

## Research Article

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# Morphological diversity of ancho chile pepper landraces from Mexico

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### Abstract

Ancho (width) chile peppers have economic, social, culinary and cultural importance in Mexico and worldwide. This chile type considers divergent subtypes that altogether have not been analysed and therefore their morphological diversity has not been systematically described. The objectives were to describe the morphological diversity of ancho pepper landraces from Mexico, to identify groups of similarity and to define the traits with the higher contribution to the total variation. Eighty-six landraces of ancho chile peppers (red, ‘mulatos’, ‘miahuatecos’, ‘cristalinos’ and ‘huacle’), collected in six states of Mexico, and two commercial controls were evaluated in two localities, in a simple randomized complete block experimental design. We recorded 76 morphological traits. Statistical analysis included a combined ANOVA, Pearson’s correlation coefficient, discriminant analysis, principal components and clusters. The morphological diversity in ancho chile peppers was mainly made up of fruit width, fruit wall thickness, stem diameter, corolla length, seed weight per fruit, plant height, stem length and pubescence. We defined four groups, which made it possible to differentiate ancho chile peppers of Puebla and the huacle chile pepper of Oaxaca from populations collected in the north and ‘Bajío’ (midland) parts of Mexico. Ancho chile peppers of Mexico showed wide morphological differences according to the type of chile pepper and seed collection regions. The traits that contributed the greatest morphological diversity were fruit width, fruit wall thickness, stem diameter, corolla length, seed weight per fruit, plant height, stem length and pubescence.

### Introduction

Plant genetic resources and their diversity constitute the basis for food production and represent an opportunity in the face of the impacts of climate change and adverse factors such as drought, heat, frost, floods, pests and diseases (FAO, 2011, 2018); Furthermore, they are indispensable for food security, sustainable development, resilience and the supply of vital ecosystem services (FAO, 2019).

Chile peppers are part of the native plant genetic resources of Mexico, which are the second economically important crop in this country, after maize (SIAP-SADER, 2019). During the process of domestication and continuous selection in the *Capsicum* genus, fruit characteristics such as size, shape, mass, colour, appearance, flavour and spiciness have been modified (Kraft *et al.*, 2014; Pickersgill, 2016; Barchenger *et al.*, 2019). Domestication generated a large number of morphological variants and diversification within and between species (Massot *et al.*, 2016; Carvalho *et al.*, 2017; Velázquez-Ventura *et al.*, 2018). In addition to domestication, Taitano *et al.* (2019) indicate that the genetic diversity in landraces of chile pepper *C. annuum* L. and *C. frutescens* are related to the techniques of its cultivation and fresh or dry use.

*Capsicum annuum* L., whose centre of domestication and genetic diversity is Mexico (Kraft *et al.*, 2014; Pickersgill, 2016), has more than 50 cultivated morphotypes (*C. annuum* var. *annuum*) and their wild relatives (*C. annuum* var. *glabriusculum*) in this country (Pickersgill, 2016; Martínez *et al.*, 2017). In particular, the ancho chile peppers are cultivated on 31 thousand hectares, whose production corresponds to 15% of the national green chile



pepper production, and 24.5% of the national dried chile pepper production (SIAP-SADER, 2019). In general, these chile peppers have economic and culinary importance in Mexico, since traditional dishes are prepared with them, such as 'chiles en nogada', 'mole poblano', 'mole negro' of Oaxaca, 'adobos', sauces, among others (Aguilar *et al.*, 2010; Vera-Guzmán *et al.*, 2017). It makes necessary its assessment and preservation.

The ancho morphotypes considers several subtypes (Fig. 1) called mulatos (brown at ripen), anchos (red), cristalinos (orange-red), huacles (dark brown), miahuatecos (brown), Sweet Bell of Yucatan (red) and pasilla (brown) of Oaxaca. In the states of Guanajuato, Durango, Zacatecas, Querétaro, Aguascalientes, San Luis Potosí (SLP) and Puebla mulatos can be found as landraces. In Zacatecas, Durango, SLP and to a lesser extent in Puebla the ancho subtype can be found. In Guanajuato and Durango, cristalinos are grown. The huacles and pasilla are exclusive to the state of Oaxaca. The miahuatecos are originally from Miahuatlán, Puebla. The sweet bell peppers are located in the states of Yucatán, Campeche and Tabasco (Aguilar *et al.*, 2010).

The several morphological diversity studies in cultivated and wild chile peppers from Mexico have been developed locally or regionally (Ballina-Gómez *et al.*, 2013). Cultivated and wild chile peppers from Tabasco (Narez-Jiménez *et al.*, 2014). Cultivated and wild chile peppers from Oaxaca (Castellón *et al.*, 2014). Poblano chile pepper landraces (Toledo-Aguilar *et al.*, 2016). Wild chile peppers from Tabasco (Velázquez-Ventura *et al.*, 2018). Piquín chile pepper from Querétaro and Guanajuato (Ramírez *et al.*, 2018). Guajillo chile peppers from Zacatecas, Durango and Puebla (Moreno-Ramírez *et al.*, 2019). Chile pepper landraces from Yucatan Peninsula (Castillo-Aguilar *et al.*, 2021). However, a study that encompasses the existing subtypes of chile ancho peppers of Mexico to determine their morphological diversity has not been carried out; consequently, the traits with the greatest contribution to the total variation in these chile peppers are unknown. Therefore, the objectives of the present research were (i) to characterize the morphological diversity of ancho chile peppers landraces of Mexico using morphological descriptors, (ii) to visualize the degree of diversity, (iii) to identify morphologically similar groups, (iv) to relate the diversity with the seed collection region and (v) to define the traits that provide more diversity to these chile pepper populations.

## Materials and methods

### Plant material

A total of 86 landraces were collected in six states of Mexico. It included the ancho, mulato, miahuateco, cristalino and huacle

subtypes (Fig. 1). We used the commercial hybrids Capulín and Abedul (Harris Moran Seed Company<sup>®</sup>) as controls (Table 1).

### Ecological information

The ecological information of Table 1 was integrated by climate and soil type. Climate information for the seed collection plot was obtained from the official website of the National Commission for the Knowledge and Use of Biodiversity (CONABIO, for its acronym in Spanish) (CONABIO, 2023). From this page, files in vector format were downloaded for the averages of total annual precipitation, and minimum, average and maximum temperatures for the period 1910–2009. For soil type, the vector layer of the National Soil Data Set Series II of the National Institute of Statistics and Geography (INEGI, for its acronym in Spanish) (INEGI, 2023) was downloaded. Seed collection plot in each locality was geographically located. The map with the plots was elaborated using the QGIS 3.22.14 program (QGIS Development Team, 2023). The overlapping of the vector layers of climate and soils made it possible to identify the values of the variables for each plot.

### Experimental design, localities of evaluation and experimental unit

The sowing was carried out in styrofoam trays of 200 cavities. The seedling production was carried out in a greenhouse. The seedlings were transplanted 66 and 69 days after sowing (das) in the fields of cooperating farmers. Treatments were evaluated in a randomized complete block experimental design, with two replications. The localities of evaluation were (a) Rancho Grande, Fresnillo, Zacatecas, located at 23° 27' N and 02° 57' W, with an altitude of 2024 m, phaeozem soil type, mean annual rainfall 400–500 mm, minimum annual temperature 2–4°C, mean annual temperature 16–18°C and maximum annual temperature 28–30°C, and (b) Santa Cruz, Nombre de Dios, Durango, located at 25° 50' N and 104° 10' W, with an altitude of 1840 m, kastañozem soil type, mean annual rainfall 400–500 mm, minimum annual temperature 2–4°C, mean annual temperature 16–18°C and maximum annual temperature 32–34°C. The experimental unit consisted of 52 plants, distributed in two rows with a length of 3.6 m and a separation of 0.9 m. The density was 80,246 plants per ha.

### Registration of morphological traits

A total of 76 morphological and agronomic traits were recorded, of which 67 correspond to the manual for *Capsicum* descriptors



**Figure 1.** Ancho chile peppers of México. From left to right: mulato (brown), ancho (red), cristalino (orange-red), miahuateco (brown) and huacle (dark-brown).

**Table 1.** Ancho chile peppers landraces of Mexico and the ecological information of their seed collection plot

State of collection	Type of chile pepper	Colour at ripen	ID	Number of populations	Soil type	Precipitation range (mm)	Min and max temperature (°C)	Altitude (m)
Guanajuato	Mulato	Brown	GM01-GM11	11	Ph, D	400–500	2 to 5 / 28 to 32	1859–2037
	Ancho	Red	GA01-GA04	4	Ph	400–500	2 to 5 / 28 to 32	1885–2017
Jalisco	Mulato	Brown	JM01	1	D	500–600	2 to 4 / 28 to 30	2147
	Ancho	Red	JA01	1	D	500–600	2 to 4 / 28 to 30	2147
	Cristalino	Orange-Red	JC01-JC03	3	D	500–600	2 to 4 / 28 to 30	2147
Zacatecas	Ancho	Red	ZA01-ZA12	12	Ph, R, Lp	300–600	–2 to 5 / 24 to 30	2042–2152
	Mulato	Brown	ZM01-ZM02	2	Cl, D	400–500	0 to 4 / 28 to 32	1900–2173
Durango	Ancho	Red	DA01-DA12	12	Ch, K, S	400–500	2 to 4 / 32 to 34	1879–1983
	Cristalino	Orange-Red	DC01-DC04	4	K, S	400–500	2 to 4 / 32 to 34	1885–1922
San Luis Potosí	Ancho	Red	SA01-SA08	8	Ch, Cl	300–400	4 to 5 / 30 to 32	1605–1642
	Mulato	Brown	SM01-SM02	2	Cl	300–400	4 to 5 / 30 to 32	1640
Puebla	Mulato	Brown	PM01-PM22	22	Ar, Cm, Lx, Ph	800–1000	2 to 4 / 24 to 28	2234–2463
	Miahuateco	Brown	PMi1-PMi3	3	Ch, Ac	600–1000	2 to 4 / 26 to 28	1814–1896
Oaxaca	Huacle	Dark brown	OH01	1	Ph	400–500	12 to 14 / 34 to 36	597

Soil type. Ph, phaeozem; D, durisol; R, regosol; Lp, leptosol; Cl, calcisol; Ch, chernozem; K, kastañozem; S, solonetz; Ar, arenosol; Cm, cambisol; Lx, lixosol; Ac, acrisol. The seed of hybrids Abedul and Capulín were not collected.

(IPGRI *et al.*, 1995). Additionally, the following traits were recorded: immature fruit colour (dark green, medium green and lemon green), number of branches after the first bifurcation, number of bifurcations, number of primary branches, number of petals and seed weight per fruit. Moreover, we estimated the indices (length/width) of cotyledonous leaf, mature leaf, plant and fresh fruit. Traits were recorded in 10 plants selected during plant maturity, inflorescence, fruiting and fruits of the second harvest (Supplementary Table S1).

### Statistical analysis

Using the SAS V9.4 program (SAS Institute, 2012), we carried out a combined analysis of variance through localities ( $P \leq 0.05$ ), Pearson's correlation coefficient between traits with statistical significance ( $P < 0.05$  and  $r \geq 0.7$  or  $r \geq -0.7$ ) and discriminant analysis with Stepdisc. These analyses allowed a reduction of traits with a greater contribution to the total variation. The resulting variables were used in the analysis of principal components and conglomerates, that was performed with R Statistical Software V4.2.0 (R Core Team, 2022) and the factoextra package (Kassambara and Mundt, 2022). Dendrogram was performed with Euclidean distances and Unweighted Pair Group Method with Arithmetic Mean method (UPGMA).

## Results

### Morphological diversity of ancho chile peppers landraces

We detected 10 invariable characteristics among all the populations and individuals analysed, that were not included in the analysis of variance. The traits were green cotyledonous leaf,

cylindrical stem, scarce pubescence in mature leaves, one flower per axil, pale blue anthers, white filament, null male sterility, absence of annular constriction of the calyx, absence of the neck at the base of the fruit and persistence of the pedicel in the fruit.

In the combined analysis of variance across localities, we identified statistically significant differences in 67% of the traits recorded between populations. The highest number of significant traits has relation to the fruits, while the flower traits have less variation. In general, there is little pubescence in the hypocotyl, leaves and stems, with the exception of the huacle chile pepper landrace of Oaxaca. Regarding flower traits, their arrangement on the plant can be erect, intermediate and sloping, with an open or bell-shaped corolla, with an exerted stigma and a mostly toothed calyx. Likewise, there is variation in the coloration of the fruit in immature, intermediate and mature stage, shape of the fruit, shape of the base and apex of the fruit, type of epidermis and transverse wrinkling. In addition, there is anthocyanin presence in the hypocotyl, stem, plant nodes, flowers, green fruits and calyx, mostly detected in collections from Puebla (Supplementary Table S2).

### Relationship between populations

With Pearson's correlation analysis ( $r \geq 0.7$ ) and the Stepdisc discriminant analysis model, 31 traits with the greatest contribution to morphological variation in ancho chile peppers morphotypes were obtained. Of the 31 variables 22 of them showed a greater association with the first five principal components (PC) that contain 61.8% of the total variation. PC1, with 30.6% of the total variation, was mainly composed of fruit width, fruit wall thickness, stem diameter, corolla length and seed weight per fruit. PC2, which contributed with 10.4%, was mainly influenced by stem

length, plant height and stem pubescence. PC3 contributed 8.0% of the total variation and was made up of traits of fruit coloration in immature, intermediate and mature stage. PC4, with 6.8% of the total variation, was made up of the cotyledonous leaf length-width index and filament length. PC5, with 6.0% of the total variation, was composed of fruit length/width index and days to fruiting (**Table 2**).

The dispersion of the populations, according to the first two principal components, shows that the mulato chile peppers of Puebla, miahuatecos and huacle (Groups 1 and 4) are separated from the rest of the populations of the north and Bajío parts of Mexico (**Fig. 2**). The mulato chile peppers from Puebla presented a lower fruit wall and plant stem thickness, shorter corolla length, related to smaller fruit size and lower seed weight per fruit. With regard to these traits, the opposite was observed in the mulato, ancho and cristalino chile peppers collected in northern states and Bajío (Group 2). On the other hand, and in relation to PC2, within the cristalino and ancho chile peppers there is variation in plant height, stem height and null pubescence in the stem. The mulato chile peppers from the north of Mexico (Group 3) showed less plant size and greater pubescence. The miahuateco chile peppers obtained higher plant and stem height than the mulatos of Puebla (Group 1). In the same sense, the population from Oaxaca presents wider chile peppers than the poblanos, but with thinner fruit wall, lower plant size and very pubescent stem.

The separation in terms of the coloration of the fruits in immature, intermediate and mature stage is observed in PC3 (not shown), which are the traits with the greatest influence on this PC.

The association between the ancho chile peppers of the present study is shown in **Fig. 3**. Four groups were delimited from the generated dendrogram. As with the principal component analysis, this analysis also shows a differentiation with the mulato chile peppers from Puebla (Group III).

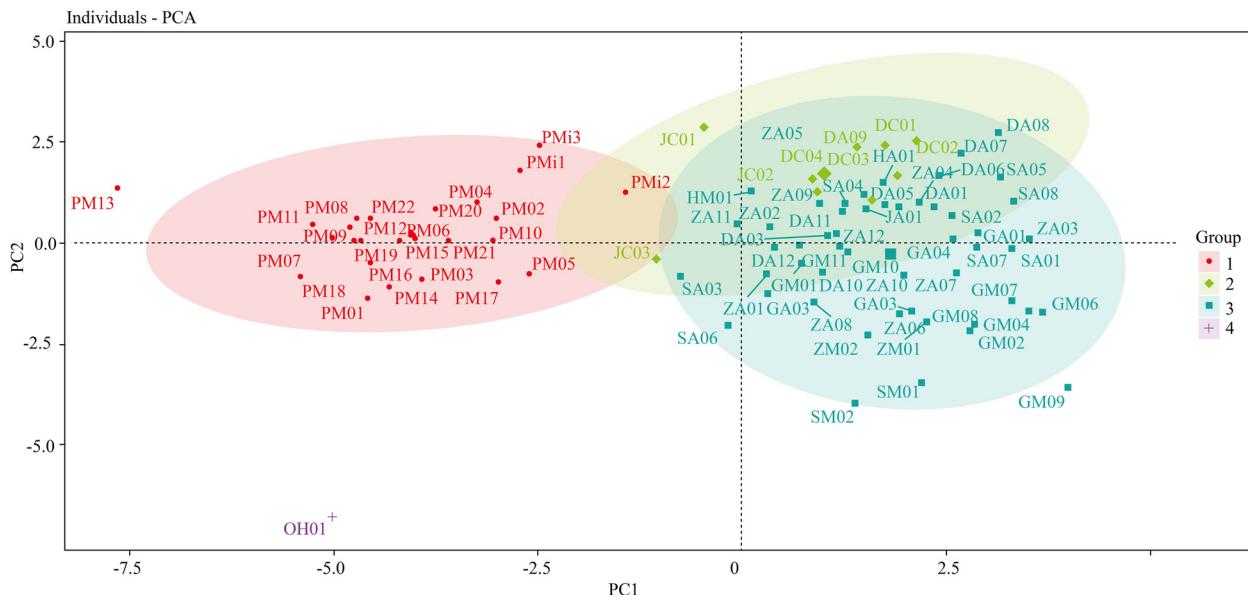
Group I was integrated by 53 populations of mulato and red ancho chile peppers from Guanajuato, Zacatecas, San Luis Potosí and Durango along with two commercial hybrids (Capulín and Abedul). The commercial hybrids showed morphological similarities with mulato chile peppers of Guanajuato. The characteristics of this group are fruits of 6.2 cm wide, 3.44 mm of fruit wall thickness, medium and dark green colour in immature and intermediate stage and red and brown in mature stage. Stem diameter of 9.9 mm, with scant pubescence, corolla length of 13.1 mm, filament length of 4.11 mm, dry seed weight per fruit of 1.86 g, plant and stem height of 74.0 and 26.8 cm and cotyledonous leaf length/width index of 4.51.

Group II, made up of nine populations of cristalino and ancho chile peppers from Durango, has fruits of 5.6 cm wide, 3.20 mm of fruit wall thickness, lemon green colour in immature stage, orange in intermediate stage and red at maturity. Stem diameter of 10.1 mm, scant pubescence, plant and stem height of 71.1 cm and 25.6 cm, corolla length of 13.0 mm, filament length of 4.05

**Table 2.** Eigenvectors of first five principal components (PC) of morphological diversity analysis of ancho chile peppers landraces of Mexico

Variable	PC1	PC2	PC3	PC4	PC5
Cotyledonous leaf length/width index	0.1700	0.1210	-0.0252	<b>-0.5864</b>	0.5302
Stem pubescence	-0.1559	<b>-0.5292</b>	0.0575	0.1158	-0.2959
Plant height	0.6700	<b>0.5554</b>	0.1246	0.0440	-0.1101
Stem length	-0.0017	<b>0.7495</b>	0.2883	0.1019	0.0118
Stem diameter	<b>0.7528</b>	0.2357	-0.0817	0.0383	-0.1358
Days to flowering	0.6870	-0.4981	-0.0774	0.2992	0.2157
Corolla length	<b>0.7294</b>	-0.0449	-0.2153	0.3321	0.2422
Filament length	0.5180	0.2837	-0.1454	<b>0.5588</b>	0.1693
Days to fruiting	0.6842	-0.4909	-0.1368	0.2126	<b>0.3250</b>
Immature fruit colour	-0.5046	0.0146	<b>0.6278</b>	0.4108	0.0704
Fruit colour at intermediate stage	0.1060	-0.0815	<b>-0.7621</b>	-0.1984	-0.2519
Fruit-bearing period	-0.6869	0.4699	0.0879	-0.2331	-0.3173
Fruit colour at mature stage	-0.5056	-0.0268	<b>0.5338</b>	0.3679	-0.1440
Fruit length	0.5910	0.5121	-0.0122	-0.0663	0.2712
Fruit width	<b>0.9023</b>	0.0111	0.2129	-0.0514	-0.1521
Fruit length/width index	-0.6253	0.4239	-0.2204	-0.0019	<b>0.3860</b>
Fruit pedicel length	0.6025	0.3067	0.0260	0.4241	-0.1134
Fruit wall thickness	<b>0.8887</b>	0.1651	0.1034	-0.0882	-0.0908
Fruit shape at pedicel attachment	0.5452	-0.1494	-0.0970	0.0802	-0.0975
Fruits number per plant	-0.6991	0.1799	-0.2733	0.0255	-0.2267
Seed diameter	0.6458	-0.0385	0.3533	-0.3196	0.2675
Seed weight per fruit	<b>0.7048</b>	-0.0351	0.2777	-0.2454	-0.2486

Bold numbers in the table indicate the largest contribution of the original variables on each principal component.

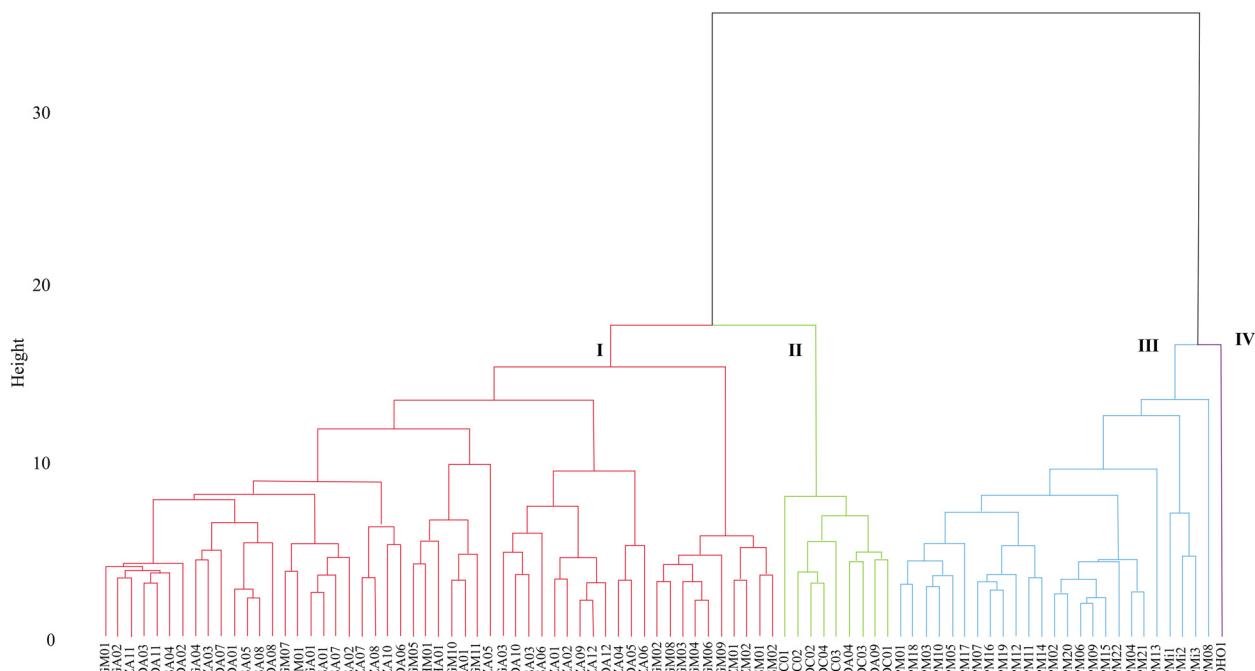


**Figure 2.** Dispersion of ancho chile pepper landraces of Mexico in the first two principal components.

mm, weight of dry seed per fruit of 1.68 g and cotyledonous leaf length/width index of 4.52.

Group III, made up of 25 populations of mulato and miahuateco chile peppers from Puebla, has fruits of 5.6 cm wide, 3.20 mm of fruit wall thickness, lemon-green colour in immature stage, orange in intermediate stage and red at maturity. Stem diameter of 10.1 mm, with little pubescence, plant and stem height of 71.1 cm and 25.6 cm, length of corolla of 13.0 mm, filament length of 4.05 mm, weight of dry seed per fruit of 1.68 g and length/width index of the cotyledonous leaf of 4.52.

The huacle chile pepper population has distinct morphological characteristics from the other three groups described and was located in group IV. This chile pepper has fruits 5.4 cm wide, with a very thin fruit wall (1.95 mm), very dark green colour in immature and intermediate stage and black at maturity. Stem diameter of 8.3 mm, with medium pubescence (the most pubescent in this study). Plant and stem height of 56.1 cm and 21.2 cm, corolla length of 12.2 mm, filament length of 3.56 mm, weight of dry seed per fruit of 1.59 g and length/width index of the cotyledonous leaf of 3.56.



**Figure 3.** Groups of ancho chile pepper landraces from Mexico generated with Euclidean distances and UPGMA grouping method.

### Geographical distribution of the landraces groups

The map of the geographic distribution of the groups is presented in Fig. 4. According to Table 1, the ecological conditions associated with the four groups are different.

Group I, comprising 53 landraces, covers the largest geographical area. Four types of soils predominate in this area: phaeozem in Guanajuato, calcisol in San Luis Potosí and regosol and leptosol in Zacatecas. Additionally, in Guanajuato, the annual variation in rainfall is 400 to 500 mm, minimum temperature 2 to 5, average 16 to 18 and maximum 28 to 32; in San Luis Potosí rainfall is 300 to 400 mm, minimum temperature 4 to 5, average 16 to 20 and maximum 30 to 32; in Zacatecas rainfall is 300 to 600 mm, minimum temperature -2 to 5, average 14 to 18 and maximum 24 to 32. In the Group II, comprising nine landraces, the predominant soils are kastañozem and solonetz. In this area, the annual variation in rainfall is 400 to 600 mm, minimum temperature 2 to 4, average 16 to 18 and maximum 28 to 34. In the Group III, comprising 25 landraces, the predominant soil is arenosol. In this area, the annual variation in rainfall is 800 to 1000 mm, minimum temperature 2 to 4, average 14 to 16 and maximum 24 to 28. Finally, in the group IV the soil type is phaeozem, the annual variation in rainfall is 400 to 500 mm, minimum temperature 12 to 14, average 16 to 18 and maximum 34 to 36.

### Discussion

#### The major morphological diversity in ancho chile pepper landraces

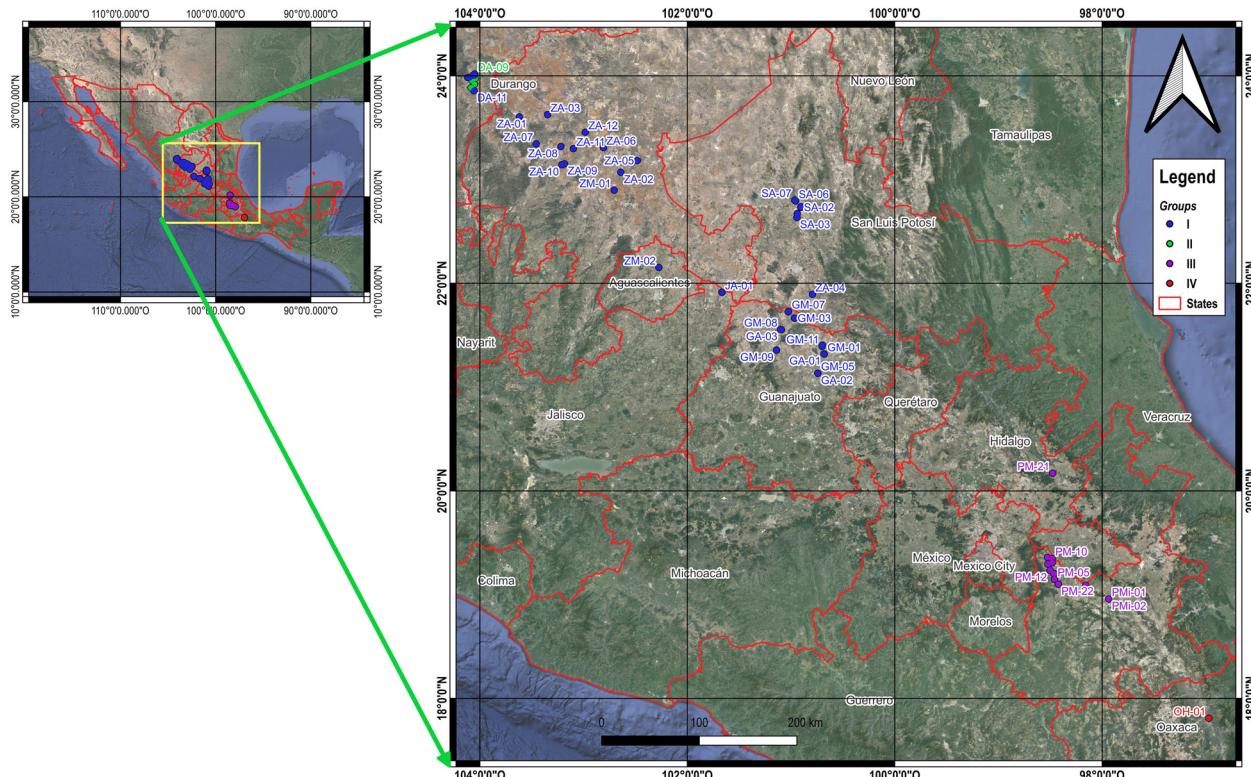
In chile peppers, the domestication and selection process have mainly modified fruit traits (Pickersgill, 2016). In ancho chile peppers, the conical shape of the fruit, width, colour and

pungency are important characteristics due to the uses of this fruit in food dishes. The variation in the coloration found in immature fruits (light green to dark green), intermediate (green and orange) and mature (shades of red, brown and black), is related to processes of mutation and selection (Guzman *et al.*, 2010; Li *et al.*, 2013). The brown or black coloration of the ripe fruit is the result of the presence of chlorophyll and carotenoids or only carotenoids in red fruits (Lippert *et al.*, 1966). The presence of anthocyanin in mulato chile pepper plants of Puebla (Toledo-Aguilar *et al.*, 2016) is due to the fact that these populations are sown at altitude above 2100 m and this characteristic is probably a mechanism to adapt to conditions of low temperatures or ultraviolet rays (da Ferreira *et al.*, 2012; Luo *et al.*, 2019).

The major variability in fruit traits has been reported in studies of domesticated chile peppers of the *C. annuum* L. species in Mexico (Castellón *et al.*, 2014; Toledo-Aguilar *et al.*, 2016; Moreno-Ramírez *et al.*, 2019). Another characteristic associated with the fruit is the persistence of the fruit with the pedicel, which does not occur in the wild populations of *C. annuum* var. *glabriusculum*, where the fruit is easily detached from the pedicel to facilitate its dispersal by birds (Pickersgill, 2016; Kumar *et al.*, 2018).

#### Differences in the morphological diversity among ancho chile pepper groups

Within ancho chile pepper landraces of Mexico, the width fruit trait is one of the characteristic with the greatest importance in PC1. For example, there are differences between chile peppers of Puebla and Oaxaca with those from the north and Bajío of Mexico. Chile peppers from the north and Bajío show greater fruit thickness and seed weight, which is desirable by producers to obtain higher yields. In



**Figure 4.** Map of the landraces seed collection plots and their groups.

regard to the plant, the chile pepper of the north and Bajío have the greater diameter of the stem, a characteristic related to support greater weight of the fruits and avoid lodging or tearing of the plants. The flower traits had the lowest variation in this study. The empirical selection that farmers have been carried out on ancho chile peppers in their niche has given rise to the morphological variability found in landraces (Nicolaï *et al.*, 2013), process that have facilitated their adaptation to specific environments.

### ***Ecological influence on morphological diversity among ancho chile pepper groups***

The genetic diversity of ancho chile in Mexico has been generated under different soil and climate conditions. The most restrictive soils (calcisol, regosol and leptosol) are in the northern part of the country, a region associated to Group I. On the other hand, Phaeozems soils (associated to Group III and IV), Cambisols and Arenosols (associated to Group III) and Kastañozems (associated to Group II) are very fertile and suitable for cultivation. Research on how soil influences the generation of genetic diversity has been focused only on microbial activity associated with plants (Bashan and Holguin, 1995; Dalmastri *et al.*, 1999; Carelli *et al.*, 2000; Manoharan *et al.*, 2017); so it is necessary to generate research on the influence of soil on plant genetic diversity.

In regard to climate, this also presents differences in the regions where the genetic diversity of ancho chile has been generated. With respect to rainfall, the region with the least amount is associated with Group I and the highest amount with Group III. The lowest temperature has been recorded in the region associated with Group I and the highest in the region associated with Group IV. When comparing some morphological characteristics of landraces between both groups, we see that in group I there is a greater expression in plant height, fruit width and thickness and seed weight. The way in which climate influences the generation of genetic diversity is through the direct effect on physiological processes, that condition plant growth and development (Shao and Halpin, 1995), manifested by variation in phenotypic plasticity (Akman *et al.*, 2020). Nooryazdan *et al.* (2010) and Moles *et al.* (2014) have demonstrated the effect of climate on plant morphology. In this regard, Romero-Higareda *et al.* (2023) found a positive correlation between climate and vegetative variables, fruit shape, larger reproductive traits, earlier flowering, specific leaf area, fruit shape and smaller plants of *C. annuum* var. *glabriuscum*. It is important to emphasize that not only ecological conditions influence the generation of morphological diversity; ethnobotany also influences plant morphological diversity.

### ***Ethnobotany associated with the genetic diversity of ancho chile pepper***

Chiles are fundamental in Mexican food, where their use is widely diversified (Aguilar-Meléndez *et al.*, 2018; Lascurain-Rangel *et al.*, 2022). They have been used as a condiment, medicine and in ceremonies (Janick, 2013). The cultural and traditional practices of farmers have a direct impact on the current gene pool in Mesoamerican crops, including chiles (Güemes and Aguilar-Meléndez, 2018). In addition, a strong cultural connection has been established with chile, which is even considered a sacred plant for communicating with the gods in religious ceremonies of native peoples. Indigenous and rural farmers are the custodians of that biocultural heritage, for continuing to cultivate traditional

morphotypes and promoting genetic variability (Aguilar-Meléndez *et al.*, 2018).

Ancho chile peppers grown in the North (Zacatecas and Durango) and Bajío of Mexico (Guanajuato and Jalisco) (Groups I and II) have a commercial connotation, which has probably led to the selection of genotypes with larger and heavier fruits. Dried chiles are marketed mainly in Mexico City. Chile mulato is grown in Puebla State in a traditional production system, characterized by less than 1 ha under cultivation, inadequate use of technology and, consequently, low fruit yields (Herrera, 2016; Lozano *et al.*, 2021). The green fruit is used in the food dish ‘chiles en nogada’ and the ripe and dry fruit in the ‘mole poblano’, a food dish widely used in social and religious celebrations in the region (Pérez *et al.*, 2017). The seeds and knowledge of the traditional cultivation system are inherited from generation to generation (Rodríguez *et al.*, 2007).

The miahuateco chile is cultivated in the Tecamachalco-Tehuacán region of Puebla State. Traditional practices are also carried out on this type of chile. Its main use is in the dishes ‘mole’ and ‘chile relleno’ (Aguilar *et al.*, 2010). Huacle chile is grown in the canyon region of Oaxaca, located in the Tehuacán-Cuicatlán Biosphere Reserve. This chile is cultivated by ‘Chinantecas’ and ‘Cuicatecas’ indigenous people, also in a traditional system. Its selection and the environment in which it is grown have given it unique characteristics in flavour, colour, aroma and fruit shape. The fruit is the main ingredient of Oaxaca ‘mole negro’, a regional food of Oaxaca. It is also offered in religious ceremonies, such as Day of the Dead and social festivities (García-Gaytán *et al.*, 2017).

As conclusions, the morphological diversity present in the populations of ancho chile peppers landraces of Mexico is expressed mostly by characteristics of fruit width, fruit wall thickness, stem diameter, corolla length, seed weight per fruit, plant height, stem length and stem pubescence. These characteristics allowed the separation of the red ancho chile peppers of the Bajío and north of Mexico from the peppers of Puebla and Oaxaca, according to divergent patterns. The morphological diversity in landraces of ancho chile peppers of Mexico suggests that human selection exerted on these populations is related to the different ecological niches where they have been cultivated, since they show morphological differences according to the type of chile pepper and its seed collection regions.

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### **References**

- Aguilar-Meléndez A, Vásquez-Dávila MA, Katz E and Hernández CMR (2018) *Los chiles que le dan sabor al mundo: contribuciones multidisciplinarias*. Xalapa, México: Universidad Veracruzana, ISBN: 978-607-502-699-2, <https://doi.org/10.25009/uv.2185.1087>.
- Aguilar RVH, Corona TT, López LP, Latournerie ML, Ramírez MM, Villalón MH and Aguilar CJA (2010) Los chiles de México y su distribución. SINAREFI. Colegio de Postgraduados, INIFAP, IT-Conkal, UANL, UAN Texcoco, Estado de México, México. ISBN: 978-607-7533-68-9.
- Akman M, Carlson JE and Latimer AM (2020) Climate explains population divergence in drought-induced plasticity of functional traits and gene expression in South African *Protea*. *Molecular Ecology* **30**, 255–273.

- Ballina-Gómez H, Latournerie-Moreno L, Ruíz-Sánchez E, Pérez-Gutiérrez A and Rosado-Lugo G (2013) Morphological characterization of *Capsicum annuum* L. accessions from southern Mexico and their response to the *Bemisia tabaci*-Begomovirus complex. *Chilean Journal of Agricultural Research* 73, 329–338.
- Barchenger DW, Naresh P and Kumar S (2019) Genetic resources of capsicum. In Ramchiary N and Kole C (eds), *The Capsicum Genome*. Switzerland: Springer Nature Switzerland AG, pp. 9–23, [https://doi.org/10.1007/978-3-319-97217-6\\_2](https://doi.org/10.1007/978-3-319-97217-6_2).
- Bashan Y and Holguin G (1995) Inter-root movement of Azospirillum brasilense and subsequent root colonization of crop and weed seedlings growing in soil. *Microbial Ecology* 29, 269–281.
- Carelli M, Gnocchi S, Fancelli S, Mengoni A, Paffetti D, Scotti C and Bazzicalupo M (2000) Genetic diversity and dynamics of *Sinorhizobium meliloti* populations nodulating different alfalfa cultivars in Italian soils. *Applied and Environmental Microbiology*, 66, 4785–4789. <https://doi.org/10.1128/AEM.66.11.4785-4789.2000>
- Carvalho SIC, Bianchett LB, Ragassi CF, Ribeiro CSC, Reifschneider FJB, Buso GSC and Faleiro FG (2017) Genetic variability of a Brazilian *Capsicum frutescens* germplasm collection using morphological characteristics and SSR markers. *Genetics and Molecular Research* 16, gmr16039689.
- Castellón ME, Carrillo-Rodríguez JC, Chávez-Servia JL and Vera-Guzmán AM (2014) Variación fenotípica de morfotipos de chile (*Capsicum annuum* L.) nativo de Oaxaca, México. *Revista Internacional de Botánica Experimental* 83, 225–236.
- Castillo-Aguilar CC, López CLC, Pacheco N, Cuevas-Bernardino JC, Garruña R and Andueza-Noh RH (2021) Phenotypic diversity and capsaicinoid content of chilli pepper landraces (*Capsicum* spp.) from the Yucatan Peninsula. *Plant Genetic Resources: Characterization and Utilization* 19, 159–166.
- CONABIO (2023) Geoportal del Sistema Nacional de Información sobre Biodiversidad. Consultation date 07/06/2023. Available at [www.conabio.gob.mx/informacion/gis/](http://www.conabio.gob.mx/informacion/gis/).
- da Ferreira SP, Paulo L, Barbaflava A, Elisei F, Quina FH and Macanita AL (2012) Photoprotection and the photophysics of acylated anthocyanins. *Chemistry European Journal* 18, 3736–3744.
- Dalmastri C, Chiarini L, Cantale C, Bevivino A and Tabacchioni S (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. *Microbial Ecology* 38, 273–284.
- FAO [Food and Agriculture Organization of the United Nations] (2011) *Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture*. Rome, Italy: Commission of Genetic Resources for Food and Agriculture. Available at <https://www.fao.org/3/a-i2624e.pdf>.
- FAO [Food and Agriculture Organization of the United Nations] (2018) *Biodiversity for Sustainable Agriculture CA2227EN/1/11.18*. Rome, Italy: Commission of Genetic Resources for Food and Agriculture. Available at <http://www.fao.org/3/CA2227EN/ca2227en.pdf>.
- FAO [Food and Agriculture Organization of the United Nations] (2019) *The estate of the world's biodiversity for food and agriculture – In brief*. Rome, Italy: Commission of Genetic Resources for Food and Agriculture. Available at <http://www.fao.org/3/ca3229en/CA3229EN.pdf>.
- García-Gaytán V, Gómez-Merino FC, Trejo-Téllez LI, Baca-Castillo GA and García-Morales E (2017). The chilhuacle chili (*Capsicum annuum* L.) in Mexico: description of the variety, its cultivation, and uses. *International Journal of Agronomy* 2017, 5641680.
- Güemes JR and Aguilar-Meléndez A (2018) Etnobotánica nahua del chile en la Huasteca meridional. In Aguilar-Meléndez A, Vásquez-Dávila MA, Katz E and Hernández-Colorado RM (eds), *Los chiles que le dan sabor al mundo, contribuciones multidisciplinarias*. Xalapa, Veracruz, México: Universidad Veracruzana, Editorial IRD, Marsella, Francia, pp. 236–259, <https://doi.org/10.25009/uv.2185.1087>
- Guzman I, Hamby S, Romero J, Bosland PW and O'Connell MA (2010) Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. *Plant Science* 179, 49–59.
- Herrera FEF (2016) El sistema de producción de chile “poblano”: características y fitomejoramiento de germoplasma local sobresaliente (MsC Thesis). Colegio de Postgraduados, Campus Puebla, Puebla, México.
- INEGI (2023) Conjunto Nacional de Datos de Suelos Serie II. Consultation date 07/06/2023. Available at <https://www.inegi.org.mx/temas/edafologia/>.
- IPGRI, AVRDC, CATIE [International Plant Genetic Resources Institute, Asian Vegetable Research and Development Center, Centro Agronómico Tropical de Investigación y Enseñanza] (1995) Descriptors for Capsicum (*Capsicum* spp.). IPGRI, AVRDC, CATIE. Available at [https://www.bioversityinternational.org/fileadmin/user\\_upload/online\\_library/publications/pdfs/345.pdf](https://www.bioversityinternational.org/fileadmin/user_upload/online_library/publications/pdfs/345.pdf).
- Janick J (2013) Development of New World crops by indigenous Americans. *HortScience* 48, 406–412.
- Kassambara A and Mundt F (2022) Factor extra: Extract and visualize the Results of Multivariate Data Analyses. R. Package Version 1.0.7. Available at <https://CRAN.R-project.org/package=factoextra>.
- Kraft KH, Brown CH, Nabhan GP, Luedeling E, Luna RJ, d'Eeckenbrugge GC, Hijmans RJ and Gepts P (2014) Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. *Proceedings of the National Academy of Sciences of the United States of America* 111, 6165–6170.
- Kumar S, Shieh HC, Lin SW, Schafleitner R, Kenyon L, Srinivasan R, Wang JF, Ebert AW and Chou YY (2018) Peppers (*Capsicum* spp.): domestication and breeding for global use. In Mandal D, Shukla AC, Siddiqui MW (eds), *Sustainable Horticulture: Diversity, Production, and Crop Improvement, Part III. Crop Improvement and Biotechnology*. Waretown: Apple Academic/CRC Press, pp. 387–400, <https://doi.org/10.1201/b22429>.
- Lascurain-Rangel M, Avendaño-Reyes S, Tan R, Caballero J, Cortés-Zárraga L, Linares-Mazari E, Bye-Boettler R, López-Binnquist C and De Ávila A (2022) Plantas americanas utilizadas como condimento en la cocina mexicana. *Revista Mexicana de Biodiversidad* 93, e933949.
- Li Z, Wang S, Gui XL, Chang XB and Gong ZH (2013) A further analysis of the relationship between yellow ripe-fruit color and the capsanthin-capsorubin synthase gene in pepper (*Capsicum* sp.) indicated a new mutant variant in *C. annuum* and a tandem repeat structure in promoter region. *PloS One* 8n, e61996.
- Lippert LF, Smith PG and Bergh BO (1966) Cytogenetics of the vegetable crops. Garden pepper, *Capsicum* sp. *The Botanical Review* 32, 24–55.
- Lozano GMD, Kuri GGD and Prado HRI (2021) Diseño de una estrategia de comunicación para fomentar la preferencia del chile poblano criollo. *Regiones y Desarrollo Sustentable* 41, 15–38.
- Luo H, Li W, Zhang X, Deng F, Xu Q, Hou T, Pang X, Zhang Z and Zhang X (2019) In planta high levels of hydrolysable tannins inhibit peroxidase mediated anthocyanin degradation and maintain abaxially red leaves of *Excoecaria Cochinchinensis*. *BMC Plant Biology* 19, 315.
- Manoharan L, Kushwaha SK, Ahrén D and Hedlund K (2017) Agricultural land use determines functional genetic diversity of soil microbial communities. *Soil Biology and Biochemistry* 115, 423–432.
- Martínez M, Vargas-Ponce O, Rodríguez A, Chiang F and Ocegueda S (2017) Solanaceae family in Mexico. *Botanical Sciences* 95, 131–145.
- Massot PHK, Vasconcelos SC, Branco VJC, Valgas RA and Barbieri RL (2016) Agronomic evaluation and morphological characterization of chili peppers (*Capsicum annuum*, Solanaceae) from Brazil. *Australian Journal of Basic and Applied Sciences* 10, 63–70.
- Moles AT, Perkins SE, Laffan SW, Flores-Moreno H, Awasthy M, Tindall ML, Sack L, Pitman A, Kattge J, Aarssen LW, Anand M, Bahn M, Bionder B, Cavender-Bares J, Cornelissen HC, Cornwell WK, Díaz S, Dickie JB, Freschet GT, Griffiths JG, Gutiérrez AG, Hemmings FA, Hickler T, Hitchcock TD, Keighery M, Kleter M, Kurokawa H, Leishman MR, Liu K, Niinemets Ü, Onipchenko V, Onoda Y, Peñuelas J, Pillar VD, Reich PB, Shiodera S, Siebert A, Sosinski Jr EE, Soudsilvskaia NA, Swaine EK, Swenson NG, van Bodegom PM, Warman L, Waihler E, Wright IJ, Zhang H, Zobel M and Bonser SP (2014) Which is better predictor of plant traits: temperature or precipitation? *Journal of Vegetation Science* 25, 1167–1180.
- Moreno-Ramírez YR, Santacruz-Varela A, López PA, López-Sánchez H, Córdova-Téllez L, González-Hernández VA, Corona-Torres T and López-Ortega R (2019) Morphological diversity of Zacatecas Guajillo chile landraces is broad and is given mainly by fruit traits. *Emirates Journal of Food and Agriculture* 31, 440–448.
- Narez-Jiménez CA, Cruz-Lázaro E, Gómez-Vázquez A, Castañón-Nájera G, Cruz-Hernández A and Márquez-Quiroz C (2014) La diversidad

- morfológica *in situ* de chiles silvestres (*Capsicum* spp.) de Tabasco, México. *Revista Fitotecnia Mexicana* **37**, 209–215.
- Nicolaï M, Cantet M, Lefebvre V, Sage-Palloix AM and Palloix A** (2013) Genotyping a large collection of pepper (*Capsicum* spp.) with SSR loci brings new evidence for the wild origin of cultivated *C. annuum* and the structuring of genetic diversity by human selection of cultivar types. *Genetic Resources and Crop Evolution* **60**, 2375–2390.
- Nooryazdan H, Serieys H, Baciliéri J, David J and Bervillé A** (2010) Structure of wild annual sunflower (*Helianthus annus* L.) accessions based on agro-morphological traits. *Genetic Resources and Crop Evolution* **57**, 27–39.
- Pérez CLJ, Tornero CMA, Escobedo GJS and Sandoval CE** (2017) El chile poblano criollo en la cultura alimentaria del Alto Atoyac. *Estudios Sociales* **49**, 49–66.
- Pickersgill B** (2016) Chile Peppers (*Capsicum* spp.). In Lira R, Casas A and Blancas J (eds), *Ethnobotany of Mexico, Interactions of People and Plants in Mesoamerica*. New York: Springer Science, pp. 417–438, [http://dx.doi.org/10.1007/978-1-4614-6669-7\\_17](http://dx.doi.org/10.1007/978-1-4614-6669-7_17).
- QGIS Development Team** (2023) QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at <https://qgis.org>.
- Ramírez NUI, Cervantes OF, Montes HS, Raya PJC, Cibrián JA and Andrio EE** (2018) Diversidad morfológica del chile piquín (*Capsicum annuum* L. var. *glabriusculum*) de Querétaro y Guanajuato, México. *Revista Mexicana de Ciencias Agrícolas* **9**, 1156–1170.
- R Core Team** (2022) *R: A Language and Environment for Statistical Computing*. Viena, Austria: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>.
- Rodríguez R, Peña OBV, Gil MA, Martínez CB, Manzo F and Salazar LL** (2007) Rescate *in situ* del chile ‘poblano’ en Puebla, México. *Revista Fitotecnia Mexicana* **30**, 25–32.
- Romero-Higareda C, Hernández-Verdugo S, Pacheco-Olvera A, Retes-Manjarrez JE, Osuna-Enciso T and Valdez-Ortiz A** (2023) Phenotype differentiation of *Capsicum annuum* var. *glabriusculum* of three regions in Mexico and its relation to climate. *Botanical Sciences* **101**, 744–760.
- SAS Institute** (2012) *SAS/STAT User’s Guide: Software Version 9.4*. Cary, North Carolina, USA: Statistical Analysis System Institute.
- Shao G and Halpin PN** (1995) Climatic controls of eastern North American coastal tree and shrub distributions. *Journal of Biogeography* **22**, 1083–1089.
- SIAP-SADER [Servicio de Información Agroalimentaria y Pesquera – Secretaría de Agricultura y Desarrollo Rural]** (2019) Servicio de Información Agroalimentaria y Pesquera. Available at <http://www.siap.gob.mx/agricultura-produccion-anual>.
- Taitano N, Bernau V, Jardón-Barbolla L, Leckie B, Mazourek M, Mercer K, McHale L, Michel A, Baumler D, Kantar M and Van der Knaap E** (2019) Genome-wide genotyping of a novel Mexican chile pepper collection illuminates the history of landrace differentiation after *Capsicum annuum* L. domestication. *Evolutionary Applications* **12**, 78–92.
- Toledo-Aguilar R, López-Sánchez H, López PA, Guerrero-Rodríguez JD, Santacruz-Varela A and Huerta-de la Peña A** (2016) Diversidad morfológica de poblaciones nativas de chile poblano. *Revista Mexicana de Ciencias Agrícolas* **7**, 1005–1015.
- Velázquez-Ventura JC, Márquez-Quiroz C, Cruz-Lázaro E, Osorio-Osorio R and Preciado-Rangel P** (2018) Morphological variation of wild peppers (*Capsicum* spp.) from the state of Tabasco, Mexico. *Emirates Journal of Food and Agriculture* **30**, 115–121.
- Vera-Guzmán AM, Aquino-Bolaños EN, Heredia-García E, Carrillo-Rodríguez JC, Hernández-Delgado S and Chávez-Servia JL** (2017) Flavonoid and capsaicinoid contents and consumption of Mexican chili pepper (*Capsicum annuum* L.) landraces. In Justino J (ed.), *Flavonoids - From Biosynthesis to Human Health*. London: IntechOpen, pp. 405–437, <https://dx.doi.org/10.5772/680.76>.