

## Detection of genetic diversity of seven maize races from the high central valleys of Mexico using microsatellites

Mario Rocandio-Rodríguez<sup>1</sup>, Amalio Santacruz-Varela<sup>1\*</sup>, Leobigildo Córdova-Téllez<sup>1</sup>, Higinio López-Sánchez<sup>2</sup>, Fernando Castillo-González<sup>1</sup>, Ricardo Lobato-Ortiz<sup>1</sup>, J Jesús García-Zavala<sup>1</sup>

<sup>1</sup>Colegio de Postgraduados Campus Montecillo. Km 36.5. Carr México-Texcoco. 56230, Texcoco, Edo de México, México

<sup>2</sup>Colegio de Postgraduados, Campus Puebla. Km 125.5. Carr Fed. México-Puebla. 72760. Santiago Momoxpan, Cholula, Puebla, México

\*Corresponding author: E-mail: [asvarela@colpos.mx](mailto:asvarela@colpos.mx)

### Abstract

In Mexico there is a broad diversity of maize. To design schemes of genetic improvement and germplasm conservation, this diversity must first be assessed. In this context, an analysis of microsatellites was conducted to estimate the degree of variation and to analyze the structure and genetic diversity of seven maize (*Zea mays* L) landraces from the High Central Valleys of Mexico (Arrocillo Amarillo, Cacahuacintle, Chalqueño, Cónico, Elotes Cónicos, Palomero Toluqueño and Purépecha) as well as the teosinte races Chalco [*Zea mays* ssp. *mexicana* (Schrader) Iltis] and Balsas [*Zea mays* ssp. *parviglumis* (Iltis and Doebley)]. Seed from 107 accessions kept in Mexican germplasm banks was used. We analyzed 31 SSR loci to estimate genetic variation based on the number of alleles per locus, proportion of polymorphic loci and index of expected heterozygosity, and genetic structure using Wright F statistics. Races were grouped based on principal component and cluster analyses. A total of 636 alleles were identified, averaging 20.52 alleles per locus, 92.75% of which were polymorphic loci. Also found were 100 alleles exclusive of some of the studied populations. Occurrence of these alleles was low, representing 16% of the total alleles found. It was determined that 76.3% of the genetic diversity of the cultivated landraces of the High Valleys of Mexico resides within populations and the remaining 23.7% is between populations. Well-defined groups of the races Cacahuacintle and Purépecha, as well as two groups of the Chalqueño race, were observed. The Purépecha race formed a compact group separate from the rest, while a large sample of the Elotes Cónicos race group was placed intermediately among one of the groups of the Chalqueño race.

**Keywords:** *Zea mays* L, plant genetic resources, germplasm, molecular markers

### Introduction

Mexico is the center of origin, domestication and one of the centers of diversification of maize (*Zea mays* L). Mexico has greatly varied orographic and edaphic conditions that interact with elements of climate, resulting in broad environmental diversity and ecological niches. Moreover, man has given the species different uses over millennia (Toledo and Ordoñez, 1993; Romero and Muñoz, 1996). This has resulted in broad morphological and genetic diversity of maize populations. This situation has led to the need to use formal classification of maize types that would be applicable for designing schemes for its genetic improvement and germplasm conservation. Classification has been based on the concept of race, which has been used to assess genetic diversity (Anderson and Cutler, 1942; Wellhausen et al, 1952; Sánchez et al, 2000; Perales et al, 2003).

Given the importance of the race concept, different means have been used for classification. Some examples are morphological characterization, interactions genotype × environment, chromosome constitution and isoenzymatic markers (Goodman and Brown, 1988; Sánchez et al, 2000). However, more

precision in racial classification is still required.

The relatively ambiguous concept of race, combined with the large diversity of Mexican maize, has often made classifying maize extremely difficult requiring considerable effort to classify discrete races. Moreover, traditional protocols that use morphological type variables for characterization necessarily face problems in which environment affects trait expression. In this context, microsatellites (SSRs), or repeated simple DNA sequences, are a useful tool that have been shown to be reliable in generating genome fingerprints and in describing and systemizing diversity between and within maize populations, thus overcoming some of the difficulties present in traditional methodologies.

Besides its higher precision, microsatellites are preferred because there is public information referring to the nucleotide sequences that individually flank numerous loci and can be used as primers for their amplification by polymerase chain reaction (PCR), generating genotypic information that can be processed by modern statistical tools. These tools have the capacity to discriminate among populations of diverse origin whether or not one belongs to

a given taxonomic group with a margin of error duly quantified. Thus, the objectives of this study were to i) conduct an analysis of genetic diversity of the seven most cultivated maize races of the High Valleys of Mexico using SSR; ii) define the population structure and degree of genetic differentiation existing within and among populations and iii) to determine similarity and phylogenetic relationships among populations of the studied races.

## Materials and Methods

### Plant material

One hundred seven accessions representative of seven maize races of the High Valleys of Mexico were analyzed: 10 Arrocillo Amarillo, 11 Cacahuacintle, 22 Chalqueño, 23 Cónico, 14 Elotes Cónicos, 8 Palomero Toluqueño, and 19 Purépecha. Also, one population of teosinte Chalco race [*Zea mays* ssp. *mexicana* (Schrader) Iltis] and another of the Balsas race [*Zea mays* ssp. *parviglumis* (Iltis and Doebley)] were used as an external group in the phylogenetic analysis. Seed for this study was acquired from the germplasm banks of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Universidad Autónoma Chapingo, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) and

Colegio de Postgraduados. Experts selected seed visually on plants and ears to obtain the most representative populations of each race.

### Microsatellite analysis

Thirty-one microsatellite loci (Table 1), distributed along the 10 maize chromosomes, on which there is abundant published information in the Maize Genetics and Genomics Database (MaizeGDB) available online at <http://www.maizegdb.org/ssr.php#> were analyzed. Genomic DNA was extracted from 100 mg mesocotile and coleoptile tissue and young leaves from 25 seedlings per accession. A commercial DNA extraction kit (ChargeSwitch® gDNA Plant, Invitrogen) was used with an extraction robot (King Fisher Flex®, ThermoScientific, Waltham, MA).

To quantify DNA concentration, 260 and 280 nm absorbance readings were done in an ultra-low volume spectrophotometer (NanoDrop 2000, ThermoScientific, Waltham, MA). The microsatellite regions were amplified by PCR with primers marked with fluorescent labels (6-FAM, ROX or HEX) at the 5' end for detection in a DNA sequencer. Amplification in multiple PCR was done in volumes of 25 µL containing 10 mM nucleotides, 25 mM MgCl<sub>2</sub>, 5x buffer, 100 ng DNA, 1 unit Taq DNA polymerase and 4 pmol of each primer. The PCR amplification procedure consisted

**Table 1** - Microsatellite loci and primers used for amplification of SSRs in populations of maize landraces of the High Valleys of Mexico.

Locus	Bin	Fragment size (bp)	Forward primer//Reverse primer
phi127	2.07	113-132	ROX-atatgcattgcctggaactggaagga//aattcaaacacgcctcccagtggt
phi051	7.06	131-143	6-FAM-gcgaagcgaacgacaacaactct//acatcgtagattatattgcagacca
phi115	8.03	291-308	HEX-gctccgtgttcgcctgaa//accatcacctgaatccatcaca
phi015	8.08	73-109	HEX-gcaacgtaccgtaccttccga//acgctgcattcaattaccgggaag
phi033	9.02	234-266	6-FAM-atcgaaatgcagcggatggttct//atcgagatgttctacgcctgaagt
phi053	3.05	170-214	ROX-ctgcctctcagattcagagattgac//aacccaacgtactccggcag
phi072	4.01	127-164	6-FAM-gtgcattgataatttctccagcct//gacagcgcgcaaatggattgaact
phi093	4.08	275-290	ROX-gtgcctcagcttcatgcctacaag//ccatgcatgcttgcaacaatggatata
phi024	5.01	354-373	HEX-ctccgcttccactgttcca//tgtccgctgcttctacca
phi085	5.06	231-265	6-FAM-agcagaacggcaagggtact//ttggcacaccacgacga
phi034	7.02	121-159	HEX-tagcgacaggtggcctctt//ggggagcagccttctgtct
phi121	8.04	93-104	6-FAM-agggaaatggagccggtgaacca//ttggtctggaccaagcacatacac
phi056	1.01	236-259	ROX-actgttgcctgctgctgac//cgcacaccactcccagaa
phi064	1.11	65-115	HEX-cgaattgaaatagctgcgagaact//acaatgaacggtggttatcaacacgc
phi050	10.03	79-93	ROX-aacatgccagacacatacggacag//atggcttagcgaagcgtagag
phi96100	2.01	232-299	6-FAM-agggagacccaactcctg//ttgcacgagccatcgat
phi101249	?	111-160	6-FAM-ttctctcactgctc//aagaacagcgaagcagagaag
phi109188	5.03	145-175	HEX-aagctcagaagccggagc//ggatcaagctctctgatcg
phi029	3.04	144-176	ROX-tcttctctccacaagcagcga//ttccagttgccaccgcaagaactt
phi073	3.05	184-200	HEX-gtgcgagaggttgaccaa//aagggtgagggcgaggaa
phi96342	10.02	230-251	6-FAM-gtaatcccacgtcctatcagcc//tccaactgaacgaactcctc
phi109275	1.03	119-149	6-FAM-cggtcatgctagctctgc//gttggctgtgtgtgtg
phi427913	1.01	118-145	ROX-caaaagctagtcgggtca//attgtcagatcacactacgc
phi265454	1.11	216-242	6-FAM-caagcactcaactctctg//tccacgctgctcacctc
phi402893	2.XX	203-247	HEX-gccaagctcaggtcaag//cacgagcgttattcgtgt
phi346482	1.XX	114-152	HEX-gcatcacactcacacaaca//gtggaataggaggcgagagag
phi308090	4.04-4.05	185-226	6-FAM-cagtctgccacgaagca//ctgctggttctgctctt
phi330507	5.02-5.06	131-151	ROX-gtaaagtagatgctcctccc//cgggtagagagaggtgtg
phi213398	4.01-4.04	285-312	6-FAM-gtgacctaactggcagacc//caagaggtacctgcatggc
phi339017	1.03	139-166	HEX-actgctgtggggtaggg//gcagctgagcaggaagc
phi159819	6.00-6.08	121-146	6-FAM-gatgggcccctagaccagct//gcctctccatctctcgtt

**Table 2** - Analysis of diversity of seven maize and two teosinte races based on 31 SSR loci.

Race	num accessions	num alleles	alleles per locus	exclusive alleles	polymorphic loci (%)	He
Chalco (Teosinte)	1	218	7.030	14	100.0	0.730
Balsas (Teosinte)	1	166	5.35	9	100.0	0.644
Arrocillo Amarillo	10	359	11.58	7	95.16	0.714
Cacahuacintle	11	361	11.64	4	97.36	0.710
Chalqueño	22	472	15.22	21	87.68	0.738
Cónico	23	466	15.03	23	84.43	0.729
Elotes Cónicos	14	414	13.35	13	86.17	0.717
Palomero Toluqueño	8	327	10.54	3	91.93	0.704
Purépecha	19	387	12.48	6	92.02	0.707
Total	109	636	-	100	-	-
Average	-	-	20.52	-	92.75	0.710

He: expected heterozygosity.

of one initial 4 min denaturation at 95 °C, followed by 25 cycles of one min at 95 °C, 2 min at 55 °C, 2 min at 72 °C and a final 60 min extension at 72 °C.

#### Electrophoresis and detection

PCR products were assessed by capillary electrophoresis in a DNA sequencer (Genetic Analyzer ABI 3130®, Applied Biosystems, Foster City, CA) using LIZ-500 as the standard internal marker. Data files of to the allele content of the markers were generated for each of the populations with GeneMapper® V. 4.0 software (Applied Biosystems, 2005), which constituted the input for the statistical analysis of the study.

#### Statistical analysis

Allele frequencies were obtained from the populations, and diversity parameters, such as number of alleles per locus, exclusive alleles, proportion of polymorphic loci, index of expected heterozygosity, were determined, as well as the genetic structure of the populations, estimated with the Wright (1965) F statistics, which hierarchically describe the degree of endogamy effects, within populations ( $F_{IS}$ ), between subpopulations ( $F_{ST}$ ) and within the entire population ( $F_{IT}$ ). These calculations used comparisons between observed and expected heterozygosity, assuming that Hardy-Weinberg equilibrium exists at the different hierarchical levels. To estimate these parameters, POPGENE 1.31 (Yeh et al, 1999) software was used.

To avoid problems of distancing between accessions and the corresponding interpretation, which occurs with low frequency or with exclusive alleles, in the cluster analysis alleles that had significant differences ( $p \leq 0.05$ ) between populations were selected using a one-way analysis of variance and allele frequency above 2%. With the selected alleles, a principal component analysis was conducted based on the matrix of correlations using SAS V. 9.0. (SAS Institute, 2002).

With the selected alleles, a phylogenetic analysis between populations was performed using the Neighbor-Joining method (Saitou and Nei, 1987) with the software NTSYSpc V. 2.21c (Rohlf, 2009), with the matrix of modified Rogers genetic distances.

## Results and Discussion

### Analysis of diversity

One hundred nine accessions, belonging to seven maize races of the High Valleys of Mexico and two teosintes, were analyzed on the basis of polymorphism of the microsatellites. The total set of populations yielded 636 alleles in the 31 loci analyzed, with an average of 20.52 alleles per locus. This result contrasts with other previous studies, such as Reif et al (2006), who found 7.84 alleles per locus in 24 Mexican maize races, and Reif et al (2005), who obtained 5.9 alleles per locus in five European crystalline maize varieties, or Labate et al (2003), who found 6.5 alleles per locus in 57 accessions, which included dent maize from the Corn Belt, Northern Flints and Southern Dents. These were analyzed with a subgroup of the same markers as those used in our study. In those studies, the parameter was lower than that found in our study, possibly because Reif et al (2005), Reif et al (2006) and Labate et al (2003) genotyped fewer than 500 individuals in a few accessions, while in our work, 2,725 plants from 109 accessions were genotyped. Moreover, 100 exclusive alleles were detected in different maize populations, with a frequency  $< 0.05$ , representing 16% of the total number of alleles found. These were observed in all but three ( $\phi 024$ ,  $\phi 121$  and  $\phi 265454$ ) of the 31 loci analyzed, indicating the need to conserve these populations that are carriers of exclusive alleles (Table 2).

The percentage of polymorphic loci in the set of 109 accessions belonging to seven maize and two teosinte races was 92.7%. Cónico was the least polymorphic (84.43%), while the most polymorphic were Cacahuacintle (97.35%) and Arrocillo Amarillo (95.16%), indicating larger diversity for these two races, both of which are closely related and share similarities (Wellhausen et al, 1952; Doebley et al, 1985; Sánchez and Goodman, 1992). The results of our research show that polymorphism in accessions belonging to races of the High Valleys was higher than other studies conducted in Mexico: Sánchez et al (2000) found percentages of polymorphic loci of 75.7 for Arrocillo Amarillo, Palomero Toluqueño, Cacahuacintle and

**Table 3** - F-statistics calculated from 31 microsatellite loci for seven maize and two teosinte races from the High Valleys of Mexico.

Race	$F_{IS}$	$F_{IT}$	$F_{ST}$
Chalco	0.381	0.381	-
Parviglumis	0.314	0.314	-
Arrocillo Amarillo	0.164	0.327	0.194
Cacahuacintle	0.144	0.276	0.153
Chalqueño	0.142	0.370	0.266
Cónico	0.108	0.376	0.300
Elotes Cónicos	0.150	0.391	0.282
Palomero Toluqueño	0.130	0.341	0.243
Purépecha	0.106	0.305	0.222
General	0.108	0.342	0.237

$F_{IS}$  - endogamy within populations;  $F_{IT}$  - endogamy within the entire population;  $F_{ST}$  - endogamy between subpopulations.

Elotes Cónicos, and 81.1 for Chalqueño and Cónico. In another study, López et al (2009) estimated 58% polymorphism in populations of the Zapalote Chico race. The high values found in our study may be due to the broad variation detected in the populations studied, as well as to the larger number of representative accessions we assessed per race. Based on this information, it is evident that in situ conservation schemes should be developed for these resources since they are potential sources of genes that can be used in conventional and participative genetic improvement programs.

The values obtained for expected heterozygosity ( $H_e$ ) for the different races show broad genetic diversity, with an average of 0.710 (Table 2).

Santacruz-Varela (2001) studied 39 populations of popcorn with the same primers as those used in our study and obtained 0.522 for  $H_e$ . Labate et al (2003) found a value of 0.53 in 57 populations of the Corn Belt in the United States. These estimated  $H_e$  values are lower than those found in our study, thus confirming broad genetic diversity and variability of the evaluated materials of Mexican races cultivated in the High Valleys of Mexico.

#### Genetic differentiation

The Wright F-statistics show that the populations within races are not in Hardy-Weinberg equilibrium, because selection produces a bottleneck for some specific loci, besides, there is a linkage between loci, and epistasis (Flint-García, 2003). The  $F_{IS}$  values indicate a deficiency of heterozygotes; with larger disequilibrium in populations of the teosinte races Chalco and Balsas (Table 3) and less in the other races assessed (0.106 for Purépecha and 0.164 for Arrocillo Amarillo). The lowest  $F_{IT}$  value was observed in the Cacahuacintle race, suggesting lower losses of heterozygotes.

Within each race, very broad genetic differentiation was found. Cónico, Elotes Cónicos, Chalqueño, Palomero Toluqueño and Purépecha races had the highest  $F_{ST}$  values, while differentiation in Arrocillo Amarillo and Cacahuacintle was moderate (Table 3), with an overall average value of 0.237, indicating re-

**Table 4** - Eigenvalues and proportion of explained and accumulated variance for the first 20 principal components using 211 alleles of 31 SSR loci in 109 maize accessions.

Principal component	Eigen value	Proportion of explained variance	Proportion of accumulated variance
1	17.62	8.35	8.35
2	10.44	4.95	13.30
3	8.24	3.90	17.20
4	7.54	3.57	20.78
5	7.23	3.43	24.20
6	6.20	2.94	27.14
7	5.74	2.72	29.86
8	5.05	2.39	32.25
9	4.84	2.29	34.55
10	4.73	2.24	36.79
11	4.60	2.18	38.97
12	4.29	2.03	41.00
13	3.95	1.87	42.87
14	3.89	1.85	44.72
15	3.68	1.74	46.46
16	3.54	1.68	48.14
17	3.51	1.66	49.81
18	3.41	1.61	51.42
19	3.28	1.55	52.98
20	3.16	1.50	54.47

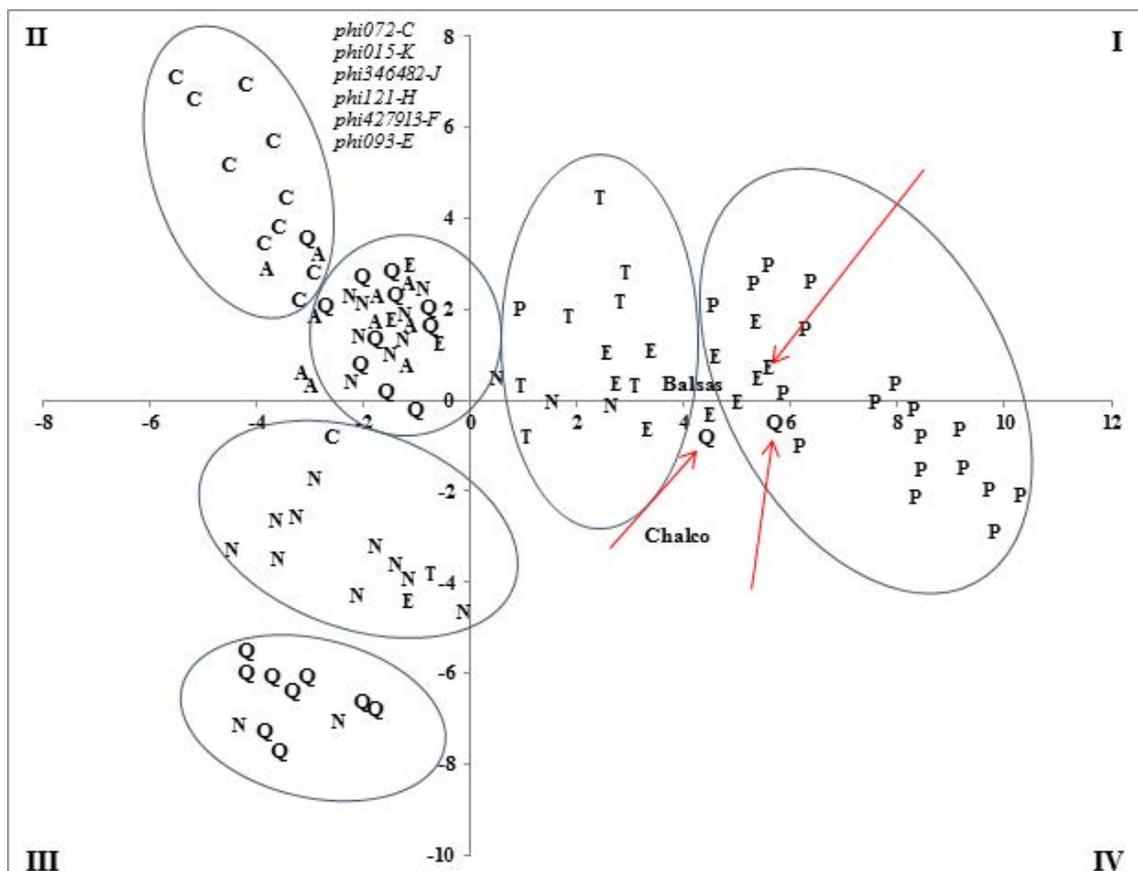
duced genetic flow among accessions of each race. Sánchez et al (2000) found lower  $G_{ST}$  (analogous to  $F_{ST}$ ) values for the same races than those obtained in our study. A general value of 0.237 for all the races indicates that only 23.7% of the total variation resides between the populations, while 76.3% is found within them. One of the possible reasons that high  $F_{IT}$  and  $F_{ST}$  values were found is that the genotypes used in our work are from seed stored in germplasm banks where the process of regeneration is isolated and occurs outside the original environments. Therefore, there is only recombination within the same populations, causing the phenomena genetic drift and endogamy because the samples used are small (Doebley et al, 1985).

#### Relationships among the studied races

A principal component (PC) analysis was conducted with frequencies of the 211 selected alleles in each population. Of the total variance, 54.47% was explained with the first 20 PC; PC1 contributed 8.35% and PC2 4.95% of the total variation (Table 4).

Dispersion of the maize accessions on the scatter plot determined by PC1 and PC2 (Figure 1) showed broad variation, with populations distributed in the four quadrants; six groups were identified. In the integration of PC1, there was greater contribution from the alleles phi115-C, phi033-L, phi265454-M, phi127-M, phi96100-N, phi265454-P, and phi053-G, while in PC2 the largest contributors were phi072-c, phi015-K, phi346482-J, phi121-H, and phi427913-F.

The accessions belonging to the Chalqueño race formed Groups 3 (with populations predominantly from states at higher latitudes such as Durango, Guanajuato, Jalisco, Zacatecas, Michoacán, Querétaro, Morelos and México) and 5 (with populations from the central part of the country such as Hidalgo, Estado de México, Puebla and Tlaxcala) located in the



**Figure 1** - Dispersion of 109 maize accessions based on the first two principal components derived from 211 alleles of 31 SSR (Q: Chalqueño, N: Cónicos, E: Elotes Cónicos, T: Palomero Toluqueño, P: Purépecha, C: Cacahuacintle).

lower part of quadrants II and III, respectively. Group 4 comprises the populations of the Cónico race located between Groups 3 and 5 in the upper part of quadrant III. This interspersing of populations of the Cónico races among those of Chalqueño race is possibly due to constant genetic flow between the two races; thus placement of these populations in well-defined groups (Reif et al, 2006) was not possible since their geographic distribution is almost identical.

The populations of the Elotes Cónicos race were included into Groups 1, 2, 4, and 5 in the four quadrants. The populations of Purépecha (Group 1) and Cacahuacintle (Group 6) were the most differentiated, forming well-defined groups (Figure 1), as occurred with their morphology (data not shown).

Group 1 comprised accessions of Purépecha, Elotes Cónicos and one of Chalqueño located in quadrants I and IV. This suggests a genetic group different from Chalqueño, as proposed by Romero et al (2002) and confirmed by Mijangos-Cortés et al (2007). Group 2 integrated seven accessions of the Palomero Toluqueño race, four of the Elotes Cónicos race, and one Purépecha. The Balsas teosinte race was placed between Groups 1 and 2, probably indicating genetic similarity to these groups. In the same context, the accessions Mexi-192 and 7202 of the

Chalqueño race was placed near the predominant group of the Purépecha race.

The phylogenetic tree (Figure 2) constructed from data of 211 SSR alleles shows the Balsas teosinte race as the immediate ancestor of cultivate maize, as has been reported in different studies (Matsuoka et al, 2002; Doebley, 2004; Vigouroux et al, 2008). Four accessions (Pur-44, Pur-39, Pur-33, Pur-15) of the Purépecha race and six (Tlax-251, Pueb-454, Pueb-163, Pueb-510, Tlax-255, Guan-160) of the Elotes Cónicos race are closer to the Balsas teosinte race, suggesting that the diversification of these races began in the Balsas River basin where teosinte (*Zea mays* ssp. *parviglumis*) is distributed naturally.

The grouping in Figure 2 defined, in general, nine groups. Group 1 comprises 10 accessions of the races Arrocillo Amarillo, Cachacuacintle, Cónico, and Chalqueño. This grouping is defined by geographic origin since nine of them are from the state of Puebla, and only the Chalqueño race accession is from the state of Morelos. Group 2 included 22 accessions, for which it was not possible to obtain a pattern of geographic distribution or racial relationship since accessions of the seven races studied were found. Of these races, accessions whose geographic origin is in the states of Mexico and Puebla predominated.

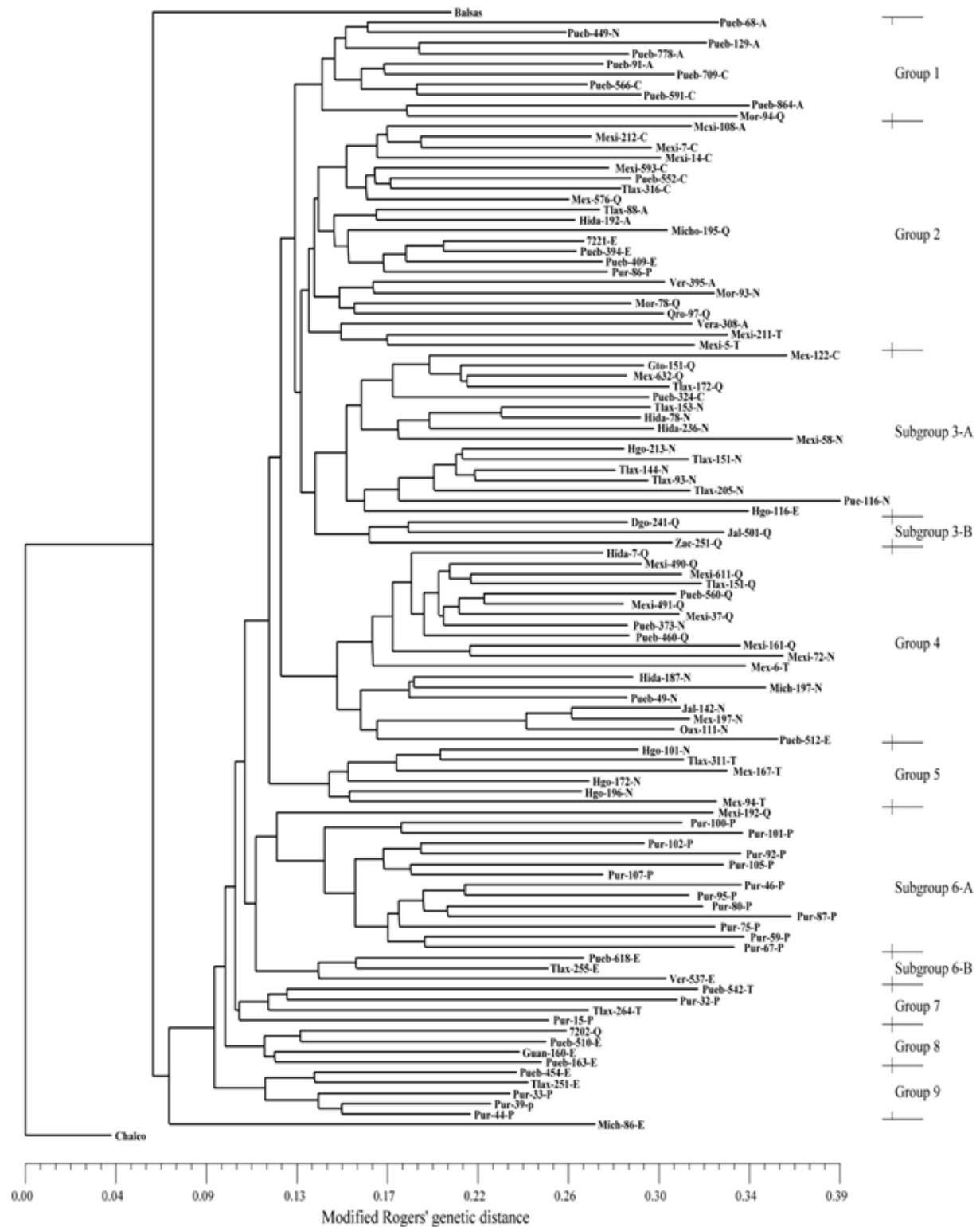


Figure 2 - Phylogenetic tree produced with the Neighbor-Joining method, based on modified Rogers' genetic distance from 211 SSR alleles.

In Group 3, two subgroups can be distinguished: subgroup 3A, formed mostly by accessions of the Cónico race from the states of Tlaxcala, Hidalgo, Puebla, and México, and subgroup 3B in which three accessions of the Chalqueño race from the Northern and Western regions of the country (Durango, Zacatecas, and Jalisco).

Group 4 comprised populations of the Chalqueño, Cónico and Palomero Toluqueño races from the states of México, Puebla, Hidalgo, Oaxaca, Michoacán, and Jalisco. Group 5 included three accessions of the Palomero Toluqueño race (two from the state of Mexico and one from Tlaxcala) and three accessions of Cónico (originating in the state of Hidalgo). Group 6 was composed of two subgroups: subgroup 6A, with 13 Purépecha accessions and one Chalqueño, and subgroup 6B with three accessions of the Elotes Cónicos race. Accessions of the Purépecha, Palomero Toluqueño and Elotes Cónicos races, which are genetically closer to the Balsas teosinte race were found in Groups 7, 8, and 9 and the accession Mich-86 of the Elotes Cónicos race was placed separately. These results reveal that the principal races of the High Valleys share a common origin with a diffuse genetic base that extends into all. This is indicated by the presence of common alleles of markers that are selectively neutral, as are microsatellites. Therefore, it is inferred that differentiation of these races has been influenced mainly by selection pressure relatively recently.

### Conclusions

Broad genetic diversity exists among the races of the High Valleys of Mexico. This conclusion is based on the count of 636 alleles, with an average of 20.5 alleles per locus, 92.75% of which are polymorphic loci, and an expected heterozygosity of 0.710. We identified 100 exclusive alleles in maize populations; these occurred at low frequency and represented 16% of the alleles found. It was determined that 76.3% of the genetic diversity in races cultivated in the High Valleys of Mexico resides within populations and the remaining 23.7% between populations. Defined groups of the Cacahuacintle and Purépecha races and two groups of the Chalqueño race were observed. In this latter race it is possible to distinguish accessions originating at higher latitudes from those originating at the central High Valleys of the country. Accessions of the Cónico race overlapped with the Chalqueño race, indicating greater genetic complexity. The Purépecha race formed a compact group separate from the rest, while a large sample of the Elotes Cónicos race clustered intermediately with one of the Chalqueño race groups.

### References

- Anderson E, Cutler HC, 1942. Races of *Zea mays*: I. Their recognition and classification. *Ann Missouri Bot Gard* 29: 69-88
- Applied Biosystems, 2005. GeneMapper® Software Version 4.0. Reference and Troubleshooting Guide. Applied Biosystems Inc, Foster City
- Doebley JF, Goodman MM, Stuber CW, 1985. Isozyme variation in the races of maize from México. *Am J Bot* 72: 629-639
- Doebley J, 2004. The genetics of maize evolution. *Annu Rev Genet* 38: 37-59
- Goodman MM, Brown WL, 1988. Races of corn, pp. 33-79. In: *Corn and Corn Improvement*. Sprague GF, Dudley JW eds. 3<sup>rd</sup> Edition. American Society of Agronomy. Madison
- Flint-Garcia SA, 2003. Structure of linkage disequilibrium in plants. *Annu Rev Plant Biol* 54: 357-374
- Labate JA, Lamkey KR, Mitchell SE, Kresovich S, Sullivan H, Smith JSC, 2003. Molecular and historical aspects of Corn Belt Dent diversity. *Crop Sci* 43: 80-91
- López RG, Santacruz VA, Muñoz OA, Castillo GF, Córdova TL, Vaquera H H, 2009. Perfil isoenzimático de maíces nativos del Istmo de Tehuantepec, Oaxaca, México. II. Variación dentro de grupos. *Rev Fitotec Mex* 32: 177-188
- Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez GJ, Buckler E, Doebley J, 2002. A single domestication for maize shown by multilocus microsatellite genotyping. *Proc Nat Acad Sci USA* 99: 6080-6084
- Mijangos-Cortés JO, Corona-Torres T, Espinosa-Victoria D, Muñoz-Orozco A, Romero-Peñaloza J, Santacruz-Varela A, 2007. Differentiation among maize (*Zea mays* L) landraces from the Tarasca Mountain Chain, Michoacan, Mexico and the Chalqueño complex. *Genet Resour Crop Evol* 54: 309-325
- Perales RH, Brush SB, Qualset CO, 2003. Landraces of maize in central México: an altitudinal transect. *Econ Bot* 57: 7-20
- Reif JC, Hamrit S, Heckenberger M, Schipprack W, Maurer HP, Bohn M, Melchinger AE, 2005. Genetic structure and diversity of European flint maize populations determined with SSR analyses of individuals and bulks. *Theor Appl Genet* 111: 906-913
- Reif JC, Warburton ML, Xia XC, Hoisington DA, Crossa J, Taba S, Muminović J, Bohn M, Frisch M, Melchinger AE, 2006. Grouping of accessions of Mexican races of maize revisited with SSR markers. *Theor Appl Genet* 113: 177-185
- Rohlf FJ, 2009. NTSYSpc: numerical taxonomy system. Ver. 2.21c. Exeter Software, New York
- Romero PJ, Muñoz OA, 1996. Varietal pattern and maize varietal selection for the agricultural systems in the Tierra Caliente región. *Agrociencia* 30: 63-73
- Romero PJ, Castillo GF, Ortega PR, 2002. Cruzas de poblaciones nativas de maíz de la raza Chalqueño: II. Grupos genéticos, divergencia genética y heterosis. *Rev Fitotec Mex* 25: 107-115

- Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406-425
- Sánchez GJJ, Goodman MM, 1992. Relationships among the Mexican races of maize. *Econ Bot* 46: 72-85
- Sánchez GJJ, Goodman MM, Stuber CW, 2000. Isozymatic and morphological diversity in the races of maize of Mexico. *Econ Bot* 54: 43-59
- Santacruz-Varela A, 2001. Genetic diversity of North American popcorn and its relationship with Mexican and South American popcorns. Ph.D. Dissertation. Iowa State University, Ames, Iowa
- SAS Institute 2002. SAS/STAT User's Guide, Software Version 9.0. Cary, NC
- Toledo MV, Ordoñez MJ, 1993. Biodiversity scenario of Mexico: A review of terrestrial habitats, pp. 757-777. In: *Biological Diversity of Mexico: Origins and distribution*. Ramamoorthy TP, Bye R, Lot A, Fa J eds. Oxford University Press, New York
- Vigouroux Y, Glaubitz JC, Matsuoka Y, Goodman MM, Sánchez GJ, Doebley J, 2008. Population structure and genetic diversity of New World maize races assessed by DNA microsatellites. *Am J Bot* 95: 1240-1253
- Wellhausen EJ, Roberts LM, Hernández XE, Mangelsdorf PC, 1952. *Races of Maize in Mexico, Their Origin, Characteristics and Distribution*. The Bussey Institution. Harvard University. Cambridge, MA
- Wright S, 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395-420
- Yeh FC, Yang R, Boyle T, 1999. POPGENE Version 1.31. Microsoft Windows-based Free-ware for Population Genetic Analysis. Quick User Guide. University of Alberta and Centre for International Forestry Research, Edmonton, Canada. available at: <http://www.ualberta.ca/~fyeh/popgene.pdf>