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

The development of modern plant breeding tech-
niques has greatly facilitated wider use of a wealth of diver-
sity from many sources including landraces, and especially,
has allowed food production to keep up with population
growth (Wood and Lanné, 1999). Currently, the genetic di-
versity 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 mol-
cular markers used in maize breeding programs. More re-
cently, 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 ge-
netic 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 tech-
nique related to desired objectives. RAPD markers are
commonly used because they are quick and simple to ob-
tain, 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 con-
sidered (Dos Santos et al., 1994). Similar results were re-
ported 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 ge-
netic distance among segregant lines (Marsan et al., 1993)
to predict the best crosses among lines for hybrid develop-
ment (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 land-
races and two commercial varieties. This will contribute in
identifying efficient strategies for the sustainable manage-
ment of the genetic resources of the landraces in study.

Materials and Methods

Plant material

Plant material consists of 81 maize accessions, com-
prising two commercial varieties, developed at the Instituto
Agronômico do Paraná (IAPAR), and 79 landraces ob-
tained from the Assessoria e Serviços a Projetos em Agri-
cultura 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          |
|------------------|-----------------|-------------|------------------------|-------------------|------------------|-----------------------|---------------------|
| 1 - Asteca       | yellow-orange   | dent        | 74                     | medium            | medium           | short                 | Rio Azul/PR         |
| 2 - Asteca antigo do Prestupa | yellow-orange   | dent        | 78                     | medium            | medium           | short                 | Bituruna/PR         |
| 3 - Asteca BaixoSabugo Fino | yellow          | dent        | 73                     | medium            | medium           | short                 | Porto União/SC       |
| 4 - Asteca Sabugo Fino | yellow-orange   | dent        | 76                     | medium            | medium           | short                 | São João do Triunfo/PR |
| 5 - Astecão Antigo | yellow          | dent        | 75                     | medium            | long             | medium                | Bituruna/PR         |
| 6 - BR 473⁵      | yellow-orange   | semi-dent   | 69                     | medium            | medium           | medium                | Porto União/SC       |
| 7 - BR106⁵       | yellow-orange   | dent        | 73                     | medium            | long             | medium                | Bituruna/PR         |
| 8 - Cabo Roxo⁶    | 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 AG³  | 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 |
|------------|----------------|-------------|------------------------|-------------------|------------------|-----------------------|------------|
| 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 | Palmela/PR |
| 22 - Ouro Verde | yellow-orange | dent | 73 | medium | medium | medium | Irati/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 | Palmela/PR |
| 27 - Azcril | yellow | dent | 76 | medium | long | medium | Cruz Machado/PR |
| 28 - Cabo Roxo | 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 | yellow-orange | dent | 75 | medium | medium | medium | Bituruna/PR |
| 31 - IAPAR 51 | yellow | dent | 73 | medium | medium | medium | IAPAR, Lon- drina/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 Döwiete | yellow | dent | 74 | medium | medium | medium | Porto União/SC |
| 35 - Amarelão Antigo do Valdivino | yellow-orange | dent | 80 | medium | long | medium | Bituruna/PR |
| 36 - Amarelão do Tião | yellow-orange | dent | 79 | medium | long | short | Rebozuca/PR |
| 37 - Amarelão Graudo | yellow-orange | dent | 77 | medium | long | medium | Rio Azul/PR |
| 38 - Amarelão Taguari | yellow-orange | dent | 79 | medium | long | medium | Rio Azul/PR |
| 39 - Antigo 30 anos | yellow-orange | dent | 82 | medium | long | medium | Irati/PR |
| 40 - Antigo Linha 5 | yellow-orange | dent | 80 | medium | long | short | Irati/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 | Irati/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 | Palmela/PR |
| 46 - Milho Antónios I | yellow-orange | dent | 80 | medium | long | medium | Irati/PR |
| 47 - Milho Caixoeira | yellow-orange | dent | 78 | medium | long | medium | São João do Triunfo/PR |
| 48 - Milho Fabricio 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 | Palmela/PR |
| 51 - Milho Pires | yellow-orange | dent | 78 | long | medium | medium | Cruz Machado/PR |
| 52 - Palha Roxa Alichtig | yellow | dent | 78 | medium | medium | medium | São João do Triunfo/PR |
| 53 - Pirulim do Tadeu | yellow-orange | dent | 80 | long | medium | medium | Bituruna/PR |
| 54 - Indiga | yellow | dent | 83 | medium | medium | medium | Cruz Machado/PR |
| 55 - IAPAR 50 | yellow-orange | dent | 78 | medium | long | medium | IAPAR, Lon- drina/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 | Rebozuca/PR |
| 59 - BR 451 (QPM) | white | dent | 71 | medium | medium | medium | Rio Azul/PR |
| 60 - Branco Comum | white | dent | 75 | medium | long | medium | Rio Azul/PR |
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 blotted 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).

Table 1 (cont.)

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

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

Figure 1 - Geographic localities of maize collection used in this study. 1, Londrina, PR (accessions 5 and 50); 2, Irati (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, Cruze 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, Reboucas, 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 leaf 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 MgCl₂, 20 mM (NH₄)₂SO₄, 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% Metaphor (FMC Bioproducts) agarose in 1x 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 unreplicable 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.

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., 1998).
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.

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.

### 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   | ACCTGCCGTG       | 6  | 4  | 2004 1462                              |
| OPAM-01   | TACGTACGG        | 9  | 7  | 1948 709                               |
| OPAR-02   | CACCTGCTGA       | 9  | 9  | 1931 1364                              |
| OPAR-04   | CACGGAGAAG       | 7  | 7  | 1789 979                               |
| OPAR-05   | CACACCTGCC       | 9  | 9  | 2059 1481                              |
| OPAR-11   | GGGGAAGACGG      | 4  | 1  | 1809 1464                              |
| OPAR-15   | ACACCTTGCC       | 4  | 4  | 1909 1628                              |
| OPAR-16   | CCTTGCCGCTC      | 7  | 6  | 2000 1240                              |
| OPAT-08   | TCTCTGCTGG       | 10 | 10 | 1441 774                               |
| OPAU-12   | CACCTGCTCT       | 7  | 5  | 1682 854                               |
| OPAV-03   | TGTAGCCGTG       | 6  | 5  | 2064 1318                              |
| OPAV-13   | CTCCTCCTGCT      | 8  | 7  | 2071 1427                              |
| OPAV-19   | CTCGATCACC       | 6  | 6  | 1309 381                               |
| OPAW-07   | AGCCCCCAAG       | 8  | 4  | 1667 174                               |
| OPAW-08   | CTTCTCTGGG       | 6  | 4  | 1500 813                               |
| OPAW-10   | GTTGTTGAGG       | 8  | 8  | 2004 1513                              |
| OPAW-11   | CTGCCACAGG       | 10 | 5  | 1576 861                               |
| OPAW-14   | GTTTCTGCTC       | 7  | 3  | 1987 794                               |
| OPAW-19   | GGACACAGAG       | 8  | 6  | 1375 693                               |
| OPAX-07   | AGCGACAGA        | 8  | 3  | 1261 104                               |
| OPAX-10   | CAGGGCTGAGG      | 8  | 5  | 1619 481                               |
| OPP-05    | CCCCGTGTAAC      | 11 | 9  | 1252 1153                              |
| OPP-14    | CACCGCCGAC       | 4  | 2  | 1600 742                               |
| OPE-18    | GACTGCTGAGA      | 8  | 6  | 1198 843                               |
| OPW-08    | GACTGCCCTCT      | 10 | 8  | 1532 968                               |
| OPW-09    | GTGACCGAGT       | 8  | 5  | 1941 524                               |
| OPW-13    | CACAAGCGACA      | 10 | 6  | 2270 925                               |
| OPY-04    | GGCTGCAATG       | 13 | 6  | 2044 673                               |
| OPY-09    | AGCAGCGCAC       | 8  | 3  | 1934 686                               |
| OPY-10    | CAAACGGTGGG      | 11 | 8  | 1757 534                               |

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

![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.](image-url)
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 Togueri, 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 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 Diviuetz, 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 an 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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