (comparing with genetic estimates made among genotypes from different species, where more diversity is seen). The genetic similarity estimates among the 96 maize inbred lines varied from 0.345 (IA606 $\times$ L110) to 0.891 (L171 $\times$ PM129), with a mean of 0.543. The high similarity observed between lines L171 and PM129 was not expected since these lines came from different genetic pools (Pop. 28 from CIMMYT, which is characterized by yellow dents, and race Tuxpeño, which is composed of white flints, respectively). This high similarity may be explained on the basis of wrong line identification, or on the probability of alikeness in state instead of identity by descent.

A dendrogram based on the similarity values was constructed using the UPGMA method to illustrate genetic relationships among the different genotypes (Fig. 1). The cophenetic coefficient, which shows the approximation of the dendrogram to the similarity matrix, was 0.77. In the dendrogram, precise separation of the groups was not observed. The absence of major genetic differences among these lines, which is reflected by the moderate mean similarity value, may explain this result. Most CIMMYT-derived lines joined in a big initial group in the dendrogram (Group A), whereas IAC "AL" and "IP" lines formed another group (Group C) (Fig. 1). The fact that these lines were found to be well-separated from the CIMMYT-derived lines group is an indication that good hybrids can be obtained from crosses between them, once they may have significant divergence among each other. Separation of these groups in the dendrogram may have reflected their distinct germplasm pool origins.

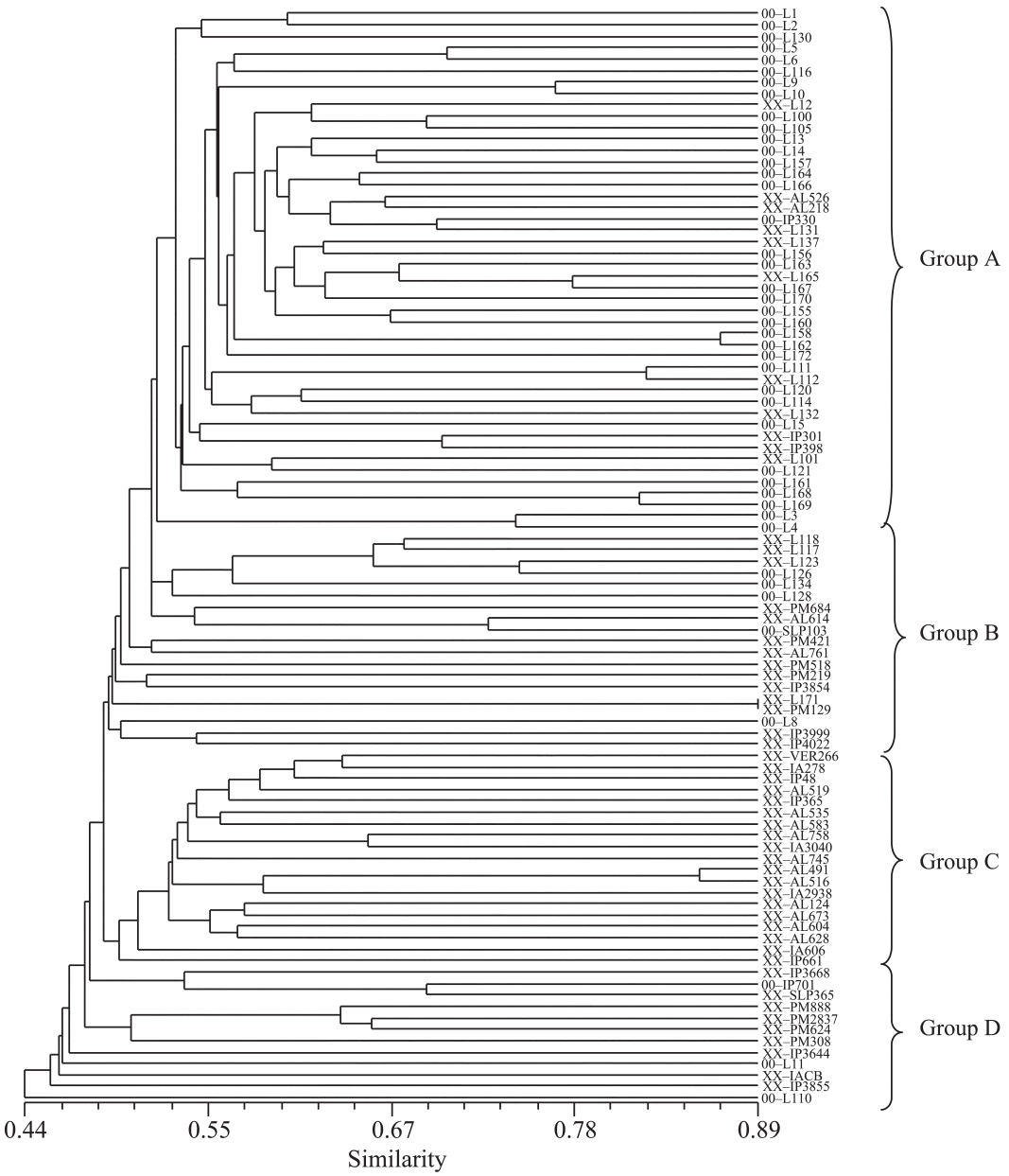

Fig. 1. Dendrogram of the 96 tropical maize lines revealed by UPGMA cluster analysis method and Jaccard's similarity coefficient ($r_{cof} = 0.77$). "00" indicates the precocious lines (50–62 days); "XX" indicates normal and late lines (62–77 days).

A third group could be observed in the dendrogram (Group B), joining some other CIMMYT-derived lines, which differ from group A lines, and IAC "IP" and "PM" lines. Besides, a fourth group, which seems to diverge much from the others, was observed, gathering together lines from different origins (Group D).

Classification of an elite germplasm into heterotic groups and assignment of inbred lines to these established groups are major decisions in any maize hybrid program (Hallauer et al. 1988). Studies with the U.S. maize (Melchinger et al. 1990) and with the European maize (Lübberstedt et al. 2000) corroborate the utility of molecular markers in the allocation of lines in different heterotic groups, emphasizing the efficiency of genetic similarities to a more precise establishment of groups.

With the aim of allocating the lines in more defined groups, Tocher's optimization procedure was employed as a complementary tool. Considering that one of the main objectives of this study is to determine genetic diversity among the lines and to predict the

Table 3. *Different groups of lines obtained using Tocher's optimization procedure. Precocious lines in italic. Normal and late lines in bold.*

Groups	Lines
1	*L1 L2 L3 L5 L6 L9 L10* **L12** *L13 L14 L15 L100 L111* **L112** *L116 L120* **L123** *L126 L128* **L137** *L155 L156 L157 L158 L162 L163 L164* **L165** *L166 L167 L170* **L171 PM129 PM888 VER266 IA278 IP301 IP365 IP398 AL491 AL614 AL218 L101 L117** *L121 L160 L161 L168 L169 SLP103 L105 L114 L130* **L132** *IP330* **IA2938 IA3040 L131** *L172* **AL526 PM421**
2	*IP701* **SLP365 IP3668**
3	**PM2837 PM624**
4	**IP48 AL519 AL535 AL758 AL745 AL673 AL628 AL604 AL124 AL583 IA606 AL516 AL761**
5	**L118** *L134*
6	**IP3999 IP4022**
7	**PM518 PM684**
8	**PM219 IP3854**
9	*L4*
10	*L8*
11	*L11*
12	**IP661**
13	**IP3644**
14	**IACB**
15	*L110*
16	**PM308**
17	**IP3855**

best crosses to produce more vigorous hybrids, a more precise determination of the groups was evaluated. This analysis allowed the identification of 17 different groups among the 96 lines studied (Table 3). The groups were separated so that there was homogeneity into groups and heterogeneity among them. The most homogeneous were groups 1 and 4; group 1 was composed basically of the CIMMYT-derived lines and, group 4 was composed mainly of the IAC "AL" lines.

The dendrogram among these groups is presented in Fig. 2, where better visualization of the results is presented. In addition, Table 3 shows the allocation of all lines to each of the 17 groups obtained. Breeders, therefore, can base themselves on this assembly to determine the genotypes to be crossed, aiming at the best line combinations.

Relationships between groups and flowering time

Flowering time is considered to be quantitatively inherited, and different studies have identified loci that affect this trait in maize (Beavis et al. 1991). Several investigations have used molecular markers to identify quantitative trait loci (QTL) controlling flowering time (Koester et al. 1993; Berke and Rocheford 1995; Austin and Lee 1996). Data concerning this trait in our inbreds (Table 1) were also used in diversity analysis. Lines with flowering times between 50 and 62 days were considered early and lines with flowering times between 62 and 77 days were considered normal to late.

A moderate relationship was observed between the groups formed on the basis of similarity analysis and flowering times. In general, early lines corresponded to lines of group 1, while the other lines, which are normal and late, came across dispersed among the other groups (Table 3).

Like the information obtained about the lines, it is known that the ones derived from CIMMYT are lines introduced around 1995, while "IP" and "AL" lines are older lines, that date from nearly 20 to 30 years ago. The dendrogram (Fig. 1) shows separation of lines in basically two groups: (1) lines with more recent origin, clustering like those with early maturity and (2) older lines, clustering like lines with late cycles.

Most CIMMYT-derived lines presented cycles with early maturity and were allocated in group 1. However, some lines such as L134 (group 5), L4 (group 9), L8 (group 10), L11 (group 11) and L110 (group 15), all considered early, were separated into different groups. Thus, these lines are very useful in breeding programs because they can act as good parents for crossing with any other early line of group 1. Coincident flowering times and divergent genotypes would make the pollination procedures easier and would also enhance the possibilities of producing good hybrids. Paterniani et al. (2000), in a study with hybrids resulting from crosses between some of these lines, observed that the hybrids derived from the cross between line L4 (group 9) and line L10 (group 1) and also hybrids resulting from the cross between line L10 (group 1) and line L11 (group 11) presented the

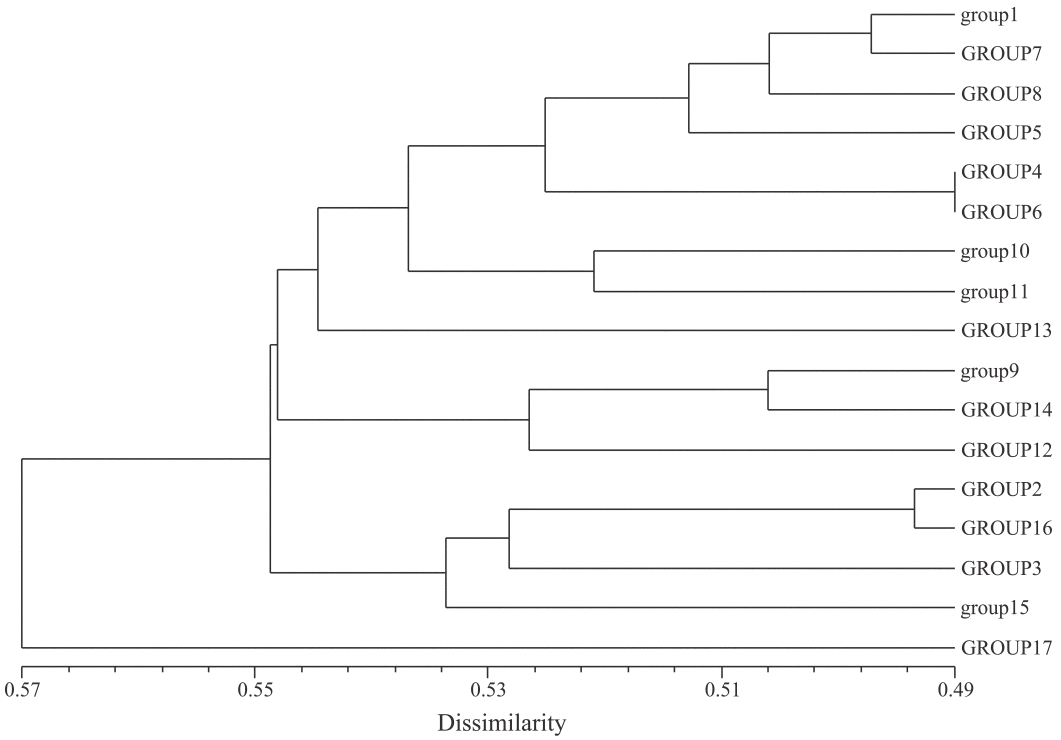

Fig. 2. Dendrogram (UPGMA) of the 17 different groups obtained through Tocher's optimization procedure. Lower case letters represent groups in which the majority of the lines are recent. Capital letters represent groups in which the majority of the lines are normal/late.

best performance in all localities studied, confirming the great heterosis and adaptability of these genotypes, in addition to the good grain predictability. The hybrids deriving from the crosses between lines of the same group (group 1) had the worst performance, the probable reason being the low genetic divergence between lines and the high susceptibility to diseases.

The tropical maize inbred lines analyzed presented considerable divergence, which was observed in the similarity matrix. Therefore, many crosses can be planned based on the results of this study. Molecular information of genetic distance is showing to be important in tropical maize germplasm analysis. Our results can help the genetic base management during the process of breeding programs. Besides, they can also help the evaluation of germplasm collection redundancies and deficiencies, creating data about efficiency of the collection process, maintenance, management and enlargement of a germplasm bank.

Besides presenting a profile of the genetic diversity among lines of the tropical germplasm, the results obtained in this study will also be of great help to the breeding programs that will use the lines of the IAC germplasm bank, because they supply information about the genetic diversity and about their allocation in putative different heterotic groups.

Temperate material has been exploited for several decades and many generations of selection have been completed, resulting in a highly productive germplasm (Hallauer et al. 1988). In general, temperate populations are composed of synthetics made up of a few inbred lines, whereas tropical maize populations are usually composites made up of crosses of several populations (Lanza et al. 1997), constituting genotypes with a wide genetic base that are the principal material for maize breeding programs in tropical countries.

Literature presents little work about the diversity among tropical maize genotypes. Initial work using molecular markers for a small number of inbreds revealed great diversity in the tropical material (Lanza et al. 1997; Benchimol et al. 2000). The present study, with 96 lines developed at different ages and from different germplasm sources, disclosed another important genetic pool available in the tropical germplasm.

As previously discussed, the tropical maize germplasm did not have the same systematic development of the temperate germplasm, where a great number of populations and inbred lines was developed from three principal heterotic groups, since the beginning of the 40's (Smith et al. 1985).

Whereas genetic diversity analyses employing molecular markers have only been confirming the allocation of temperate maize lines to already known groups, studies carried out with tropical germplasm have been uncovering a complex genetic organization, allocating lines in previously unknown groups.

Thus, the results obtained indicate that the use of molecular markers is an interesting way of evaluating and establishing different tropical maize genotypes. Such proceedings will allow a quick allocation of the different genotypes of heterotic groups that will greatly help the choice of crosses to be carried out in order to obtain hybrids. Also, it will decrease the costs and will increase the efficiency of maize breeding programs developed in the tropics.

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