

Characterization of cultivated varieties of *Capsicum pubescens* in Michoacán, Mexico

Ana Karen Escalera-Ordaz¹
Héctor Guillén-Andrade^{2§}
Ma. Blanca Nieves Lara-Chávez²
Clemente Lemus-Flores¹
Javier German Rodríguez-Carpena¹
Roberto Valdivia-Bernal¹

¹Academic Unit of Agriculture-Autonomous University of Nayarit. Tepic-Compostela Highway km 9, Xalisco, Nayarit, Mexico. CP. 63780. Tel. 5212112478. (ana.karen.escalera.ordaz@gmail.com; clemus23@gmail.com; germencillo@yahoo.com.mx; beto49-2000@yahoo.com.mx). ²Faculty of Agrobiolgy President Juárez-Advanced Research Unit in Agrobiotechnology-Michoacana University of San Nicolás de Hidalgo. Paseo Gral. Lázaro Cárdenas and Berlin s/n, Col. Viveros, Uruapan, Michoacán, Mexico. CP. 60170. Tel. 452 5236474. (chavez12001@yahoo.com.mx).

§Corresponding author: hguillenandrade@gmail.com.

Abstract

The state of Michoacán has diverse homogeneous agro-ecological zones that favor the wide diversity of plant species, which includes the cultivation of chili, considered one of the main crops worldwide. The cultivation of *C. pubescens* has gained greater importance in recent years due to the increase in its production. This species has broad genetic variability that can be used in breeding programs. However, the variability in the state of Michoacán has not been sufficiently documented, so the genetic potential it represents is unknown. In the present work, the collection and morphological characterization of cultivated varieties of *C. pubescens* in different agroecological zones was proposed in order to evaluate the morphological variability and relate it to the different existing agro-ecological conditions. The in situ morphological characterization was made based on the descriptors proposed by IPGRI, AVRDC and CATIE and UPOV, the evaluation included 27 characteristics of leaf, fruit and seed. The results obtained indicate that there is a wide variability in the cultivated varieties of *C. pubescens*. Likewise, the fruit characteristics were identified as the most important to discriminate the varieties with potential to be used, as quality parameters, in breeding programs. Finally, no relationship was found between the morphological variability of *C. pubescens* and the sites of origin of the cultivated varieties.

Keywords: genetic resources, perón chili.

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Introduction

The cultivation of chili is one of the most important horticultural crops in the world (de la Cruz-Lázaro *et al.*, 2017). It is distinguished by the pungency of its fruits, mainly caused by three capsaicinoids: nordihydro-, dihydro- and capsaicin (Sánchez-Sánchez *et al.*, 2010). It presents a wide variability of shapes, colors, aromas, flavors and sizes that constitute a valuable contribution of Mexico to the culture, food and gastronomy of the world since pre-hispanic times (Alonso *et al.*, 2012; Hernández-Verdugo, 2014; Pérez *et al.*, 2015), with great relevance in Mexican cuisine, being the main spice (Zegbe *et al.*, 2012).

The chemical-food value is important because of the content of valuable antioxidant compounds in the prevention of chronic and degenerative diseases (López-López *et al.*, 2015). Likewise, its economic value is increased in the process industry and extraction of dyes and oleoresins (Arreguin *et al.*, 2015). In Mexico, the production of chili is 1 276 123 t ha⁻¹, the main producing states are: Sinaloa, Sonora, Baja California Sur, Jalisco, San Luis Potosí, Veracruz, Tamaulipas, Guanajuato and Michoacán.

The latter contributes with a planted surface of 448 ha of the national surface and with a yield of 11 095 t ha⁻¹ (SIAP, 2019). However, one of the main species of chili cultivated in the states of Michoacán, Veracruz, State of Mexico and Puebla is *C. pubescens* (Barrios-Puente *et al.*, 2014), with great importance in national and foreign markets, and that its production has been increased by the high yields obtained, fruit quality and high profitability, the price fluctuates from \$10.00 to \$80.00 and is directly related to the season, size and fruit color.

Due to the above, there is a potential demand in growth for its consumption and production (Espinosa-Torres and Ramírez-Abarca, 2016). This species is characterized by being mainly rainfed, where the most significant months for harvesting are from August to December (Barrios-Puente *et al.*, 2014). In Mexico, *C. pubescens* is commonly known as manzano chili, rocoto, canario or chili wax and in the state of Michoacán, it is recognized as perón chili (Espinosa-Torres and Ramírez-Abarca, 2016) and has wide genetic variability that can be used in genetic improvement programs as a result of the wide diversity of existing environments (Bobadilla-Larios *et al.*, 2017).

In this state, there are diverse homogeneous agroecological zones, which are generally favorable for the development of avocado cultivation (Gutiérrez-Contreras *et al.*, 2010) and some favor the development of perón chili, since good adaptation is observed in areas of shade where warm air mixes with cold wind, under the avocado trees (SAGARPA, 2013).

However, *C. pubescens* is known only under cultivation and the lack of knowledge about diversity and phylogenetic relationships limit its genetic improvement (Pérez *et al.*, 2015). In this sense, the related phylogenetic resources acquire great relevance because of the genetic potential they present and because they are the basis for obtaining improved varieties (Moreno-Pérez *et al.*, 2011).

According to the above, there is a need to know the distribution, variability and genetics of the materials retained by the producers in their plots (Martínez-Sánchez *et al.*, 2010) to avoid the loss due to genetic erosion as a consequence of disasters caused by factors adverse effects such as demographic changes, droughts, destruction of natural areas, substitution of landraces, pests and diseases, among others (CONABIO, 2011; Toledo-Aguilar *et al.*, 2016).

In this sense, it is necessary to rescue and characterize (IBPGR, 1983; UPOV, 2006) the genetic diversity cultivated with the purpose of generating information on the variability of this species that prevails in different regions and agroecological zones of the state of Michoacán. It is also important to explore alternatives that allow us to know and conserve important characteristics to lay the foundations for the genetic improvement of the species.

In the present research work the collection of perón chili morphotypes in different agroecological zones of the state of Michoacán was proposed in order to evaluate the existing morphological variability, as well as the identification of important morphological characteristics to be used in breeding programs of the species.

Materials and methods

The research was carried out in the Phylogenetic Resources Laboratory (LAREFI) of the Advanced Research Unit in Agrobiotechnology (UIIA) of the 'Presidente Juárez' School of Agrobiology, located in the city of Uruapan, Michoacán, Mexico. The germplasm collection was carried out through field trips made from February 2016 to August 2017, due to the association of *C. pubescens* with avocado cultivation, mainly in the area known as the avocado producer fringe. Michoacán, located in the physiographic province of the transversal volcanic system between the parallels 18° 45' and 20° 6' north latitude and the meridians 101° 47' and 103° 13' west longitude, where ten homogeneous agro-ecological zones predominate (Gutiérrez-Contreras *et al.*, 2010).

In total, five fruits and five leaves of 31 cultivated varieties were taken at random. The characterization of fruit, leaf and seed was made based on the morphological descriptors proposed for *Capsicum* spp. (CATIE-IPGRI-AVRDC, 1995, UPOV, 2006). In total, 27 variables were considered, six of leaf: color, shape, margin of leaf blade, pubescence, length and width; 16 of fruit: volume, presence of anthocyanin stains or stripes, color, shape, length, width, weight, length of the fruit pedicel, thickness of the wall, shape of the fruit in union with the pedicel, neck at the base of the fruit, shape of apex of fruit, appendix in the fruit, transverse wrinkling, number of locules and type of epidermis and five of seed: color, texture, size, weight and number of seeds per fruit.

The experimental design used was completely randomized with five repetitions. For the analysis of qualitative variables, double-state and multi-state data were given to which nominal values were assigned with logical sequence and quantitative variables were standardized to whole numbers with two decimals except for the number of locules and number of seeds per fruit. Subsequently, to determine the existence or not of statistical differences between the characters evaluated, a one-way analysis of variance was performed with the Proc Anova procedure and to evaluate significant statistical differences between cultivated varieties, the Tukey test was used ($\alpha \leq 0.05$).

In order to select the variables that provided the most information to explain the total variance among the cultivated varieties, 24 variables were selected that presented significant statistical differences, on this list a discriminant analysis of Stepwise was practiced (Romano and Wolf, 2005). Subsequently, to eliminate the correlated variables and avoid problems of collinearity in the matrices, a multiple correlation analysis was made (Pearson and Filon, 1898).

In order to determine the similarity between the cultivated varieties, multivariate statistics tools were used, such as principal component analysis (PCA) and cluster analysis (AC) with the Ward grouping method, with the pseudo F test was determined on degree of reliability of the groups.

For the analyzes described, the SAS computation program version 9.2 was used (SAS, 2012). Finally, to determine the possible relationship of the morphological characteristics with the origin of the cultivated varieties, the georeferencing information of the origin of the cultivated varieties was transformed (FCC, 2018) to construct a matrix of geographical distances (DG), which was correlated with the matrix of Euclidean distances (DE) of morphological characteristics, by means of the Mantel test with the Genalex statistical package (Peakall and Smouse, 2012).

Results and discussion

In four homogeneous agroecological zones (Gutiérrez-Contreras *et al.*, 2010) of the Michoacán avocado producer fringe, it was possible to identify perón chili, in total, 31 cultivated varieties of perón chili with contrasting morphological characteristics. The cultivated varieties were located between 1 623 and 2 282 meters above sea level, height ranges that coincide with those reported for the cultivation of this species (Yáñez *et al.*, 2015; Espinosa-Torres and Ramírez-Abarca, 2016; Caballero-Gutiérrez *et al.*, 2017).

Perón chili is identified as a species that has genetic variability that can be used in breeding programs (Arias *et al.*, 2017). In this study, the cultivated varieties of perón chili showed significant statistical differences ($\alpha < 0.05$) for 20 variables. In Table 1, simple statistics are presented for each of the variables analyzed. The coefficients of determination (R^2) obtained ranged from $R^2 = 0.29$ (texture of the seed) to $R^2 = 0.82$ (fruit length), indicating that the data fit the model used for the analysis of the information, in addition, most of the variables presented high predictive capacity of the variance.

Table 1. Simple statistics for 27 variables evaluated in 31 cultivated varieties of *C. pubescens* from the state of Michoacán, Mexico.

Variable	R^{2*}	CV ¹	DE ²	Mean	Pr> F ³
Leaf color	0.54	17.52	0.58	3.32	0.0001
Leaf shape	0.51	20.05	0.52	2.58	0.0001
Leaf blade margin	0.39	29.26	0.5	1.7	0.0001
Leaf pubescence	0.49	16.82	0.39	2.29	0.0001
Leaf length	0.72	15.96	2.23	13.96	0.0001

Variable	R ^{2*}	CV ¹	DE ²	Mean	Pr> F ³
Leaf width	0.64	17.85	0.91	5.08	0.0001
Fruit volume	0.75	20.55	9.12	44.39	0.0001
Spots or streaks	0.37	91.68	0.44	0.48	0.0004
Fruit color	0.73	27.95	1.09	3.9	0.0001
Fruit shape	0.3	18.33	0.82	4.46	0.015
Fruit length	0.82	8.81	4.58	52.01	0.0001
Fruit width	0.81	9.25	4.66	50.34	0.0001
Fruit weight	0.81	19.75	8.17	41.38	0.0001
Pedicle length	0.57	15.42	4.63	30.02	0.0001
Wall thickness	0.36	24.32	1.35	5.56	0.0005
Fruit shape at the junction with the pedicel	0.63	13.58	0.51	3.74	0.0001
Neck at the base of the fruit	0.56	61.27	0.36	0.59	0.0001
Fruit apex shape	0.39	13.18	0.37	2.79	0.0001
Fruit appendix, vestige of flowering	0.49	95.3	0.39	0.41	0.0001
Cross wrinkle	0.55	28.6	0.54	1.88	0.0001
Number of locules	0.32	21.69	0.54	2.48	0.007
Epidermis type	0.37	28.57	0.33	1.14	0.0004
Seed color	0.34	23.26	0.53	2.26	0.002
Seed texture	0.29	25.23	0.51	2.03	0.0207
Seed size	0.4	27.49	0.5	1.81	0.0001
Seed weight per fruit	0.49	28.98	0.33	1.14	0.0001
Number of seeds	0.4	26.91	17.31	64.33	0.0001

*= coefficient of determination; ¹= coefficient of variation; ²= standard deviation; ³= statistical probability of F.

The standard deviation (SD) in most of the collections was low, except for the variables: fruit weight (SD= 8.17), fruit volume (SD= 9.12) and number of seeds per fruit (SD= 17.31). On the other hand, the variability of the evaluated characteristics and interpreted by the coefficient of variation (CV) showed that the measured characters have different levels of dispersion, the characters with the greater variation between collections were: appendix of fruit, vestige of flowering (CV= 95.30) and presence or absence of anthocyanin spots or streaks (CV= 91.68), in contrast to length (CV= 8.81) and fruit width (CV= 9.25).

Similar results have been obtained when characterizing populations of *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* (Palacios and García, 2009). In this sense, when analyzing collections of *C. annuum*, *C. frutescens* and *C. chinense* CV greater than 25% were obtained for 75% of the evaluated descriptors (Villota-Ceron *et al.*, 2012), in collections of *C. annuum* (timpinchi) the highest CV values were determined for stem length and crown diameter with a CV greater than 50% (Alonso *et al.*, 2012).

The wide variability of morphological characters presented by the genus *Capsicum* has been observed in different works (Pardey *et al.*, 2009). Although different species belonging to the genus *Capsicum* share traits in common, each species has its own characteristics, of importance in the selection processes (Palacios and García, 2009). However, the variables evaluated in fruit are mainly considered to discriminate useful genetic variability in breeding programs (Pardey *et al.*, 2006).

For example, the thickness of pulp, weight, length and volume of fruit have been reported as important characteristics in the explanation of the variation that exists within the genus *Capsicum* (Medina *et al.*, 2006; Castañon-Najera *et al.*, 2008; Bozokalfa *et al.*, 2009; Singh *et al.*, 2017). The results obtained from the stepwise discriminant analysis indicate that the variables that do not provide information on the variability between the cultivated varieties are the volume of fruit and type of epidermis.

Additionally, the results obtained from the stepwise discriminant analysis indicated that the variables: volume and thickness of the fruit wall, do not provide useful information to explain the variation between the cultivated varieties of manzano chili. Likewise, when reviewing the Pearson correlation values, these variables showed a high correlation with the weight and width of the fruit.

In Table 2, there are 27 significant correlations ($r > 0.05$) identified among the 27 variables evaluated in leaf, fruit and manzano chili seed. Of the 27 correlations, five correlations were identified with values of $r \geq 0.8$, the fruit weight with volume and width of fruit ($r = 0.9521$ and $r = 0.8383$, respectively), width of fruit, with volume of fruit ($r = 0.8197$), number of seeds with the weight of seeds ($r = 0.8044$) and width of the leaf with leaf length ($r = 0.7841$). 20 correlations with value $r \leq 0.5$.

In contrast, two correlations with negative value, length of fruit with shape of fruit apex ($r = -0.3108$) and fruit color with fruit width ($r = -0.3114$) were identified. Similarly, variables with high values of correlation between leaf and fruit characteristics in collections of the genus *Capsicum* have been identified (Villota-Ceron *et al.*, 2012).

Table 2. Pearson correlation coefficients identified among the variables evaluated in 31 cultivated varieties of *C. pubescens* from the state of Michoacán, Mexico.

Variable 1	Variable 2	Correlation coefficient	Variable 1	Variable 2	Correlation coefficient
VF	PF	0.9521	LF	CBF	0.3871
ACHF	PF	0.8383	ACHF	FFUP	0.3811
VF	ACHF	0.8197	LF	ARRT	0.3765
PS	NS	0.8044	PF	EP	0.3739
LH	ACHH	0.7841	VF	FFUP	0.3732
PF	PS	0.4894	ACHF	EP	0.3528
LF	LH	0.4868	VF	EP	0.3503

Variable 1	Variable 2	Correlation coefficient	Variable 1	Variable 2	Correlation coefficient
VF	PS	0.4588	FAF	NS	0.3205
PF	NS	0.4473	NL	NS	0.3182
VF	NS	0.4146	NL	PS	0.3175
ACHF	PS	0.4128	LF	CH	0.3116
LF	LP	0.4097	LF	FAF	-0.3108
PF	FFUP	0.4091	CF	ACHF	-0.3114
ACHF	NS	0.3919			

Fruit volume (VOL); fruit weight (PF); fruit width (ACHF); seed weight (PS); leaf length (LH); fruit length (LF); fruit apex shape (FAF); number of locules (NL); fruit color (CF); number of seeds (NS); wall thickness (EP); fruit shape at the junction with the pedicel (FFUP); transverse wrinkle (ARRT); leaf width (ACHH); seed color (CS); leaf color (CH); pedicel length (LP); and neck at the base of the fruit (CBF).

In hybrids of *C. pubescens* the close association of the variables height of the plant, length and width of the leaf is demonstrated, in addition, the effect on the bearing and vigor of the plant as well as on the size of the fruit is pointed out (Arias *et al.*, 2017). The weight of the fruit is a dominant characteristic, related to the size of the fruit and the thickness of the pericarp; these characteristics are important due to consumer demand, since the latter is attracted by large and showy fruits, and fruits with thicker pericarp are more resistant in post-harvest handling (Lannes *et al.*, 2007).

However, the susceptibility to fruit rot caused by *Phytophthora* was correlated with the thickness of the pericarp; that is, chili with thicker pericarp tend to be more susceptible to *P. capsici* for this reason, small fruits are considered in breeding programs for their characteristics of resistance to insects and diseases (Naegele *et al.*, 2016). The results obtained from the analysis of principal components (CP) indicated that the total variance explained, with the first three components was only 45.89%, while with nine, 80% is explained.

Similar results have been obtained in works of morphological characterization in situ in the genus *Capsicum*, since it has been observed that in this type of work it is not possible to explain more 80% of the variance with the first three components (Castañon-Najera *et al.*, 2008; Martínez-Sánchez *et al.*, 2010; Alonso *et al.*, 2012; Villota-Ceron *et al.*, 2012; de la Cruz-Lázaro *et al.*, 2017). Similar results in this type of work in other species have been reported (Medina-Torres *et al.*, 2015, 2016; Toledo-Aguilar *et al.*, 2016).

In the Figure 1 shows the distribution of 31 cultivated varieties from four homogenous agro-ecological zones based on three main components. When analyzing the eigenvectors with values above 0.3, it is determined that the CP1 is a function of the presence of the neck at the base of the fruit (CBF), leaf length (LH) and fruit length (LF), the CP2 is determined according to the weight of the seed (PS), fruit volume (VOL), width (ACHF) and fruit weight (FP), finally, CP3 is a function of fruit color (CF), transverse wrinkling (ARRT), number of locules (NL) and seed color (CS).

Similar results have been reported in others in other species, for example, in *C. annuum* 74% of the total variance is indicated, explained with the first three main components (Martínez-Sánchez *et al.*, 2010) as a function of days to flowering and fruiting, pericarp thickness, corolla length and width. The most important features to explain the variability between cultivated varieties of different species of the *Capsicum* genus are those related to the fruit and the architecture of the plant (Castañon-Najera *et al.*, 2008; de la Cruz-Lázaro *et al.*, 2017), these results coincide with those obtained in the present investigation, since the width and length of leaf, shape, length, width and fruit weight have also been identified among the main morphological characteristics that favor the explanation of variability between collections.

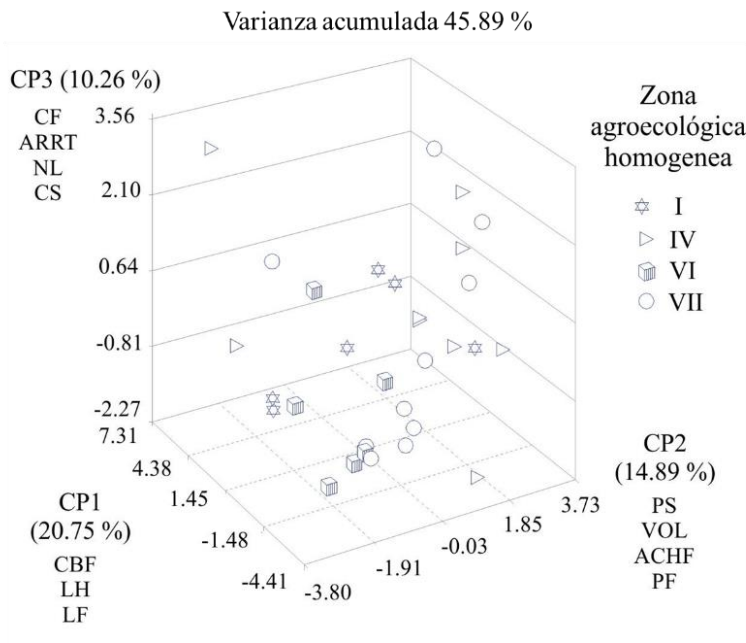


Figure 1. Dispersion of cultivated varieties of perón chili from four homogeneous agro-ecological zones of the state of Michoacán; through, of the first three components. Variables associated with CP1= neck in the fruit base (CBF); leaf length (LH) and fruit (LF); variables associated to CP2= seed weight (PS); volume (VOL); width (ACHF) and fruit weight (PF); variables associated with CP3= fruit color (CF); transverse wrinkling (ARRT); number of locules (NL); and seed color (CS).

In the dendrogram generated from the cluster analysis, groups of cultivated varieties from the same locality were identified, as well as the association of varieties cultivated by the agroecological zone of the place of origin (Figure 2). In the germplasm collected there is a wide variability, these differences may be related to the places of origin. It has been identified that the temperature and the quantity of water available during the growth and reproduction of the plants are important factors for the differentiation of the populations of wild chili that grows in natural conditions (Hernández-Verdugo *et al.*, 2012).

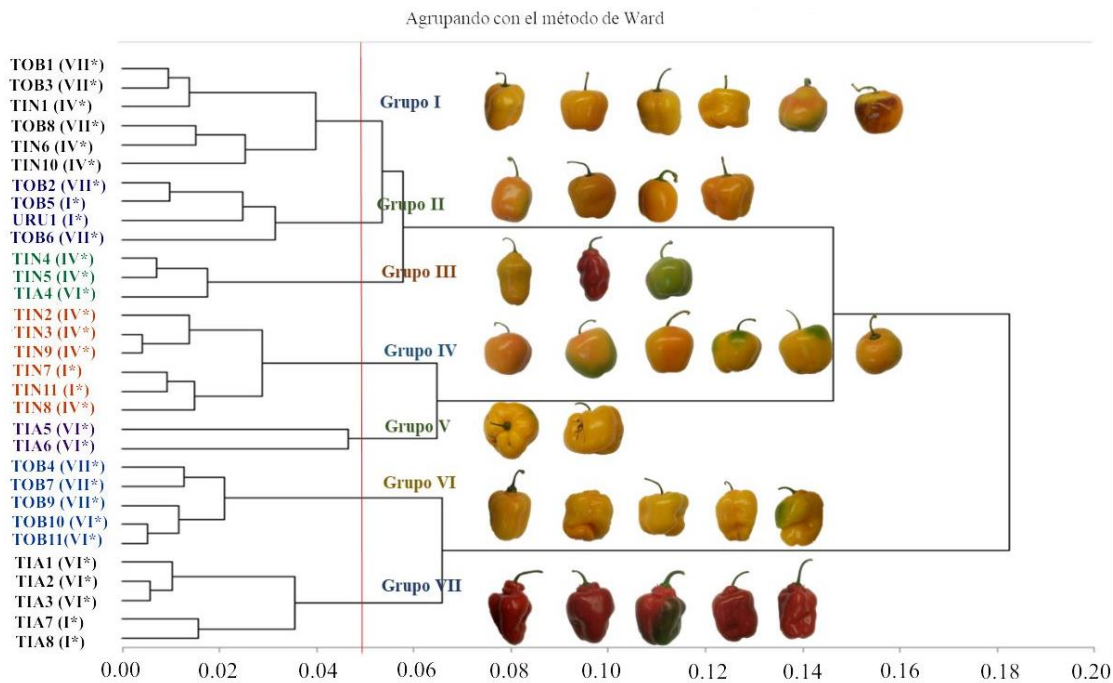


Figure 2. Grouping of 31 cultivated varieties of *C. pubescens* from four homogeneous agroecological zones* of the state of Michoacán; Toreo el bajo (TOB); Tingambato (TIN); Tiamba (TIA); and Uruapan (URU).

In contrast, the grouping of materials shows no relationship between species or collection environments (Villota-Ceron *et al.*, 2012). The results obtained on the variability and its relation with the origin of the cultivated varieties coincide partially with those indicated by the cited authors, in this work, the existence of groups of cultivated varieties clearly related to the agroecological zones was determined, although they were also identified groups without any apparent relationship to their place of origin. The results obtained in this study corroborate that the existing variability in *C. pubescens* is mainly explained by characteristics evaluated in the fruits (Table 3).

Table 3. Description of groups of cultivated varieties of *C. pubescens* based on nine variables that provide the most information to explain the existing total variance.

G ¹	MOR ²	CF ³	FF ⁴	AF ⁵ (mm)	PF ⁶ (g)	FFUP ⁷	CBF ⁸	ATF ⁹	NS ¹⁰
1	present	yellow to orange	bell-shaped and bell-shaped in block	46-48	34-42	truncated, cordate and lobed	present and absent	slightly corrugated to intermediate	50-79
2	present and absent	orange	bell-shaped and bell-shaped in block	35-46	21-33	truncated	absent	slightly corrugated	46-73
3	absent	yellow orange and red	almost round, elongated and bell-shaped in block	36-54	21-43	cordate	present	slightly corrugated	45-61

G ¹	MOR ²	CF ³	FF ⁴	AF ⁵ (mm)	PF ⁶ (g)	FFUP ⁷	CBF ⁸	ATF ⁹	NS ¹⁰
4	absent	yellow to orange	almost round	51-61	42-62	lobed	absent	slightly corrugated	60-78
5	present and absent	yellow	bell-shaped in block	63-77	60-100	lobed	absent	intermediate to very corrugated	88-100
6	present	yellow to orange	bell-shaped and bell-shaped in block	45-65	34-43	truncated and cordate	present	intermediate to very corrugated	56-73
7	present and absent	red	triangular and bell-shaped	40-49	25-45	cordate	present	intermediate to very corrugated	52-78

¹= group; ²= anthocyanin stains or streaks; ³= color of fruit; ⁴= width of fruit; ⁵= fruit weight; ⁶= form of the fruit at the junction with the pedicel; ⁷= neck at the base of the fruit; ⁸= transverse wrinkling; ⁹= number of seeds per fruit.

Finally, the results obtained from the Mantel test indicate that there were no significant correlations between the origin of the cultivated varieties and the morphological characteristics evaluated (Figure 3); nevertheless, the data used to correlate the variability with the origin are: latitude north and longitude west, without taking into account the agroecological characteristics of the sites of origin. Therefore, the variability identified among cultivated varieties of *C. pubescens* is not directly related to geographic location but may be influenced by homogeneous agroecological zones of provenance.

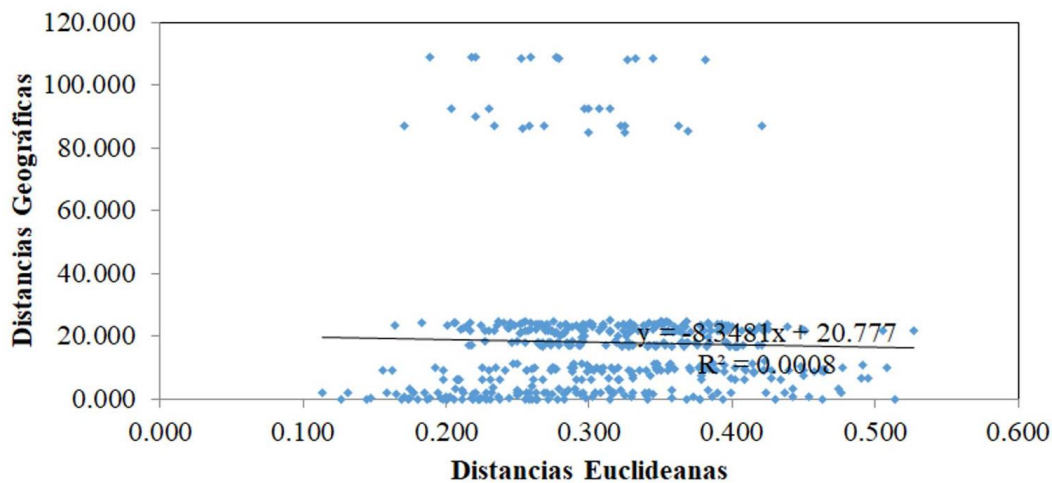


Figure 3. Mantel test, linear correlation between distribution of 31 cultivated varieties of *C. pubescens* based on Euclidean distances generated by morphological characteristics and geographical distribution according to the geographic coordinates of origin.

In species of the *Capsicum* genus, the variability within and between species is not necessarily related to the zones of origin (Bobadilla-Larios *et al.*, 2017). Therefore, the grouping of the varieties cultivated in the present study may be influenced by gene flow since the appearance of new forms, new genotypes or hybrids is mainly due to the exchange of germplasm among farmers (Pérez *et al.*, 2015).

Conclusions

The cultivated varieties of perón chili from four homogeneous agro-ecological zones of the state of Michoacán presented a wide morphological variability in leaf tissue, fruit and seed. The characteristics evaluated in the fruit stood out as the most important variables to explain the total variation identified, in addition, that these characteristics can be used as parameters of fruit quality of perón chili.

On the other hand, it was not possible to establish any relationship between the morphological variability and the sites of origin of the cultivated varieties of perón chili evaluated. However, the idea that the morphological characteristics are influenced by the agro-ecological zones of the site of origin and by the gene flow due to the exchange of germplasm among farmers is not discarded.

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