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Genetic diversity in accessions of 10 Mexican races of intermediate altitude maize

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Abstract

The diversity of maize (Zea mays L.) in Mexico is wide and there is still a need to carry out works that allow to discern the variation within and between racial groups and establish collections of racial reference for their understanding. The morphological diversity of representative populations of 10 mexican maize races was assessed. In the spring-summer cycle of 2010, experiments were established with 88 representative accessions of Bofo, Celaya, Coscomatepec, Dulce de Jalisco, Elotes Occidentales, Mushito, Palomero de Jalisco, Serrano de Jalisco, Tablilla de Ocho, and Zamorano Amarillo races, in the localities of Roque, Guanajuato and Ciudad Guzmán, Jalisco, under an experimental design of incomplete blocks, 30 morphological characters were measured. The analysis of variance indicated significant differences among the accessions for all the characters (variables). The repeatability analysis showed that 16 characters had a coefficient \geq 3.0, these were the least affected by the environment. In the analysis of main components and conglomerates, variation within and between races was observed, with a continuous dispersion, but which allowed the identification of four racial complexes. Group I was made up of accessions mainly from Bofo, Celaya II, Dulce de Jalisco III and Elotes Occidentales IV, the remaining races are mostly added in group II as subgroups. This study confirmed clustering of accessions in welldefined races and represented in germplasm banks. It is necessary to conduct deeper studies in races with few accessions to achieve a better racial definition.

Keywords: morphological characters, multivariate analysis, racial groups.

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Introduction

Mexico is recognized as the center of origin and domestication of maize, so there is a great diversity of types that have been adapted to specific environmental and sociocultural conditions (Matsuoka *et al.*, 2002). In Mexico, the genetic variability of maize is the result of human interaction for thousands of years with wild relatives and the environment (CONABIO, 2013). In this process, the mechanisms of dispersion are given, the genetic recombination by natural crosses between maize and teosinte (Z. *mays*: ssp. *huehuetenanguensis*, ssp. *mexicana* and ssp. *parviglumis*, among others); through, the geography of the country and the wide diversity of human groups, who make selection according to their customs in specific ecological niches (Orozco-Ramírez *et al.*, 2016).

To understand genetic diversification, morphological differentiation among native maize populations has been studied by applying the concept of race (Anderson and Cutler, 1942). The physiological and morphological reproductive traits have been used, mainly as a tool to assess diversity (Wellhausen *et al.*, 1952; Hortelano *et al.*, 2008; Ángeles *et al.*, 2010; Rocandio-Rodríguez *et al.*, 2014). To achieve an accurate racial assessment, it is necessary to know the variation between variants within the races (Castillo, 1993), with the purpose of designing the use of some forms within the regional genetic diversity of the species.

To date, approximately 62 maize races have been described, a number that increases or decreases slightly according to the considerations of each study (Sánchez *et al.*, 2000; Vielle-Calzada and Padilla, 2009; Hortelano *et al.*, 2012). Many of the native maize populations collected in Mexico are intermediate variants between races; however, once the characteristics of the variants described as representative of races are known, possible routes of racial diversification can be proposed (Wellhausen *et al.*, 1952).

Studies of maize diversity in Mexico have included few representative samples of the reported breeds (Wellhausen *et al.*, 1952), or have focused on diversity in specific geographic areas (Herrera-Cabrera *et al.*, 2004; Mijangos-Cortes *et al.*, 2007; Ángeles *et al.*, 2010). Knowledge of genetic diversity is essential to: expand the sources of germplasm, try to minimize the risks of genetic vulnerability, increase the probability of detecting favorable alleles and conserve plant genetic resources (Bellon, *et al.*, 2009).

The contributions of the Wellhausen *et al.* (1952); Hernández and Alanis (1970); Ortega (1979); Sanchez and Goodman (1992); Sánchez *et al.* (2000), among others, in breeds such as Bofo, Celaya, Coscomatepec, Dulce de Jalisco, Elotes Occidentales, Mushito, Palomero de Jalisco, Serrano de Jalisco, Tablilla de Ocho and Zamorano Amarillo indicate that the diversity of maize has been studied step to bring together existing racial genetic variability, intensively in different ecological and ethnic regions of the country, as well as their specialized forms of consumption.

This has laid the basis for current racial classifications by means of morphological, physiological, genetic, biochemical and molecular characters; however, the racial characterizations of some accessions in germplasm banks have been identified and classified in collections that are typical and do not correspond to the racial descriptions proposed by these authors.

In this context, the present work aimed to study the genetic diversity of representative accessions of ten maize races with adaptation to low to intermediate altitudes (100-2 200 m) through morphological characters to estimate genetic diversity between and within breeds, that allows to classify them by their intrinsic characteristics and to determine the condition of the type accessions in the germplasm banks.

Materials and methods

Genetic material

88 maize accessions were characterized, cataloged according to their passport data as belonging to 10 breeds in Mexico: Bofo (8 accessions), Celaya (15), Coscomatepec (7), Dulce de Jalisco (12), Elotes Occidentales (23), Mushito (5), Palomero de Jalisco (1), Serrano de Jalisco (3), Tablilla de Ocho (7) and Zamorano Amarillo (7). The seed was provided by the germplasm banks of the International Maize and Wheat Improvement Center (CIMMYT), the Autonomous University of Chapingo (UACH), the National Institute of Agricultural and Livestock Forestry Research (INIFAP) and the Postgraduate College (CP).

Experimental sites and cultivation cycles

Two experiments were established in the spring-summer 2010 cycle, the first one in the Experimental Field of the Technological Institute of Roque, Celaya, Guanajuato (20° 34' 55'' North latitude, 100° 49' 33'' West longitude, altitude of 1 766 meters above sea level) and the second at Rancho Los Paredones, Cd. Guzmán, Jalisco (19° 43' 09'' North latitude, 103° 29' 57'' West longitude, altitude of 1 515 m), both with irrigation.

Design and experimental unit

The experiments were established under an experimental design in incomplete blocks 9 x 10, with two repetitions, in which the 88 accessions were included. The experimental unit consisted of two furrows 5 m long and 0.85 m wide. Three seeds per plant were planted 0.50 m apart, six weeks after sowing, thinning to leave two plants per plant for a population density of 47 000 plants ha⁻¹ (Rocandio-Rodríguez *et al.*, 2014).

Measured characters

From each plot, five plants with complete competence were randomly labeled, in which 30 variables were measured following the procedures indicated by Sánchez *et al.* (1993).

Vegetative and phenological characters: in the final stage of the vegetative development (when the last leaf appears at the apex of the plant), the plant height (AP), height at the ear (AMz), length of the cob leaf (LHMz) and width of the maize husk (AHMz), these expressed in cm, number of total leaves (HojP), leaves above the ear (HAMz) and of buds per plant (HijP). In the flowering stage, the days of female flowering (FF) and male flowering (FM) were recorded, days after sowing, the first when 50% of the plants of the experimental unit presented exposed stigmas and the second when 50% of the plants presented pollen emission, floral synchrony (SF, FF-FM) and the AMz/AP ratio.

Spike characters: one week after anthesis the total length of the spike (LE), the peduncle (LPE), of the central branch (LRCE, central axis from the insertion point of the highest lateral branch) was measured), and of the branched section (LTRE), these variables were measured in cm, the number of primary branches (RPE), and the LTRE/LE ratio were also evaluated.

Characteristics of the ear: length (LMz), diameter (DMz, in the center of the ear), peduncle length (LPMz), ear diameter (OD) and number of rows (HMz), were measured The LMz/DMz ratio was estimated.

Grain characters: the ears were individually shelled and from the central part a sample of 10 grains per ear was taken to measure the width (AGr), length (LGr), thickness (EGr), the three variables in mm and AGR/LGr ratio, another sample of 100 grains was taken, to measure the weight (P100Gr, g), volume (V100Gr, cm³) and the coefficient of shelling in percentage (CD= [(weight of grain) x (100)/total weight of the cob]).

Statistical analysis

An analysis of variance was applied through localities. The variance components for genotypes (σ^2_{gen}) , localities (σ^2_{loc}) and their interaction $(\sigma^2_{gen \times loc})$ were estimated and the repeatability coefficient $r = [\sigma^2_{gen} / (\sigma^2_{loc} + \sigma^2_{gen \times loc})]$, was estimated. used to perform a selection of variables, it was taken as a criterion to accept the characters with $r \ge 3$ (Goodman and Paterniani, 1969). The selected variables were used to perform the analyzes described below.

A principal components analysis was applied based on the correlation matrix, the dispersion of the accessions was plotted in the plane determined by the first two components (biplot, described by Arellano *et al.*, 2014) and a cluster analysis in the that the Euclidean distance coefficient and the grouping method of the unweighted arithmetic mean (UPGMA) were used.

The choice of the cut of the dendrogram was based on the visual comparison of the groupings obtained in the analysis of main components, which was based on significant correlations ($\alpha \leq 0.05$) in addition, that the repeatability analysis ensures the use of uniform variables with greater variability for the formation of groups; with this information, the decision of the cut was established by the researcher which required knowledge of the plant material studied and an adequate base of assumptions that help in the definition of groups and distances of similarity (Núñez-Colin *et al.*, 2004). Statistical statistical analysis system (SAS) version 9.3 packages for Windows (SAS Institute, 2002) and NTSYSpc version 2.21 h (Rohlf, 2009) were used.

Results and discussion

Variance analysis

The analysis of combined variance of the 88 genotypes across localities identified significant statistical differences in the totality of the evaluated variables, which indicates the presence of ample genetic diversity among the accessions of the 10 maize races studied (Table 1). These results

agree with those reported by Herrera *et al.* (2000); Chávez-Servia *et al.* (2011); Hortelano *et al.* (2012) and those who point out that the statistical differences between native maize is an indicator of genetic diversity in them for the character under analysis.

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V		Madia	CV					
variable -	Loc	Rep/Loc	Gen	Gen x Loc	Error	Media	(%)	
gl	1	2	89	89	178			
FF	2992.9**	253.25**	642.86**	67.26^{*}	49.26	83.61	8	
FM	1886.04**	396.01**	665.69**	71.27^*	52.07	82.33	9	
SF	513.61**	8.62 ^{NS}	8.03**	6.48^{**}	3.2	5.08	35	
HijP	0.53^{*}	0.05^{NS}	0.3**	0.17^{*}	0.12	0.44	80	
AP	25959.3^{**}	579 ^{NS}	10527.57**	1041.71^{**}	389.92	245.95	8	
AMz	88304.13**	204.75^{NS}	8360.16**	922.63**	280.96	146.38	11	
AMz/AP	0.59^{**}	0^{NS}	0.01^{**}	0^{NS}	0	0.58	7.0	
HojP	235.38^{**}	10.13**	15.62^{**}	1.7^{**}	0.96	13.87	7	
HojAMz	72.63**	0.36 ^{NS}	1.27^{**}	0.26^{**}	0.16	5.34	8	
LHMz	7758.08^{**}	114.25 ^{NS}	484.15^{**}	57.41 ^{NS}	51.66	95.57	8	
AHMz	0.09^{NS}	1.74^*	4.11**	0.86^{**}	0.54	8.5	9	
LE	246.18^{**}	14.41 ^{NS}	70.7^{**}	22.36 ^{NS}	17.35	43.6	10	
LTRE	711.07**	1.19 ^{NS}	29.72^{**}	7.92^{**}	5.12	14.48	16	
LTRE/LE	0.23^{**}	0^{NS}	0.01^{**}	0^{**}	0	0.33	18	
LPE	24.08^{NS}	11.56 ^{NS}	30.66**	11.87 ^{NS}	10.89	25.71	13	
LRCE	130.3**	8.99 ^{NS}	40.98^{**}	21.47 ^{NS}	16.62	29.37	14	
RPE	409.17^{**}	1.24^{NS}	40.49^{**}	12.01 ^{NS}	9.07	15.46	19	
LMz	38.12**	1.23 ^{NS}	24.49^{**}	3.54**	1.84	18.32	7	
DMz	0.53^{*}	0.01 ^{NS}	0.61^{**}	0.11^{*}	0.08	4.56	6	
LMZ/DMz	0.15^{NS}	0.01 ^{NS}	1.35^{**}	0.34^{*}	0.25	4.07	12	
HMz	53.38**	0.31 ^{NS}	17.04^{**}	2.02^{**}	0.99	12.36	8	
LPMz	2220.95^{**}	19.93 ^{NS}	37.72**	16.57^{**}	9.77	14.42	22	
DO	0.00^{NS}	0^{NS}	0.3**	0.06^{*}	0.04	2.42	9	
LGr	15.57^{**}	0.25 ^{NS}	4.92^{**}	0.73 ^{NS}	0.62	12.55	6	
AGr	11.26**	0.25 ^{NS}	7.81^{**}	0.55^*	0.36	9.74	6	
AGr/LGr	0.21^{**}	0^{NS}	0.05^{**}	0^{NS}	0	0.78	11	
EGr	4.01^{**}	0.18^{NS}	1.02^{**}	0.22^{*}	0.15	4.72	8	
P100Gr	2026.79^{**}	0.26^{NS}	375.02**	55.35**	31.37	42.04	13	
V100Gr	8120.2**	35.73 ^{NS}	979.02^{**}	136.31**	72.99	65.03	13	
CD	323.88^{**}	43.13 ^{NS}	49.75^{**}	24.82 ^{NS}	22.13	83.77	6	

 Table 1. Mean squares of the analysis of variance combined through localities, for 30 morphological characters.

*, **, ^{NS} = significant difference α = 0.05, α = 0.01, not significant, respectively; gl= degrees of freedom. Loc= localities; Rep/Loc= repetitions nested in localities; Gen= genotypes; Gen × Loc= genotype interaction by location.

In the interaction genotypes by locality, 70% of the total of the variables were statistically significant, indicating that at least one of the accessions presented a differential response in the characters when changing locality, these results agree with those reported by Rocandio-Rodríguez *et al.* (2014).

Selection of variables

The repeatability analysis (r) showed very marked differences, with values between 0.09 and 11.78 for the characters studied and of the total of them, 16 had a value of $r \ge 3$, 63% of these correspond to cob characters (Table 2), which is in agreement with that reported by Hortelano *et al.* (2012).

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Variable	$\sigma^2_{\text{Gen x Loc}}$	σ^2_{Gen}	$\sigma^2_{ m Loc}$	r
Flowering female (FF)	9	143.9	15.12	5.97
Male flowering (FM)	9.6	148.6	8.17	8.36
Floral synchrony (SF)	1.64	0.39	2.79	0.09
Buds by plant (HijP)	0.02	0.03	0	1.24
Plant height (AP)	325.9	2371.46	137.38	5.12
Cob height (AMz)	320.83	1859.38	485.88	2.3
AMz/AP ratio	0	0	0	1.17
Total leaves per plant (HojP)	0.37	3.48	1.25	2.15
Leaves above the ear (HAMz)	0.05	0.25	0.4	0.57
Length of the cob leaf (LHMz)	482.67	2541.86	4009.55	0.57
Width of the cob leaf (AHMz)	0.16	0.81	-0.01	5.32
Total length of the spike (le)	2.51	12.08	1.26	3.21
Length of the branched section of the spike (ltre)	1.4	5.45	3.93	1.02
LTRE/LE ratio	0	0	0	0.65
Length of the spindle peduncle (LPE)	0.49	4.7	0.06	8.52
Length of the central branch of the spike (LRCE)	2.42	4.88	0.65	1.59
Primary branches of the spike (RPE)	1.47	7.12	2.25	1.91
Length of ear (LMz)	0.85	5.24	0.2	5.02
Diameter of ear (DMz)	0.02	0.12	0	6.08
LMz/DMz ratio	0.04	0.25	0	5.75
Rows per maize (HMz)	0.52	3.75	0.29	4.65
Length of the cob peduncle (LPMz)	3.4	5.29	12.19	0.34
Diameter of the olote (DO)	0.01	0.06	0	7.53
Grain length (LGr)	0.05	1.05	0.08	7.63
Grain width (AGr)	0.09	1.82	0.06	11.78
Agr/LGr ratio	0	0.01	0	5.22
Thickness of grain (EGr)	0.04	0.2	0.02	3.47
Weight of 100 grains (P100Gr)	11.99	79.92	11.13	3.46
Volume of 100 grains (V100Gr)	31.66	210.9	44.56	2.77
Grain with respect to the ear (% GrMz)	1.34	6.23	1.54	2.16

Table 2. Estimators of the components of variance and repeatability value (r).

 σ^2_{Gen} = component of variance for genotypes; σ^2_{Loc} = for localities; $\sigma^2_{Gen \ x \ Loc}$ = for interaction between genotypes and localities and r= repeatability coefficient.

The characters with $r \ge 3$ correspond mostly to reproductive descriptors, which are less affected by environmental factors, in this sense, these characters reflect high genetic variability and contribute in a better way to their explanation (González *et al.*, 2013; Rocandio-Rodríguez *et al.*, 2014).

Principal component analysis

The principal components analysis indicated that the first five components explain 88% of the total observed variation and the first two components close to 60% (data not shown). Ferraz *et al.* (2013) mention that a high proportion explained with a low number of components, allows better interpretation of the variation and studies carried out with characters related to yield in maize, show that the models that involve the biplot graphs are useful to determine the intensity and nature of native maize populations under certain environmental conditions (Martínez-Sánchez *et al.*, 2016).

The variables that showed the highest absolute values in the coefficients of the eigenvectors of the first two components, and that determine in a relevant way the racial dispersion, as well as the formation of groups were: for the component one FM, LMz, AGr and P100Gr and for component two AHMz, HMz, AGr/LGr and EGr (Table 3).

Variable	Vector own					
variable	CP-1	CP-2				
Days to feminine flowering (FF)	0.298	0.242				
Days to male flowering (FM)	0.301	0.223				
Plant height (AP)	0.27	0.268				
Width of the cob leaf (AHMz)	0.23	0.343				
Length of the spike (LE)	0.296	0.069				
Length of the spindle peduncle (LPE)	-0.021	-0.105				
Length of the ear (LMz)	0.346	0.033				
Diameter of the ear (DMz)	0.086	0.321				
LMz/DMz ratio	0.277	-0.157				
Number of rows of cob (HMz)	-0.262	0.333				
Diameter of the olote (DO)	0.136	0.189				
Grain length (LGr)	0.103	0.295				
Grain width (AGr)	0.331	-0.226				
AGr/LGr ratio	0.246	-0.387				
Thickness of the grain (EGr)	0.187	-0.316				
Weight of 100 grains (P100Gr)	0.316	-0.155				

Table	3.	Own	vectors	associated	with	the	first	two	main	components	of	the	analysis	of	16
variables, in 88 type accessions of 10 maize races.															

The dispersion of the accessions according to the first two main components makes it possible to differentiate the following four groups (Figure 1): group I: composed of 100% of the accessions of the Bofo breed, 57% Zamorano Amarillo, 43% Tablilla de Echo and 17% of Elotes Occidentales, which share morphological characteristics related to LPE (26.97 cm), EGr (5.01 mm), AGr (10.9 mm), P100Gr (49.46 g) and the LMz/DMz (4.62) and AGr/LGr (0.9) ratios.



Figure 1. Dispersion of 88 representative accessions of 10 maize races based on the first two main components. B= Bofo; CE= Celaya; CO= Coscomatepec; D= Dulce de Jalisco; E= Elotes Occidentales; M= Mushito; P= Palomero de Jalisco; S= Serrano de Jalisco; T= Tablilla de Ocho; Z= Zamorano Amarillo.

The total grouping of the accessions of the Bofo breed is indicative of a marked genetic differentiation of these. The low number of accessions of the other breeds in this group is possibly due to the fact that these accessions share a certain geographical area in their distribution (100 to 1400 meters above sea level) and greater variability, although it is also possible that *a priori* racial identification does not It has been successful. These races were classified by Sánchez and Goodman (1992); Sánchez *et al.* (2000) within a group of maize called 'Ocho Hileras' and in particular Zamorano Amarillo as a subgroup within it.

According to Wellhausen *et al.* (1952) and Hernández and Alanis (1970), the Bofo and Tablilla de Ocho breeds share the Tabloncillo breed as a common ancestor, which also partly explains the location of both breeds in this group.

Group II: integrates 100% of the accessions of Mushito, 86% of Celaya, 86% of Coscomatepec, 43% of Zamorano Amarillo, 33% of Serrano de Jalisco and 8% of Elotes Occidentales. The morphological characteristics that distinguish this group are: HMz (13.13 rows), DMz (4.8 cm), LGr (13.11 mm), AHMz (9.20 cm), AP (261.04 cm), FF (86.81 days) and FM (84.79 cm).

Considering the number of accessions, in this group the Celaya race predominates, which is a well-defined race (Wellhausen *et al.*, 1952; Sánchez and Goodman, 1992), while Mushito is mentioned as an ill-defined race (Wellhausen *et al.*, 1952; Bretting and Goodman, 1989), which also does not have a number of considerable and representative accessions in germplasm banks,

like the rest of the breeds in this group and even when they have some resemblance to the Celaya breed, its characteristics suggest that they constitute different genetic groups, which need to be studied in greater depth, this agrees with Hernández and Alanis (1970); Sánchez and Goodman (1992).

Group III: 92% of the accessions of Dulce de Jalisco, 33% of Serrano de Jalisco, 28% of Tablilla de Ocho, 14% of Coscomatepec, 6% of Celaya and 4% of Elotes Occidentales. In general they are the earliest populations, low in height of plant and ear, reduced size of spike, ear and grain (72.57 days at FF, 71.51 days at FM, 194.95 cm from AP, 13.45 HMz, 7.38 cm from AHMz , 39.03 cm of LE, 25.52 cm of LPE, 15.68 cm of LMz, 36.19 g in P100Gr, 4.45 cm of DMz, 11.55 mm of LGr, 8.95 mm of AGr and 4.88 mm of EGr), the group is located predominantly in the third quadrant of Figure 1; that is, the accessions generally have negative values of the first two main components.

Group IV: composed of 48% of Elotes Occidentales accessions which are characterized by being late FF and FM (106.45, 106.68 days), with large spike and ears (LMz, 21.10 cm, LE, 49.07 cm), heavy grain (P100Gr, 57.96 g) and LMz/DMz of 4.60, which indicates own slender ears for fresh consumption.

The Palomero de Jalisco breed was not integrated into any group, due to the low representativeness of accessions that occurred in the study. These results agree with those reported by Vega-Alvárez *et al.* (2017), who mention that a small number of accessions in studies of group differentiation in maize, can affect the formation of these.

Cluster analysis. In the dendrogram (Figure 2) there is a continuous differentiation from lower to greater Euclidean distance between accessions and groups. In this regard, Orozco-Ramírez *et al.* (2016) report that the maize races are grouped in a continuum with respect to a geographical area, environmental variation, morphological characters and the relationship with the social origin of the maize races in southern Mexico.

The diversity found in this analysis is manifested in four groups from a Euclidean distance of 5.16, which coincide between 68 and 100% with the trends of groupings in the analysis of major components (Figure 1), confirming that the best-defined breeds are for group one Bofo, for two, Celaya with the rest of the races in small groups or under a continuum; for the three Dulce de Jalisco and for the four Elotes Occidentales. Perales *et al.*, (2005) and mention that using morphological characteristics that are under the selection of farmers, it has been demonstrated that maize populations are maintained at different regional scales much smaller; likewise, Wellhausen *et al.* (1952) and later Vega-Alvárez *et al.* (2017) mention that studies based on morphological characteristics indicate that some of the local varieties arise from the hybridization of other previously existing breeds.

Results similar to those of this research are reported by Rocandio-Rodríguez *et al.* (2014), in a study of morphological and agronomic characterization of seven maize races of the High Valleys of Mexico, where they confirmed that the groupings and racial interrelations are more precise than other studies, due to the large sample sizes used.



Figure 2. Dendrogram of 88 accessions of 10 maize races, constructed with the UPGMA method using Euclidean distances considering 16 morphological variables. B= Bofo; CE= Celaya; CO= Coscomatepec; D= Dulce de Jalisco; E= Elotes Occidentales; M= Mushito; P= Palomero de Jalisco; S= Serrano de Jalisco; T= Tablilla de Ocho; Z= Zamorano Amarillo.

Conclusions

There is a high phenotypic diversity for the characters evaluated in the accessions of the ten maize races studied. The representation of the type accessions was confirmed in the differentiation of the races Bofo, Celaya, Elotes Occidentales and Dulce de Jalisco, while those of Coscomatepec, Mushito and Zamorano Amarillo, tend to group in small conglomerates as a continuum. Serrano de Jalisco, Palomero de Jalisco, Tablilla de Ocho, are not defined in any grouping, so it is necessary to deepen this type of studies for these breeds with a greater number of accessions and with additional strategies such as molecular markers.

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