Morphological diversity of wild and semi-wild chili populations of Tabasco and the north of Chiapas States, Mexico
Diversidad morfológica de poblaciones silvestres y semi-silvestres de chile de los estados de Tabasco y norte de Chiapas, México

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Abstract. The research was conducted with the aim to identify the variability in situ of wild and semi-wild morphotypes of Capsicum spp. that were found growing in different places of Tabasco and the north of Chiapas States. Morphotypes included “Amashito” (five types), “Pico de paloma” (two types), “Garbanzo”, “Ojo de sapo”, “Ojo de cangrejo”, “Colmillo de lagarto” and “Corazón de pollo”. Such characterization is important because there is an extensive variability of forms cultivated in the country, resulting from a wide range of agroecological diversity as well as diverse forms, colours, flavors and sizes that constitute a valuable collection of genes and a valuable contribution to gastronomy. We measured qualitative traits like leaf colour, leaf shape, calyx margin, stem colour, stem shape, plant growth habit, branching habit, flower position, fruit colour and fruit shape. Quantitative variables such as plant height, stem diameter, number of flowers per axil, fruit length, fruit width and number of seeds per fruit were also registered. From the first Principal Component Analysis (PCA), nine variables were selected as the most discriminant. A second PCA was performed with these selected variables and a cluster analysis (CA) was also performed. The three first principal components explained 58.27% of the total variation. The cluster analysis ordered the population of chilies in contrasting groups. These were grouped by species, locality of identification and the superiority of any (or some) traits that were common in every group.

Keywords: Morphological characterization; Collection; Wild chilies; Plant genetic resources; Diversity.

Resumen. La investigación se realizó con el objetivo de identificar la variabilidad in situ de morfotipos de Capsicum spp. que se encontraban en estado silvestre y semi-silvestre en diferentes lugares de Tabasco y el norte de los estados de Chiapas. Se colectaron morfotipos de “Amashito” (cinco tipos), “Pico paloma” (dos tipos), “Garbanzo”, “Ojo de sapo”, “Ojo de cangrejo”, “Colmillo de lagarto” y “Corazón de pollo”. La caracterización es importante porque existe una gran variedad de formas cultivadas en el país, producto de una amplia gama de diversidad agroecológica, así como de diversas formas, colores, sabores y tamaños que constituyen una valiosa colección de genes y una valiosa contribución a la gastronomía. Se midieron los caracteres cualitativos como color de la hoja, forma de la hoja, margen del cáliz, color del tallo, forma del tallo, hábito de crecimiento de la planta, hábito de ramificación, posición de la flor, color del fruto y forma del fruto. Las variables cuantitativas medidas fueron altura de planta, diámetro de tallo, número de flores por axila, longitud del fruto, ancho de fruto y número de semillas por fruto. Del primer análisis de componentes principales (ACP), se seleccionaron las nueve variables que resultaron significativas, con las cuales se realizó un segundo ACP, y un análisis de conglomerados (AC). Los primeros tres componentes principales del segundo (ACP) explicaron 58.27% de la variación total. El análisis de conglomerados (AC) ordenó las poblaciones de chile en grupos contrastantes; primero, las colectas se agruparon por especie, luego por localidad y finalmente por algunos caracteres que fueron comunes en los grupos.

Palabras clave: Caracterización morfológica; Chiles silvestres; Recursos fitogenéticos; Diversidad.

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INTRODUCTION

Mexico is considered the origin and domestication center of a number of cultivated plants, including species belonging to the genus *Capsicum* commonly known as chilies (Ortega, 1991; Eshbaugh, 1993). They were later introduced in Spain, and thereafter distributed to the rest of the world (Aguilar et al., 2006). The Mexican population has considered the *Capsicum* spp. as an important food in their diet. There are more than 100 morphotypes of wild and cultivated chili, which are distributed in the Mexican territory. They are highly consumed and requested in the market of urban and rural communities (Castellón-Martínez et al., 2014). The five domesticated species are *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* Jacq., *Capsicum pubescens* Ruiz & Pavon and *Capsicum baccatum* L. (Morán et al., 2004; Milla, 2006). In terms of the area sown and the high demand worldwide of these five species, *C. annuum* is the most important crop. This species presents the largest variation regarding shape, size, color of the fruit, flavor and pungency for the elaboration of diverse typical dishes (e.i., the different types of chili) (Hernández-Verdugo et al., 1998; Martínez-Sánchez et al., 2010). Many studies of morphological characterization of these types of chili have been reported (Latournerie et al., 2002; Martinez-Sánchez et al., 2010; Salinas et al., 2010; Moreno-Pérez et al., 2011; Nsabiyera et al., 2013; Occhiuto et al., 2014; Ramírez-Meraz et al., 2015; Toledo-Aguilar et al., 2016). In accordance with Bosland (1996), González & Pita (2001) and Bosland & Votava (2012), *C. annuum* is the species with the largest morphologic, genetic and plant architecture variability. Because of this, Gunn (2004) established that the *annuum* species has been the basis to generate the highest number of improved varieties of chili, in comparison to other species of the same genus. As a result, *Capsicum* is very important in the southeast of Mexico, specifically in the states of Tabasco and the north of Chiapas. The objective of this work was to identify the genotype that establish morphological relationships among wild and semi-wild chili variants collected during exploration trips across different localities from both states.

MATERIALS AND METHODS

The study area was established in the states of Tabasco and north of Chiapas, located between 17° 15' 00" - 18° 38' 45" N and 90° 58' 08" - 94° 07' 00" W and (INEGI 2011). The continental surface area of Tabasco is 24738 km²; 95% of it has warm, humid climate while 4.5% has warm, sub-humid climate. There are heavy rains in summer, and the average annual temperature is 27 °C (Ruiz-Alvarez et al., 2012). Chiapas State has 7331 km² of continental surface area (Hernández

| Subregión (Vicente Guerrero) | Municipality | Average annual precipitation (mm) | Soil type of wetland with respect to Tabasco states and north of Chiapas | Characteristic of soil and use |
|-----------------------------|--------------|----------------------------------|------------------------------------------------------------|---------------------------------|
| Sierra (Vicente Guerrero)   | Teapa        | 3711                             | Lacustrine 0.37% Marsh 5.82% Riparian 0.74% Agriculture 12.25% | Gleysol and histosol inland lagoons; use livestock mainly agricultural and forest to a lesser extent. |
| Sierra (Ejido Cerro Blanco) | Tacotalpa    | 4014                             | Lacustrine 0.37% Marsh 5.82% Riparian 0.74% Agriculture 12.25% | Gleysol and histosol inland lagoons; use livestock mainly agricultural and forest to a lesser extent. |
| Chontalpa (Miahuatlan)      | Cárdenas     | 1225                             | Coastal 7.70% Lacustrine 0.40% Marsh 12.38%                | Flooded and fertile soil types histosol and gleysol, solonchack e histosol influence marina; agricultural use, forest to a lesser degree. |
| Pantanos y Ríos (Ejido Corralillo) | Macuspana | 3186                             | Lacustrine 3.36% Marsh 4.480% Riparian 1.66/                  | Soils suitable for agriculture as well as grassland |
| Llanuras aluviales del norte (Chiapas) | Reforma | 2000 a 3000                     | Litosol 4.38% Regosol 0.23% Cambisol 14.51% Gleysol 15.13% | Cultivated grassland and temporary agriculture |
| Llanuras aluviales del norte (Chiapas) | Macayo   | 2000 a 3000                     | Cambisol 14.51% Gleysol 15.13%                             | Land of crops |

Table 1. Main features of surface hydrology, soil and land use in the municipalities of Tabasco state and northern Chiapas.

Tabla 1. Principales características de hidrología superficial, edáficas y uso de suelo en los municipios del estado de Tabasco y norte de Chiapas.
54% of its territory has warm, humid climate, 40% is warm, sub-humid; 3% is temperate humid, and 3% has temperate sub-humid climate. Some soil features of both study localities are shown in Table 1.

Ten fruits and ten flowers were measured per plant on a total of 134 plants, surveyed at the field. Measurements were made from November 2015 and February 2016 from the localities of Vicente Guerrero (VG), Ejido Cerro Blanco (ECB), and Ranchería El Porvenir (RPV).

### Table 2. Sites and codes of the morphotypes collected during exploration of the species Capsicum spp.

| ENTRADA | TIPO                  | Descripción         |
|---------|-----------------------|---------------------|
| VICENTE GUERRERO |                        |                     |
| 1       | Amashito              | VGAM                |
| 2       | Amashito Redondo      | VGAR                |
| ECB |                        |                     |
| 3       | Amashito              | ECBA                |
| 4       | Pico de Paloma        | ECBPP               |
| 5       | Ojo de sapo           | ECBO                |
| RANCHERÍA EL PORVENIR |                  |                     |
| 6       | Corazón de Pollo      | RPVCP               |
| 7       | Pico de Poloma        | RPVPP               |
| MIAHUATLÁN |                        |                     |
| 8       | Amashito Morado       | MIAM                |
| 9       | Amashito Redondo      | MIAR                |
| 10      | Amashito Blanco       | MIAB                |
| 11      | Pico de Paloma        | MIIPP               |
| 12      | Ojo de Cangrejo       | MIOP                |
| REFORMA |                        |                     |
| 13      | Pico de Paloma        | REPP                |
| 14      | Amashito              | REA                 |
| 15      | Amashito Bolita       | REABO               |
| MACAYO |                        |                     |
| 16      | Amashito Alargado     | MAAL                |
| 17      | Pico de Paloma        | MAPP                |
| 18      | Amashito Grande       | MAAG                |
| 19      | Amashito              | MAA                 |
| 20      | Garbanzo              | MAG                 |
| PORVENIR |                        |                     |
| 21      | Garbanzo Blanco       | PVGB                |
| 22      | Colmillo de Lagarto   | PVCL                |
| 23      | Corazón de Pollo      | PVCP                |
| 24      | Amashito              | PVA                 |
| 25      | Pico de Paloma        | PVPP                |
| 26      | Pico de Paloma Delgado| PVPPD               |
| CORRALILLO |                        |                     |
| 27      | Pico de Paloma        | CMPP                |
| 28      | Pico de Paloma Blanco | CMPPB               |
| 29      | Colmillo de Lagarto   | CMCL                |

Descriptors and scales of measurement according to IPGRI (1995).
(ECB), Miahuatlán (MI), El Porvenir (PV), Ranchería El Porvenir (RPV) and Corralillo Macupana (CM) in Tabasco State. Sampling was also made on the localities of Reforma (RE) and Macayo (MA) in the north of Chiapas State (see Table 2). For each collection, 16 variables were measured using the Morphologic Descriptors Manual for Capsicum (IPGRI, 1995) shown in Table 3.

**Statistical analysis.** With the obtained data a Principal Component Analysis (PCA) and UPGMA (Unweighted pair Group Method with Aritmetic Mean) cluster analysis were effected standardizing the information to \( \mu=0 \) and \( \sigma^2=1 \), in such a way that the measured variables contribute more proportionally to the similarity estimation (Lévy & Varela, 2003). All the analysis were realized with SAS (Statistical Analysis System V9.0 2004). From the results obtained of this analysis, nine variables were selected according to Pla (1986). Five of them were qualitative (leaf colour LC, leaf shape LS, calyx margin CM, stem shape SS, fruit shape FS) and four were quantitative (fruit length FL, number of seeds per fruit NSF, plant height PH, stem diameter SD). With those nine variables, another PCA was performed standardizing the information to \( \mu=0 \) and \( \sigma^2=1 \). The significance of eigenvalues and eigenvectors obtained with the second PCA were determined following the indications of Kaiser (1960).

### RESULTS

The Principal Component Analysis (PCA) (Table 4) showed that the total variance explained by the first three principal components (PC1, PC2 and PC3) was 58.27%; in accordance with Kaiser (1960), the eigenvalues of these components resulted significant. The principal component 1 (PC1), explained 22.5% of the total variance, and showed that the eigenvectors of the fruit shape and fruit length variables, as well as number of seeds per fruit and stem diameter resulted positive, significant results (and with greater weight); while the stem shape resulted significant but, with a negative sign.

The PC2 with a eigenvalue of 1.8654 and 20.73% of the variance explained, was only present high values for the leaf colour, fruit shape, fruit length and stem shape traits; and for values with a negative, while for FS and SS the significance resulted with a positive sign. The PC3 showed an eigenvalue of 1.3539 and contributed with 15.04% to the explanation of the total morphological variation. This principal component presenting relevant values related to the variables: leaf shape (negative sign), calyx margin and plant height; both positive sign.

Figure 1 shows the collections distribution according to the first two principal components (PC1 and PC2). Note that in the quadrant I, the highest values of plant height, stem diam-

| PC1     | PC2      | PC3     |
|---------|----------|---------|
| Eigenvalues | 2.0245 * | 1.8654 * | 1.3539 * |
| Proportion of variance | 0.2250 | 0.20732073 | 0.1504 |
| Cumulative variance (%) | 22.50 | 43.22 | 58.27 |
| Traits | Eigenvectors |         |
| Leaf Colour | 0.1361 | -0.5456* | 0.0887 |
| Leaf shape | -0.0582 | 0.1352 | -0.4813* |
| Calyx margin | -0.1689 | -0.1961 | 0.6016* |
| Fruit shape | 0.3491* | 0.3924* | -0.0623 |
| Fruit length (cm) | 0.3441* | -0.4525* | -0.1360 |
| Number of seeds per fruit (number) | 0.4732* | 0.1662 | -0.1247 |
| Stem shape | -0.3340* | 0.4030* | 0.0753 |
| Plant height (cm) | 0.2194 | 0.2932 | 0.5755* |
| Stem diameter (cm) | 0.5707* | 0.1040 | 0.1541 |

* Significative values (Kaiser, 1960).
eter and number of seeds per fruit were grouped, along with fruit shape. In the quadrant II we distinctively grouped the variants “Pico de paloma” (PP), “Corazón de pollo” (CP), and two types of “Amashito Blanco” (AB) and “Amashito Redondo” (AR); the variables with the highest incidence in this group were: leaf shape, calyx margin and fruit shape. The majority of the Amashito types were placed in quadrant III, the variables involved to group them here were: fruit length, calyx margin, stems shape and leaf shape. These traits make easy to identify the “Amashito” types from any other of types of chili collected.

The collections that presented lower average frequency (data not showed) in terms of leaf colour, calyx margin and fruit length were grouped in quadrant IV; for these three variables the “Amashito” and “Garbanzo” can be observed. Whereas for plant height and number of seeds per fruit. “Ojo de sapo” (OS) and “Pico de paloma” (PP) can be observed (quadrant IV). Figure 2 shows the distribution of the collections according to the components 1 and 3 (PC1 and PC3). Note that there is certain similarity in the distribution of collections as observed in Figure 1. However, there was a change in the distribution and placement of some accessions. For instance, the accessions “Ojo de sapo” ECB-OS collected at Ejido Cerro Blanco (ECB). The G1 collections correspond to higher values than average in terms of leaf colour, calyx margin and stem diameter. The group 2 (G2) at a distance of 0.63, includes six collections: two of “Amashito grande” (AG) and amashito spotted (AM) and four of “Pico de paloma”; the variables that presented a higher value than the average in this group (G2) were: leaf colour, leaf shape, calyx margin, plant height and stem diameter. The group 3 (G3). They joined at a distance of 0.68; the variables that exceeded the general average were: leaf colour (LC), leaf shape (LS), calyx margin (CM) and stem diameter (SD). In group 4 (G4) two accessions of “Corazon de pollo” (CP) and one of “Pico de paloma” (PP) joined at a distance of 0.59; six variables defined this group (G4): leaf colour (LC), leaf shape (LS), calyx margin (CM), number of seeds per fruit (NSP), plant height (PH) and stem diameter (SD). The group 5 was formed with the highest number of accessions (7), this group was characterized by clustering two types of variants: Amashito (A) and Garbanzo (G); the variables that differentiated this group from the rest were: leaf colour (LC), leaf shape (LS), calyx margin (CM) and stem diameter (SD). The groups from 6 to 10 (except for group 9 that was formed by two accessions were clustered in just one collection, and are characterized by the variables: plant height (PH), stem diameter (SD), stems shape (SS) and number of seeds per fruit (NSP).
When the variables that contributed little or nothing to the first and second principal components stem colour (SC), growth habit plant (GHP), branching habit (BH), flower position (FP), fruit colour (FC), and fruit shape (FS) and number of flowers per axil (NFA). The Principal Component Analysis (PCA) explanation improved; however, it did not reach the 80% suggested by Pla (1986) as a preset limit for the PCA to better explain the variability of the germplasm evaluated. A possible explanation about why only 58.7% of the variation was explained by the first three principal components in our research can be that the morphotypes collected, some of them could be natural crossbreeds in the localities of collection the different plants could have grown close by or even, studies have been carried out new forms and found, new variants, the intra or interspecific breeding Pérez-Castañeda et al. (2015). Too, in this regard, Onus & Pickersgill (2004) mention that there is a unilateral incompatibility among some Capsicum species, meaning that there is a one direction crossbreeding but not in the opposite direction, as it has been observed with the “Pico de paloma” (Azurdia, 2014). Working with Pico de paloma Capsicum frutescens, De la Cruz et al. (2017) reported that the first three principal components explained 37.43% of its variability. On the other hand, working with guajillo chili and found that 58.0% of the variation was explained by the first three principal components, Barbosa et al. (2010) worked with four chili fruit traits and found that...
firsts two components explained 94.36% from total variation. While Pardey et al. (2006) studied Capsicum and found that the first four principal components explained 73.0% of the total variation; Martínez-Sánchez et al. (2010) studied chili the explained an 85% of the variation total; Toledo-Aguilar et al. (2016) in chili poblano with four components only explained 56% of the total variation. However, the validity of our study is based on the proposals by Trejos (2007), which indicates that if the data is standardized, then all the principal components associated to eigenvalues equal or greater than 1.0, should be taken into consideration to explain the variation, and that was done in our research. Then, when using the principal components, the species studied have to be taken into consideration, to define the amount of principal components and better explain the variation of the measured traits in collections or the morphotypes evaluated. The collections distribution based on the principal component analysis agrees with the reporting by Latournerie et al. (2002), who found that the measured variable of Capsicum spp. that mostly contributed in each principal component was the leaf shape (LS), just as what was found in our research. As well, Barbosa et al. (2010); Villota et al. (2012) found that the fruit length (FL) in chili were the most important traits that explained the morphological variation of their collections evaluated, similar results was obtained in our research. The collections grouped in Figure 3 correspond to the type that these correspond as well as the variables that are similar among them. Similar results were reported by Hernández-Verdugo et al. (2006), Castañón-Najera et al. (2008), Moreno-Pérez et al. (2011) and Hernández-Verdugo et al. (2012); who indicated that the wild species of their collections were grouped due to stem diameter (SD), plant height (PH), fruit weight (FW) and number of seeds per fruit (NSF) variables, similar to our research. Martínez-Sánchez et al. (2010) performed greenhouse chili collections and found that the main variables used to group them were: plant height (PH) and fruit length (FL), with higher averages of each variable than those in our work.

CONCLUSIONS

Of the 29 collections of chili collected in the study regions one of them is a new variant of Amashito, while the variants locality known as “Ojo de sapo” (OS) and Colmillo de la lagarto (CL) were found for the first time in the region of Tabasco. In the north of Chiapas, only two variables of Amashito (normal the morphological characteristics of this type of variant are ripe fruits lightly oval red-orange colour and bolita is a ripe fruit of a red colour of a very round shape) were collected. The principal component analysis and cluster analysis showed that the grouping tendency of the collections was according to the type of species, and secondarily according to the collection site. Although we only considered nine morphological traits, the percentage of the total variation explained by the three principal component selected to nine variables, five qualitative (leaf colour LC, leaf shape LS, calyx margin CM, stem shape SS, fruit shape FS) and four quantitative (fruit length FL, number of seeds per fruit NSF, plant height PH, stem diameter SD). One of the reasons of great importance for the conservation of wild and semi-wild plant genetic resources is because they have an agglomeration of genes.

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