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Morphological diversity of piquin chilli (*Capsicum annuum* L. var. *glabriusculum*) from Querétaro and Guanajuato, Mexico

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Abstract

In the present investigation, the morphological variability of 11 populations of wild piquin chili (*Capsicum annuum var. glabriusculum*) of the Mountain range Gorda and Semidesert of Queretaro (Municipalities of Arroyo Seco, Jalpan of Serra, Toliman and Cadereyta de Montes) and Guanajuato (Municipality of Xichu). The characterization was carried out under greenhouse conditions at the Technological Institute of Roque (ITR) located in Celaya, Guanajuato, Mexico. The 44 characters were recorded in seedling, plant, flowering, fruit maturity and seed. The principal component analysis (CP) showed that the first three components explained 56.6% of the total morphological variability. The CP1 was explained by the characteristics of weight (0.326), width (0.301) and fruit length (0.271), leaf density (0.277) and seed diameter (0.297). In CP2, the variables that contributed most to the expression of the variation were width (0.329) and leaf pubescence (0.317) and finally, in CP3, the shape of the fruit (0.344) and color of the anthers (-0.308) were the most important characters. On the other hand, the cluster analysis generated four groups, group I included the G3 (Rosa of Castilla), G2 (Palmitas The Tanque), G1 (The Tanque) populations, all of the Mountain range Gorda in Guanajuato. Group II was made up of population Q7 (The Patol, Querétaro). Group III was formed by population Q5 (Presa Jalpan, Querétaro) and finally, group IV was integrated by six populations, Q4 (San Antonio Tancoyol), Q8 (Higuerillas stream), Q6 (Higuerillas The Shote), Q2 (The Refugio solar-huerta), Q3 (Tancoyol Road to Tancoyol) and Q1 (The Chilar Refuge), all of the State of Querétaro. The morphological diversity found in this species tells us that this native plant should be conserved and that it can be easily integrated into a program of sustainable use of the flora of the region.

Keywords: morphological characterization, native plant, variation, wild populations.

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Introduction

The chili piquin is recognized as the closest ancestor of the cultivated variety, it is of natural occurrence and wide distribution in Mexico. The fruit is harvested and generates important income during the collection. The commercial use of chili piquin has been explored under diverse agronomic criteria with little success, due to the phenotypic and genotypic variation, which reduces the ecological establishment and cultivation of the plant (Vázquez-Dávila, 1996; Ramírez-Meraz *et al*., 2003; Rodríguez del Bosque *et al*., 2003).

In Mexico, there is great variability of chilis in shape, size, color, taste and itching, with a distribution from sea level to 2 500 m in height (Hernandez-Verdugo *et al*., 1999). Wild chili piquin [*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser and Pickersgill], is distributed from the south of USA to almost all of Mexico, Central and South America. In Mexico, it is found from Sonora to Chiapas along the Pacific coast and from Tamaulipas to Yucatan along the coastal strip of the Gulf of Mexico, although it develops to a greater degree from the Huasteca Tamaulipeca, located in the south of Tamaulipas, towards the north-central part of the same. State, as well as towards San Luis Potosí and Nuevo Leon, at altitudes from sea level to 600 m in height. This variational geographic location has given the chili piquin a genetic breadth and diverse uses, although it stands out as a natural resource of great importance for the rural communities of the northeast of Mexico (Laborde and Pozo, 1982; Hernández-Verdugo *et al*., 2001; Medina *et al*., 2001; Villalon *et al*., 2002; Rodríguez del Bosque *et al*., 2004).

The wild relatives of the cultivated plants are an important genetic resource that constitutes a collection of primary genes, which can help to solve problems of the current agriculture, such as tolerance or resistance to plagues and diseases and increase the quality and quantity of the production (Hernández-Verdugo *et al*., 1998). In this sense, knowledge of morphological variation and its geographic distribution patterns is of interest to understand the evolution of local plant species, their phenotype, possible use and work in their conservation (Solis-Neffa, 2010).

Among the geographical factors that influence the differentiation of populations are climate, latitude and altitude. Climate is one of the main factors that affect the distribution and variation of plant species, because it can act directly in the physiological processes of growth and reproduction or indirectly; through, of ecological interactions, such as competition for resources (Shao and Halpin, 1995).

To locate the places with the possibility of finding the chili piquin, you must have some references about the characteristics of the environment where they live. The plants of wild chili (*C. annuum* var. *glabriusculum*), commonly known as 'chiltepines' or 'piquines' are perennial, herbaceous or climbing, the fruits are small, red when ripe and spicy. The chili piquin plants are usually associated with native vegetation, from shrub plants in submontane and mountain forest, to shrubs and cacti in arid zones.

In arid environments, they are also located on dry river beds or streams, undisturbed sites of low deciduous forest, banks of roads, orchards, pastures and low remaining vegetation on the shores of agricultural fields (Hernández-Verdugo *et al*., 1999; Medina *et al*., 2001; Medina *et al*., 2002; Villalon *et al*., 2002; Medina *et al*., 2006). Although some efforts have been made, chili piquin is the only chili that has not been domesticated (Bañuelos *et al*., 2008). In this way, the wild species is an important source of genes for plant breeding through genetic engineering (González-Cortés *et al*., 2015).

Based on the above, the objective of this study was to morphologically characterize 11 chili piquín populations of the Mountain range Gorda of the states of Querétaro and Guanajuato.

Materials and methods

Collection of oblations

The fruit samples were collected from 11 chili piquin populations from four municipalities of Querétaro and one from Guanajuato (Table 1) belonging to the Mountain range de Querétaro and Guanajuato (Figure 1). Route were made through the different points with a history of existence of these plants. In each population, healthy and well-behaved plants were selected, with mature fruits and trying to be uniform in size, the geographic location of the collection site was registered by means of global positioning system (GPS). In addition, a survey was carried out to some collectors and inhabitants of the region, about the distinctive and important characteristics for them of the fruits they use.

Table 1. Populations of chili piquin used in the study and its place of origin.

	Town	State	Altitude (m)	Collection place	Municipality
1	Q1	Qro.	912	The Refugio (The Chilar)	Arroyo Seco
2	Q2	Qro.	1024	The Refugio (solar- huerta)	Arroyo Seco
3	Q3	Qro.	870	Tancoyol (carretera to Tancoyol)	Jalpan of Serra
4	Q4	Qro.	1120	San Antonio Tancoyol	Jalpan of Serra
5	Q5	Qro.	680	Presa Jalpan	Jalpan of Serra
6	Q6	Qro.	1588	Higuerillas (The Shote)	Cadereyta of Montes
7	Q7	Qro.	1833	The Patol	Toliman
8	Q8	Qro.	1504	Higuerillas (arroyo)	Cadereyta of Montes
9	G1	Gto.	1300	The Tanque	Xichu
10	G2	Gto.	1300	Palmitas (The Tanque)	Xichu
11	G3	Gto.	1460	Rosa of Castilla	Xichu

Establishment of greenhouse plants

The morphological characterization was carried out at the facilities of Technological Institute of Roque (ITR, for its acronym in Spanish) located in Celaya, Guanajuato, Mexico, which according to Google Earth data is located at 20° 32’ 05’’ north latitude and 100° 50’ 00’’ west longitude at an altitude of 1 752 meters above sea level. The climate of this region is semi warm with an average annual rainfall of 400 to 700 mm. The thermal regime is 18 to 20 °C. The soils are of the pelic vertisol type, which are characterized by being clayey of dark coloration (García, 1973).

Figure 1. Location of the collection sites of 11 populations of chili piquin (*Capsicum annuum* L. var. *glabriusculum*) of Queretaro and Guanajuato, Mexico (Google Maps).

Once the collections were obtained in the field, the seeds were extracted from the fruits and conditioned to produce the seedlings in the greenhouse, for this purpose a seeding was made in 200-well unicel trays and 'Peat Moss Peat was used as a substrate of Sphagnum' PREMIER®. When the seedlings reached an average height of 10 to 15 cm they were transplanted in 40 x 40 cm plastic bags with a capacity of 15.14 liters, using common agricultural soil as a substrate (texture: sand 17.48%, clay 25.80% and silt 56.72%) and it were placed inside a medium technology plastic greenhouse. The application of fertilizers was in the irrigation water, diluting 20 g of N, 10 g of P and 20 g of K per 100 liters of water, as a source of N was used urea; MAP for P and potassium nitrate for K (Rodríguez del Bosque *et al*., 2004). Applied once a month to maximize the development of the plant.

Morphological characterization

In the seedling, flowering and fruit maturity stages, the morphological data were recorded; once the fruits reached maturity they were harvested and the seed was extracted to later characterize them. Characterization was carried out according to the descriptors recommended by the International Plant Genetic Resources Institute (IPGRI, AVRDC and CATIE, 1995). The description was made for each population of piquin chili considering the following characters:

In seedling, hypocotyl color (CDH), hypocotyl pubescence (PDH), cotyledonous leaf color (CHC), cotyledonous leaf shape (FHC), cotyledonous leaf length (LHC) and cotyledonous leaf width (AHC) were determined. Plant registered: life cycle (CV), stem color (CT), stem anthocyanin (AT), stem shape (FT), stem pubescence (PT), plant height (A), growth habit (HC), plant width (AP), tillering (M), leaf density (DH), leaf color (CH), leaf shape (FH), leaf pubescence (PH), mature leaf length (LHM), width of the leaf (AH). In inflorescence, it was measured; number of days to flowering (NDF), number of flowers per armpit (NFA), position of the flower (PDLF), color of the anthers (CA), color of the filament (CF). In fruit; days to fructification (DF), anthocyaninic spots or streaks (MRA), fruiting period (PDF), fruit shape (FF), ripe fruit color (CFM), fruit length (LF), fruit width (AF) , weight of fruit (PF), length of the pedicel (LP), shape of the fruit in the union with the pedicel (FFUP), neck in the base of the fruit (CBF), shape of the apex of the fruit (FAF) and finally, in seed the following characteristics were recorded; seed color (CS), seed surface (SS), seed size (TS), seed diameter (DS), weight of one thousand seeds (PMS) and number of seeds per fruit (NSF).

Data analysis

The morphological data were subjected to a multivariate analysis, which included the use of principal components (ACP) and cluster analysis (AC), for which the statistical package SAS® Ver. 9.0 (SAS Institute, 2002) was used.

Results and discussion

Principal component analysis (ACP)

In the Table 2 shows the results of the principal components analysis (ACP) for the morphological characterization of 11 chili piquin populations from the states of Queretaro and Guanajuato, where it is shown that three main components (CP) explain 56.6% of the total variability that exists between the chili piquin populations; in this sense, CP1 explains 25.6%, CP2, 17.5% and CP3, 13.4% of this total variability. In this context, CP1 is explained by the characteristics of weight (0.326), width (0.301) and fruit length (0.271), leaf density (0.277) and seed diameter (0.297). In CP2, the variables that contributed most to the expression of the variation were width (0.329) and leaf pubescence (0.317) and finally, in CP3 the variables that were most important were the shape of the fruit (0.344) and color of the anthers (-0.308).

In the Figure 2 shows the diversity of the variables among the chili piquin populations studied, where it can be seen that the fruit weight (PF), fruit width (AF), seed diameter (DS), leaf density (DH), fruit length (LF), weight of one thousand seeds (PMS), leaf width (AH), leaf pubescence (PH), hypocotyl color (CH), fruit shape (FF) and color of the anthers (CA), are the characteristics that contribute most in the variability between populations. These results are similar to those obtained by Kadri *et al.* (2009) who conducted a study with 48 populations of *Capsicum annuum* in Turkey, where they obtained that the first six main components (CP) explained 54.3% of the total variation, also concluded that the CP1 was formed by the fruit and fruit variables and CP2 by fruit length and pedicel variables.

Table 2. Characteristic vectors of the variables of greatest descriptive value, with respect to their main component in 11 populations of *C. annuum* of Queretaro and Guanajuato.

Variable	Characteristic vector		
	CP1	CP2	CP3
Fruit weight	0.326*	0.058	-0.051
Fruit width	0.301*	0.128	-0.089
Diameter of seed	0.297*	0.113	0.063
Density of leaves	0.277*	0.052	0.157
Fruit length	0.271*	0.048	-0.203
Sheet width	0.1	0.329*	0.114
Leaf pubescence	-0.038	0.317*	0.046
Shape of the fruit	-0.018	0.03	0.344*
Color of the anthers	0.058	-0.05	-0.308*
Characteristic value	8.73	5.96	4.57
Proportion of variance (%)	25.6	17.5	13.4
Proportion of the cumulative variance (%)	25.6	43.2	56.6

*= greater descriptive value.

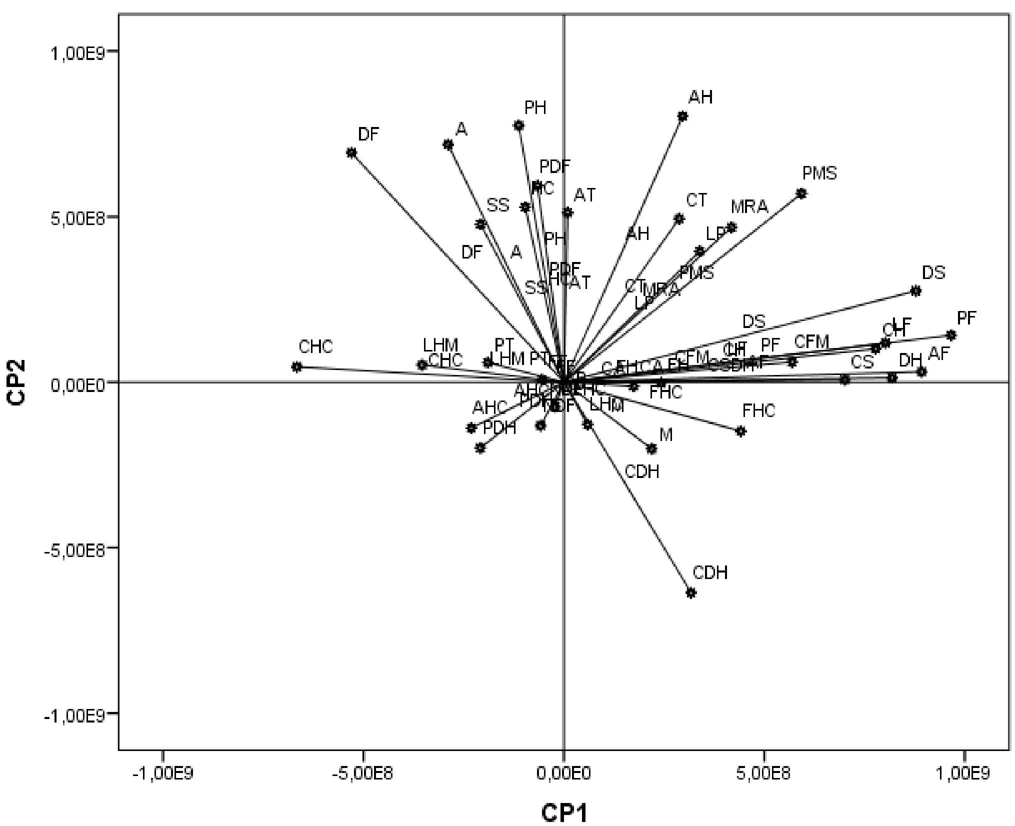

Figure 2. Distribution of morphological variability based on 44 characteristics in 11 chili piquin populations of Querétaro and Guanajuato.

In the same way, Toledo (2010) in a study of morphological diversity in native chili poblano varieties, concluded that fruit weight and color are important characteristics in phenotypic diversity. In this topic, Narez-Jiménez *et al*. (2014) in its study of in situ morphological diversity of wild chili in the state of Tabasco found that CP1 explained 43.61% of the total variation and is explained by fruit variables. Another study reported by Pardey *et al*. (2006) state that the variability between chili populations is given by fruit characteristics, plant architecture, flower structure and number of flowers per armpit.

Other investigations affirm that the color and shape of the fruit in mature state have shown importance in the classification of several collections of *C*. *annuum* from several regions of the country (Corona, 2000, Hernández, 2000, Martínez, 2000, Pérez, 2000; Bañuelos, 2003), it has also been concluded that the variation in these characteristics is a hallmark of this species (Hernández *et al*., 1999, De, 2003). Similarly, Toledo (2010) points out that in native varieties of chili poblano the total variation was explained by variables related to the quality of the fruit; likewise, Moreno-Pérez *et al*. (2007) in studies of morphological diversity in chili guajillo found variation in leaf length and width characteristics, as well as petiole length; whereas, Moran (2008) in a study of 43 populations of creole chilis from the south of the state of Puebla concluded that there is a strong variability in vegetative characters, such as the habit of growth and leaf size.

Observing the resulting means of each of the characteristics that contributed most to the variability in all populations, it was found that the highest fruit weight (0.211 g) was for G1 (The Tanque) and the lowest weight (0.072 g) resulted population Q2 (The Refugio solar-huerta), for fruit width, population G1 (The Tanque) obtained the highest value (0.70 cm) and Q3 (Tancoyol road to Tancoyol) the lowest (0.45 cm); in seed diameter, the genotype G1 (The Tanque) obtained the largest diameter (3.18 mm) and Q6 (Higuerillas The Shote) obtained the lowest value (2.52 mm); in density of leaves, the majority of Queretaro's populations [Q2 (The Refugio solar-huerta), Q4 (San Antonio Tancoyol), Q5 (Jalpan Dam), Q6 (Higuerillas The Shote), Q8 (Higuerillas Arroyo)] were in the intermediate density range (5), while the populations of Guanajuato [G1 (The Tanque), G2 (Palmitas El Tanque), G3 (Rosa of Castilla)] and the population Q7 (El Patol, Qro.) for the dense range (7) and Q1 populations (El Chilar Refuge) and Q3 (Tancoyol road to Tancoyol) resulted in the low density range (3).

For fruit length, the G1 population (The Tanque) with the longest length (1.22 cm) and Q2 (The Refugio solar-huerta) with the lowest value (0.64 cm) stood out again; in leaf width Q7 (The Patol) obtained the highest result (3 cm) and Q2 (The Refugio solar-huerta) the lowest response (1.3 cm); on the other hand, most of the populations [Q1 (The Chilar Refuge), Q3 (Tancoyol road to Tancoyol), Q6 (Higuerillas The Shote), Q7 (The Patol), G1 (The Tanque) and G2 (Palmitas The Tanque]] showed a leaf pubescence in the intermediate range (5), while Q2 (The Refugio solar-huerta), Q4 (San Antonio Tancoyol), Q8 (Higuerillas arroyo) and G3 (Rosa of Castilla) showed low pubescence (3) and only Q5 (Presa Jalpan) was consistent with dense pubescence (7); for the fruit form, all the populations were almost round (2) except Q4 (San Antonio Tancoyol) that presented an elongated fruit shape (1), for the color of the anthers the populations of Guanajuato G1 (The Tanque), G2 (Palmitas The Tanque), G3 (Rosa of Castilla) and some of Querétaro [Q1 (The Chilar Refugio), Q2 (The Refugio solar-huerta), Q3 (Tancoyol road to Tancoyol), Q4 (San Antonio Tancoyol), Q5 (Presa Jalpan) presented purple anthers (5), while the populations Q6 (Higuerillas The Shote), Q7 (El Patol) and Q8 (Higuerillas arroyo) anther blue color.

The results of this investigation are similar to those of Hernández-Verdugo *et al*. (2012) who studied 19 populations of wild *C. annuum* and found significant differences in all recorded characteristics (plant height, branch length, stem diameter and peduncle length). Also, Murillo-Amador *et al*. (2015) in their study show a high morphometric variability among wild *C. annuum* populations in three sites near two biosphere reserves in Baja California Sur, Mexico and comment that the phenotypic diversity and undoubtedly the genetic diversity of the wild *Capsicum* in each of these populations they are affected by geography, climate, ecology and human intervention.

Correlation analysis

When correlating the fruit weight (PF) with the outstanding morphological variables and with the main components (Table 3) positive and significant correlations are observed with the fruit width (0.94), seed diameter (0.79), leaf density (0.74), fruit length (0.87) and leaf color (0.8), for leaf width (0.46) and leaf pubescence (0.12) showed a positive but low correlation. On the other hand, CP1 showed a strong and positive correlation with fruit weight (0.96), fruit width (0.89), seed diameter (0.87), leaf density (0.81) and fruit length (0.8). The CP2 correlated strongly and positively with leaf width (0.8) and leaf pubescence (0.77); while CP3 showed low correlation and negative fruit length (-0.43) and leaf color (-0.31) but low and positive leaf density (0.33).

In this sense, García (2006) reported correlations in width, weight and fruit length characters, as those variables that contribute most in the first component. The high correlation value may indicate redundancy in the information; that is to say, that evaluating only one of the highly-correlated characters does not lose global information about the variation present in the group, saving time and money. However, the high index of correlation between characters can also be associated with a high degree of relationship within the group (Roa, 1997).

Table 3. Correlation of variables with main components of 11 populations of *C. annuum* from Querétaro and Guanajuato.

	PF	AF	DS	DH	LF	CH	AH	PH
PF	1	0.94	0.79	0.74	0.87	0.8	0.46	0.12
CP1	0.96	0.89	0.87	0.81	0.8	0.77	0.29	-0.11
CP2	0.14	0.31	0.27	0.12	0.11	0.1	0.8	0.77
CP3	-0.1	-0.19	0.13	0.33	-0.43	-0.31	0.24	0.09

CP1= main component 1; CP2= main component 2; CP3= main component 3; FP= fruit weight; AF= fruit width; DS= seed diameter; DH= leaf density; LF= length of fruit; CH= leaf color; AH= sheet width; PH= leaf pubescence.

Conglomerate analysis

The cluster analysis (AC) is presented in Figure 3, where the formation of four groups is observed, group I included the populations G3 (Rosa of Castilla), G2 (Palmitas El Tanque), G1 (The Tanque), all populations belong to the region of the Mountain range Gorda in Guanajuato. Group II, was only made up of population Q7 (The Patol, Querétaro). Group III was constituted only by population Q5 (Jalpan Dam, Querétaro) and finally, group IV was integrated by six populations of Querétaro State Q4 (San Antonio Tancoyol), Q8 (Higuerillas arroyo), Q6 (Higuerillas The Shote), Q2 (The Refugio solar-huerta), Q3 (Tancoyol road to Tancoyol) and Q1 (The Refugio The Chilar).

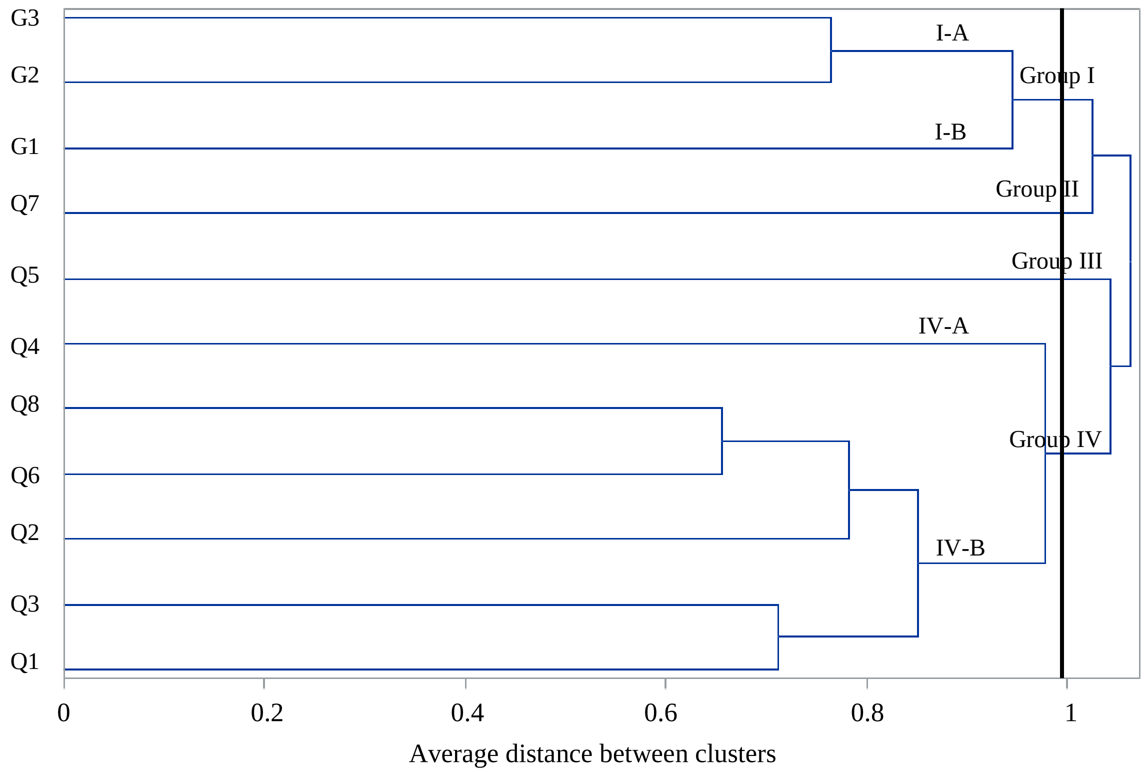

Figure 3. Dendogram of 11 populations of chili piquin de Querétaro and Guanajuato.

On the other hand, in the relationship between the groups generated, the association of group I and group II can be observed; which are populations of Guanajuato (G1), (G2) and (G3) and Querétaro (Q7) these populations are located near geographically. Another association between groups resulted in group III and group IV, where the population Q5 was associated with the populations Q4, Q8, Q6, Q2, Q3 and Q1. In addition, the formation of two large associations was observed, the first with populations exclusively from Queretaro and the other with populations from Guanajuato and a population from Queretaro. On the other hand, the populations that make up group I have characteristics of cotyledoneal leaf length (LHC) in group II, they distinguish stem color (CT) and stem pubescence (PT); in group III the populations had lower plant width (AP), longer days to flowering (DF) and longer fruiting period (PDF) and group IV highlighted populations with characteristics of stem anthocyanin (AT) and spots or stripes anthocyanin (MRA) lower.

The populations Q6 (Higuerillas The Shote) and Q8 (Higuerillas arroyo) were very similar in their characteristics, but the fruit characteristics were the most important, also coming from geographically close communities. Similar results were reported by Pardey (2008) who made a characterization of accessions of *Capsicum* and the analysis of conglomerates grouped populations with characteristics associated with the fruit. The same author affirms the conformation of the diverse groups with materials coming from the same geographical area. On the other hand, the populations Q7 (The Patol) and Q5 (Jalpan Dam), presented the greatest divergence, probably because they are the largest (1833) and the lowest (680) altitude (meters above sea level) of all populations.

The groups generated allow us to observe that the populations of Guanajuato (1 300- 1 460 meters above sea level) are related and/or close to only one of the Queretaro populations, the Patol (1 833 meters above sea level), which is characterized by having the highest altitude of all observed populations. In this sense, Hernández-Verdugo *et al*. (2012) mention that in populations of *C. annuum* var. *glabriusculum*, the correlation between morphological variation and climatological factors such as temperature and the amount of water available by the plants allow to differentiate the populations.

Conclusions

Morphological variability was found between and within the chili piquin populations of Querétaro and Guanajuato; so there is diversity in these populations, which can serve as a guide for further studies.

The analysis of main components allowed to explain 56.6% of the morphological diversity among the studied populations based on three of them.

The characteristics that most influenced the explanation of the total variation among the populations were those related to the fruit and the leaf.

Based on the registered variables it is possible to differentiate the populations depending on their geographical origin.

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