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Genetic diversity of the floury race of maize Avati Morotî from the Guaraní tribe in Paraguay

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Abstract

Avati Morotî is a race of floury maize widely used by the Guarani people in South America, whose variability and potential value for breeding has been neglected so far. The objective of this research was to explore the genetic variability within the main Paraguayan race Avati Morotí. We studied the genetic variability available in the 20 accessions of Paraguayan Avati Morotî included in the South American core collection made by CIMMYT. Thirty individuals per accession were genotyped with 30 SSR (simple sequence repeat); we determined genetic diversity and made a cluster analysis in order to define genetic relationships among accessions. Mean of polymorphic loci (0.96), alleles per locus (3.57), alleles per polymorphic locus (3.65), expected (0.48) and observed (0.43) heterozygosity, and coefficient of consanguinity (0.12) revealed that Avati Morotî contains a genetic diversity comparable to the most variable maize races of maize. The cluster analysis classified the 20 populations in 8 groups, five of them with a single accession, and a large group representing a central pool of germplasm. These results indicate that there is a large variability available in this race, and encourage the collection of more samples of Avati Morotî, particularly in marginal areas that were scarcely sampled.

Additional key words: *Zea mays*; germplasm; biodiversity; heterozygosity; polymorphism.

Abbreviations used: A (alleles per locus); Ap (alleles per polymorphic locus); CIMMYT (Centro Internacional de Mejora de Maíz y Trigo); F_{st} (coefficient of consanguinity); GD (genetic distance); GDA (genetic data analysis); H_e (expected heterozygosity) H_o (observed heterozygosity); P (polymorphic loci); PCR (polymerase chain reaction); SSR (simple sequence repeat); UPGMA (unweighted pair group method with arithmetic mean)

Introduction

Several millennia ago, maize (*Zea mays* L.) originated in Central America and spread through the Americas; the vehicles for that expansion were the Native Americans looking for new lands and crops. The relationship between people and maize created a large diversification of maize into races and varieties. After the irruption of Europeans in America five centuries ago, a large displacement of germplasm occurred throughout the Americas (Sauer, 1966). As a consequence, most of the autochthonous maize races hybridized and loosed their peculiarities so deeply that we cannot be confident about the genuineness of the currently representatives of the ancient genetic pools.

However, some of the ancient peoples and races have managed to survive relatively unpolluted; such might be the case of the Guarani people and their main maize race Avati Morotî in the center of South America, far from the coasts and the commercial routes. Maize was probably introduced in the central area of South America, including Paraguay, through the Andean Mountains and the coast of Brazil (Vigouroux *et al*., 2008), while the Guarani people arrive through the Amazonian Forest. Perhaps both have a Caribbean origin, and they started a lasting relationship that endures until today.

According to Salhuana & Machado (1999) there are 10 races of maize in Paraguay: the floury types Avati Morotî, Avati Tî, Avati Guapy and Avati Mitâ; the flint races Tupi Pytâ with orange grain and Tupi Morotî with white grain; the semident races Sape Pytâ (orange grain) and Sape Morotî (white grain); and the popcorn races Pichinga Aristado and Pichinga Redondo. Sánchez *et al.* (2007) made an isozyme study of South American maize and concluded that the populations from the highlands had the lowest genetic diversity. Nevertheless, Sánchez *et al.* (2006) found that the highest genetic diversity is concentrated in Mesoamerican and Andean races compared to the maize populations from North America, the Caribbean area or the south cone of America.

Avati Morotî belongs to the racial complex called Coroico, typical of the lowlands in the East of the Andes (Goodman & Brown, 1988). This racial complex includes the races Piricinco and Entrelazado. Sánchez *et al.* (2006) stated that this complex called "Amazonian Interlocked Flours" is the most used maize in the area. The interlaced characteristic is common in those genotypes, although it is not always present in Avati Morotî. The varieties of the race Avati Morotî have been made by the natives after selection for adaptation and local preferences. Brieger *et al.* (1958) considered this race as the most representative of the South American maize races and suggested that it could have been involved in the origin of other regional races (Robert *et al.*, 1957). Paterniani & Goodman (1978) found that, although other races were more productive, Avati Morotî had large variability and was more promising for selection. This is the race most commonly used for food either as bread (chipa), boiled or toasted in different forms or in Paraguayan soup, and is also fermented for producing a type of beer called chicha (Salhuana & Machado, 1999). Avati Morotî is highly appreciated by the natives; therefore, most of the local farmers cultivate Avati Morotî in their fields with a growth cycle of 140 days and yields around 4.690 kg/ha in 2009 (MAG, 2010).

Avati Morotî is the most widespread race in Paraguay, being present in most of the small farms (Salhuana & Machado, 1999). This race has been maintained in relative isolation due to its tolerance to early sowings and relative late flowering and maturation (Salhuana & Machado, 1999). It is cultivated throughout the whole country but originally it was cultivated mainly in the eastern side of Paraguay. However, nowadays these local varieties are being replaced by modern hybrids.

On the other hand, a major consequence of the European irruption into America was that maize spread towards the rest of the World. Spanish conquerors soon realized that maize was an interesting crop and introduced the new grain into Spain, Portugal and the Old World. Most authors believe that the origins of such introductions were mainly the Caribbean area and North America (Revilla *et al.*, 1998, 2003; Rebourg *et al.*, 2003).

The history of maize has neglected important genetic pools based on the presumption that their incidence on the genetic resources available worldwide was marginal (Revilla *et al.*, 2003). Indeed, some of the comprehensive studies of maize germplasm paid little or null attention to South-American genetic resources; *e.g.* Vigouroux *et al.* (2008) only included five entries from Paraguay, and Rebourg *et al.* (2003) completely skipped maize from this country. However, some of those germplasm pools could provide decisive information about the historical relationships and the potential inputs that we can still expect from unexplored variability. Furthermore, to the best of our knowledge, no reports of genetic diversity among floury open-pollinated populations have been published. The objective of this research was to explore the genetic variability within the main Paraguayan race Avati Morotî.

Material and methods

Genetic materials

The accessions of the maize race Avati Morotî included in this study came from the South American core collection made by CIMMYT in 2003 (unpublished data). That collection was made from 335 accessions of Avati Morotî, including 182 from Paraguay, 114 from Argentina, 28 from Brazil, 9 from Bolivia and 2 from Uruguay, according to the distribution of the Guaraní people. The study made by CIMMYT analyzed days to anthesis and silking, plant and ear height, number of leaves, days to senescence of ear leaf, grain moisture, proportion of grain in the ear, ear health, lodging, ear length and diameter, kernel length and width, number of ear rows, grain yield and yield index (unpublished data). They obtained a collection with 20% of the accessions. In our study, the 20 Paraguayan accessions of Avati Morotî from the core collection were included (Table 1); they were collected from 1953 to 1998 in the areas where the richness of maize races was the highest. The 20 accessions were sent by Dr. Suketoshi Taba to the research station "Centro de Investigación de Capitán Miranda", where the accessions were multiplied from 2006 to 2008 by crossing plant to plant for obtaining at least 120 ears.

SSRs markers characterization

For each of the 20 Avati Morotî accessions, 50 grains were germinated for five days at 25 °C without light, and DNA was extracted from 30 coleoptiles following the procedure described by Liu & Whittier (1994). Thirty microsatellites (SSRs) distributed throughout the 10 maize chromosomes (Table 2) were used for characterizing the 20 accessions (Table 1). We used a set of SSR markers previously selected for a study with Spanish germplasm because they had good performance in our laboratory, they had a convenient distribution along the maize genome, and they were polymorphic for the Spanish populations (Romay *et al.*, 2012). PCR was carried out with a thermocycler MyCycler (BIO-RAD Laboratories, Inc.). The final mixture included 50 ng/µL of genomic DNA (2 µL), 2 × PCR buffer with *Taq* DNA polymerase (Promega) and dNTPs (10 µL), 4 ng/µL primers F and R (http://www.maizegdb.org/) (2 × 0.8 µL) and 7.4 µL purified H_2O. The PCR program involved an initial phase at 95 °C for 5 min, followed by 30 cycles (denaturation at 95 °C: 30 s, hybridization at 56 °C: 30 s and extension at 72 °C: 30 s), then a final extension phase at 72 °C for 10 min, and conservation of final products at 4 °C until utilization.

After amplification, SSR products were separated by electrophoresis along with a weight marker (Hyper-Ladder V) using 1 × TBE on a 6% non-denaturing gel of acrylamide with ethidium bromide at 250 V for 3h in a vertical system (DASG-400-50, C.B.S. Scientific Co.). By using a UV table, we photographed each gel.

Statistical analysis

From the photographed gels, fragments were manually identified by their molecular weight. Fragments were numbered from low to high weight. The number of alleles was determined for the genotypes in order to calculate allelic frequencies, number of alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o), coefficient of consanguinity (F_{st}) (Nei, 1978; Wright, 1978) and genetic distances (Nei & Li, 1979). Analysis of allele frequencies were obtained from the program GENEPOP 3.3 (http://genepop.curtin.edu.au). The statistical program used for analyzing heterozygosity and coefficient of inbreeding was Genetic Data Analysis (GDA) version 1.1 developed by Lewis & Zaykin (2002).

For calculating genetic distance (GD) among the 20 Avati Morotî accessions, we used the method of Nei & Li (1979) according to the formula:

$$GD_{NL}=1 - [2N_{11}/(2N_{11}+N_{10}+N_{01})]$$

where N_{11} is the number of alleles present in both individuals, N_{10} is the number of alleles present only in genotype 1, N_{01} is the number of alleles present only in genotype j.

Table 1. Data of the 20 accessions from the race Avati Morotî from Paraguay, provided by CIMMYT, that have been characterized in this study

Accession	Collection date	Collection place	Department	Latitude	Longitude	Altitude	Grain types[a]	Grain colors[b]
PAZM2035	17/06/1998	General Elizardo Aquino, Barrio Niño Jesús	San Pedro	24º13'12"	56º22'48"	160	A	B
PAZM2040	17/06/1998	General Elizardo Aquino, Barrio Niño Jesús	San Pedro	24º12'36"	56º22'48"	170	A, F	A
PAZM2087	19/06/1998	Choré	San Pedro	24º04'12"	56º20'24"	190	A	B
PARA107	01/01/1954	Natalicio Talavera	Guairá	24º27'36"	56º15'36"	366	A	B, D
PAZM4022	12/03/1998	Natalicio Talavera, Barrio Panatey	Guairá	25º25'12"	56º09'00"	190	A	B, F
PAZM14084	15/07/1987	Ype Jhu	Canindeyú	23º32'24"	55º12'00"	220	F, A	B, A
PARA119	01/01/1953	Unknown	Misiones	27º00'00"	57º00'00"	400	A	B, A
PARA100	01/01/1954	Coronel Oviedo	Caaguazú	25º18'00"	56º18'00"	400	A	B, A
PARA108	01/04/1953	Natalicio Talavera	Guairá	25º27'36"	56º15'36"	400	A	B, D
PARA117	01/05/1953	Asunción	Unknown	25º15'36"	57º24'36"	200	A	B
PAZM4033	12/03/1998	Independencia, Yroisa	Guairá	25º28'48"	56º03'36"	200	A, F	B, J
PAZM6037	21/04/1998	Solalinde	Gaazapá	26º06'36"	56º15'00"	210	A, F	B, A
PAZM7097	06/04/1998	San Pedro de Paraná, Guazu Ygua	Itapúa	26º32'24"	56º00'00"	310	A, F	B, A
PAZM9044	10/03/1998	Ybytymi, Potrero Garay	Paraguari	25º32'24"	57º01'12"	200	A	B
PARA124	01/01/1954	Unknown	Misiones	27º00'00"	57º00'00"	274	A	A, B
PARA766	01/01/1954	Unknown	Unknown	Unknown	Unknown	122	A	A, B
PAZM1034	19/07/1987	Loreto	Concepción	23º09'36"	57º06'36"	95	A, F	B, J
PAZM14091	15/07/1987	Ype Jhu	Canindeyú	23º32'24"	55º12'00"	180	A	B, A
PAZM14121	07/05/1998	Corpus Christi, Santa Librada	Canindeyú	24º08'24"	55º03'00"	270	A, F	B, J
PAZM2082	18/06/1998	Lima	San Pedro	23º34'48"	56º15'00"	285	A	B

[a] Grain type: primary and secondary types are shown sequentially A = floury, F = flint. [b] Grain color: primary and secondary colors are shown sequentially A = white, B = yellow, D = variegated, F = orange, J = red

Table 2. Microsatellites (SSR) used for characterizing the maize accessions of the race Avati Morotî from Paraguay

SSR	Position (Bin)	SSR	Position (Bin)	SSR	Position (Bin)
umc1222	1.01	bnlg1520	2.09	umc1545	7.00
umc1403	1.03	phi036	3.04	phi114	7.03
umc1335	1.06	umc1682	4.01	umc1327	8.01
umc1147	1.07	umc1963	4.04	umc1984	8.03
umc1725	1.11	umc1329	4.06	bnlg1812	8.05
umc1165	2.01	phi128	5.07	phi028	9.01
umc1265	2.02	umc1225	5.08	phi027	9.03
phi109642	2.03	umc1887	6.03	umc1505	9.08
phi083	2.04	umc1424	6.06	phi059	10.02
phi127	2.08	bnlg1740	6.07	umc1930	10.04

Diversity was analyzed with the software GENEPOP 3.3. The cluster analysis was made using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with the program GDA (Ward, 1963).

Results

The characterization of 30 plants from the 20 accessions of Avati Morotî from Paraguay with 30 SSR yielded 251 alleles (Table 3). There were 3.57 alleles per locus (A) on average and 3.65 alleles per polymorphic locus. The proportion of polymorphic loci was 0.96, the expected heterozygosity 0.48, the observed heterozygosity 0.73 and the coefficient of consanguinity 0.12. The variability within the race was not homo-geneously distributed, as the accession PAZM14091 with the largest number of alleles per locus (A = 5) and alleles per polymorphic locus (A$_p$ = 5) had twice the value of the accession with the smallest value (PARA107, with A = 2.27 and A$_p$ = 2.65, respectively). The proportion of polymorphic loci was highest (P = 1) for half of the accessions and lowest for PARA107. The accession PAZM14091 had the highest expected and observed heterozygosity (H$_e$ = 0.57 and H$_o$ = 0.52), PAZM4033 had the same observed heterozygosity, while PARA766 and PARA107 had the lowest (H$_e$ = 0.34) and the lowest observed heterozygosity was for PARA119 (H$_o$ = 0.28). Conversely, the coefficient of consanguinity was highest for PARA119 (F$_{st}$ = 0.21) and lowest for PAZM4033 (F$_{st}$ = 0.05).

Table 3. Proportion of polymorphic loci (P), alleles per locus (A), alleles per polymorphic locus (A$_p$), expected (H$_e$) and observed (H$_o$) heterozygosity, and coefficient of consanguinity (F$_{st}$) for 20 maize accessions of the race Avati Morotî from Paraguay

Accession	P	A	A$_p$	H$_e$	H$_o$	F$_{st}$
PAZM14121	0.97	3.67	3.76	0.51	0.46	0.11
PARA766	0.97	3.27	3.34	0.34	0.29	0.13
PARA124	0.97	3.27	3.34	0.44	0.38	0.14
PAZM6037	1.00	3.50	3.50	0.53	0.48	0.09
PAZM2082	0.97	3.57	3.66	0.49	0.45	0.08
PAZM7097	1.00	4.00	4.00	0.53	0.50	0.06
PAZM14091	1.00	5.00	5.00	0.57	0.52	0.09
PAZM1034	1.00	4.23	4.23	0.56	0.51	0.09
PARA117	0.87	2.80	3.08	0.41	0.37	0.11
PARA108	1.00	3.60	3.60	0.53	0.44	0.16
PARA9044	0.97	4.07	4.17	0.54	0.47	0.14
PAZM4033	1.00	4.20	4.20	0.54	0.52	0.05
PARA100	0.97	3.67	3.76	0.49	0.46	0.06
PARA107	0.77	2.27	2.65	0.34	0.29	0.14
PAZM4022	1.00	2.97	2.97	0.46	0.37	0.19
PAZM2035	1.00	3.27	3.27	0.47	0.43	0.08
PAZM2040	1.00	3.73	3.73	0.52	0.45	0.14
PAZM14084	1.00	4.50	4.50	0.56	0.45	0.20
PAZM2087	0.97	3.20	3.28	0.46	0.40	0.14
PARA119	0.87	2.67	2.92	0.35	0.28	0.21
Mean	0.96	3.57	3.65	0.48	0.43	0.12

The cluster analyses based on the genetic distances of Nei & Li (1979) revealed that some of the accessions were closely related in small groups: 1) PAZM7097, PARA108 and PAZM9044; 2) PAZM2082 and PAZM4033; 3) PAZM14084, PAZM2035 and PAZM2040; and 4) PAZM4022 and PAZM2087 (Fig. 1). On the other side, PARA119 and PARA107 were distant from the rest of accessions. The first small group clustered in a main group of accessions including PAZM9044, PARA108, PAZM7097, along with PAZM14091, PAZM6037 and PAZM14121 that are close to small cluster 2) PAZM4033 and PAZM2082, and to PARA100 and PAZM1034. These 10 accessions come from the center and north of the maize area of Paraguay (Table 1). The small cluster 3) including PAZM14084, PAZM2040 and PAZM2035 comes from the north of the area. The cluster involving PAZM4022 and PAZM2087 is geographically diverse and PAZM4022 comes from the center and the second from the north of the area. All accessions coming from the north of the maize area were clustered in two groups that were genetically distant between them and with the other accessions. The most genetically distant accessions (PARA119, PARA107 and PARA766) have diverse geographic origins, as the first one comes from the south and the second one from the center of the maize area. The geographic and genetic distances are related, *e.g*. PARA107 and PARA119 are geographically and genetically distant, and PAZM2040 and PAZM2035 are geographically and genetically close; however, there are not linear relationships as PARA119 and PAZM2087 come from the same location but are genetically distant, and PAZM2087 and PAZM2040 come from the same place but are genetically distant. However, geographic distances do not imply climatic differences because the maize area of Paraguay has homogeneous climatic conditions.

Accession PARA119 was collected in 1953 while PAZM2087 was collected in 1998; similarly, PARA107 was collected in 1954 and PAZM4022 in 1998. There could have been genetic drift during conservation of accessions PARA119 and PARA107, causing low genetic diversity (Table 3) and large genetic distance with accession collected in adjacent areas, as PARA119 and PAZM2087 or PARA107 and PAZM4022. Nevertheless, the accession PAZM14084 was collected recently and has high coefficient of inbreeding.

Discussion

Current plant breeding programs use only a small proportion of the genetic material available in germplasm banks; thus, reducing the ability to respond to challenges generated by stresses and the expected gain in long term selection programs (Smith, 1988). Commercial varieties include mainly elite genotypes that have been previously used in commercial programs. This short term strategy causes a bottleneck that limits future breeding success. Local varieties are potential sources of favorable genes for adaptation, tolerance to stresses, pests and diseases, and present and future human needs. However, local varieties have often low yields and agronomic performance compared to modern hybrids (Wietholter *et al*., 2008). Saving, valorizing and incorporating local varieties in breeding programs could increase crop sustainability and added value. The Paraguayan race Avati Morotî deserves our interest because it is a floury race of maize widely used in South America and has been adapted during centuries to a variety of environments and uses as food.

This study of a core collection of Paraguayan Avati Morotî accessions reports the variability available within this pool and the genetic relationship among accessions in order to allow its inclusion in future breeding programs. Molecular markers reveal the genetic variation without environmental effects; particularly SSR have been used for investigating the genetic variability available in germplasm collections (Hoxha *et al*., 2004; Beyene *et al*., 2006; Warburton *et al*., 2008). In the present study, 30 SSR were used to characterize 575 individuals from 20 accessions yielding 394 alleles. Altogether we found 13.13 alleles per locus, which is higher than the value reported by Aci *et al*. (2013) among Algerian populations or Romay *et al*. (2012) among Spanish germplasm with the same SSR set of loci. The number of alleles per locus was also higher than that reported in other American origins with different SSRs (Reif *et al*., 2003b, 2004, 2005, 2006; Wiethotler *et al*., 2008; Bracco *et al*., 2012).

The mean number of alleles per accessions in the Avati Morotî collection was 3.57, and varied from 2.27 to 5.00, higher than the values reported by Aci *et al*. (2013) for Algerian populations or by Romay *et al*. (2012) for Spanish germplasm, and similar to the alleles per accession found in Mexican germplasm by Reif *et al*. (2006). Therefore, the variability found in Avati Morotî was higher than that found in Africa or Europe and similar to the variability reported in Mexican races that are the most variable because they are closer to the center of origin of maize. The reasons for this high variability could be the wide sources of Avati Morotî used by the Guarani people, the interchange of germplasm made by the diverse communities of the Guarani people or by mutations occurring during selection for adaptation to diverse environments and uses. The African or European germplasm has less diversity than the American maize (Romay *et al*., 2012; Aci *et al*.,

2013) as expected given that maize originated in America thousands of years ago and only a small part of the variability was introduced in Africa less than 500 years ago.

Our results also show that the variability was lower for the accessions collected in the 1950's (PARA766, PARA124, PARA117, PARA107 and PARA119) with 3.07 alleles per accession on average, except accessions PARA100 and PARA108 with 3.67 and 3.60 alleles per accession, respectively, that have similar values than the accessions collected later, whose mean number of accessions was 3.87. The proportion of polymorphic loci overlapped between the oldest accessions (from 0.87 to 1.00) and the newest ones (0.97 to 1.00) and both were in the upper limit of the values found by other authors (Romay *et al*., 2012; Aci *et al*., 2013). Consequently, the number of alleles per polymorphic locus was also lowest for older accessions such as PARA107 and PARA119 with 2.65 and 2.92, respectively, and highest for the newer accessions PAZM4022 and PAZM14091 with 2.97 and 5.00, respectively.

The mean expected heterozygosity was 0.48 and varied from 0.20 to 0.55, values that are above those reported by Aci *et al*. (2012) for Algerian populations and below the heterozygosity found by Romay *et al*. (2012) in Spanish germplasm. These values are within the ranges published for other American origins (Reif *et al*., 2003a, 2006) Once again, the values of Avati Morotî are similar to those of the races with highest variability. Besides, the older races (PARA107, PARA766, PARA119, PARA117 and PARA124) have lower values tan the newer ones except PARA100 and PARA108. Differences in variability between accessions collected in the 1950's and later could be due to reduced sample size in the collections or during multiplications. The mean observed heterozygosity was 0.43. As H_o was slightly lower than H_e, there are an excess of homozygotes. The mean coefficient of inbreeding (Wright, 1978) was $F_{st} = 0.12$ and was variable among accessions, indicating high diversity within the race Avati Morotî. These results indicate larger variability within Paraguayan accessions compared to Algerian (Aci *et al*., 2013) or Spanish accessions (Romay *et al*., 2012) who reported F_{st} around 0.3.

The number of alleles per locus and alleles per polymorphic locus were very similar because all loci were polymorphic. The mean number of alleles per locus was 13.1 and varied from 4 to 30, which is higher than the values reported by Romay *et al*. (2012) and Aci *et al*. (2013). Once again, the variability within Avati Morotî was higher than in other races.

The cluster analysis classified the 20 populations in 8 groups (Figure 1) with some single-accession clusters

such as accessions PARA119, PARA107, PARA766, PARA124, and PARA117 that correspond to the clusters 1 to 5, respectively. The sixth cluster contains 10 accessions divided in three subgroups. The seventh cluster has three accessions and the eighth has two. This number of clusters is large for 20 accessions compared to other authors who have found a smaller ratio cluster / accession, for example, Aci *et al*. (2013) identified three clusters among 15 Algerian populations. The five first clusters contain Avati Morotî accessions collected in the 1950' with low yield and limited genetic variability. Although there is no clear association between genetic distance and geographical situation, all populations located in single-accession clusters came from the south of the maize growth area, even
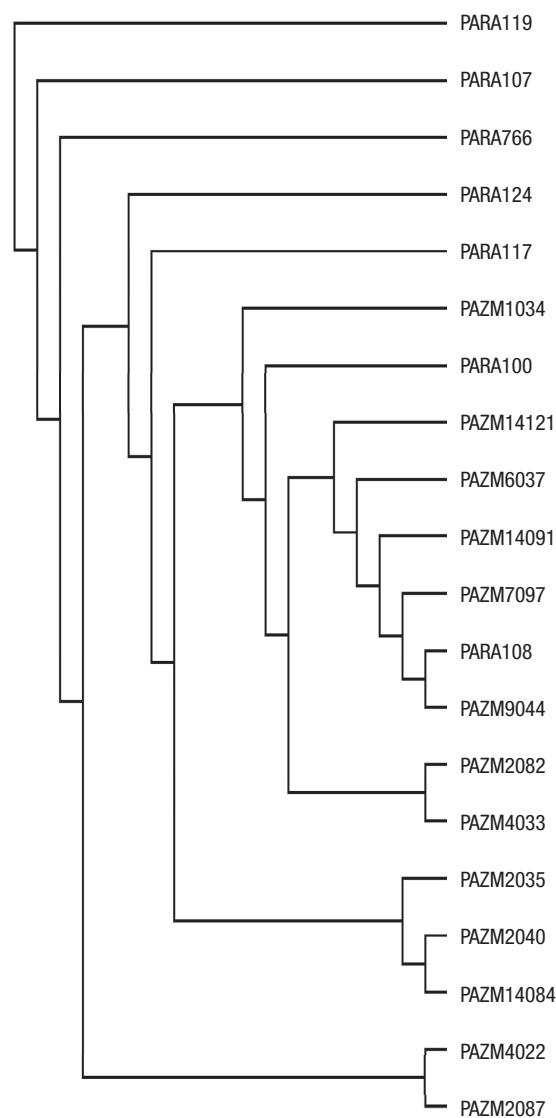

Figure 1. Cluster analysis of 20 maize accessions of the race Avati Morotî from Paraguay using the UPGMA method and the genetic distance of Nei & Li (1979) from the genetic characterization of 30 individuals per accession with 30 SSR.

though there were southern populations in all clusters except the seventh. The 10 accessions of the sixth cluster came from all over the area and none of the clusters have a consistent geographic origin. The two accessions coming from the meridional department of Misiones were genetically diverse; and the four accessions from Guairá were located in different clusters, except PARA108 and PAZM4033 that were in different subgroups of cluster six. On the other hand, two of the three accessions from Canindeyú were quite close in cluster six; and two of the four accessions from San Pedro were very close in cluster seven. Contrarily, other authors have found a relationship between clusters based on molecular markers and geographic distribution (Beyene *et al*., 2006; Aci *et al*., 2013).

These results suggest that the genetic diversity of the accessions collected in the first period could be caused by genetic drift that originated inbreeding depression, and encourages the collection of more samples of this race, particularly in marginal areas that were scarcely sampled. As conclusion, the Paraguayan germplasm of the race Avari Morotî has large genetic variability, even higher than some Mexican races, that is conserved by the Guarani people nowadays in a vast area of South America, and deserves further attention.

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