Morphological variability of native maize (*Zea mays* L.) of the west highland of Puebla and east highland of Tlaxcala, Mexico

Variabilidad morfológica del maíz nativo (*Zea mays* L.) del altiplano poniente de Puebla y altiplano oriente de Tlaxcala, México

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Abstract

The objective of the present study was to analyze the morphological variability of native maize of the western highlands of Puebla and east of Tlaxcala, Mexico, in order to, in addition to defining it, relate it to races, commercial varieties and the altitude of the localities of collection. The genetic material evaluated were 134 populations collected in 34 localities, in addition to 10 witnesses. The experiments were established in three localities, using a 12 x 12 lattice. A total of 32 variables were evaluated, of which 27 presented highly significant differences, reflecting high variability at the level of morphological characters, many of them of agronomic interest. From the analysis of variance, 16 variables were selected for use in a cluster analysis using the Modified Localization Method, which concentrated the populations in six groups, most of them in group 1, with morphological characteristics of late-cycle varieties: tall plants, with more primary ramifications of the spike, ears of greater length and diameter and with greater length and thickness of grain. The conclusions indicate that the morphological variability of the populations is not associated with the altitude of the localities where they were collected and that these have a greater relationship with the Chalqueño race, little with the Cónico race, null with the Cónico Norteño and Palomero Toluqueño races and almost null with the commercial varieties.

Keywords
Zea mays L. • creole maize • plant genetic resources • germplasm • *in situ* conservation

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Resumen

El objetivo del presente estudio fue analizar la variabilidad morfológica de los maíces nativos del altiplano poniente de Puebla y oriente de Tlaxcala, México, para, además de definirla, relacionarla con razas, variedades comerciales y con la altitud de las localidades de colecta. El material genético evaluado fueron 134 materiales colectados en 34 localidades, además de 10 testigos. Los experimentos se establecieron en tres localidades, mediante un Látice12 x 12. Un total de 32 variables fueron evaluadas, de las cuales 27 presentaron diferencias altamente significativas, lo que refleja alta variabilidad a nivel de caracteres morfológicos, muchos de ellos de interés agronómico. Del análisis de varianza se seleccionaron 16 variables para utilizarse en un análisis de agrupamiento mediante el Método de Localización Modificado, el cual concentró a las poblaciones en seis grupos, la mayoría de ellas en el grupo 1, con características morfológicas de variedades de ciclo tardío: plantas altas, con más ramificaciones primarias de la espiga, mazorcas de mayor longitud y diámetro y con mayor longitud y grosor de grano. Las conclusiones indican que la variabilidad morfológica de las poblaciones no se asocia con la altitud de las localidades donde fueron colectadas y que estas tienen mayor relación con la raza Chalqueño, poca con la raza Cónico, nula con las razas Cónico Norteño y Palomero Toluqueño y casi nula con las variedades comerciales.

Palabras clave

Zea mays L. • maíz criollo • recursos fitogenéticos • germoplasma • conservación *in situ*

Introduction

The study of the genetic diversity of maize (*Zea mays* L. ssp. *mays*) in traditional agricultural production systems is important, since it is the primary source of food and nutrition for the rural population, based mainly on native varieties, both in Mexico and in other regions, such as southeastern Europe (41). Corn is not a typical result of natural selection, it was literally invented by the ethnic groups of Mexico (46). Over time, corn has evolved *in situ*, thanks to the joint intervention of men and women (26); the woman improves quality characters, while the man preserves the plant and increases its yield, especially in adverse conditions for the crop, such as drought.

Corn is the most important crop in Mexico, since, according to data from the Food and Agriculture Organization, in the period 2007-2017, an average of 23.4 million t was produced in an average annual area of 7.0 million hectares (8); In addition, it is the crop that involves a greater number of farmers (around 3.2 million out of a total of four million farmers in the country).

The state of Puebla participates with an approximate production of 10% of the national total, in an area that comprises 5% of the national total sown.

In the state, the Rural Development District of Libres is the one with the highest production, since in the 2007-2017 period it produced 28.9% of the state total, with average yields of 4.7 and 2.3 t ha^{-1} in irrigation and in rainfall conditions, respectively. However, the area sown in rainfall conditions in the same period represents 94% (approximately

102,788 ha), while in irrigation it is only 6% (approximately 6,221 ha) (40).

In the state of Puebla, research has been carried out that describes the morphological variability of maize populations: Mixteca Alta (2), part of the plateau (9, 17), Puebla Valley (16) and humid tropics (20), without an investigation to date indicating the morphological variability of the races planted in the Rural Development District of Libres, despite its importance in maize production.

The studies that could give an idea of the races are those of Wellhausen *et al*. (1951) and Cervantes and Mejía (1984), who mention that populations belonging to the Chalqueño and Cónico races are planted in the Valley of Puebla, adjacent to the District.

Despite the engrained tradition of the District of Libres as a producer of maize, a fluctuation in the area (ha) planted (123,298, 118,685, 127,841, 121,254, 118,910, 118,157, 116,678, 134,366, 122,413, 120,564 and 130,646 ha) has been observed (2007-2017 period), with a significant decrease in the years 2008, 2011, 2012 and 2013 (40), a situation that has serious implications from the point of view of the preservation of the native germplasm, in addition to the fact that the presence of generations with more field experience and knowledge about plant genetic resources is decreasing due to natural aging, without replacing new generations in agricultural activities, due to the marked process of emigration.

The inadequate preservation of germplasm can lead to genetic erosion, understood as the loss of a crop, of varieties within the crop or alleles within the variety (43). The importance of preventing such erosion lies in that the genetic diversity is a support for sustainable food production systems (6, 18) and for the generation of improved varieties that increase food production that will be demanded by the increasingly growing world population, estimated to exceed 9 billion in 2050 (14).

It is therefore urgent to document and evaluate the existing plant genetic resources (1, 3), in order to propose appropriate strategies for future use in the event of climate change and the incidence of biotic and abiotic adverse factors. In this context, the present study was conducted with the objective of analyzing the morphological variability of the native maize that originated in the corridor that includes the western highlands of Puebla (Libres and Mazapiltepec) and the eastern highlands of Tlaxcala (Huamantla), to know their main morphological characteristics and the relationship they still have with the maize races and commercial varieties grown in the region, as well as establishing some relationship of morphological variation with altitudinal patterns of the localities where the seed was collected.

Materials and methods

Description of the study area, the localities of collection and evaluation of localities

Three ecological niches were defined based on the criteria of Muñoz (1988): Libres (NE1), Mazapiltepec (NE2) and Huamantla (NE3), in the states of Puebla and Tlaxcala, respectively. In each ecological niche, a random stratified sampling was carried out (27) in order to select a representative number of municipalities and localities for the seed collection of maize populations, obtaining a total of 13 municipalities and 34 localities (table 1, page 220).

Table 1. List of localities of seed collection.
Tabla 1. Lista de las localidades de la colecta de semilla.

Ecological Niche	Locality	Key	Municipality	Altitude (m a. n. m.)
Libres	Buenavista de Guerrero	BdeG	Cuyoaco	2646
Libres	Emiliano Zapata	EZ	Cuyoaco	2490
Libres	Texcal	TEX	Cuyoaco	2493
Libres	Tepeyahualco	TEPE	Tepeyahualco	2337
Libres	Cuyoaco	CUY	Cuyoaco	2439
Libres	Temextla	TEM	Cuyoaco	2462
Libres	Ocotepec	OCO	Ocotepec	2445
Libres	Payuca	PAY	Cuyoaco	2382
Libres	Tehuatzingo	TEHUA	Libres	2412
Libres	El Fuerte	ELFUE	Tepeyahualco	2344
Libres	El Sabinal	ELSAB	Libres	2398
Libres	Juan Sarabia Pizarro	JSP	Tepeyahualco	2336
Libres	Miravalles	MIRA	Oriental	2395
Libres	Oriental	ORI	Oriental	2355
Libres	Pedernales	PED	Libres	2790
Libres	S. Antonio Virreyes	SAV	Oriental	2368
Mazapiltepec	S. José Ozumba	SJO	S. José Chiapa	2360
Mazapiltepec	Vicencio	VIC	S. José Chiapa	2376
Mazapiltepec	Nopalucan	NOP	Nopalucan	2456
Mazapiltepec	Sta. María Ixtiyucan	SMI	Nopalucan	2456
Mazapiltepec	Soltepec	SOL	Soltepec	2431
Mazapiltepec	Mazapiltepec	MAZA	Mazapiltepec	2353
Mazapiltepec	Álvaro Obregón	AO	Soltepec	2386
Mazapiltepec	Eréndira	ERÉN	Nopalucan	2379
Huamantla	Máximo Serdán	MS	Lara Grajales	2402
Huamantla	Col. Lázaro Cárdenas	CLC	Altzayanca	2704
Huamantla	Lomas de Junguito	LdeJ	Altzayanca	2495
Huamantla	S. Fco. Cuexcontzin	SFC	Cuapiaxtla	2433
Huamantla	S. José Xicoténcatl	SJX	Huamantla	2465
Huamantla	Benito Juárez	BJ	Huamantla	2476
Huamantla	Ignacio Zaragoza	IZ	Huamantla	2539
Huamantla	Los Pilares	PIL	Huamantla	2695
Huamantla	Zitlaltepec	ZITLA	Zitlaltepec	2588
Huamantla	Barrio San Lucas	BSL	Huamantla	2513

Altitude. Source Google Earth 7.1.2.2041.2013 / Altitud. Fuente Google Earth 7.1.2.2041. 2013.

Information on the localities where the experimental material was evaluated is presented below. Buenavista de Guerrero: NE1, 19°38'07" N L, 97°30'32" W L, altitude 2646 m a. s. l., rain 516 mm, average maximum and minimum annual temperature 23 and 4°C, planting April 14th; Máximo Serdán: NE3, 9°16'36" N L, 97°48'19" W L, altitude 2402 m.a.s.l., rain 598 mm, average annual temperature 21 and 5°C, planting April 21st; Mazapiltepec: NE2, 19°07'14" N L, 97°39'56" W L, altitude 2359 m a. s. l., rain 665 ml, average annual temperature 22 and 6°C, planting May 4th. The planting in the 3 localities was in the 2007 spring summer cycle.

Genetic material

134 native populations collected in the study area were evaluated, where the racial witnesses Cónico (Criollo del Mezquital), Cónico Norteño (Zac 58), Chalqueño Crema (7CSM), Chalqueño Palomo (Col-6583), Chalqueño del Valle de Toluca (Méx-158) and Palomero Toluqueño (Méx-5) were added.

Racial witnesses were used for the purpose of relating populations to any of them. Additionally, four commercial witnesses (Sintético Serdán, 32D06, Halcón and Z60) were used to see the agronomic potential of corn populations.

Design and experimental unit

The 144 materials were evaluated in a simple 12x12 lattice design at each experimental site. The experimental unit consisted of two rows of 5 m long and 0.8 m wide, where three seeds were sown every 50 cm to later leave two plants, generating a population density of 51,754 plants per hectare.

Each experiment was surrounded by 2 rows of plants on the sides and 10 m of plants in rows at the front and at the end of the experiment. The experimental units were separated by one meter in between them.

Crop management

The fertilization was carried out in two applications: during the first weeding all the phosphorus and 1/3 of the nitrogen were applied and during the second weeding the rest of the nitrogen. In Buenavista de Guerrero the fertilization dose was 100-30-00, in Máximo Serdán 100-40-00, and for Mazapiltepec 110-50-00. Weeding was carried out periodically and herbicide was applied once (2,4-D amine) at a rate of 1 L ha^{-1}.

Registered traits

The following traits were recorded: 1)- days to male flowering (DMF) and 2)- days to female flowering (DFF), counted from the day of planting until 50% of the plants presented dehiscent anthers and exposed stigmas, respectively, 3)- floral asynchrony (FAS), considered as the difference between DFF and DMF, recording these three traits at the experimental unit level; five random plants were subsequently selected to record the traits 4)- plant height (PH), from the stem base to the base of the spike and 5)- ear height (EH), from the base of the stem to the knot of insertion of the ear, both in cm, 6)- height index (HI), as the ratio EH/PH, 7)- number of leaves above the ear (LAE), 8)- number of primary ramifications of the spike (PRS), 9)- spike peduncle length (SPL) in cm, 10)- length of the branched spike section (LBSS) in cm, 11)- length of the spike's central branch (LPCB) in cm, 12)- total length of the spike (TLS) in cm, 13)- number of plants (NP), 14)- percentage of plants with more than one ear (PPM1E), 15)- percentage of sterile plants (PSP), 16)- plant-to-harvest aspect (PHA) in visual scale 1-5, where 1 corresponds to plants with good appearance and 5 to plants with bad appearance, 17)- number of harvested ears (NHE), 18)- ear rating (ER) in scale 1-5, where 1 corresponds to a good aspect and 5 the opposite case, 19)- field weight (FW) in g, 20)- weight of five ears in g, 21)- grain yield per hectare (GYPH) in t ha^{-1}, using the formula:

$$\text{Yiel} = \frac{(\text{FW x \% humidity})\text{x \%dry matter x SF}}{\text{Number of plants}} \text{x } 45000/1000$$

Additionally, five-plant ear characters were recorded per experimental unit: 22)- ear diameter (ED) in cm, 23)- ear length (EL) in cm, 24)- number of rows (NR), 25)- grains per row (GPR), 26)- ear length/diameter ratio (ELDR). From each ear 10 grains were taken to measure 27)- width (W), 28)- length (L) and 29)- grain thickness (GT), all of them in mm, and 30)- grain length/width ratio (GLWR); In addition, the five ears were shelled and 31) - grain weight (GW) and 32)- cob weight (CW) in g were taken to calculate 33) - the shelling factor (SF) using the ratio GW/(GW + CW).

Statistical analysis

In order to assess the stability of the expression of the traits in the three ecological niches, a combined analysis of variance between environments was performed using the PROC GLM procedure of the SAS package (Statistical Analysis System) version 9.1 (38); The linear model used was as follows:

where:

Yijkl= the observation of the i-th collection in the j-th environment of the k-th repetition and the l-th block

μ= the general mean and constitutes a constant common to all observations

α_i= the effect of the I-th observation of the collection

γ_j= the effect of the j-th environment

δ_{ij}= the interaction effect of the i-th collection with the j-th environment

$B(L)_{l(kj)}$= the effect of the l-th block nested in the k-th repetition of the j-th environment

ε_{ijkl}= the experimental error associated with the experimental unit $Y_{ij(k)}$.

Subsequently, in order to detect and eliminate multicollinearity, defined as the linear dependence between traits and which implies singularity (the matrix is not full range) in the Mahalanobis variance-covariance matrix, in the multivariate post-analysis, a Pearson's simple correlation matrix was elaborated, using the PROC CORR procedure of SAS, eliminating highly correlated traits (r ≥ 0.7); For example, between days of male and female flowering, which were highly correlated, only the one with the greatest biological significance was selected for later analysis. Traits were also debugged using the PROC STEPDISC procedure of SAS, applying the STEPWISE sequential method by which the discriminatory power of the traits involved in the analysis is analyzed.

$$Y_{ijkl} = \mu + \alpha_i + \gamma_j + \delta_{ij} + B(L)_{l(kj)} + \varepsilon_{ijkl,} \quad i = 1,2,.........,144, \quad j = 1,2,3, \quad k = 1,2, \quad l = 1,2,.........,12$$

STEPWISE is a multivariate technique that is applied when the traits have the property of having a normal multivariate distribution and have a common covariance matrix. This technique basically consists in the selection of a subset of informative traits or with greater discriminatory capacity to identify differences between the units of analysis.

However, this technique alone does not ensure a good selection of discriminant traits and should be complemented with other techniques and with the experience of the researcher to choose those traits that are as informative as possible of the existing variation (36, 37). Based on the F statistic, traits at 15% significance were selected.

The modified localization model or method (MLM) was used, which is a two-stage classification strategy (7, 10, 11, 12): in the initial stage, groups are defined using a hierarchical grouping method (UPGMA-unweighted pair group method with arithmetic mean), the location model is then applied to the groups formed, in which significant differences between and within groups will be verified using the Mahalanobis distance criterion, which estimates the distances and the parameters that define the groups in diversity studies (21).

The MLM has the advantage of combining all categorical traits into a single multinomial trait, which in turn can then be used in a single matrix, along with continuous traits; it is even possible to combine common grouping methods, such as Ward's method, with this technique (24).

Results and discussion

Variance analysis

In the analysis of combined variance FAS, SPL, PPM1E and PSP did not show significant differences for genotypes, while the remaining 28 traits were significant ($p \leq 0.01$), which is considered as an indicator of diversity (9, 17).

In most genetic diversity studies of native populations of maize races, high diversity is reported, mainly intrapopulation (19, 44, 45), although in some cases, such as in the Cacahuacintle race, it is minimal (13).

With respect to environments, PPM1E and PSP showed no statistical significance, FAS and HI were significant with $p \leq 0.05$, while the remaining 28 traits were significant with $p \leq 0.01$. As in this study, others have reported significant differences between environments in most of the traits evaluated in the native populations of maize races (2, 20).

Regarding the interaction genotypes × environments PH, EH, HI, LPCB, ED, GPR and GLWR did not show statistical significance (table 2, page 224), which implies that these traits respond in a similar way to changes in the environment between the various genotypes.

Trait selection

Multicollinearity is defined as the linear dependence between traits, its presence implies singularity (the matrix is not of full range) in the Mahalanobis variance-covariance matrix (exponential part of the multivariate normal distribution), which estimates the distances and parameters that form and define the groups in diversity studies.

One way to detect and eliminate multicollinearity is to perform a simple correlation analysis (ρ), followed by a method of trait selection.

Table 2. Mean squares, level of significance and coefficients of variation of morphological data of maize landraces tested in three localities of the Libres-Mazapiltepec-Huamantla región.

Tabla 2. Cuadrados medios, niveles de significancia y coeficientes de variación para datos morfológicos de maíces nativos evaluados en tres ambientes de la región Libres-Mazapiltepec-Huamantla, 2007.

Traits	Genotypes		Environments		Genotype × Environment		CV (%)
GYPH (t ha^{-1})	3414150.3	**	478346720	**	1981653.61	**	27
DMF (días)	200.84	**	127949	**	28.59	**	3.4
DFF (días)	195.07	**	145376	**	20.37	**	3.3
FAS (días)	10.72	NS	881	*	14.04	**	6.8
PH (cm)	1848.89	**	339529	**	74.41	NS	6.5
EH (cm)	1194.48	**	147327	**	55.5	NS	9.6
HI (cm)	0.01	**	0.04	*	0	NS	6
LAE	0.77	**	29.61	**	0.14	**	7.5
PRS (cm)	13.92	**	197	**	5.76	**	25.3
SPL (cm)	11.4	NS	9893	**	9.24	**	11.7
LBSS (cm)	18.76	**	10783	**	12.11	**	9.2
LPCB (cm)	3.84	**	152	**	1.48	NS	18.4
TLS (cm)	41.04	**	46124	**	24.31	**	6.8
ED (cm)	50.21	**	859	**	4.73	NS	4.6
EL (cm)	5.42	**	19	**	1.95	**	10.1
NR	9.17	**	35	**	1.31	**	7.1
GPR	20.91	**	1529	**	8.43	NS	11.4
ELDR	24.77	**	583	**	11.14	**	10.9
W (mm)	1.52	**	62	**	0.29	**	6
L (mm)	3.2	**	746	**	0.72	**	5.4
GT (mm)	0.32	**	3	**	0.11	**	6.8
GLWR	0.11	**	2.82	**	0.02	NS	7.2
GW (g)	23462.21	**	10303666	**	11658.35	**	20.1
CW (g)	841.66	**	33979	**	146.64	**	19.4
SH	0	**	0.29	**	0	**	2.2
NP	57.17	**	6829	**	41.5	**	12.3
PPM1E (%)	58.22	NS	2024	NS	44.3	**	12.8
PSP (%)	137.16	NS	10469	NS	123.26	**	14.2
PH (1-5)	1.04	**	16	**	0.31	**	17.4
NHE	61.05	**	18509	**	45.78	**	16.3
ER (1-5)	0.28	**	6	**	0.27	**	16.6
FW (g)	4381195.4	**	37898670	**	1178050.74	**	21

** Significance P≤0.001, * Significance P≤0.05, NS there is no significance.

** Significancia P≤0,001, * Significancia P≤0,05, NS No existencia de significancia.

Trait names are in Material and Methods.

Los nombres de las variables están en Materiales y Métodos.

According to Romero *et al*. (2002) and Mijangos-Cortés *et al*. (2007), of the pairs of traits that presented correlations greater than 0.75 in absolute value ($r < -0.75$ and $r > 0.75$), 9 traits with less agronomic importance were eliminated: DMF-DFF (0,98), PH-EH (0,94), DMF-TLS (-0,79), DFF-TLS (-0,78), DMF-SPL (-0,77), DFF-SPL (-0,78), SPL-TLS (0,92), LBSS-TLS (0,92) and EL-ELDR (0,79).

When applying the method of sequential selection of STEPWISE traits, the selected traits were HI, NR, ED, GLWR, DFF, PH, LAE, PRS, W, L, SH, LBSS, LPCB, EL, GT and PHA (table 3). Most of the selected traits are consistent with previous diversity studies (15, 19, 30, 31, 35).

Grouping analysis

The grouping analysis, using the Modified Localization Method, in its first classification strategy defined four groups. When applying the second classification strategy, the optimal number of groups that were formed by estimating the likelihood profile and obtaining the probabilities of belonging *a posteriori* to each of the 144 materials evaluated with maximum restricted likelihood was determined (39).

The likelihood test ran 14 iterations before converging on the value -1098,4327, after which it assembled 6 groups (figure 1, page 226).

When calculating the *a posteriori* probabilities for each of the 144 evaluated materials, they fluctuated between 0,88 and 1,00, which represented a good classification of each of the 144 materials under study (information not shown). It was observed that there are significant differences between groups and within groups using the distance criterion of Mahalanobis (21), which allowed to form 6 groups.

Additionally, and considering the large number of populations and the variability that the materials presented within Group 1, it was considered convenient to form subgroups within this group using the Modified Localization Method (MLM), thus integrating Subgroups 1a, 1b and 1c of figure 1 (page 226).

Table 3. Selected traits using the STEPWISE method.

Tabla 3. Variables seleccionadas utilizando el método STEPWISE.

CT	Partial R^2	Pr>F	CC	Pr> ASCC	CT	Partial R^2	Pr>F	CC	Pr> ASCC
HI	0.644	<.0001	0.005	<.0001	AG	0.306	<.0001	0.026	<.0001
NR	0.618	<.0001	0.009	<.0001	LG	0.642	<.0001	0.03	<.0001
ED	0.618	<.0001	0.013	<.0001	FD	0.311	<.0001	0.031	<.0001
GLWR	0.437	<.0001	0.016	<.0001	LM	0.293	<.0001	0.033	<.0001
DFF	0.452	<.0001	0.017	<.0001	LTRE	0.275	<.0001	0.035	<.0001
PH	0.378	<.0001	0.019	<.0001	GG	0.269	<.0001	0.037	<.0001
LAE	0.4	<.0001	0.021	<.0001	LRCE	0.25	<.0001	0.038	<.0001
PRS	0.358	<.0001	0.024	<.0001	ASF	0.207	<.0213	0.039	<.0001

VS: chosen trait, CC: Canonic correlation.

VS: Variable Seleccionada, CC: Correlación Canónica.

Trait names are in Material and Methods (page 219).

Los nombres de las variables están en Materiales y Métodos (pág. 219).

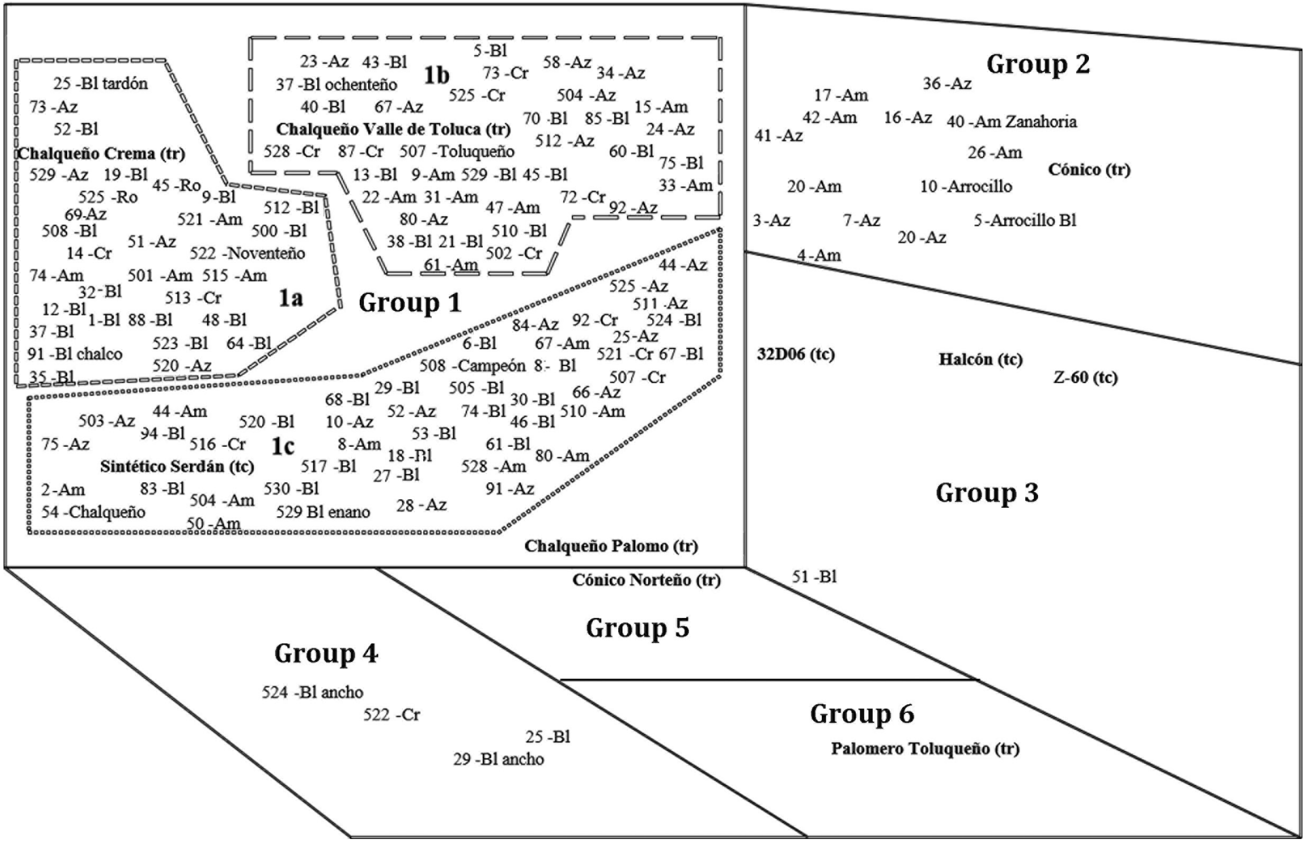

Figure 1. Groups and subgroups obtained by the Modified Localization Method.

Figura 1. Grupos y subgrupos ensamblados mediante el Método de Localización Modificado.

In table 4 (page 228), the morphological characteristics of each of the groups can be observed, as follows: Group 1 was made up of 119 materials, of which 116 are native populations, two racial witnesses belonging to the Chalqueño breed (Chalqueño Crema and Chalqueño del Valle de Toluca) and an improved variety (Sintético Serdán).

The materials of this group are late flowering, higher, greater number of PRS, occupies the third place in regards to NR, longer ears with larger diameter, longer and thicker grains, and it is the second group with wider grains. Within Group 1 is Subgroup 1a, consisting of 31 materials, of which 30 are native, plus the racial witness Chalqueño Crema. This subgroup presents the latest flowering varieties, the largest PH, and the highest number of PRS, longer ears with the largest diameter and less thick and less wide grains.

Subgroup 1b was integrated with 37 materials, 36 of which are native and one is the Chalqueño del Valle de Toluca racial witness. This subgroup is the earliest flowering of the three, the second with the highest PH, lowest number of PRS, highest NR, less wide grains and smaller thickness. Subgroup 1c was made up of 51 materials, 50 native and the witness Sintético Serdán; it is the second with the highest number of DFF and lowest PH, the second with the highest number of PRS and lowest NR, the second with L and with the highest W and with greater GT.

Group 2 was made up of 15 materials, 14 native and the Cónico racial witness. This group is the earliest flowering of all, the third with the highest PH, an average of 6,9 PRS, 15,2 rows per ear (NR). It ranks third place in terms of EL and ED, second in terms of W, third with respect to L, fourth in W, and third in GT. This group was characterized by grouping only yellow, blue and "arrocillo" grain materials, not including white color materials.

Group 3 was integrated with three commercial witnesses (Z-60, Halcón and 32D06) plus a native material, from Oriental, Puebla. These materials are late flowering, not very tall. They have a greater number of rows, longer ear length, and occupy the penultimate place in regards to PRS, second in ED, third in L, fourth in W, and last place in regards to GT.

Group 4 was made up of four native materials: 522 Cr and 524 Bl wide, from the localities of Los Pilares and Barrio San Lucas of the region of Huamantla, Tlaxcala and 25 Bl and 29 Bl wide, from Ocotepec and Payuca in the region of Libres, Puebla. These materials are of intermediate flowering and plant height. They are the ones with the lowest NR, and occupy second place in EL and fourth place in ED.

Group 5 was formed by the racial witness Cónico Norteño, which was the earliest flowering of all, the smallest in size, the penultimate in regards to NR, lower EL, the penultimate with respect to ED, lower L and second place with respect to W.

Group 6 was represented by a single material, the racial witness Palomero Toluqueño, which occupied the second place in EH, the penultimate in PH. It is the one who presented a higher NR, and occupied the penultimate place in EL, lower ED, narrower and thinner grains and had the penultimate place in L.

Table 4. Morphological data by group of 134 landraces, four control cultivars and six control maize races grouped by the modified localization method.

Tabla 4. Datos morfológicos por grupo de 134 poblaciones nativas, 4 testigos comerciales y 6 testigos raciales ensamblados mediante el Método de Localización Modificado.

Trait	Formed Groups																	
	Group 1		SUB1a		SUB1b		SUB1c		Group 2		Group 3		Group 4		Group 5		Group 6	
	n=119		31		37		51		15		4		4		1		1	
DFF (days)	114.8	a	116.6	a	113.6	b	114.5	b	104.6	b	114.4	b	104.6	b	90.8	d	97.8	c
FAS (days)	6.8	a	6.5	a	6.8	a	6.9	a	7.1	a	5.8	a	7	a	4.7	a	5.2	a
PH (cm)	179.4	a	187.2	a	177.3	b	176.1	b	153.6	b	130.3	c	153.6	b	96.9	d	130	c
HI	0.6	a	0.6	a	0.6	a	0.59	b	0.6	a	0.5	c	0.6	a	0.4	d	0.57	b
LAE	4.5	b	4.6	a	4.5	b	4.6	a	4.2	c	5.5	a	4	c	3.6	d	4.3	b
PRS	8.6	a	9	a	8.2	a	8.6	a	6.9	b	5.2	b	8	a	7.7	a	4.2	c
LBSS (cm)	29.6	a	30	a	29.6	a	29.3	a	27.7	a	29.2	a	28.2	a	24.7	c	26.1	b
LPCB (cm)	6.42	a	6.7	a	6.2	b	6.4	b	5.3	b	5.2	b	6.1	b	5.3	b	4.3	c
NR	14.6	b	14.5	b	14.9	a	14.3	b	15.2	a	15.8	a	9.9	d	12.1	c	19.6	a
EL (cm)	12.53	a	12.71	a	12.32	b	12.57	a	11.34	b	13.45	a	12.92	a	8.91	d	10.07	c
ED (mm)	47.4	a	47.8	a	47.1	a	47.3	a	43.5	b	46.8	a	40.1	c	36.1	d	34.5	d
L (mm)	14.5	a	14.8	a	14.4	b	14.4	b	13.3	b	12.4	c	13.2	b	11.4	d	11.7	c
W (mm)	7.9	b	7.9	b	7.7	c	8.1	a	7.2	c	7.7	c	9.5	a	7.8	b	4.7	d
GT (mm)	4.34	a	4.29	b	4.31	b	4.4	a	4.2	b	4.1	b	4.5	a	4.1	b	3	c
GLW	1.8	b	1.9	a	1.9	a	1.8	b	1.9	a	1.6	c	1.4	d	1.5	d	2.6	a
SH	0.88	b	0.9	a	0.9	a	0.9	a	0.89	b	0.83	d	0.89	b	0.86	c	0.92	a
GYPH (t/ha)	4850	a	4832	a	4892	a	4830	a	4064	b	5101	a	3827	c	1412	d	2003	d

Trait names are in Material and Methods. / Los nombres de las variables están en Materiales y Métodos.

Means with same letter by row are not different statistically. / Medias con la misma letra en cada fila son iguales estadísticamente.

Based on the above, the characteristics of the majority (86.6%) of the populations are related to the Chalqueño race, few (10.4%) with the Cónico race, none with the Cónico Norteño and Palomero Toluqueño races. It is worth mentioning that the Chalqueño race, which is distributed mainly in the eastern part of the state of Mexico (15) is not considered as one of the six main races of maize in Mexico, contrary to Cónico and Cónico Norteño races that are considered the second and fourth most common, respectively (28). Only 0.7% share characteristics with commercial varieties and 2.98% do not resemble any races or commercial varieties. Therefore, the majority of the populations are of late flowering cycle, of higher plants, with more primary ramifications of the spike, ears of greater length and diameter, and with greater grain length and thickness. Even though there is a high morphological variability in the populations, it is important to highlight the low relationship with maize races, since only a relationship was found with 2, despite having conducted the study in 34 locations of three ecological niches located in the state of Puebla and Tlaxcala.

In another study carried out in several locations in the central region of Mexico, the presence of up to 8 races of maize at the local level is reported (32), which can be explained based on the fact that this is considered one of the six regions of Mexico with the highest diversity of races (34).

The greater relationship of the populations with the Chalqueño race indicates that the adaptation area has been expanded, because the *in situ* conservation of the genetic diversity of the crops is dynamic (31), since the extension is due to the fact that each race has a new area of potential distribution, where proper management can favor the conservation of its diversity (42).

Regarding the relationship of the groups with grain color (white, cream, blue, yellow and red) of the populations, there is no direct relationship between the two, since all were present in Subgroup 1a, and, with the exception of red, in Subgroup 1b and Subgroup 1c, which grouped 83% of the populations. In the other groups, for having fewer populations and for being integrated by the commercial witnesses, white color prevailed.

The grouping of genetic diversity through multivariate statistical methods and the modified localization method (MLM) have been used in various studies on genetic diversity of maize races. Just to mention an example, Ortiz *et al*. (2008) formed eight groups with populations of 8 maize races from the High Valleys of Peru.

Relationship between locations and groups

In figure 2 (page 230), it can be seen that most of the seed collection localities were integrated in Group 1, associated with native materials genetically close to the racial witnesses of the Chalqueño race. When performing the breakdown in subgroups, it is observed that Subgroup 1a was associated with the localities of Álvaro Obregón (AO, 2386 m a. s. l.), Nopalucan (NOP, 2456 m a. s. l.), Sta. María Ixtiyucan (SMI, 2456 m a. s. l.), Zitlaltepec (ZITLA, 2588 m a. s. l.), Pedernales (PED, 2790 m a. s. l.), Ignacio Zaragoza (IZ, 2539 m a. s. l.) and Benito Juárez (BJ, 2476 m a. s. l.), indicating that these localities have a closer relationship with native materials associated with the Chalqueño Crema subrace. On the other hand, Subgroup 1b was associated with the localities of Temextla (TEM, 2462 m a. s. l.), Soltepec (SOL, 2431 m a. s. l.), S. Fco. Cuexcontzin (SFC, 2433 m a. s. l.), and S. Antonio Virreyes (SAV, 2368 m a. s. l.), which indicates that these localities are mostly associated with native materials and the Chalqueño del Valle de Toluca witness; Subgroup 1c had a strong relationship with the localities of Buenavista de Guerrero (BdeG, 2619 m a. s. l.), Lomas de Junguito (LdeJ, 2495 m a. s. l.), Tehuatzingo (TEHUA, 2412 m a. s. l.), El Fuerte (ELFUE, 2344 m a. s. l.), Mazapiltepec (MAZA, 2413 m a. s. l.), Eréndira (ERÉN, 2379 m a. s. l.), S. José Xicoténcatl (SJX, 2465 m a. s. l.), Tepeyahualco (TEPE, 2337 m a. s. l.) and Col. Lázaro Cárdenas (CLC, 2704 m a. s. l.).

Group 2, which was strongly related to the Cónico racial witness, presented an association with the localities of Cuyoaco (CUY, 2439 m a. s. l.) and Emiliano Zapata (EZ, 2490 m a. s. l.).

On the other hand, Group 3 was strongly related to commercial witnesses and shared together with Group 5 the locality of Juan Sarabia Pizarro (JSP, 2336 m a. s. l.).

Group 4 was associated with the localities of Barrio San Lucas (BSL, 2520 m a. s. l.), Payuca (PAY, 2382 m a. s. l.), Los Pilares (PIL, 2695 m a. s. l.), Texcal (TEX, 2493 m a. s. l.) and Miravalles (MIRA, 2395 m a. s. l.). It should be clarified that the native materials of this group had no relationship to any racial witness.

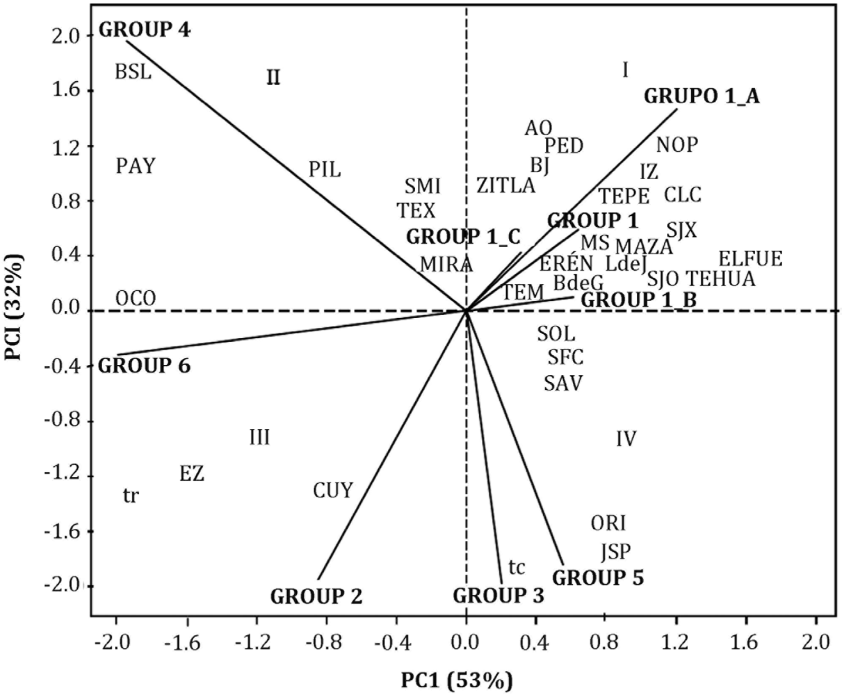

Figure 2. Relation among localities and the groups formed by the Modified Localization Method. The meaning of codes are in table 1 (page 220).

Figura 2. Relación entre las localidades y los grupos ensamblados mediante el Método de Localización Modificado. El significado de las claves está en la tabla 1 (pág. 220).

Group 5, which was related to the Cónico Norteño racial witness, but without native materials, was associated with the localities of Oriental (ORI, 2355 m a. s. l.) and part of Juan Sarabia Pizarro (JSP, 2336 m a. s. l.), while Group 6 was related to Ocotepec (OCO, 2445 m a. s. l.).

When relating the groups and subgroups to the altitude of the localities, it is observed that this ranges from 2386 to 2790 m in Subgroup 1a, from 2368 to 2462 m in Subgroup 1b, from 2337 to 2704 m in Subgroup 1c, from 2439 to 2490 m in Group 2, 2336 m in Group 3 and from 2382 to 2695 m in Group 4; in Group 5 and 6 there were no populations.

Therefore, the maximum altitudinal difference between the localities was 454 m, a difference that was not sufficient to associate the variation of the populations with an altitudinal pattern.

In other studies with a greater altitudinal difference, such as that of Diego-Flores *et al*. (2012) and that of Mercer *et al*. (2008), a relationship was found between the origin of the populations and the altitudinal pattern of evaluation.

Romero *et al*. (2002) mention that one of the effects of altitude on populations is associated with the temperature it generates and the effect on the regulation of genes responsible for flowering. The same authors, in a very large study on allelic diversity in flowering time in maize and its local adaptation, report 366 genes with significant association to altitude and 881 and 883 genes with significant association at days of female and male flowering, respectively.

Conclusions

There is a wide morphological variability among native maize grown in the study area, based on the statistical differences between vegetative, reproductive and yield traits, and in the 6 groups and the 3 subgroups of group 1 where the populations were located.

Most populations have vegetative, reproductive and yield characteristics typical of long or late flowering-cycle plants: tall plants, with more primary ramifications of the spike, ears of greater length and diameter, and with greater grain length and thickness, which is why they were mainly related to the Chalqueño race, to a lesser extent with the Cónico race, minimal or almost null with the commercial varieties and null with the Cónico Norteño and Palomero Toluqueño races.

The morphological variation of the populations is not associated with an altitudinal pattern, due to the little altitudinal difference between the localities.

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