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Research Article

Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers

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Abstract

The genetic relationships among 81 maize accessions consisting 79 landraces and two improved varieties, maintained by farmers in southern Brazil were investigated using Random Amplified Polymorphic DNA (RAPD). Thirty-two highly informative primers amplified 255 markers of which 184 (72.2%) were polymorphics. Based on the RAPD markers, a dendrogram was constructed using the UPGMA method. The range of genetic similarity was from 0.78 to 0.91. The molecular data grouped the accessions into two main clusters, which were correlated according to kernel colors. Small clusters were seen associated to characteristics, such as kernel morphology. The analysis of the molecular data revealed that maize management adopted by small-scale farmers has contributed to the maintenance of genetic variability and since field isolation is a regular practice, variety identities have been preserved. These results will be useful to establish and maintain a germplasm collection of landrace maize and may guide us in designing strategies that maximize the utility of maize genetic resources.

Key words: corn landrace, genetic variability, molecular genetic markers, RAPD.

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Introduction

Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm characterization of plant materials. Therefore, it is imperative to rationalize conservation and use of genetic resources to guide in the establishment of strategies that ensure the maintenance of genetic variability that is essential in plant breeding. World collections of maize comprise about 12,000 accessions that are represented in 256 races, of which about 30 are in the process of extermination (Machado *et al*., 1998). It is estimated that only around 2% of the maize germplasm is utilized in breeding programs and an important fraction is cultivated and conserved by small landholder farmers. While most of the genetic variability is represented within and between landraces maintained by the traditional family farming systems (Marshall, 1977), there was agreement, during the IV International Conference of Plant Genetic Resources in Germany (1996), that the main reason for accelerated genetic erosion was the substitution of maize landraces for a small number of improved varieties. Data reported by the Food and Agriculture Organization (FAO) in 1996 indicated that 20% of maize varieties from Mexico have disappeared since 1930. In addition, 91% of maize varieties used in USA in the beginning of the 20th century have also disappeared and today all production is based on less than ten hybrids.

Many racial complexes are considered important for maize improvement, including Dents of México (Tuxpeño, Vandeño, Tepecintle, Zapalote, Zapalote Chico, Grande and Celaya), Dents of the Corn Belt of the United States (Reid, Lancaster and Krug), Dents of Caribbean (Tusóns), Flints of Caribbean (Coastal, Tropical Flints, Comuns and Costeños), Catetos (flint orange colored maize from Brazil, Argentina and Uruguay), and Flint and Floury (maize from Northern United States and Southern Canada) (Paterniani *et al*., 2000). Today, maize germplasms are represented by 3,800 accessions of which approximately 288 are introductions, 222 are populations with some genetic improvement, and 1,783 are assessments from different Brazilian regions (Abadie *et al*., 2000).

Maize landraces and creolized varieties have been broadly and independently cultivated throughout Brazilian regions and they are of relevant socio-economic importance for the family farming systems. As a result, different accessions are developed and selected for different environments and morphological characteristics (Paterniani *et al*., 2000). The genetic diversity of landraces is, therefore, the most immediately useful part of maize biodiversity. However, more consistent agronomic and genetic knowl-

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edge about these collections is still lacking and it is a serious limitation to utilizing, managing, and conserving the landrace maize gene pools (Nass *et al*., 1993).

The development of modern plant breeding techniques has greatly facilitated wider use of a wealth of diversity from many sources including landraces, and especially, has allowed food production to keep up with population growth (Wood and Lanné, 1999). Currently, the genetic diversity of plants has been assessed more efficiently after the introduction of methods that reveal polymorphism directly from the biochemical and DNA levels. Markers based on isoenzymes (Prince *et al*., 1986; Lankey *et al*., 1997) and RFLP (Lee *et al*., 1989; Bernardo, 1994) were the first molecular markers used in maize breeding programs. More recently, markers based on polymerase chain reaction (PCR), such as random amplified polymorphic DNA or RAPD (Williams *et al*., 1990) have been used in analysis of genetic distance in several plant species (Sharma *et al*., 1995; Gunter *et al*., 1996; Lashermes *et al*., 1996; Samec and Nacinec, 1996; Irvin *et al*., 1998; Colombo *et al*., 2000). Comparisons among the different types of markers have contributed to the selecting of the most appropriate technique related to desired objectives. RAPD markers are commonly used because they are quick and simple to obtain, enabling genetic diversity analysis in several types of plant materials, such as natural populations, populations in breeding programs and germplasm collections (Ferreira and Grattapaglia, 1996). When compared with markers based on RFLP, RAPD markers have been shown to be equivalent in determining intraspecific genetic diversity among genotypes of *Brassica oleracea* L. However, RAPD markers were superior when simplicity and cost were considered (Dos Santos *et al*., 1994). Similar results were reported for estimating the genetic relationships among and within cruciferous species (Thormann *et al*., 1994). In maize, RAPD markers have been used in the analysis of genetic distance among segregant lines (Marsan *et al*., 1993) to predict the best crosses among lines for hybrid development (Lanza *et al*., 1997), and to assess genetic diversity among collections of native maize (Moeller and Schall, 1999).

The goal of this research was to investigate the level of genetic diversity in 79 cultivated accessions of maize landraces and two commercial varieties. This will contribute in identifying efficient strategies for the sustainable management of the genetic resources of the landraces in study.

Materials and Methods

Plant material

Plant material consists of 81 maize accessions, comprising two commercial varieties, developed at the Instituto Agronômico do Paraná (IAPAR), and 79 landraces obtained from the Assessoria e Serviços a Projetos em Agricultura Alternativa (AS-PTA), a non-governmental organization that coordinates a program for collection and conservation of maize landraces (Table 1). According to Nass and Paterniani (2000), only 14% of the accessions of maize germplasm maintained in Brazil are used and little is known about the genetic variability of the different collec-

Table 1 - Accessions of maize studied, morphological characteristics and locality.

Accessions	Endosperm color	Kernel type	Flowering times (days)	Length kernel (mm)	Width kernel (mm)	Thickness kernel (mm)	City/State[10]
1 - Asteca	yellow-orange	dent	74	medium	medium	short	Rio Azul/PR
2 - Asteca antigo do Prestupa	yellow-orange	dent	78	medium	medium	medium	Bituruna/PR
3 - Asteca Baixo Sabugo Fino	yellow	dent	73	medium	medium	short	Porto União/SC
4 - Asteca Sabugo Fino	yellow-orange	dent	76	medium	medium	medium	São João do Triunfo/PR
5 - Astecão Antigo	yellow	dent	75	medium	long	medium	Bituruna/PR
6 - BR 473[6]	yellow-orange	semi-dent	69	medium	medium	medium	Porto União/SC
7 - BR106[7]	yellow-orange	dent	73	medium	long	medium	Bituruna/PR
8 - Cabo Roxo[4]	yellow	dent	74	long	medium	medium	São João do Triunfo/PR
9 - Caiano	yellow-orange	dent	78	medium	medium	medium	Bituruna/PR
10 - C 408 x AG[8]	yellow-orange	dent	71	medium	medium	medium	Rio Azul/PR
11 - Carioca	yellow	dent	75	medium	medium	medium	Bituruna/PR
12 - Comum Antigo x Sabugo Fino	yellow-orange	dent	73	medium	long	medium	Rio Azul/PR
13 - Cravinho do Prestupa	yellow-orange	dent	76	short	medium	short	Bituruna/PR
14 - Cravinho Sabugo Grosso	yellow-orange	dent	78	medium	medium	medium	Cruz Machado/PR
15 - Cunha Amarelo	yellow-orange	dent	73	medium	long	medium	Rio Azul/PR
16 - Dente de Cotia	yellow	dent	76	medium	medium	medium	Cruz Machado/PR
17 - Ivo Agostiniak	yellow-orange	dent	76	medium	long	medium	Cruz Machado/PR
18 - Macaco	yellow-orange	dent	75	medium	long	medium	Porto União/SC

Table 1 (cont.)

Accessions	Endosperm color	Kernel type	Flowering times (days)	Length kernel (mm)	Width kernel (mm)	Thickness kernel (mm)	City/State[10]
19 - Maia	yellow-orange	dent	76	medium	long	medium	Cruz Machado/PR
20 - Milho Faxinal	yellow-orange	dent	73	medium	medium	medium	São Mateus do Sul/PR
21 - Milho Sem Nome	yellow-orange	semi-dent	74	medium	medium	medium	Palmeira/PR
22 - Ouro Verde	yellow-orange	dent	73	medium	medium	medium	Iratí/PR
23 - Palha Roxa	yellow-orange	dent	74	long	long	medium	Porto União/SC
24 - Palha Roxa	yellow	dent	73	long	long	short	São João do Triunfo/PR
25 - Sete Variedades	yellow-orange	dent	73	medium	medium	medium	Porto União/SC
26 - Sol da Manhã	yellow-orange	semi-dent	69	short	medium	medium	Palmeira/PR
27 - Azcril	yellow	dent	76	medium	long	medium	Cruz Machado/PR
28 - Cabo Roxo[5]	segregant	dent	76	medium	long	medium	São João do Triunfo/PR
29 - Pintado	yellow-orange	dent	72	medium	long	medium	Porto União/SC
30 - Sangue do Adão[2]	yellow-orange	dent	75	medium	medium	medium	Bituruna/PR
31 - IAPAR 51[1]	yellow	dent	73	medium	medium	medium	IAPAR, Londrina /PR
32 - Amarelão Antigo	yellow	dent	77	medium	long	medium	Porto União/SC
33 - Amarelão Bazonni	yellow-orange	dent	80	long	long	medium	Porto União/SC
34 - Amarelão Diwietz	yellow	dent	74	medium	medium	medium	Porto União/SC
35 - Amarelo Antigo do Valdivino	yellow-orange	dent	80	medium	long	medium	Bituruna/PR
36 - Amarelo do Tião	yellow-orange	dent	79	medium	long	short	Rebouças/PR
37 - Amarelo Graudo	yellow-orange	dent	77	medium	long	medium	Rio Azul/PR
38 - Amarelo Taguari	yellow-orange	dent	79	medium	long	medium	Rio Azul/PR
39 - Antigo 30 anos	yellow-orange	dent	82	medium	long	medium	Iratí/PR
40 - Antigo Linha 5	yellow-orange	dent	80	medium	long	short	Iratí/PR
41 - Cravinho do Zeno	orange	dent	79	medium	medium	short	Cruz Machado/PR
42 - Dente de Rato	yellow-orange	dent	80	medium	medium	medium	Iratí/PR
43 - Encantilado	yellow-orange	dent	83	medium	long	medium	Cruz Machado/PR
44 - Linha Paraná	yellow-orange	dent	81	long	long	medium	Cruz Machado/PR
45 - Milho Antigo	yellow-orange	dent	78	medium	long	medium	Palmeira/PR
46 - Milho Antônios I	yellow-orange	dent	80	medium	long	medium	Iratí/PR
47 - Milho Caxoeira	yellow-orange	dent	78	medium	long	medium	São João do Triunfo/PR
48 - Milho Fabrício Darci	yellow-orange	dent	78	medium	long	medium	São João do Triunfo/PR
49 - Milho Ferrinho	yellow-orange	semi-dent	77	short	medium	medium	União da Vitória/PR
50 - Milho Gropires	yellow-orange	dent	78	medium	long	medium	Palmeira/PR
51 - Milho Pires	yellow-orange	dent	78	long	medium	medium	Cruz Machado/PR
52 - Palha Roxa Alicheski	yellow	dent	78	medium	long	medium	São João do Triunfo/PR
53 - Pirulim do Tadeu	yellow-orange	dent	80	long	medium	medium	Bituruna/PR
54 - Indígena[3]	yellow	dent	83	medium	medium	medium	Cruz Machado/PR
55 - IAPAR 50[1]	yellow-orange	dent	78	medium	long	medium	IAPAR, Londrina/PR
56 - Antigo	segregant	dent	78	medium	long	medium	Rio Azul/PR
57 - Antigo Venglarek	segregant	dent	75	medium	long	medium	São Mateus do Sul/PR
58 - Asteca Branco Sabugo Fino	white	dent	75	long	long	short	São João do Triunfo/PR
59 - BR 451 (QPM)[9]	white	dent	71	medium	medium	medium	Rebouças/PR
60 - Branco Comum	white	dent	75	medium	long	medium	Rio Azul/PR

Table 1 (cont.)

61 - Branco do Ferraz	white	dent	78	medium	medium	medium	São Mateus do Sul/PR
62 - Bromado	white	dent	71	medium	medium	medium	São João do Triunfo/PR
63 - Bugre Branco	white	dent	75	medium	very long	medium	Rio Azul/PR
64 - Casano	white	dent	79	short	very long	medium	Rio Azul/PR
65 - Cinquentinha	white	dent	75	medium	medium	medium	Cruz Machado/PR
66 - Milho Branco do Vicente Huk	white	dent	73	medium	medium	medium	Rebouças/PR
67 - Oito Carreiras	white	dent	76	medium	very long	medium	Cruz Machado/PR
68 - Tostão Oito Carreiras	white	dent	75	short	very long	medium	Rio Azul/PR
69 - Asteca Branco	white	dent	81	medium	medium	medium	Rio Azul/PR
70 - Astecão Branco	segregant	dent	80	medium	long	medium	São João do Triunfo/PR
71 - Branco	segregant	dent	84	medium	long	medium	Rio Azul/PR
72 - Branco de Cercado	white	dent	83	medium	long	medium	Palmeira/PR
73 - Branco do Norte	white	dent	81	medium	long	medium	Iratí/PR
74 - Branco dos Borges	white	dent	83	medium	long	medium	Rebouças/PR
75 - Branco Mexicano	segregant	dent	82	medium	long	medium	Palmeira/PR
76 - Branco Lastek Dinart	white	dent	83	medium	long	medium	São João do Triunfo/PR
77 - Cunha Branco	white	dent	83	medium	long	medium	Iratí/PR
78 - Maizena	white	farinaceous	80	medium	long	medium	Cruz Machado/PR
79 - Milho Branco Palha Roxa	segregant	dent	81	medium	long	medium	Cruz Machado/PR
80 - Milho Mexicano	white	dent	81	medium	long	medium	Cruz Machado/PR
81 - Tostão	white	dent	79	short	very long	medium	Rebouças/PR

[1]Improved variety developed at Instituto Agronômico do Paraná (IAPAR).
[2]Variety with red aleurone.
[3]Accessions with blue aleurone.
[4,5]Varieties with the same name, cultivated in same city, but planted by different local communities.
[6,7,8,9]Commercial varieties submitted to massal selection by local farmer for several years.
[10]Origin of maize accessions. PR indicates Paraná State and SC indicates Santa Catarina State.

tions. This is a limiting factor in the use of these germplasm in breeding programs.

The present collection, which represents a small part of the Brazilian maize germplasm, comprises 79 maize landraces cultivated by small regional landholder farmers of Paraná and Santa Catarina States (Figure 1) and maintained in reproductive isolation by traditional agriculture for many years of farmer-directed selection. This collection includes the dent and floury kernel types and also different kernel colors that are conditioned by endosperm (yellow, orange, yellow-orange, and white endosperm) or by pericarp and aleurone colors (colorless, red, and blue, and blotched in many tones) (Table 1). According to Nass and Paterniani (2000), color and kernel types and flowering time can be used in the germplasm evaluation. Six kernel types (dent, flint, popcorn, floury, sweet and waxy) have been described in maize (Bandel, 1987).

In maize, flowering time generally occurs between 40 and 100 days after germination, even though it is highly influenced by environmental conditions (Goodman and Smith, 1987). The maize landraces used in this manuscript showed a smaller variation in flowering time. They were evaluated in a same locality and the flowering time occurred between 69 and 84 days (Figure 1, Table 1).

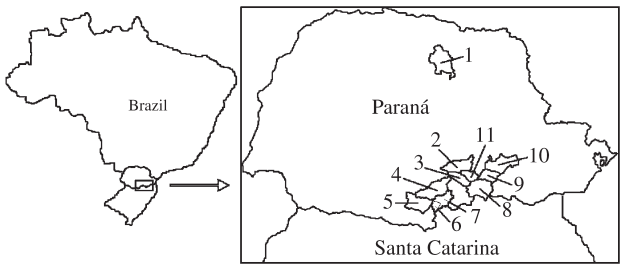

Figure 1 - Geographic localities of maize collection used in this study. **1**, Londrina, PR (accessions 5 and 50); **2**, Iratí (accessions 22, 39, 40, 42, 46, 73 and 77); **3**, Rio Azul, PR (accessions 1, 10, 12, 15, 37, 38, 56, 60, 63, 64, 68, 69 and 71); **4**, Cruz Machado, PR (accessions 14, 16, 17, 19, 27, 41, 43, 44, 51, 54, 65, 67, 78,79 and 80); **5**, Bituruna, PR (accessions 2, 5, 7, 9, 11, 13, 30, 35, and 53); **6**, Porto União, SC (accessions 3, 6, 18, 23, 25, 29, 32, 33 and 34); **7**, União da Vitória, PR (accession 49); **8**, São Mateus do Sul, PR (accessions 20, 57 and 61); **9**, São João do Triunfo, PR (accessions 4, 8, 24, 28, 47, 48, 52, 58, 62, 70 and 76); **10**, Palmeira, PR (accessions 21, 26, 45, 50, 72 and 75); **11**, Rebouças, PR (accessions 36, 59, 66, 74 and 81). The numbers are listed in Table 1. PR = Paraná State and SC = Santa Catarina State.

DNA extraction and amplification

Total DNA was extracted from bulked leaves containing equivalent proportions of leave tissue from 15 plants for each population, for a total of 81 bulks. The DNA was extracted using the CTAB procedure (Doyle and Doyle, 1989). DNA samples were quantified in a fluorometer (DyNA-Quant-200, Hoeffer-Pharmacia) and the concentration adjusted to 10 ng/µL. RAPD reactions were done in a volume of 15 µL containing 1x PCR buffer (75 mM Tris-HCl pH 9.0, 50 mM KCl, 2.0 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 0.3 mM of each dNTP (dCTP, dGTP, dTTP, dATP), 0.4 µM of 10-mer primers (Operon Technologies), 0.7 U of *Taq* polymerase (Biotools), and 20 ng of template DNA. Amplifications were carried out in a PTC-100 Thermocycler (MJ Research) with the following program: 1 initial denaturation step at 94 °C for 2 min followed by 47 cycles at 94 °C for 1 min, 38 °C for 1.45 min, and 72 °C for 2 min and a final cycle at 72 °C for 7 min. The amplified products were separated by electrophoresis in 1.4% Methaphor (FMC Bioproducts) agarose in 1 x TAE buffer (Tris-acetate 0.04 M and EDTA 0.01 M pH 7.5), containing 0.15 µg/µL of ethidium bromide. The gels were photographed under UV light and the images transferred to a microcomputer for future analysis. A 100 base pairs DNA ladder (GIBCO BRL) was included in the gels as standard molecular weight.

Morphological and molecular data analysis

The morphological characteristics listed in Table 1 were evaluated in a randomized complete-block design with five replications. Each plot consisted of two 5 m rows spaced at 0.9 m between the rows, with a total of 40 plants. The experiment was conducted in the experimental field of IAPAR during the years 2000/2001.

Each RAPD product was assumed to represent a single locus and data were scored as the presence (1) or absence (0) of a DNA band. Only those fragments consistently amplified were considered for analysis. Genetic similarities were calculated according to the simple matching coefficient (Gover, 1985) and a dendrogram was created based on the UPGMA (unweighted pair-group method using arithmetical averages) method (Sneath and Sokal, 1973) of the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System for personal computers), Version 2.1 package (Rohlf, 2000). Estimates of the bootstrap standard deviation were based on 1000 samples using a simple matching coefficient (Gover, 1985). The calculations were performed with the Dboot version 1.1 (Coelho, 2001).

Results and Discussion

RAPD marker analysis

Preliminary studies involved screening 212 primers against the DNA of five plants from the maize landraces to test their ability to produce polymorphism. One hundred and eighty primers (85%) yielded either monomorphic or unreproducible fragments (data not shown). The remaining 32 primers provided reliable and consistent polymorphic bands and were then used to amplify genomic DNA of the 81 maize accessions (Table 1). A total of 255 fragments, in a range of 104 (OPAX-07) to 2270 (OPX-13) base pairs, were scored with an average of 8 fragments per primer (Table 2). Strong and weak bands were produced in the RAPD reactions. Weak bands result from low homology between the primer and the pairing site on the DNA strand (Thormann *et al*., 1994). The weak bands were, therefore, disregarded to increase analysis precision. The pattern of amplified products generated with OPW-08 primer is shown in Figure 1.

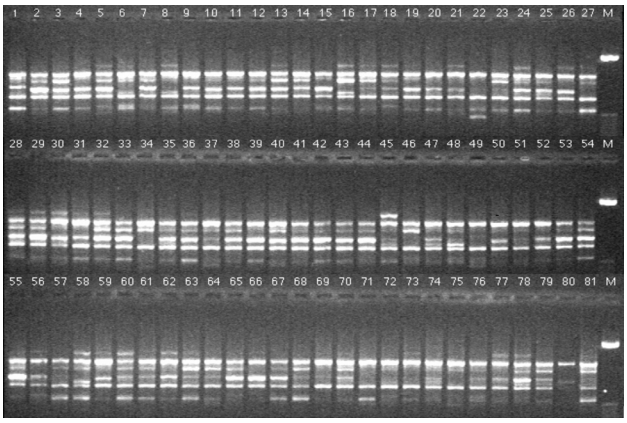

Figure 2 - RAPD gel profile with fragments generated by primer OPW-08 in 81 accessions of maize. Lines 1 through 81 refer to the collection listed in Table 1. M is 100 bp DNA size marker (Gibco BRL).

The percentage of polymorphism (72%) was similar to the values observed in the genetic analysis of endogamic maize lines (Lanza *et al*., 1997), in the study of maize hybrids (Heun and Helentjaris, 1993), and in the evaluation of genetic polymorphism among varieties of native American maize as reported by Moeller and Schall (1999). RAPD markers have also been extensively used in assessing genetic variation in other plant species such as *Colocasia esculenta* (Irvin *et al*., 1998), *Vigna* species (Santala *et al*., 1998), and potato germplasms (Forapani *et al*., 1999).

Dendrogram stability is an important consideration in genetic variability studies. In this study, the number of bands necessary to obtain a stable classification of all accessions was estimated using bootstrap analysis. The calculations of bootstrap showed that the rate of decrease was comparatively minimal beyond 150 markers (Figure 3) suggesting that 255 markers are adequate for assessing the genetic variation present in the 79 landraces and two improved varieties (coefficient of variation = 2.8%). Similar results have been revealed in other maize studies. Analysis of genetic diversity involving endogamic maize lines showed that 150 polymorphic fragments were sufficient to stabilize the dendrogram (Lanza *et al*., 1997; Pejic *et al*.,

Table 2 - Decamer oligonucleotide primers (Operon Technologies Inc.) selected for RAPD analysis of 81 accessions of maize including number of fragments for each primer and number and size of polymorphic fragment produced.

Primers	Sequence (5'-3')	Nf	Np	Fragment size in base pair	
				Larger	Smaller
OPAD-06	AAGTGCACGG	9	6	1359	826
OPAD-14	GAACGAGGGT	8	7	1653	978
OPAK-15	ACCTGCCGTT	6	4	2004	1462
OPAM-01	TCACGTACGG	9	7	1948	709
OPAR-02	CACCTGCTGA	9	9	1931	1364
OPAR-04	CCAGGAGAAG	7	7	1789	979
OPAR-05	CATACCTGCC	9	9	2059	1481
OPAR-11	GGGAAGACGG	4	1	1809	1464
OPAR-15	ACACTCTGCC	4	4	1909	1628
OPAR-16	CCTTGCGCCT	7	6	2000	1240
OPAT-08	TCCTCGTGGG	10	10	1441	774
OPAU-12	CCACTCGTCT	7	5	1682	854
OPAV-03	TGTAGCCGTG	6	5	2064	1318
OPAV-13	CTGACTTCCC	8	7	2071	1427
OPAV-19	CTCGATCACC	6	6	1309	381
OPAW-07	AGCCCCCAAG	8	4	1667	174
OPAW-08	CTGTCTGTGG	6	4	1500	813
OPAW-10	GTTGTTTGCC	8	8	2004	1513
OPAW-11	CTGCCACGAG	10	5	1576	861
OPAW-14	GGTTCTGCTC	7	3	1987	794
OPAW-19	GGACACAGAG	8	6	1375	693
OPAX- 07	ACGCGACAGA	8	3	1261	104
OPAX- 10	CCAGGCTGAC	8	5	1619	481
OPP- 05	CCCCGGTAAC	11	9	1252	1153
OPP-14	CCAGCCGAAC	4	2	1600	742
OPE-18	CGACTGCAGA	8	6	1198	843
OPW-08	GACTGCCTCT	10	8	1532	968
OPW-09	GTGACCGAGT	8	5	1941	524
OPW-13	CACAGCGACA	10	6	2270	925
OPY-04	GGCTGCAATG	13	6	2044	673
OPY-09	AGCAGCGCAC	8	3	1934	686
OPY-10	CAAACGTGGG	11	8	1757	534
Total		255	184		

Nf: Number of fragment.
Np: Number of polymorphic fragment.

1998). However, according to Thormann *et al*., (1994), the number of bands giving a particular variation coefficient depend on the nature of the genotypes analyzed.

Genetic relationships among maize landraces

This research was done to characterize the extent of genetic variation in 79 maize landraces and two commercial varieties grown in Southern Brazil using RAPD markers. The UPGMA dendrogram based on the similarity matrix associated the 79 landrace accessions into two major clusters. There were close relationships among accessions with yellow and yellow-orange endosperm, which clustered with a similarity coefficient of between 0.82 and 0.90. The group containing mostly accessions with white endosperm displayed a comparable range of genetic similarity. The other five accessions remained isolated in the dendrogram (Table 1, Figure 4). Carvalho *et al.* (2002) showed similar results by using inter simple sequence repeat (ISSR) markers in the same maize collection. The similarity values and polymorphic index were greater for ISSR than for RAPD markers. ISSR and RAPD markers were also used to estimate the polymorphic indexes of diploid, tetraploid, and hexaploid wheat species (Nagaoka and Ogihara, 1997) and varieties of *Oriza sativa* (Beverley *et al*., 1997). Moeller and Schaal (1999), using RAPD markers in Native American maize collections of Great Plains, showed a similarity index that varied from 0.44 to 0.80. This high level of genetic variability suggested that many maize accessions had been traded into those regions or migrated with indigenous tribes who had begun maize agriculture in other localities. The maize landraces included in this research showed low variability (0.78 to 0.91), in comparison to American maize of the Great Plains, possibly because it represents just a small fraction of the Brazilian maize core collection.

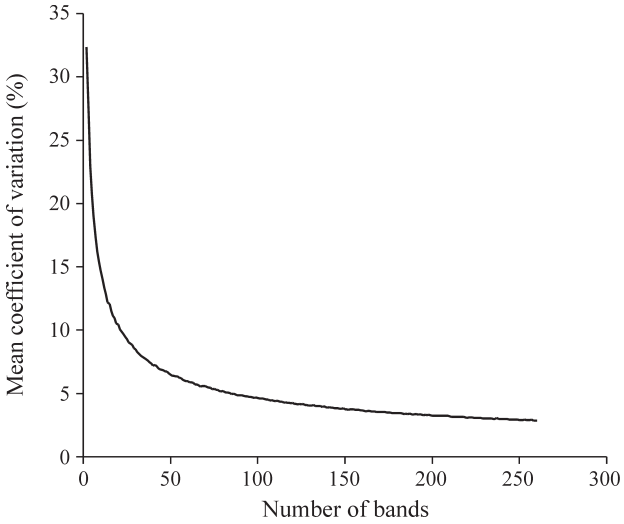

Figure 3 - Sample variance of genetic similarity estimation for 81 maize accessions as depicted by the relationship between the mean coefficient of variation (%) and number of bands derived from a bootstrap procedure.

Studies comprising 28 open pollinated varieties of maize (Parentoni *et al.*, 2001) showed that there was an association between the dendrogram obtained by RAPD markers and morphological characteristics. The author found that flint and semi-flint genotypes as well as the dent and semi-dent germplasm were placed in different groups by RAPD markers.

Good agreement between known pedigree obtained by morphological data and phylogeny among open pollinated varieties estimated by RAPD have been reported by Yu and Pauls (1993) and Kongkiatngan *et al.* (1996). The associations revealed in cluster 1 show a high genetic similarity between the yellow and yellow-orange maize accessions studied (Figure 4). The Asteca Antigo do Prestupa, Astecão Antigo, and Asteca Baixo Sabugo Fino landraces, all of Aztec origin, formed a small group, which also display similar kernel morphology and flowering time (Table 1). The same pattern is observed for the Antigo 30 anos, Antigo Linha 5, Milho Antigo, Comum Antigo x Sabugo Fino, and Amarelão Antigo landraces. These landraces have characteristics that were found in the antique germplasm.

Accession C 408 x AG was found isolated in the dendrogram. It was the result of crossing two commercial hybrids that were maintained by small landholder farmers for an extended period of time. The Maia landrace and the improved variety IAPAR 50 were associated with high genetic similarity coefficient (0.87). This association is consistent with their common origin since both the Maia and IAPAR 50 accessions contain the Maya gene pool.

The Cunha Amarelo, Sangue de Adão, Amarelo Graudo, Dente de Cotia, Ivo Agostiniak, Amarelão Basonni, Amarelo Taguari, Cabo Roxo and Pintado landraces were associated in a small group. These accessions display similar kernel characteristics and flowering time, and except for the Sangue de Adão landrace, which shows red seeds that are conditioned by red pericarp, all others have yellow seeds (Figure 4, Table 1).

Genetic associations in cluster 2 reveal high similarity among the accessions. In this cluster, seven accessions grouped together, of which five (Branco Comum, Bugre Branco, Casano, Oito Carreiras and Tostão) showed similar kernel characteristics and flowering time and two (Antigo Venglarek and Antigo) segregate for endosperm color (Figure 4, Table 1). The Oito Carreiras and Tostão landraces were very close, with a similarity coefficient of 0.90. Both accessions display eight-row ears and very long kernel width, characteristics that are also observed in the Hickory King race introduced in Brazil from United States (Paterniani, 2000). It is possible that Oito Carreiras and Tostão are derived from the Hickory King race. Other accessions of cluster 2 that share similar kernel characteristics and flowering time were also very close by RAPDs, revealing coefficients of genetic similarities that ranged from 0.86 to 0.88 (Figure 4, Table 1).

The highest genetic similarity (0.91) was observed between the Branco Lastek Dinart and Cunha branco landraces. These accessions have been cultivated in distinct regions by unrelated small farmers and are known by different names. In contrast, two other accessions, Astecão Branco and Asteca Branco that have been treated by similar names were less related by RAPD showing a similarity of

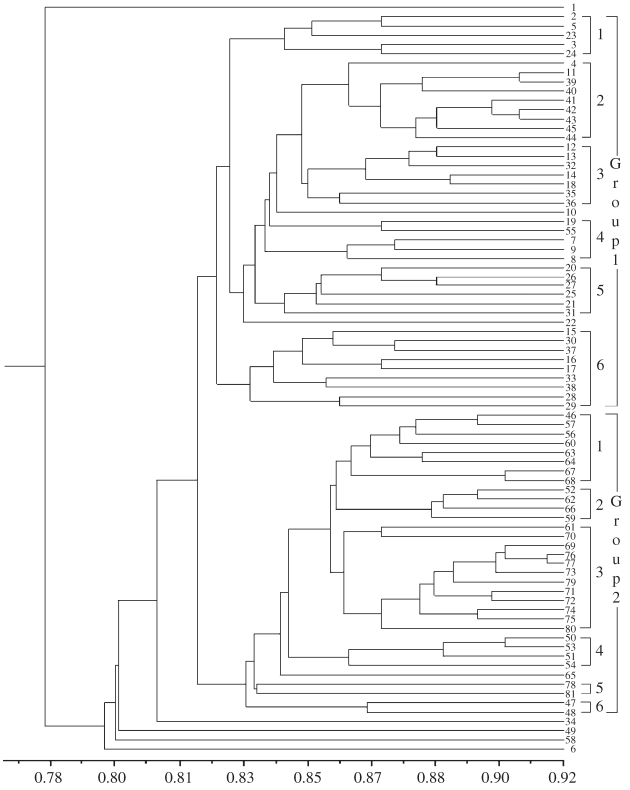

Figure 4 - Dendrogram of 81 maize accessions constructed using UPGMA method based on a similarity matrix produced with simple matching coefficients. Numbers 1 through 81 refer to the collection listed in Table 1.

0.85. Gimenes and Lopes (2000) reported similar results by using isoenzyme analysis. The authors studied 15 maize populations derived from three indigenous maize races and observed that there was no connection between the accessions name and the genetic relationships. Therefore accessions evaluation by molecular markers is important in germplasm classification to avoid the replication of genetic materials.

The Amarelão Diwietz, Milho Ferrinho, Asteca Branco Sabugo Fino, BR 473, and Asteca landraces appeared isolated from other accessions in the dendrogram (Figure 4, Table 1). The Asteca landrace, which showed the lowest similarity to the other accessions (0.78), has a uncertain origin and it seems derived from a sample collected a few years ago by small landholder farmer.

The several small groups formed into each cluster revealed some genetic divergence within the yellow and within the white landraces. This is in line with the observations of Paterniani (2000). According to this author, the Brazilian maize landraces are derived from crossing introductions from United States (at different times in the past) and maize types cultivated for an extended period of time by indigenous tribes and European colonizers after the discovery of the American Continent. In the middle of the 18th century, yellow dent germplasm was introduced to Brazil

from the United States, while the white dent germplasm was recently introduced with the Hickory King variety. Doebley *et al.* (1988) reported that yellow dent, white dent, and Hickory King are races derived from Southern United States dent types. These facts explain the separation of yellow and white landraces by RAPD.

The landraces analyzed in this research have been used by small-scale farmers according to endosperm color. The white landraces are mainly used for flour manufacturing for human consumption and the yellow accessions are generally used in animal nutrition. The farmers consider that the planting of the landraces in small areas is less expensive than the commercially improved hybrids. Therefore, genetic improvement of this germplasm is important for traditional agriculture maintenance developed by the small landholder farmers from Paraná and Santa Catarina states.

In conclusion, the simplicity of laboratory assays for RAPD is an attractive method for the analysis of genetic diversity among maize landraces. The polymorphism detected among the accessions can be used in breeding programs to maximize the use of genetic resources.

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