

## MORPHOLOGICAL DIVERSITY OF NATIVE MAIZE IN THE HUMID TROPICS OF PUEBLA, MEXICO

# [DIVERSIDAD MORFOLÓGICA DE MAÍCES NATIVOS DEL TRÓPICO HÚMEDO POBLANO, MÉXICO]

Fernando López-Morales<sup>1</sup>, Oswaldo Rey Taboada-Gaytán<sup>2\*</sup>, Abel Gil-Muñoz<sup>2</sup>, Pedro Antonio López<sup>2</sup>, Delfino Reyes-López<sup>3</sup>

<sup>1</sup>Programa de Maestría en Estrategias para el Desarrollo Agrícola Regional. Colegio de Postgraduados Campus Puebla, México.

<sup>2</sup>Colegio de Postgraduados, Campus Puebla. Km. 125.5 Carretera Federal México-

Puebla, Santiago Momoxpan, San Pedro Cholula, Puebla. CP. 72760. Tel.

01(222)285-1442. Email: toswaldo@colpos.mx

<sup>3</sup>Facultad de Ingeniería Agrohidráulica, Benemérita Universidad Autónoma de

Puebla. CP. 73695. Teziutlán, Puebla. Tel. 01 (231)312-2933.

\* Corresponding author

### SUMMARY

The most recent studies on maize in the humid tropics of Puebla were done in 1997, and consisted of agronomic evaluations. Thus, the objectives of this study were to assess the level of genetic diversity present among the native populations of maize, cultivated in this region, through their morphological characterization, and to determine the degree of association between these maize populations and the Tuxpeño race. Therefore, in the 2009-2010 autumnwinter season, 93 native maize populations, six improved varieties, and a racial control (Tuxpeño type) were evaluated in two localities, under a lattice experimental design  $10 \times 10$  with two replications. The results of the analysis of variance suggested that genetic diversity was present in the populations evaluated, represented by a large morphological diversity. With cluster and principal component analyses, such variation was grouped in six groups, based on vegetative, phenological, tassel, ear and grain traits, as well as on several indexes. The maize landraces cultivated in the humid tropics of Puebla can be regarded as part of the Tuxpeño race, since most of them kept similarity with such race.

**Key words:** Agronomic traits; morphological characterization; racial similarity; native populations; Tuxpeño race.

## RESUMEN

Los estudios más recientes sobre maíz en el trópico húmedo poblano se llevaron a cabo en 1997 a través de una valoración agronómica. Por ello, los objetivos de la presente investigación fueron el cuantificar el nivel de diversidad genética presente en las poblaciones nativas de maíz cultivadas en la región, a través de su caracterización morfológica, y precisar el nivel de asociación de tales poblaciones con la raza Tuxpeño. Para ello, en el ciclo Otoño-Invierno 2009-2010 se evaluaron, en dos localidades, 93 materiales de maíz nativo, seis variedades mejoradas y un testigo racial tipo Tuxpeño, bajo un diseño experimental látice 10×10 con dos repeticiones. Los resultados del análisis de varianza indicaron la existencia de diversidad genética en las poblaciones evaluadas, representada por una gran diversidad morfológica. La variación encontrada pudo agruparse, a través del análisis de conglomerados y de componentes principales, en seis grupos, con base en las características vegetativas, fenológicas, de espiga, de mazorca y del grano y la elaboración de varios índices. Los maíces nativos cultivados en el trópico húmedo poblano pueden considerarse como pertenecientes al tipo Tuxpeño, ya que la mayoría de los colectados mantiene similitud con esta raza.

**Palabras clave**: Características agronómicas; caracterización morfológica; correspondencia racial; poblaciones locales; raza Tuxpeño.

#### **INTRODUCTION**

In Mexico, the area annually planted with maize (*Zea mays* L.) reaches 8 million ha, 86 % of which are

located in rainfed areas in the sub-humid tropical, temperate-humid, and sub-humid regions of central and southwestern Mexico (Bellon *et al.*, 2009; Kato *et al.*, 2009). In these areas, maize is grown for self-

sustenance and tradition; thus explaining why farmers protect, maintain and use the genetic diversity that they have shaped (Ángeles *et al.*, 2010). As a result of the continuous interaction between the environmental diversity, the genetic variation present in maize and farmer's management (which includes an empirical breeding process), in several regions of the country a large genetic diversity has been generated in this crop. This diversity is perceptible through the variation of both agronomical and morphological characteristics, as well as by the large number of uses given to the products of this plant (Kato *et al.*, 2009).

Maize diversity in Mexico was initially classified by Wellhausen et al. (1951) in 25 races and several subraces. Since then, several multivariate techniques have been used to have a better classification of the races and sub-races of maize (López et al., 2005). Nowadays, the world's maize diversity has been grouped in more than 300 races (Brown and Goodman, 1988; Serratos, 2009), 59 of which have been described in Mexico (Sánchez et al., 2000; Kato et al., 2009) thus representing a 20 % of the total racial diversity. This is one of the reasons why Mexico has been considered a center of origin, domestication and diversity for this crop (Kato et al., 2009). One of the most important races from an agronomic perspective is the Tuxpeño race; this is because of its high grain yield, drought tolerance and disease and pest resistances. These attributes have turned this race into one of the most worldwide used materials in breeding programs (Brown and Goodman, 1988; Bellon et al., 2005; Wen et al., 2012). The Tuxpeño race is distributed along the coast of the Gulf of Mexico, from Yucatan to northeastern Mexico, at altitudes ranging from 0 to 500 m; however, this race has demonstrated a wide adaptation to other regions, including the humid tropics of the state of Puebla (Wellhausen et al., 1951; Muñoz, 2005).

According to Herrera et al. (2000), most of the genetic diversity of Mexico's native maize is represented by the local populations that are planted in agricultural fields throughout the country. The studies on morphological diversity of maize in the humid tropics of Puebla started in 1997; they proved the existence of a large diversity of types and colors among the native maize populations from the ecological niches of Ayotoxco, Hueytamalco and Acateno (Muñoz, 2005). For the first niche, Gil et al., (2004) found differences in grain color: 166 white, 20 yellow, 8 pinto and 4 blue. It was also found that with respect to days to silking, all materials were classified as 'ultra-early', with 60 to 77 days to silking. Regarding grain yield, it was found that the best native maize materials surpassed by 35 % the improved varieties used as controls. Strictly speaking, both works were just agronomic evaluations,

therefore it was deemed convenient to conduct a study to quantify the level of genetic diversity present in the native populations of maize grown in the region (via their morphological characterization) and to determine the degree of association between such populations of maize and the Tuxpeño race.

According to the numbers of the Sistema de Información Agroalimentaria y Pesquera (SIAP), in 2010 in the state of Puebla, 606,534 ha of maize for grain were sown, mainly under rainfed conditions (92 %). The region where the largest area is sown and where the highest yields are achieved are the high valleys; however, there are other regions, such as the humid tropics of Puebla, where maize is also important. The humid tropics of Puebla encompass an area of 1,574.95 km<sup>2</sup> which represents approximately 4 % of the state area (Yanes, 2011). The agricultural area of those municipalities with at least 35 % of their territory within the humid tropics is of 1,192.59 km<sup>2</sup> (INEGI, 2009), while the area devoted to maize production for grain is of 24,927 ha. In the municipalities of San José Acateno, Hueytamalco, Ayotoxco and Tenampulco (Figure 1), located in the northeastern portion of the state, and where this research took place, maize is produced under rainfed conditions, on an area of 4,300 ha, with a production volume of four thousand tons and an average yield of 1.3 t ha<sup>-1</sup> (SIAP, 2010).

Several studies on native maize diversity in the state of Puebla have been conducted, but none of them has been carried out in the humid tropics of Puebla, addressing both the morphological diversity of native maize and their level of association with the racial types reported for that region. Therefore, the objectives of this study were: 1) to assess the genetic diversity present in the humid tropics of Puebla through a morphological characterization of the native maize populations there cultivated; and 2) to determine whether or not a correspondence exists between those maize populations and the Tuxpeño racial type reported for that region.

# MATERIALS AND METHODS

# Plant Material

Between May and September 2008, a collection of native maize was made in the municipalities of Tenampulco, Ayotoxco de Guerrero, Hueytamalco and San José Acateno. A total of 250 accessions was obtained. The explored region is located in the humid tropics of Puebla State, Mexico, between 20° 08' and 20° 12' N and 97° 19' and 97° 23' W. The altitude of the four municipalities ranges from 10 to 1,900 m. For evaluation purposes, a set of 93 native maize populations collected from 93 to 372 masl, was selected (Table 1). These accessions were evaluated

along with an accession representative of the Tuxpeño race (Vera – 39) provided by the International Maize and Wheat Improvement Center (CIMMYT) and six improved varieties: AF09A and AF07A (CIMMYT's experimental hybrids,

recommended for the region); CP-560, CP-562 and CPVM-301, from Colegio de Postgraduados, and the hybrid PROGRANO 988, produced by 'Productores de Grano del Norte', included for its aptitude for corn husk production.



Figure 1. Climate types distribution and location of collection sites in four municipalities of the humid tropics of the state of Puebla.

Table 1. Number of accessions, maize grain color, and maximum and minimum altitudes of the collection sites in four municipalities from the humid tropics of Puebla.

Maariainalitaa		Grain	color†		Total of	Site altitude (masl <sup>1</sup> )		
Municipality	WH	YE	BL	RE	accessions	Minimum	Maximum	
Ayotoxco	14	5	5	5	29	181	352	
Hueytamalco	17	2	2	0	21	143	372	
Acateno	17	1	3	0	21	151	319	
Tenampulco	17	1	3	1	22	93	253	
Total	65	9	13	6	93			

<sup>†</sup>WH=white, YE=yellow, BL=blue, RE=red; <sup>1</sup>= meters above sea level

### Localization of experiments

In Puebla, the humid tropics can be found in two parts of the state: one in the south (11 % of the area), and the other in the north, north-east (with 89 % of the area). This study was conducted within the latter, which includes 15 municipalities. Two climate types are found: Af(m), tropical rain forest, with rains all year, and Am(f), tropical monsoon with abundant summer rains.

Two experiments were planted, one in Rancho Nuevo, municipality of Ayotoxco de Guerrero, located at 20° 06' N, 97° 25' W, and 250 masl, and another in La Virgen, municipalty of Hueytamalco, located at 19° 56' N, 97° 17' W and 420 masl (INEGI, 2010). In these communities, the average annual temperature is of 23 and 21 °C, and the average annual precipitation of 3000 and 2550 mm, respectively (INEGI, 2009). Climates are tropical rain forest, Af(m), in the first town, and semi warm with rains all year, (A)C(fm), in the second (García, 1973).

Planting dates were: December 9th and 10th, 2009 (Rancho Nuevo and La Virgen, respectively) in "year after year" fields, with a slope lower than 10 %. Sowing was made according to local practices: the stover from the previous crop was incorporated and after a moderate rain, sowing took place under the following procedure: with a 'coa' (a large stick with a sharp, pointy end in one extreme), in the place corresponding to each hill, every 50 cm, a small hole was opened in the soil (10 cm deep) and three seeds were placed. After four weeks, thinning was made in order to have 34 plants per experimental plot. The experimental plot consisted of two rows, five meters long and 0.8 m apart. Experiments were conducted exclusively under rainfed conditions. Treatment distribution was made under a lattice design 10×10 with two replications (Martínez, 1988).

# Crop management

Crop management was similar in both experiments and according to the traditional practices in the region, except for fertilization, which was applied 70 days after sowing using a 128-92-00 (N-P-K) dose. Pest control for armyworm (*Spodoptera* spp.) and cutworm (*Agrotis* spp.) was performed 20 days after sowing, using a cypermethrin insecticide, at a dose of 250 ml·ha<sup>-1</sup>. Throughout all the cycle weeds were controlled manually to avoid competition with maize.

### **Evaluated variables**

Based on the studies of Caballero and Cervantes (1990). Herrera et al. (2000) and Hortelano et al. (2008), at each experimental plot, several vegetative, reproductive, tassel, totomoxtle, grain and ear characteristics were recorded. The indexes reported by Sánchez and Goodman (1992) were also calculated. The vegetative characteristics measured in every experimental plot, on a sample of five representative plants, with complete competence were: plant height, height of insertion of top ear (cm), and number of leaves below and above the top ear. The phenological traits evaluated were: days to 50 % anthesis and to 50 % silking; considered as the number of days elapsed from sowing date up to the moment in which 50 % of the plants from the experimental plot were shedding pollen and had exposed stigmas in the upper ear, respectively; floral asynchrony was the result of subtracting days to silking from days to anthesis. Characteristics measured on the tassel were: tassel length, length of the branched segment, length of the central branch, peduncle length (all in cm), and number of primary branches. On the bract that covers the ear (corn husk), ear coverage was evaluated, considered as the distance (in centimeters) from the tip of the ear cob up to the tip of the bract; other traits measured were the length of the bract that covers completely the ear, and the number and total weight of the bracts. The variables recorded in the ear were: length, diameter, number of rows and number of grains per row in two opposite rows. All tassel and ear data were taken in the same five plants where the vegetative characteristics were measured. The grain characteristics evaluated were thickness, length and width of 10 grains taken from the central part of the ear, and weight of 100 grains. Finally, several indexes were calculated: plant height divided by upper ear height, leaves below divided by leaves above the ear, and ear length divided by its diameter. The product of multiplying kernel length by kernel width by kernel thickness provided the average kernel volume (in  $cm^3$ ).

#### Statistical analysis

A combined analysis of variance was performed for all measured variables using the software Statistical Analysis System (SAS Institute, 2002) version 9.0. Afterwards, a simple correlation analysis was conducted, which served to identify those highly correlated ( $\geq 0.7$ ) variables; from each pair, only the variable considered more informative was selected. Thus, out of the 40 variables measured, only 21 were considered for the multivariate analysis. With the average values per population across environments and the 21variables previously selected, a data matrix was generated for the principal component analysis (Jarvis et al., 2006). Based on the data generated, the populations were located in a three dimensional plane whose axes were the first three main components. Additionally, a cluster analysis was conducted (Franco and Hidalgo, 2003) using the matrix of Euclidean distances; the corresponding dendogram was obtained using Ward's Minimum Variance grouping method. To improve the graphics presentation, the determination coefficient was used as a scale of dissimilarity between clusters (Mohammadi and Prasanna, 2003).

### Description of the Tuxpeño Race

То determine whether or not there was correspondence between the native maize populations and the Tuxpeño racial type, the references were the description made by Wellhausen et al. (1951) and the data collected on the Tuxpeño racial type (Vera-39) evaluated in the experiment According to the previously mentioned authors, the Tuxpeño race has the following characteristics: plant height, 2.7 m; number of leaves above the ear, six; total tassel length, 42.6 cm; peduncle length 5.1 cm; length of the branched segment, 14.4 cm; number of primary branches, 22. The ear is cylindrical, with a length of 19.7 cm, diameter of 4.4 cm, with 12.6 rows on average and 16 bracts. The grain has a width of 9.3 mm, thickness of 3.7 mm and length of 12.8 mm. Days to 50 % silking are 118.

#### **RESULTS AND DISCUSSION**

#### **Collection composition**

From the 93 accessions evaluated (Table 1), 65 had white grain (69.9 %), 13 blue (13.9 %), nine yellow (9.7 %) and six red (6.5 %). Gil *et al.* (2004) reported that in a collection of 198 native populations conducted in 1997 in the Ayotoxco region, a predominance of white grain populations (83.8 %), was found, they were followed by yellow materials (10.2 %) and at a lesser extent, the blue (2.0 %) and pinto ones (4.0 %). This can be considered as an indicator that the pattern of variation -at least in terms of grain color- has remained relatively constant for eleven years.

### **Earliness variation**

In relation to the earliness levels, Gil et al. (2004) reported that the materials they evaluated reached 50 % silking between 60 and 77 days, hence they were classified as 'ultra-early'. For the populations evaluated in the present study, an average of 126 to 131 days to silking (DFF) was determined, which according to the scale used by the above mentioned authors, classifies them as late materials. This can be explained by the fact that in 1997, the materials were evaluated in the spring-summer season, while in the present work the populations were studied during the fall-winter season, period in which the lower temperatures cause a delay in flowering (Pérez et al., 2002). For this variable, the data are consistent with the results of Wellhausen et al. (1951), so it is inferred that they may have been evaluated in the same growing season.

### **Analysis of Variance**

The combined analysis of variance (Table 2) revealed that among localities (LOC) there were highly significant differences (P $\leq$ 0.01) in 37 variables, significant (P $\leq$ 0.05) in one (number of ear bracts) and non-significant in two. Among populations (POBL) there were highly significant differences (P $\leq$ 0.01) in 39 variables and significant (P $\leq$ 0.05) in only one (dry matter percentage). The interaction localities × populations (LOC × POBL) was highly significant (P $\leq$ 0.01) for seven variables and significant in ten (P $\leq$ 0.05); for all other variables (23), the interaction was non-significant. Table 2. Mean squares of the combined analysis of variance for 100 maize populations, evaluated in four municipalities of the humid tropics of Puebla.

VARIARIES	Mean squares					
VARIADLES	LOC	POBL	LOC*POBL	%		
Vegetative characteristics						
Plant height (ALTPL) (cm)	39209.34**	3493.53**	415.81ns	340.20	6.95	
Ear height (ALTMZ) (cm)	5019 44**	2394 07**	300.26 *	222.55	9.71	
Leaves above the ear (NHARR) (number)	1 45**	0.51**	0.10ns	0.09	5 28	
Leaves below the ear (NHABA) (number)	16.43**	3 30**	0.19ns	0.02	7.81	
Plant score (ASPI)	6.13**	0.82**	0.42113	0.40	12 73	
Far score (CALMZ)	111 16**	0.52**	0.30118	0.23	12.73	
Phenological characteristics	111.10	0.52	0.27	0.21	15.05	
Days to anthesis (DFM)	11662 24**	117 593**	25 436**	16 499	3 35	
Days to silking (DFF)	18037 34**	178 545**	34 979**	22 455	3 74	
Eloral asynchrony (ASELO) (days)	700 00**	11 085**	5 708ns	5 022	30.34	
Grain weight (PESGRANO) (g)	403829 34**	804 104**	400 016ns	323 894	25 25	
Shalling factor (EACDESCA)	0.13**	0.007**	0.001 *	0.001	4.02	
Dry matter percentage (MASECA)	0.13	0.007	0.001	0.001	5.48	
Vield (RENDIMIE) (Kg hg <sup>-1</sup> )	240441231**	1613238**	855025**	304562	J.40 13.87	
Tassal characteristics	240441231	1013238	055725	504502	43.07	
Total length (LONTOL) (cm)	5068 44**	65 66**	30 85ns	23 78	7 83	
Length of the branched segment ( $I OPARA$ ) (cm)	1890.05**	47 67**	19 84ns	17.24	9.80	
Length of the central branch (LORACE)(cm)	956 83**	24 93**	13.87 *	10.15	11 41	
Peduncle length (LONPED) (cm)	186 64**	10 94**	6.18 *	4 42	10.06	
Number of branches (NUMR)	155 8/1**	25 11**	8 21ns		13.78	
Combusk characteristics	155.04	23.11	0.21115	0.49	13.70	
Ear coverage (CUBERTO) (cm)	60.79**	5.15**	2.57ns	2.75	18.18	
Length of the bracts (LONHOJA) (cm)	1295.97**	14.28**	4.15ns	3.13	6.74	
Number of bracts (NUMBRAC)	13.12 *	6.34**	3.07ns	2.81	14.77	
Bracts weight (PESOTOT) (g)	34687.56**	155.70**	76.21 *	53.25	25.95	
Ear characteristics						
Ear length (LONMAZ) (cm)	1683.97**	5.82**	2.69 *	1.94	9.57	
Ear diameter (DIAMAZ) (cm)	45.22**	0.20**	0.06ns	0.07	7.25	
Rows per ear (HILMAZ) (number)	63.02**	3.54**	1.38 *	1.01	8.61	
Grains from opposite rows (NGRAO)	8174 84**	74 09**	23 67ns	21.34	15 79	
Grain characteristics	0171.01	/ 1.0/	20.07115	21.51	10.77	
10 grains thickness (GROSOR) (cm)	0.0005ns	0.010**	0.002ns	0.002	12.89	
10 grains length (LARGO) (cm)	2.1299**	0.016**	0.007**	0.002	6.43	
10 grains width (ANCHO) (cm)	0.2095**	0.006**	0.003**	0.001	4.92	
Weight of 100 grains (PDEGRANO) (g)	11400.9231**	35.138**	21.492**	13.427	15.44	
Indexes						
Plant height/ear height (PLANMAZ)	0.187368**	0.118**	0.013**	0.009	5.40	
Leaves below/leaves above (ABAJARRI)	1.051883**	0.089**	0.024 *	0.017	9.17	
Ear length/ear diameter (LONGDIA)	15.285470**	0.647**	0.206ns	0.213	11.59	
Grain length/grain width(LONANCH)	1.115469**	0.035**	0.006ns	0.006	6.51	
Plant height/tassel (PLANESPI)	3.941439**	0.803**	0.234ns	0.204	10.54	
Peduncle length/tassel (PEDULO)	0.034022**	0.002**	0.001ns	0.001	11.45	
Length of the central branch/ tassel (RACEL)	0.000002ns	0.003**	0.002ns	0.001	9.39	
Grain length/grain thickness (LONGROS)	12.223776**	0.739**	0.140ns	0.141	13.80	
Grain thickness/ grain width (GROSANC)	0.058289**	0.017**	0.004ns	0.004	13.68	
Grain length x width x thickness (LOANGRO)	0.460724**	0.007**	0.003 *	0.002	14.05	

\* and \*\* = Significant differences at  $P \le 0.05$  and  $P \le 0.01$ , respectively; ns = No significant differences; LOC = localities; POBL = populations; LOC\*POBL = localities × populations; CV = coefficient of variation.

In all cases, degrees of freedom for localities and populations were 1 and 99, respectively. Degrees of freedom for the LOC\*POBL interaction were 99 for DFM ASPL ALTPL ALTMZ NHARR NHABA PLANMAZ ABAJARRI LONTOL LOPARA PEDULON LORACE RACELON LONPED NUMR PLANESPI; 98 for DFF ASFLO FDESG MASECA RENDIMIE; 97 for LONH NBRAC COBTOT PTOT LONMAZ DIAMAZ NHIL GROGR LONGR ANCGR PESGRANO LONDIA LONANCH LONGRO GROANC LAANGRO NGRAO P100GR and 96 for CALMZ.

The results indicate the existence of genetic diversity in the evaluated varieties, and that they respond differently, in some variables, when the environment changes, as denoted by the 'localities × populations' interaction. According to Hortelano et al. (2008), the fact that the interaction 'localities  $\times$  populations' was not significant in a large proportion of the variables they studied, implies that even though the environments of evaluation showed variations in the level of expression of the characteristics studied, those variations were constant and similar between populations, regardless of the localities. Data on Table 2 are consistent with the reports of Hortelano et al. (2008) as to the percentage of significant differences found in the sources 'localities' and 'populations': this was not the case for the interaction 'localities  $\times$  populations'. The percentages for the sources 'localities' and 'localities × populations', are similar to those found in native maize from Molcaxac, Puebla, reported by Ángeles et al. (2010). The results from the combined analysis of variance suggest that in the humid tropics of Puebla, and especially in the region of study, there is a considerable genetic diversity (expressed through morphological attributes) among the native maize populations there cultivated.

# Principal component analysis

The principal component analysis revealed that with the first three components, 54 % of the original phenotypic variation observed in the populations was explained. The individual contribution of each component was: 22.9 % for the first component (CP1), 19.8 % for the second (CP2) and 11.2 % for the third (CP3). According to the magnitude of the eigenvectors (Table 3), the original variables with greater weight on CP1 were: days to silking, length of the branched segment and number of branches of the tassel, and the indexes plant height divided by ear height, leaves below the ear divided by leaves above the ear and ear length divided by its own diameter. In CP2, the original variables with greater weight were number of grains in opposite rows and shelling factor, along with the indexes grain thickness divided by grain width and grain volume. The CP3 was largely influenced by ear diameter and grain width.

Of the 12 most important original variables found in this study (Table 3), eight of them are directly or indirectly related with Hortelano's *et al.* (2008) findings, when they studied the morphological diversity of native maize from the Puebla valley. These variables are: days to silking, ear diameter, number of grains and grain width. The similitude was also present for some indexes, for example: plant height divided by ear height, ear length divided by ear diameter, grain thickness divided by grain width and grain volume. There was also coincidence with two of the most important original variables reported by López *et al.* (2005): plant height and days to silking, and with three reported by Ángeles *et al.*, (2010): days to silking, ear diameter and grain width. The most relevant original variables found in the four studies (including this) are: days to silking, ear diameter and grain width.

According to Herrera *et al.* (2000) the most important variables to determine differences among maize populations from the same region are the morphological characters, such as the vegetative and agronomic attributes, those of the tassel and grain, and specifically those from the ear, since they are the least affected by the environmental factors. On this matter, Goodman and Paterniani (1969) stated that the characters chosen for numerical taxonomic studies must be those that are less affected by the environment.

# **Cluster Analysis**

To define similarity relationships between native maize and controls, a dendrogram was generated in which, at a cutoff distance of 0.4 units; six groups (Figure 2) were identified. These are described next.

Group I comprised the six improved materials included in the experiment; it is evident that they conformed a group scarcely related with the native populations from the region. According to data from Table 4, this group was characterized by being the earliest (107 days), having a short length of the branched segment, and few branches on the tassel. It also had the lowest values for the following indexes: 'leaves below the ear / leaves above the ear', 'ear length / ear diameter' and grain volume, and had the highest value for 'plant height/ ear height'. In general terms, this indicates that they were plants with little growth, compared with the other materials. Group II consisted of 15 populations from the four municipalities studied: Ayotoxco (1), Hueytamalco (5), San José Acateno (7) and Tenampulco (2); most of them came from the central region; 86.8 % had white grain. The group was characterized by presenting the highest values for number of branches in the tassel, grains in opposite rows (33 grains), and shelling factor (86.6 %); nevertheless, it had one of the lowest values in grain volume. Group III was one of the largest; it consisted of 21 populations from the four municipalities: Tenampulco (1), Ayotoxco (9), Hueytamalco (5) and San José Acateno (6). In this group, white populations predominated (57.1 %), followed by the yellow ones (19 %), blue (14.2 %) and red (9.7 %). In general, this group showed intermediate values for all variables, except for the index 'grain thickness / grain width', which was the lowest, and grain volume, which also had low values. Group IV included six materials: three of them were blue grained. Five of these populations were collected in the municipality of Tenampulco and one in Hueytamalco, in communities located in the northern part of the studied region. This group was characterized by having the narrowest ears and the lowest number of grains per row, as well as the lowest shelling factor. The index 'grain thickness / grain width' was the highest among the six groups and its grain volume was one of the highest. Group V grouped 21 populations, three from Ayotoxco, one from San José Acateno, four from Hueytamalco and 13 from Tenampulco, 95 % were white-grained. In general, this group was the latest in terms of maturity (Table 4) and was among the groups with the largest values for tassel characteristics, it had the widest grains, a high index of 'leaves below / leaves above the ear' and a low index for 'plant height / ear height'. Finally, the group VI was the largest, with 30 native maize populations which came from the four municipalities: Tenampulco (1), Avotoxco (16),

Hueytamalco (6) and San José Acateno (7). Distribution in terms of grain color was: white 63.3 %, blue 20 %, yellow 10 % and red 6.7 %. In this group the Tuxpeño racial control was included, suggesting that this group of native maize was the one with the highest similarity to this race.

Table 4 shows the average values for the racial control (Tuxpe). When compared with the characteristics reported by Wellhausen *et al.* (1951) for the Tuxpeño race, it turns out that those of the former are of a lesser magnitude. Out of the six groups identified, Group VI was the one that had the highest correspondence with the Tuxpeño racial control (Vera-39), since they coincided in most of the characteristics evaluated. Materials of the Group VI are concentrated in a warm and humid area, with rains throughout all the year (García, 1973), and altitudes from 147 to 352 m, thus suggesting that this is the area where the native maize populations more related to that race can be found.

Table 3. Eigenvectors for the first three main components (CP) and 21 original variables used for the characterization of 100 maize varieties from the humid tropics of Puebla.

Original Variables	CP1 [4.82] <sup>1</sup> (22.9%) <sup>2</sup>	CP2 [4.17] <sup>1</sup> (19.8%) <sup>2</sup>	CP3 [2.36] <sup>1</sup> (11.2%) <sup>2</sup>
Days to silking (DFF)	0.352	0.020	-0.114
Leaves above ear (NHARR)	0.214	0.164	0.270
Length of the branched segment (LOPARA)	0.303	0.139	-0.205
Peduncle length (LONPED)	-0.005	-0.065	-0.216
Number of branches (NUMR)	0.310	-0.049	0.091
Ear coverage (CUBRTO)	0.102	0 249	-0.032
Number of bracts (NUMBRAC)	0.099	0.035	0.056
Ear diameter (DIAMAZ)	-0.139	-0.053	0.481
Rows per ear (HILMAZ)	-0.213	0.011	0.084
Grains from opposite rows (NGRAO)	0.122	-0.418	0.028
Grain width (ANCHO)	0.082	0.067	0.502
Shelling factor (FDESG)	0.035	-0.446	-0.029
Plant height/ ear height (PLANMAZ)	-0.384	0.125	-0.080
Leaves below/ leaves above (ABAJARRI)	0.323	-0.155	-0.099
Ear length/ ear diameter (LONDIA)	0.335	0.110	-0.164
Grain length/ grain width (LONANCH)	0.059	-0.280	-0.256
Plant height/total tassel length (PLANESPI)	0.271	-0.178	0.284
Peduncle length/total tassel length (PEDULON)	-0.238	-0.140	0.006
Length of the central branch/total tassel length (RACELON)	-0.101	0.170	-0.188
Grain thickness/ grain width (GROSANC)	0.031	0.417	-0.201
Grain length x width x thickness (LOANGRO)	0.159	0.345	0.234

<sup>1</sup>Characteristic value of the component, <sup>2</sup> percentage of variation explained by each component, <sup>3</sup>in bold, original variables with the greatest explanatory power for each principal component.



**Euclidian Distance Coefficient** 



Figure 3 shows the location of the maize populations in a three-dimensional plane, determined by the first three main components. The first aspect that stands out is that Group I was separated from the rest, situation that is explained by the fact that it included all the introduced materials, thereby evidencing the little morphological similitude with the materials from the region. Therefore, the most relevant analysis derives from the inspection of the clustering patters of the native populations and the racial control. It was observed that the accession representative of 'Tuxpeño' was part of Group VI, which indicates that the materials on this group were the ones that kept, at a larger extent, the distinctive traits of such race. It is noteworthy that around Group VI, the Goups II to V were located, denoting that these sets of native populations maintain similarity with Tuxpeño. This is consistent with the concept of race, which according to Anderson and Cutler (1942) is defined as a group of related individuals with enough characteristics in common that enable its differentiation from other groups and that, according to Muñoz (2005), pools a group of varieties relatively similar, adapted to a

particular ecological region. Thus, and supported on the results herein obtained (Figures 1, 2 and Table 4), it can be affirmed that even though on the native maize populations from the region, natural and artificial selection processes have been taking place, which have led to a certain level of morphological differentiation, in general terms they have kept the attributes proper of the Tuxpeño race.

When the collection sites of the 93 native populations were considered, it was found that most of the populations from Groups II and III were distributed along the warm humid climate with rains all year [Af(m)], the same climatic type where Group VI is found, but with slight differences. On the other hand, Groups IV and V are more associated to the warm humid climate with abundant rainfalls in summer [(A)C(fm)], most of their populations belonged to the municipality of Tenampulco and had morphological characteristics more similar to the accessions included in Group VI, where the Tuxpeño racial control was found.



Figure 3. Dispersion of 100 populations evaluated in the humid tropics of Puebla in the space defined by the first three main components (CP).

GROUP	DFF (days)	LOPARA (cm)	NUMR	DIAMAZ (cm)	NGRAO	ANCHO (cm)	FDESG (%)	PLANMAZ	ABAJARRI	LONGDIA	GROSANC	LOANGRO (cm <sup>3</sup> )
Ι	107.0c	35.0c	13.8b	4.0a	23.6bc	0.84ab	0.78cd	2.23a	1.07c	3.0b	0.48bcd	0.33c
II	127.3ab	41.9ab	18.3a	3.5bc	33.0a	0.79b	0.86a	1.71c	1.51a	3.9a	0.42cd	0.29c
III	125.4ab	39.5bc	18.1a	3.6b	31.8a	0.86ab	0.85ab	1.73c	1.47a	3.9a	0.41d	0.33bc
IV	127.8ab	44.6a	16.7ab	3.2c	21.1c	0.81ab	0.74d	1.79bc	1.45a	4.4a	0.64a	0.40a
V	132.3a	44.6a	19.9a	3.6ab	27.1ab	0.87 <sup>a</sup>	0.79bcd	1.68c	1.50a	4.2a	0.50bc	0.39ab
VI	126.8ab	43.9ab	19.0a	3.7ab	29.8a	0.84ab	0.82abc	1.73c	1.42ab	3.9a	0.47bcd	0.35abc
Tuxpe	124.0b	42.0ab	20.4a	3.6b	22.8bc	0.80b	0.83abc	1.99b	1.20bc	3.2b	0.53b	0.32c
LSD	8.16	4.81	4.22	0.37	5.85	0.06	0.05	0.23	0.22	0.59	0.08	0.06

Table 4. Means for the 12 most important original variables for the five groups identified in the dendrogram of 100 maize populations from the humid tropics of Puebla.

DFF = Days to silking, LOPARA = Length of the branched segment, NUMR = Number of branches, DIAMAZ = Ear diameter, NGRAO = Number of grains in opposite rows, ANCHO = Grain width, FDESG = Shelling factor, PLANMAZ = Plant height / Ear height, ABAJARRI = Leaves below / leaves above, LONGDIA = Ear length/ Ear diameter, GROSANC = Grain thickness/ grain width, LOANGRO = Grain volume. Tuxpe = Values for the Tuxpeño racial control evaluated in the present study. LSD= Least significant difference.

### CONCLUSIONS

In the humid tropics of Puebla there is a considerable morphological diversity among the native maize populations there grown, such variation was grouped in six groups distinguished basically by differences in days to silking, length of the branched segment of the tassel, number of branches of the tassel, ear diameter, number of grains in opposite rows, grain width, shelling factor and in the following indexes: plant height divided by ear height, leaves below divided by leaves above the ear, ear length divided by ear diameter, grain thickness divided by grain width, and grain volume.

Maize landraces grown in the humid tropics of Puebla can be regarded as part of the Tuxpeño race. Among them, the populations collected in the altitudinal strata from 147 to 352 masl, in a warm humid climate, with rains all the year, are the ones that keep the highest similarity with the aforementioned race.

### Acknowledgements

We thank the financial support from Fondos Mixtos CONACYT – Gobierno del Estado de Puebla, (Mix Research Funds from both The National Council of Science and Technology (CONACYT) and The Puebla State Government), during the years 2009 – 2010, through the project PUE-2007-01-76993 "Genetic diversity, conservation and improvement of local maize (*Zea mays* L.) populations in major producing regions of Puebla", from which the present study was derived. Likewise, for the financial support from the Consejo Estatal de Ciencia y Tecnología del Estado de Puebla (Council of Science and Technology from Puebla State, CONCYTEP) via a scholarship (Beca-Tesis 2011).

# REFERENCES

- Ángeles, G. E., Ortiz, T. E., López, P. A., López, R. G. 2010. Caracterización y rendimiento de poblaciones de maíz nativas de Molcaxac, Puebla. Revista Fitotecnia Mexicana 33 (4): 287-296.
- Anderson, E. and Cutler, H. C. 1942. Races of *Zea Mays*: I. Their Recognition and Classification. Annals of the Missouri Botanical Garden 29: 69–88.
- Bellón, M. R., Adato, M., Becerril, J., Mindek, D. 2005. Impact of Improved Germplasm on Poverty Alleviation: The Case of Tuxpeño-Derived Materials in México. México, D. F. CIMMYT.
- Bellón, M. R., Barrientos, P., A. F., Colunga, G. P., Perales, H., Reyes, A., J. A., Rosales, S.

R., Zizumbo, V. D. 2009. Diversidad y conservación de recursos genéticos en plantas cultivadas. *En*: Capital natural de México, Volumen II. Estado de conservación y tendencias de cambio. CONABIO, México. pp. 355-382.

- Brown, W. L. and Goodman, M. M. 1988. Races of corn. *En*: Sprague, G. F. and Dudley J. W. (ed.). Corn and Corn Improvement. 3° Ed. American Society of Agronomy. Madison, Wisconsin. pp. 33-79.
- Caballero, H. F. y Cervantes, S. T. 1990. Estudio genético y taxonómico de poblaciones de maíz de la raza Tuxpeño. Agrociencia serie Fitociencia 1 (2): 43-64.
- Franco, T. L. e Hidalgo, R. (eds.). 2003. Análisis Estadístico de Datos de Caracterización Morfológica de Recursos Fitogenéticos. Boletín técnico no. 8, Instituto Internacional de Recursos Fitogenéticos (IPGRI), Cali, Colombia.
- García, E. 1973. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía. UNAM. México.
- Gil, M. A., López, P. A., Muñoz, O. A., López, S. H. 2004. Variedades criollas de maíz (*Zea mays* L.) en el Estado de Puebla, México: diversidad y utilización. *En*: Chávez-Servia, J. L., Tuxill, J. y Jarvis, D. I. (eds.). Manejo de la diversidad de los cultivos en los agroecosistemas tradicionales. Instituto Internacional de Recursos Fitogenéticos (IPGRI), Cali, Colombia. pp. 18-25.
- Goodman, M. M. and Paterniani, E. 1969. The races of maize III: Choices of Appropriate Characters for Racial Classification. Economic Botany 23:265-273.
- Herrera, C., B. E., Castillo, G. F., Sánchez, G., J. J., Ortega, P. R., Goodman, M. M. 2000. Caracteres morfológicos para valorar la diversidad entre poblaciones de maíz en una región: caso la raza Chalqueño. Revista Fitotecnia Mexicana. 23: 335-354.
- Hortelano, S., R. R., Gil, M. A., Santacruz, V. A., Miranda, C. S., Córdova, T. L. 2008. Diversidad morfológica de maíces nativos en el Valle de Puebla. Agricultura Técnica en México. 34 (2): 189-200.
- Instituto Nacional de Estadística, Geografía e Informática (INEGI). 2009. Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Puebla. Disponible en Internet:

<http://mapserver.inegi.org.mx/dsist/pront uario/index2.cfm> Consultado el 11 de abril de 2012.

- Instituto Nacional de Estadística, Geografía e Informática (INEGI). 2010. Anuario estadístico de Puebla. Tomo I. INEGI-Gobierno del Estado de Puebla. INEGI. México.
- Jarvis, D. I., Myer, L., Klemick, H., Guarino, L., Smale, M., Brown, A., H. D., Sadiki, M., Sthapit, B. Hodgkin, T. 2006.Guía de capacitación para la conservación in situ en fincas. Versión 1. Instituto Internacional de Recursos Fitogenéticos (IPGRI), Roma, Italia.
- López, R. G., Santacruz, V. A., Muñoz, O. A., Castillo, G. F., Córdova, T. L.Vaquera, H. H. 2005. Caracterización morfológica de poblaciones nativas de maíz del istmo de Tehuantepec, México. Interciencia 30:284-290.
- Kato, Y., T. A., Mapes, S. C., Mera, O., L. M., Serratos H., J. L., Bye B., R. A. 2009. Origen y Diversificación del Maíz: Una Revisión Analítica. Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México.
- Martínez, G. A. 1988. Diseños experimentales. Métodos y elementos de teoría. Trillas. México.
- Mohammadi, S. A. and Prasanna, B. M. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Science 43:1235– 1248
- Muñoz, O. A. 2005. Centli-Maíz. Prehistoria e Historia, Diversidad, Potencial, Origen Genético y Geográfico, Glosario Centli-Maíz (2° edición). Colegio de Postgraduados. Montecillo, México.
- Pérez, C. A., Molina, G., J. D., Martínez, G. A. 2002. Adaptación a clima templado de razas tropicales y subtropicales de maíz de México por selección masal visual. Rendimiento, altura de planta y precocidad. Revista Fitotecnia Mexicana. 25 (4): 435-441.

- Sánchez, G., J. J. and Goodman, M. M. 1992. Relationships among the Mexican races of maize. Economic Botany. 46: 72-85.
- Sánchez, G., J. J., Goodman, M. M, Stuber, C. W. 2000. Isozimatic and morphological diversity in the races of maize of México. Economic Botany. 54: 43-59.
- Statistical Analysis System (S.A.S.) Institute. 2002. SAS User's Guide Statistics. Release 9.0. SAS Institute. USA.
- Serratos, H., J. A 2009. El Origen y la Diversidad del Maíz en el Continente Americano. Greenpeace. México. Disponible en Internet: <http://www.greenpeace.org/mexico/Glob al/mexico/report/2009/3/el-origen-y-ladiversidad-del.pdf> Consultado el 25 de julio de 2010.
- Servicio de Información Agroalimentaria y Pesquera (SIAP) 2010 SAGARPA-Secretaría de Agricultura Ganadería Desarrollo Rural Pesca y Alimentación. México. Cierre de la producción agrícola por estado. Disponible en Internet: <http://www.siap.gob.mx/index.php?optio n=com\_wrapper&view=wrapper&Itemid= 350> Consultado el 06 de septiembre de 2011.
- Wellhausen, J. E., Roberts, M. L., Hernández, X. E., en colaboración con Mangelsdorf, P. C. 1951. Razas de maíz de México, su origen, características y distribución. Xolocotzia. Tomo II. Revista de Geografía Agrícola. pp. 609-732.
- Wen, W., Franco, J., Chavez, T., V. H., Yan, J., Taba, S. 2012. Genetic characterization of a core set of a tropical maize race Tuxpeño for further use in maize improvement. PLoS ONE 7(3): e32626. Disponible en Internet: <doi:10.1371/journal.pone.0032626> Consultado el 02 de abril de 2012.
- Yanes, G. G. 2011. Diversidad del ecosistema. En: Coordinador Nacional Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). La Biodiversidad en Puebla: Estudio de Estado. México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Gobierno del Estado de Puebla. Benemérita Universidad Autónoma de Puebla. pp. 71-90.

Submitted June 19, 2012 – Accepted January 09, 2014 Revised receivedFebruary 17, 2014