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PHENOTYPIC AND GENETIC VARIATION IN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) LANDRACES CULTIVATED IN A TRADITIONAL SYSTEM OF SOUTHEASTERN MEXICO

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is an economically important crop due to its nutritional and environmental benefits. In Mexico, farmers in marginal conditions produce most of the crops using landraces classified and informally named based on the physical characteristics of the seed and the growth habit of the plant. The analysis of genetic diversity is a crucial step towards enhancing crop productivity and is accomplished by measuring variation in phenotypic and genotypic traits using morphological and molecular markers. In order to obtain valuable information for cowpea management and conservation, this study assessed the genetic variability and relationships among 14 landraces cultivated in the Yucatan Peninsula, Mexico, based on morphological traits and molecular markers (ISSR). The results contribute to our knowledge of genetic diversity in cultivated landraces of cowpea in the Yucatan Peninsula, including the first report of landraces collected in a previously unexplored region. The results indicated a higher level of diversity among varieties than within varieties. Additionally, most morphological characteristics of cowpea genotypes were very similar. The populations studied were grouped into two main clusters by a PCA based on both ancestry analysis and a dendrogram. The analysis indicated a low level of gene flow between groups.

Key words: Diversity, Genetic markers, Landraces, Milpa system, Phenotypic characterization.

Introduction

The genus *Vigna* comprises 159 species distributed worldwide and contains the subgenera *Ceratotropis, Plectotropis, Macrorhycha, Sigmoidotropis, Sigmoidotropis, Lasiosporon, Haydonia*, and *Vigna* (Vijaykumar *et al*., 2010). The subgenus *Vigna* includes 40 species, with *Vigna unguiculata* being the most important. *V. unguiculata* is native to Africa, and the West and East African gene pools stand out as the regions with the greatest diversity of landraces and cultivated cowpeas (Huynh *et al*., 2013), and the center of diversity is West Africa.

At present, cowpea is grown in tropical and subtropical regions and is considered an important crop for food security, especially in the low-income strata of the world (Alghamdi *et al*., 2019) due to its nutritional value and low cost of production (Gonçalves *et al*., 2020). Globally, cowpea production in 2019 was 8.90 million tons (Mt), with Nigeria and Niger being the countries with the highest production at 3.57 and 2.38 Mt, respectively (Anon., 2020), followed by Brazil that produced about 822,000 t on 1.6 million ha (Boukar *et al*., 2019).

In Mexico, production is carried out by farmers in marginal areas using local varieties classified and informally named according to the physical characteristics of the seed and growth habit as well as the production area and the local market (Latournerie-Moreno *et al*., 2006; Velasco-Murguia *et al*., 2021). The cowpea bean, or X'pelón as it is called in the Mayan language, is related to Mayan culture; traditional Mayan farmers conserve and develop the plant genetic resources of this species by preserving local varieties and traditional knowledge associated with crop management, primarily through the traditional slash-and-burn system, known as "milpa" (cornfield) (Arias *et al*., 2004; Morales-Morales *et al*., 2019).

Genetic and phenotypic diversity is essential for long-term crop sustainability. *Vigna* bean landraces represent a reservoir of genes that can be used for the development of conservation and crop improvement programs; therefore, understanding the characteristics and genetic variability of landraces is crucial in obtaining valuable information that can help breeders develop more suitable and productive cultivars (Govindaraj *et al*., 2015; Lopes *et al*., 2015). To achieve this, landraces can be evaluated and classified using a combination of morphological characteristics, agronomic traits, and molecular markers (Gonçalves *et al*., 2008; Mwangi *et al*., 2021).

Previous studies of morphological characters (Animasaun *et al*., 2015; Mofokeng *et al*., 2020) and genetic diversity (Lioi *et al*., 2018; Dos Santos *et al*., 2020) in cowpea have been developed separately, and both morphological and molecular markers have shown their efficiency in assessing and characterizing genetic diversity. However, the information generated by both markers can be combined to obtain more robust estimates of genetic diversity.

Molecular and morphological data are necessary for more efficient estimation of the genetic diversity of cowpea germplasm; this will allow us to generate basic information that can be used in the planning of conservation and genetic improvement programs. Among the broad types of molecular markers developed, inter simple sequence repeat (ISSR) markers stand out. These markers are dominant and

are based on DNA amplification using a single primer composed of a microsatellite sequence. ISSRs have been demonstrated to be valuable in identifying genetic variation without prior knowledge of DNA sequences (Zietkiewicz *et al*., 1994). ISSR markers have been useful in the study of genetic diversity, structure, and characterization of *Vigna unguiculata* germplasm (Desalegne *et al*., 2016; Desalegne *et al*., 2017). Therefore, the objective of this study was to evaluate the phenotypic and genetic diversity of cowpea landraces grown in the Yucatan Peninsula, Mexico, based on morphological traits and molecular markers (ISSRs).

Material and Methods

Study area: The Yucatan Peninsula is situated in southeastern Mexico and comprises the states of Yucatan, Campeche, and Quintana Roo. The vegetation of regions is diverse, including low deciduous and medium deciduous forests, as well as thorny low deciduous forests with clay soils (Duno de Stefano *et al*., 2018).

The peninsula has limited surface hydrography, and its climate is predominantly warm and sub-humid with an intermediate rainfall regime. According to INEGI (2016), the region has an annual precipitation range of 500 to 1500 mm and average temperatures ranging from 26 to 28°C. The region experiences dry seasons lasting from three to seven months and has areas at altitudes ranging from 8 to 1000 mean sea level (MSL).

Crop establishment: The study was conducted from January to May 2020 at the Instituto Tecnológico de Conkal, Conkal, Yucatán, Mexico (21° 04' 50.1" N, 0.89° 29' 53.9" E, and an altitude of 9 m). Fourteen landraces previously collected in the Yucatan Peninsula, Mexico, were analyzed (Table 1).

Thirteen seeds of each landrace were germinated in 200-cavity polyethylene trays. Once the plants formed the first two true trifoliate leaves and reached a minimum height of 20 cm, they were transplanted into 10 L pots containing local soil pH 7.4 and electrical conductivity of 1.3 dS m^{-1}, with a distance of 40 cm between plants and 90 cm between rows, in a completely randomized experimental design. Drip irrigation was used for watering, and the plants were fertilized with 46-46-60 (N-P-K). All P, K, and 50% N were applied at 25 days after sowing (das), while the missing 50% N was applied at 55 das, and nutrition was supplemented at 50 das by the application of micronutrients (Maxiquel multi® Fe, Mn, Zn, B 570 EDDHA) at doses of 1.15, 0.49, 0.16 and 0.16 mg L^{-1} respectively. The temperature during growth ranged between 18 and 45°C, with an average humidity of 84.6% measured with portable equipment (HOBO® data logger, ONSET brand).

Phenotypic characterization: During growth, 28 morphological traits related to vegetative characteristics of the plant, flower, fruit (pods), and seed were recorded, comprising 15 quantitative and 13 qualitative traits (Table 2). For the evaluation of phenotypic traits, the descriptors developed by Anon., (1983) were used, and 10 plants per landrace were evaluated. The pods were harvested when fully ripe, and yield was calculated up to 120 days of production according to the following formula:

$$Y = (NPP*NSP)*ISW$$

where NPP = Is the number of pods per plant; NSP= Number of seeds per pod, and ISW = Individual seed weight

Molecular characterization

DNA extraction: Genomic DNA extraction was obtained from 13 plants per landrace using the central leaflet of the first fully mature trifoliate leaf that was free of pests and diseases. DNA extraction was performed using the Mini Kit DNeasy® extraction kit (QIAGEN). A total of 2.5 g of leaf tissue was used for extraction. To confirm the quality of the extracted DNA, electrophoresis was performed on 1% agarose gels in 1X TBE buffer, and the gels were stained with Uview 6 × loading dye (BioRad, Hercules, CA, USA).

PCR amplification: For PCR amplification, 10 ISSR primers were tested, of which 4 were selected because they showed good amplification and high levels of polymorphism for the landraces evaluated [CTC $(GT)_8$, TACA $(GCA)_3$ G, UBC809, and UBC827]. PCR was carried out using the method described by López-Castilla *et al*., (2019). Amplification products were stained with Uview 6 × loading dye (BioRad) and separated by 1% agarose gel electrophoresis with 1X TBE buffer at a constant 110 V for 50 min. A 1 kb molecular marker standard was included in each gel, and the bands were visualized using the Gel Doc EZ Imager program (BioRad). Repeatable ISSR bands were recorded as present (1) or absent (0), and each ISSR band was considered as an independent locus. The number of different bands and the frequency of polymorphic bands were calculated for each primer.

Data analysis

Morphological data analysis: The qualitative variables were analyzed with descriptive statistics, transforming the observed ranges of each category into percentages. Quantitative data were subjected to analysis of variance and comparison of means using Tukey´s test. Qualitative and quantitative traits were subjected to principal component analysis (PCA) using a correlation matrix to examine the association between the traits analyzed and the similarity between landraces. This method considers the individual contributions of different morphological traits to the total amount of variation observed among landraces. The 28 morphological traits standardized to $\mu = 0$ and $\sigma^2 = 1$ were used to perform the PCA, with the varimax rotation criterion using the correlation matrix. The SPSS statistical program (Anon., 2016) was used for the PCA. Kaiser´s rule (1960) was used to determine the significance of the eigenvalues and eigenvectors of each component. In addition, a cluster analysis was performed using the WARD-ML method with Gower's distance as the similarity measure for the 28 traits. A *P*-value <0.05 was considered significant.

Table 1. Collection site of cowpea landraces in the Yucatan Peninsula.

Clave colecta	Nombre común	Localidad	Municipio	Estado	Altura (msnm)
OXC01	Yax pelón	Xul	Oxcutzcab	Yucatán	89
HEC02	X´pelón	San Vicente Cumpich	Hecelchakán	Campeche	39
HEC03	Chalack simin	San Vicente Cumpich	Hecelchakán	Campeche	39
OXC04	Paysin	Xul	Oxcutzcab	Yucatán	39
OXC05	Paysin	Xul	Oxcutzcab	Yucatán	89
CHE06	Espelón perón	Kuxeb	Chemax	Yucatán	25
PET07	Espelón Domingo	Peto	Peto	Yucatán	35
PET08	Espelón blanco	Peto	Peto	Yucatán	35
JMM09	Chalack simin negro	San Felipe 1	José María Morelos	Quintana Roo	35
JMM10	X´pelón	López Mateos	José María Morelos	Quintana Roo	35
PET11	X´nuuc Pelón	Xoy	Peto	Yucatán	16
CHE12	X´pelón de guía	Mucel	Chemax	Yucatán	25
FCP13	X´pelón	Polyuc	Felipe Carrillo Puerto	Quintana Roo	20
HAL14	Paysin	Halachó	Halachó	Yucatán	16

Table 2. Quantitative traits evaluated in 14 cowpea landraces collected in traditional crop system from the Yucatan Peninsula.

Descriptor	Acronym	Description and units
Quantitative		**Vegetative**
Terminal leaflet length	TLL	Terminal leaf length in the sixth week after sowing (cm).
Terminal leaflet width	TLW	The widest dimension of the terminal leaf in the sixth week after planting.
		Inflorescence and pods
Days to flowering	DF	Number of days from sowing to the stage when 50 % of the plants have started to flower.
Peduncle length	PL	Average length (cm) measured when pods are fully ripe.
Number of pods per plant	NPP	Average number of pods per plant at 120 dds.
Pods length	PL	Average length (cm) of 10 fully expanded mature pods from 10 plants.
Pods width	PW	Average width (mm) of the 10 pods.
Pods thickness	PT	Average thickness (mm) of the 10 pods.
		Seed
Number of locules per pod	NLP	Average number of locules of 10 pods per plant out of 10 plants.
Number of seeds per pod	NSP	Average number of seeds for pods.
Seed length	SL	Average of 10 ripe seeds measured parallel to hilum.
Seed width	SW	Average width of the 10 seeds measured for SL.
Seed thickness	ST	Average of the 10 seeds measured for SL, measuring perpendicular to the length and width.
Individual seed weight	ISW	Average weight of 10 seeds individually per accession.
Weight of 100 seeds	W100S	Weight of 100 seeds in g.
Qualitative		**Vegetative**
Growth habit	GH	1: determined, 2: undetermined.
Plant vigor	PV	3: Not vigorous, 5: Intermediate, 7: Vigorous, 9: Very vigorous.
Leaf color	LC	1: Intensity of green color. 3: pale green, 5: intermediate green, 7: dark green.
Terminal leaf shape	TLS	1: globose, 2: sub-globose, 3: sub-cast, 4: up toast.
		Inflorescence and pods
Flower color	FC	1: white, 2: violet, 3: mauve pink, 4: other.
Flower pigmentation	FP	0: not pigmented, 1: wing pigmented, 2: pigmented margins on wing and standard, 3: wing pigmented; standard slightly pigmented, 4: wing with pigmented upper margin; standard is pigmented, 5: completely pigmented, 6: other.
Attachment of the sheath to the peduncle	ASP	3: pendulous, 5: 30-90° from erection, 7: erect.
Pigmentation of the mature pod	PMP	1: none, 2: pigmented tip, 3: pigmented sutures, 4: pigmented valves, green sutures, 5: splashes of pigment, 6: evenly pigmented, 7: other.
Color of ripe pod	CRP	1. Pale tan or straw, 2: dark tan, 3: dark brown, 4: black or dark purple, 5: other.
Pod curvature	PC	0: straight, 3: slightly curved, 5: curved, 7: spiral, 7: spiral.
		Seeds
Seed color	SC	1: cream, 2 cream brown, 3. brown, 4: ochre brown, 5: olive brown, 6: black and white, 7: white, 8: black, 9: red.
Seed shape	SS	1: kidney, 2: ovoid, 3; cuboid, 4: globose, 5: rhomboid.
Seed texture	ST	1: smooth, 2: smooth to rough, 3: rough.

Table 3. Quantitative morphological traits of 14 cowpea landraces cultivated in traditional crop systems.

Landraces	NLP	NSP	SL	SW	ST	ISW	W100S	Y
			mm			g		
OXC01	14.78±1.3abcd	14.12±1.4ab	8.06±0.4cde	6.41±0.3ab	4.60±0.1bcd	0.13±0.01cd	13.72±0.03bc	38.08±5.2ab
HEC02	15.38±2.6abcd	14.58±2.4ab	7.43±0.4ef	6.04±0.3cd	4.22±0.3de	0.11±0.01ef	10.68±0.04c	36.72±6.4ab
HEC03	13.82±1.6cde	12.18±1.6ab	8.73±0.6abc	6.67±0.5ab	5.37±0.3a	0.23±0.02a	18.27±0.02a	36.37±3.2ab
OXC04	14.10±1.8bcde	14.68±6.6ab	8.66±0.5bc	6.70±0.3ab	4.63±0.2bc	0.17±0.01b	14.68±0.02bc	38.06±7.5ab
OXC05	14.28±1.3abcd	13.38±1.4ab	8.77±0.5abc	6.65±0.3ab	4.99±0.4ab	0.16±0.01b	16.08±0.02ab	44.43±7.9ab
CHE06	16.56±1.7ab	14.84±2.3ab	7.07±0.2f	5.62±0.2ef	4.23±0.2de	0.10±0.005f	9.92±0.03d	31.67±4.5ab
PET07	15.89±0.8abc	14.21±1.0ab	8.14±0.4bcde	6.30±0.3abc	4.52±0.2cd	0.13±0.01cde	13.44±0.02bc	58.99±3.1a
PET08	13.04±0.8de	11.78±1.0ab	6.90±0.3f	5.24±0.6f	4.07±0.2e	0.13±0.01cde	8.61±0.03e	59.04±2.8a
JMM09	12.46±2.2e	11.26±1.9b	9.55±0.4a	6.90±0.4a	5.27±0.2a	0.17±0.02b	17.56±0.01ab	30.91±5.6b
JMM10	14.94±1.8abcd	13.26±2.60b	8.91±0.7ab	6.87±0.4a	4.64±0.2bc	0.17±0.02b	15.40±0.02b	34.14±3.4ab
PET11	16.06±1.7abc	14.58±14.5ab	8.52±0.3bcd	6.19±0.2bcde	4.45±0.1cde	0.16±0.01bc	13.59±0.04bc	48.59±4.2ab
CHE12	14.96±1.7abcd	12.90±2.5ab	8.86±0.7abc	6.62±0.4abc	4.74±0.2bc	0.17±0.02b	15.39±0.03b	36.14±3.1ab
FCP13	16.82±1.7a	15.42±1.8a	7.70±0.5def	5.92±0.3de	4.36±0.2cde	0.13±0.01de	10.72±0.03c	57.34±6.2ab
HAL14	16.20±1.4abc	13.62±1.94b	8.66±0.9bc	6.48±0.3abcd	4.50±0.1cd	0.16±0.01bc	14.36±0.03bc	36.91±5.6ab
CV	13.84	20.09	11.04	9.63	9.51	23.45	18.12	22.49

CV= Coefficient of variation; NLP= Number of locules per pod; NSP= Number of seeds per pod; SL= Seed length; SW= Seed width; ST= Seed thickness; ISW= Individual seed weight; W100S= Weight of 100 seeds; Y= Yield; n=10 ± Standard deviation

Genetic structure analysis: The genetic structure was analyzed by two methods. First, the data were analyzed using an individual assignment test performed with STRUCTURE v. 2.3.4 software (Pritchard *et al*., 2000) that uses a Bayesian clustering approach to assign individual genotypes to a predefined number of *K* populations. The optimal *K* value was chosen according to the ΔK statistic proposed by Evanno *et al*., (2005) using Structure Harvester software (Earl & VonHoldt, 2012). Finally, ancestry plots for the optimal *K* value were generated using STRUCTURE v. 2.3.4 software (Pritchard *et al*., 2000). Second, an analysis of molecular variance (AMOVA) was performed to determine the variability between and within landraces using GenAlEx 6.5 (Peakall & Smouse, 2006).

Analysis of genetic relationships: Genetic relationships between cowpea landraces were analyzed using a dendrogram by the unweighted pair group method with arithmetic mean (UPGMA) with Euclidean distance as the similarity measure. Tree topology was evaluated with 1000 Bootstrap replicates using the PAST program (Hammer *et al*., 2001).

Genetic diversity analysis: Genetic diversity was assessed following the methodology described by Lopez-Castilla *et al*. (2020) at the landrace level and in observed groups with allelic richness indices using the program POPGENE v. 1.31 (Yeh & Boyle, 1999), while genetic diversity estimators such as the Shannon-Weaver diversity index (I) and mean heterozygosity (H_{bay}) were calculated using AFLPSURV v. 1.0 (Vekemans, 2002).

Results and Discussion

Evaluation of genetic diversity and morphological characterization of germplasm are important requirements for selecting the best attributes of landraces and developing strategies for conservation, utilization, and genetic improvement (Safamanesh *et al*., 2017). In this study, the morphological and molecular diversity of cowpea landraces grown in traditional systems in the Yucatan Peninsula, Mexico, were analyzed together for the first time.

Phenotypic diversity based on quantitative traits: Comparisons of the means of the quantitative morphological traits of the 14 landraces (Table 3) revealed a high level of genetic variability ($p<0.001$) in all measured traits. This finding constitutes a promising starting point for plant development programs, since it suggests the possibility of introducing new varieties and hybrids.

The characters with the greatest variation (≥ 20) included peduncle length, days to flowering, number of pods per plant, number of seeds per pod, individual seed weight, and total yield. These results were consistent with those of previous studies. Stoilova & Pereira (2013) also identified these morphological traits as those with the highest level of variation.

In contrast, the characters of seed width and seed thickness exhibited the least variation, with a coefficient ≤ 10, as reported by Mafakheri *et al*., (2017). This indicated that the seed characteristics were relatively consistent in all of the genotypes studied.

The peduncle size varied from 20.04 to 38.10 cm, with an average of 25.74 cm. The landraces OXC05, OXC04, and HEC03 had the longest peduncles. Longer peduncles are important because they allow pods to be located above the canopy, a factor that helps to prevent damage by the pod borer (*Maruca vitrata*) and reduces diseases associated with humid environments (Aremu, 2011).

A shorter period to initiate flowering can indicate good tolerance to drought and low humidity (Belko *et al*., 2014). Nkaa *et al*. (2014) noted that early-blooming varieties matured sooner. Manggoel & Uguru (2012) classified varieties that flowered in less than 45 days as early flowering. In this study, all local varieties evaluated could be classified as intermediate or late flowering. These findings align with those of Ashinie *et al*., (2020) from populations in Ethiopia, where flowering times ranged from 42 to 90 days.

Significant variation was observed in the number of pods per plant (13.40-38.10), consistent with the results obtained by Abiodun *et al*., (2020), who reported a maximum of 38.25 pods per plant. However, these results were lower than those reported by Gerrano *et al*., (2019), who found landraces with 54 pods per plant. The difference in pod number between cultivars can be attributed to several factors, including genetics, environment, and nutrition.

Landraces FCP13, CHE06, OXC04, PET11, and HEC02 produced the highest numbers of seeds per pod, with values within the range of 8.12 to 18.30 seeds per pod reported by Viswanatha & Yogeesh (2017). These results are important for farmers, as they can choose varieties that produce more seeds per pod, a critical factor in determining the final grain yield (Peksen, 2004; Atakora *et al*., 2023). Furthermore, studies have indicated that the number of seeds per pod is a heritable trait influenced by additive, dominant, and epistatic genetic effects (Drabo *et al*., 1985). Therefore, selecting cowpea varieties with a higher number of seeds per pod is crucial for maximizing yield and meeting market demand.

The size of cowpea seeds is a crucial factor that can impact their final value in the market. For example, local varieties with larger seeds are often preferred for canning (Henshaw, 2008). Additionally, seed size is a relevant factor for producers, as larger seeds tend to result in higher yields. There was significant genetic diversity observed in both the weight of individual seeds and the weight of 100 seeds. For example, CHE06 had the lowest seed weight, while the local variety HEC03 had an individual seed weight of 0.23 g and 18.27 g per 100 seeds. These values were within the range reported by Kaptso *et al*., (2008) who stated that cowpea seed weight ranged from 0.08 to 0.32 g.

Ogle *et al*., (1987) classified cowpea seeds based on weight, considering seeds weighing less than 15 g as small, 15.1 to 20 g as medium, 20.1 to 25 g as large, and seeds weighing more than 25g as numerous. Among the 9 local varieties studied, 64.28% were classified as having small seeds, and 35.72% as having medium seeds. Seed weight is directly related to size and can be an important criterion for optimizing crop performance.

The local varieties FCP13, PET07, and PET08 had the highest yield per plant, being 57.34, 58.99, and 59.04 g, respectively. Although FCP13 did not have the largest number of pods, its high yield was attributed to the highest number of seeds per pod. The values obtained from the experiments exceeded the average yield of 13.00 g per plant reported by local farmers in Tabasco, Mexico (Márquez-Quiroz *et al*., 2015). The variation in yield may be due to genetic differences between populations and environmental variation during crop growth and development.

Crucial indicators for cultivar improvement through selection include variation in flowering days, pod size, and seed yield per plant (Dareus *et al*., 2021). Hall *et al*., (2003) noted that candidate local varieties for improvement should possess additional attributes, including a short photoperiod, weed competition, small seed size, and resistance to pests and diseases in addition to the aforementioned variables.

Qualitative morphological diversity in landraces and cowpea varieties: Morphological descriptions indicated two forms of terminal leaflet, sub-hastate (64.29%) and sub-globose (35.71%), while for terminal leaf color, this was dark green in 57.14% and light green in 42.87%. The results were similar to those reported by Egbadzor *et al*., (2014), who found a higher percentage of sub-hastened and sub-globose leaves in 118 cowpea genotypes produced in Ghana.

Four landraces (HEC02, CHE06, PET08, and FCP13) exhibited a determinate growth habit; therefore, plant height and yield-related traits differed from those with indeterminate growth. The determinate-growing landraces have certain advantages, being more drought tolerant compared to the indeterminate types (Hall, 2012). However, indeterminate cowpea landraces are known to achieve higher productivity due to their prolonged maturity and the efficiency of their photosynthetic process (Silva *et al*., 2020).

The observed flower colors of the landraces used in this study indicated that about 93% produced violet flowers, while landrace PET08 (7%) produced white flowers. Of the total sample, 85.71% had pigmented wings; 7.14% had pigmented margins, and 7.14% did not have any pigmentation. In terms of pod pigmentation, 85.71% had a pigmented apex, and 7.14% did not show any pigmentation. The results agreed with those reported by Othman *et al.* (2006) and confirmed that the violet color of the flower was dominant over the white color. Additionally, there is a relationship between flower color, immature pod pigmentation, and seed coat color in cowpeas. This relationship is due to an increase in anthocyanin and melanin that are responsible for the colors of the flowers, pods, and seeds of cowpeas. According to Egbadzor *et al*., (2012), there is pleiotropic control over pigmentation in cowpea that affects flower, pod, and seed coat color. This linkage between flower color and other traits could be useful for the indirect selection of economically important traits.

In the variable fixation of the pod to the peduncle, 64.29% were pendulous, and the rest (35.71%) were considered erect because they presented an angle of inclination between 30 and 90°. Meanwhile, for pod curvature, nine landraces (64.28%) presented slightly curved pods; four (28.57%) were completely curved, and the landrace CHE06 (7.15%) was completely straight. The importance of curvature is due to the fact that the erect pods stand above the canopy and thus facilitate harvesting. However, the curved pods are longer and have a greater number of seeds than the erect pods, especially when they have long peduncles, a characteristic that contributes to a higher seed yield (Egbadzor *et al*., 2014). In this sense, it is desirable to have landraces with curved pods on relatively long peduncles. In this study, 92.85% of the landraces possessed this characteristic.

Lazaridi *et al*., (2017) reported analogous results, stating that 65.5% of the landraces were pale bronze. The morphology of the landrace seeds exhibited significant variation in terms of color, size, shape, and texture. Black seeds were the most common (85.71%), followed by white (7.14%) and cream (7.14%). Seed color and texture are important traits that influence consumer preference, along with cultural factors (Herniter *et al*., 2019). Therefore, the high percentage of black landraces in this study (85.71%) may have been influenced by the preference for consuming beans of this color in the southern region of Mexico (Rodriguez *et al*., 2010; Monge *et al*., 2019). However, cream and white landraces were also discovered, and thus could serve as alternatives for markets in central and northern Mexico (Ramírez-Jaspeado *et al*., 2020). Seed

coat color is determined by a few crucial genes that aid in the selection process during crop development (Herniter *et al*., 2019). Additionally, in some crop plants, the color of the seed coat affects physiological activities such as water absorption, gas diffusion, seed dormancy, seed quality, germination, and seedling emergence (Atis *et al*., 2011). Makoi *et al*., (2010) found a correlation between seed coat pigmentation and pest resistance.

The results indicated that cowpea bean seeds presented wide phenotypic variability. Twelve landraces had an ovoid shape (85.71%), and two (14.29%) were globose. Likewise, 78.57% had a smooth to rough texture, and 21.43% of the remaining accessions had a smooth texture (HEC02, HEC03, and HAL14). These results were similar to those reported by Doumbia *et al*., (2013) on the characterization of cowpea bean collections but differed from those reported by Stoilova & Pereira (2013), who observed 44.0% soft textured seeds and 56.0% with rough texture. Seed texture is a factor in the consumer acceptability of cowpeas. For example, in West Africa, consumers prefer rough seeds because of the ease of hulling, the swelling capacity used for processed foods, and the shorter cooking time compared to smooth textured seeds (Oladejo *et al*., 2020).

Principal component analysis of phenotypic traits: Principal component analysis (PCA) for the 28 morphological traits (qualitative and quantitative traits that showed statistically significant differences) is presented in (Table 4). The first three components explained 75.18% of the total variation. Therefore, this aligns with the suggestion by Gixhari *et al*., (2014) that values above 75.0% of the total observed variation are acceptable for the genetic characterization of legume crops.

The PCA showed that the first three vectors had latent roots greater than one, indicating a significant level of variation among the varieties evaluated for morphological characteristics. The three principal components (PC) individually contributed 48.47%, 13.68%, and 13.02% of the total cumulative variation among the landraces; according to the principle of Syafii *et al*., (2015), the first principal component accounts for the maximum variability in the data with respect to the rest; in the present study, this was fulfilled. PC1 was influenced by the variables terminal leaflet length, terminal leaflet width, days to flowering, peduncle length, number of pods per plant, pod length, pod width, pod thickness, number of locules per pod, seed length, seed width, seed thickness, individual seed weight, 100-seed weight, plant vigor, flower color, and seed color. Mafakheri *et al*., (2017) reported similar results on cowpea varieties grown in Iran. For CP2 the primary contributing factors were the number of seeds per pod, leaf color, mature pod pigmentation, and mature pod color. CP3 was predominantly influenced by qualitative variables such as growth habit, flower color, flower pigmentation, and seed shape. The variables with the highest contribution to both CP1 and CP2 were quantitative morphological characteristics. The PCA confirmed that cowpea landraces had high levels of diversity and that each of the traits contributed to the total phenotypic variability.

Table 4. Eigenvectors of the first four principal components measured in morphological traits of 14 cowpea landraces.

Variables	Component		
	1	2	3
Terminal leaflet length	0.884*	0.355	0.163
Terminal leaflet width	0.784*	0.521	0.171
Days to flowering	0.654*	0.136	-0.121
Peduncle length	0.969*	-0.083	-0.024
Number of pods per plant	-0.810*	-0.019	-0.177
Pod length	0.610*	-0.464	0.275
Sheath width	0.958*	-0.164	-0.042
Sheath thickness	0.919*	-0.127	0.080
Number of locules per pod	0.969*	-0.083	-0.024
Number of seeds per pod	-0.245	-0.633*	0.622*
Seed length	0.929*	0.250	-0.018
Seed width	0.945*	0.118	0.110
Seed thickness	0.876*	0.241	-0.163
Individual seed weight	0.817*	-0.050	-0.523
Weight of 100 seeds	0.963*	0.135	-0.130
Growth habit	0.268	-0.300	-0.630*
Plant vigor	0.613*	0.151	-0.282
Leaf color	-0.452	0.665*	0.163
Flower color	0.649*	-0.278	0.688*
Flower pigmentation	0.294	-0.254	0.727*
Attachment of the sheath to the peduncle	-0.451	0.483	0.038
Pigmentation of the mature pod	0.096	0.865*	0.323
Color of ripe pod	0.377	0.703*	0.109
Curvature of the sheath	0.655	-0.312	-0.472
Seed color	0.728*	-0.420	0.146
Seed shape	-0.170	-0.045	-0.854*
Seed texture	-0.194	0.352	0.134
Eigen value	13.08	3.69	3.51
Explained variation	48.47%	13.68%	13.02%
Accumulated variation	48.47%	62.15%	75.18%

The values with * are the significant variables for each component

(Fig. 1) shows the dispersion of the 14 landraces on the plane determined by the first two principal components and indicates the formation of two large groups that explained 62.15% of the total variation. The first group (I) was formed by the cowpea landraces HEC02, CHE06, PET07, PET08, and FCP13 that exhibited comparable characteristics in quantitative morphological traits such as seed length, width, and thickness, as well as the number of pods per plant. The findings of this investigation align with those reported by Nkhoma *et al*., (2020), whose first group was formed by the same phenological characteristics in 100 Zambian landraces. Therefore, the close relationships between the local varieties in this study can be attributed to their collection from regions characterized by analogous climate and soil types.

The second group (II) comprised the landraces OXC01, HEC03, OXC04, OXC05, JMM09, JMM10, PET11, CHE12, and HAL14. This group was characterized by qualitative morphological traits such as leaf color, mature pod color, pigmentation of the mature pods, and seed color, traits that further distinguished these landraces within the broader cowpea population. Our results agreed with those of Ghalmi *et al.* (2010) who classified Algerian landraces based on morphological characteristics such as small black and cream-brown seeds and seed texture.

The results of the cluster analysis (Fig. 2) corroborated the findings from the PCA (Fig. 1), showing 100% similarity in the obtained groupings. This analysis identified two distinct groups, with a coefficient of 21.6. Group I comprised the landraces HEC02, CHE06, PET07, PET08, and FCP13. This group exhibited identical values in terms of the length and width of the central leaf, days to 50% flowering, and length of peduncles as well as the length, width, and thickness of seeds. Additionally, the group displayed consistency in individual seed weight and weight of 100 seeds, the greater number of pods per plant, the greater number of seeds per pod, and a determined growth habit. In group I, the landrace PET08 was the variety that presented the smallest seeds among all the landraces, with smaller peduncle size, smaller individual weight, and the weight of 100 seeds, but was the variety with the highest number of pods per plant; for qualitative traits, PET08 was absent pigmentation in the flower and was the only landrace with white flowers, with globose and white seeds.

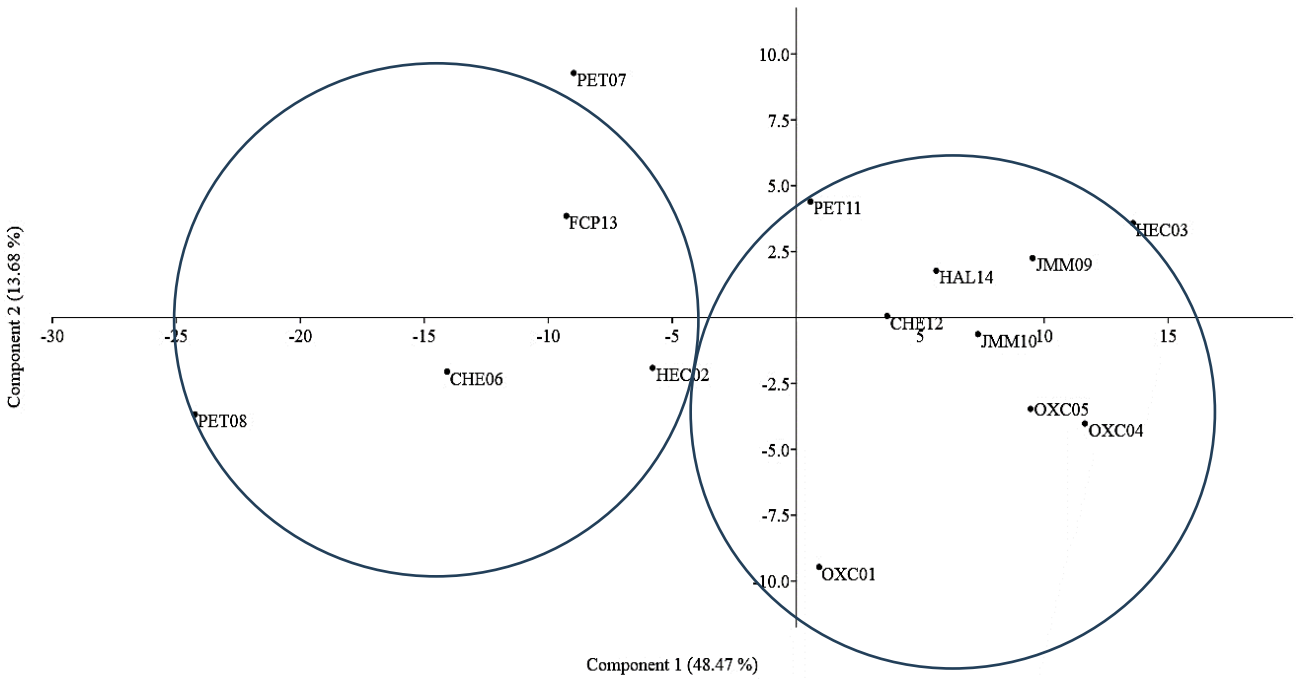

Fig. 1. Spatial distribution of the 14 cowpea landraces grown in southeastern Mexico based on the first two principal components and 28 morphological variables.

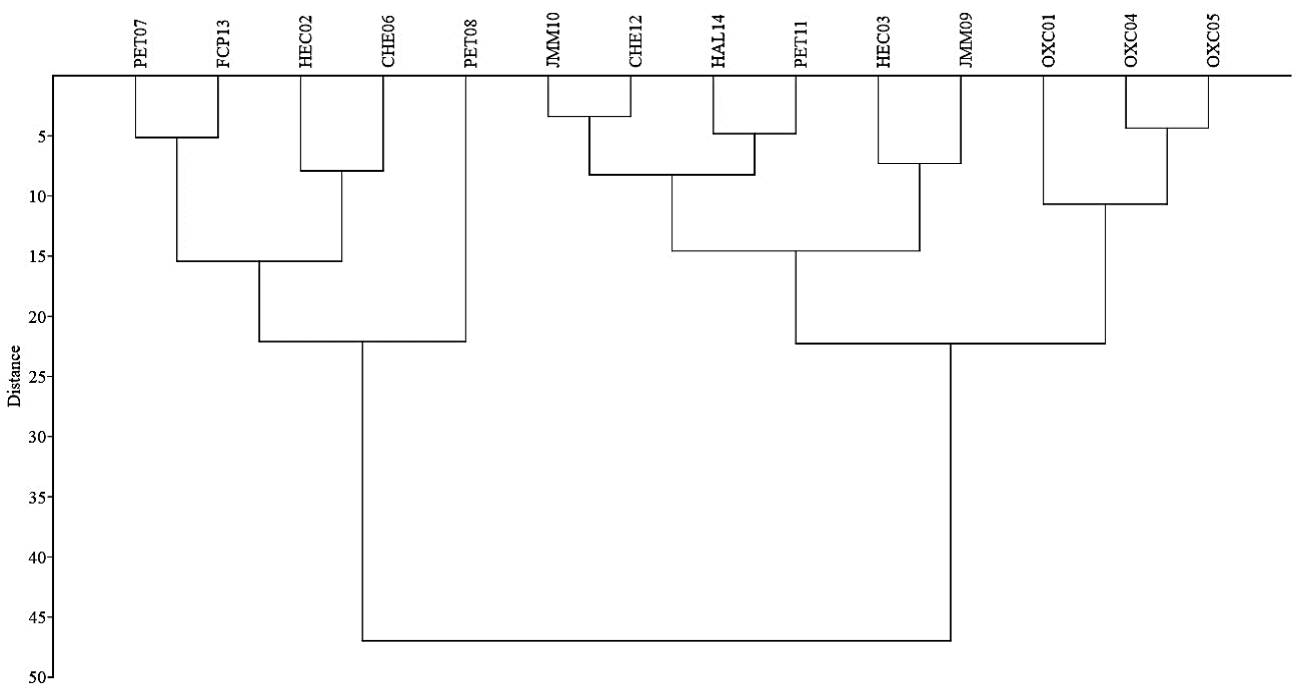

Fig. 2. Cluster analysis of 14 cowpea landraces grown in southeastern Mexico based on Gower's distance, using the WARD-ML method with morphological traits.

Group 2 comprised the landraces OXC01, HEC03, OXC04, OXC05, JMM09, JMM10, PET11, CHE12, and HAL14, all of which were characterized by larger leaves, longer peduncles, and larger seeds in length, width, and thickness. All are late flowering landraces with indeterminate growth and have flowers with pigmented wings, violet flowers, ovoid seeds, smooth to rough texture, and black seed color. The formation of this group of landraces can be attributed to the seed management practices of farmers. Seed exchange between producers or at seed fairs is common in the region. The trait pattern may also be the result of the interaction of natural selection, genetic enrichment, genetic drift, and environmental variation. In this regard, Jivani *et al*., (2013) stated that genetic drift and selection in diverse environments could lead to greater diversity. Several factors contribute to the genetic diversity and distribution of a species, including its reproductive system, habitat availability, migration patterns between populations, population size, and environmental factors (Lazaridi *et al*., 2017).

Analysis of the genetic diversity of cowpeas using molecular markers: Genetic diversity index statistics of cowpea landraces grown in the Yucatan Peninsula indicated that the 4 ISSR primers analyzed in this study exhibited a high degree of polymorphism and generated 42 loci, presenting from 8 to 29 alleles per locus, with an average of 22.71 (Table 5), with the JMM10 variety having the highest percentage of polymorphic loci (% P=69) and the CHE12 variety showing the lowest percentage of polymorphic loci (% P=19). The ranged values, akin to those reported by Massawe *et al*., (2003), varied 9 to 20 alleles, alongside high levels of polymorphism ranging from *Vigna subterranea* (L.) as determined by RAPD markers.

Table 5. Estimators of the genetic diversity of 14 landraces grown in southeastern Mexico.

Landraces	NL	NPL	% P	Na	Ne	I	HBay
OXC01	42	22	52.40	0.50	1.18	0.15	0.10
HEC02	42	25	59.50	1.00	1.33	0.28	0.18
HEC03	42	26	61.90	1.00	1.28	0.24	0.16
OXC04	42	26	61.90	1.00	1.22	0.22	0.14
OXC05	42	25	61.90	1.00	1.27	0.27	0.17
CHE06	42	25	59.50	0.50	1.08	0.10	0.06
PET07	42	25	59.50	0.75	1.22	0.16	0.11
PET08	42	21	50.00	1.00	1.27	0.26	0.17
JMM09	42	25	59.50	1.25	1.41	0.32	0.22
JMM10	42	29	69.00	1.25	1.08	0.13	0.07
PET11	42	20	47.60	1.50	1.30	0.30	0.18
CHE12	42	8	19.00	0.75	1.24	0.17	0.12
FCP13	42	22	52.40	0.50	1.00	0.00	0.00
HAL14	42	19	45.20	0.75	1.04	0.06	0.03
Media	42	22.71	54.24	0.91	1.21	0.19	0.12

NL= Number of loci; NPL= Number of polymorphic loci; % P= Percentage of polymorphism loci; Na= Number of observed alleles; Ne= Effective number of alleles; I= Shannon-weaver genetic diversity index; H_{Bay} = Nei genetic diversity with Bayesian approach

In another case study, Igwe *et al*., (2017) reported a comparable number of alleles (4 to 14) and up to 94% polymorphism in 14 cowpea landraces from Nigeria and 20 ISSR-type markers. Based on the observed results and according to Vinceti *et al*., (2013) the landraces with a high number of alleles indicated good diversity and can be used for conservation and breeding of plant material. The variance in allele counts among various authors may be attributed to factors such as the type of material, the technique used for DNA detection, or the number and type of markers used in each study.

The genetic diversity analysis revealed that Variety JMM09 exhibited the highest diversity value (I=0.31; H_{bay}=0.22). Conversely, FCP13 displayed the lowest genetic diversity as the markers used did not detect any polymorphic alleles (I=0.00; H_{bay}=0.00). This outcome could be attributed to the possibility that the sampled individuals were monomorphic for the assessed primers. Shannon's information index and the percentage of polymorphism indicate an intermediate diversity among the cowpea populations studied; however, the values were lower than that found by Desalegne *et al*., (2016), which was 0.46 for cowpea varieties grown in Ethiopia. In this regard, Dawson *et al*., (1995) indicated that the Shannon diversity index was not sensitive to the effects caused by the inability to detect heterozygotes.

The results showed a total average of 0.91 alleles observed, and landrace PET11 presented the highest number of alleles, while three landraces (OCX01, CHE06, and FCP13) presented the lowest number of alleles at 0.50. A high number of effective alleles (Ne) were observed in all the landraces, with landrace JMM09 demonstrating the highest value of 1.41. In this sense, the number of effective alleles was within the range of 1.28 to 1.78 reported by Igwe *et al*., (2017) in 18 Nigerian landraces, values that were higher than those reported by Ali *et al*., (2015) but lower than those obtained by Sarr *et al*., (2020). The high variation among cowpea landraces in southeastern Mexico is probably due to the fact that the crop has been produced for a long time.

The analysis of molecular variance (AMOVA) revealed that all sources of variation were statistically significant (p<0.005). Out of the total variation observed, 59.0% could be attributed to genetic differentiation among landraces, while 41.0% of the variation was found among individuals within varieties (Table 6). These results indicated a substantial level of genetic differentiation among the analyzed varieties, highlighting the distinct genetic characteristics present within the landraces. This result contrasts with that reported by Nkhoma *et al*., (2020), who obtained a value of 8.0% between populations, while within-population variation accounted for 92.0% of the total variance of 90 genotypes using SNP markers.

The results obtained in this study indicate that the difference between landraces is high and that populations are more homogeneous. As Ghalmi *et al*., (2010) noted, the landraces of crops with self-pollinated reproduction systems such as cowpea tend to have low intravarietal variability and this may be due to the traditional management practices that producers give to the crop, since they use the seeds of the harvest for subsequent cycles, a practice that would reduce genetic diversity.

Genetic relationships: The genetic relatedness of the varieties was analyzed to identify the optimum number of populations (K). Analysis using Evanno's method grouped the tested varieties into two main groups, with the highest ΔK value occurring at K= 2 (Fig. 3). Genetic analysis using ISSR markers categorized the test populations into two genetic groups.

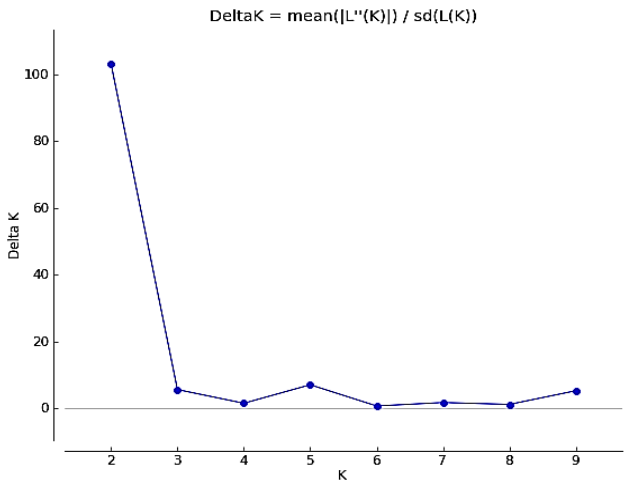

Fig. 3. Estimation of the number of genetic groups of 14 cowpea varieties based on the ΔK index with K values of 2 to 9.

Figure 4 shows the ancestry coefficients of the 182 samples analyzed from the 14 landraces studied. Two genetically differentiated groups were observed. The first group was formed by eight landraces (OXC01, HEC02, HEC03, OXC04, 0XC05, CHE06, JMM09, and JMM10), and the second group was formed by six varieties (PET07, PET08, PET11, CHE12, FCP13 and HAL14). The clusters depicted in Figure 3 showed good agreement with the ancestry analysis (Fig. 4) and resembled the groups formed in the dendrogram (Fig. 5). However, the ancestry analysis indicated gene flow among the landraces CHE06, PET07, and PET08, with low levels in the varieties CHE05 and JMM09, as reflected in the dendrogram. The populations where there was greater gene flow were generally from the same municipality or nearby; therefore, it is likely that the self-pollinating nature of the cowpea as well as environmental influences may contribute to the limited gene flow (Pasquet *et al*., 2008; Boukar *et al*., 2020). The gene flow observed within the cowpea landraces indicates limited connectivity and dispersal of genetic material between populations. This restricted gene flow can lead to genetic differentiation and population fragmentation, and thus could affect genetic diversity and the ability of species to adapt to environmental changes.

The UPGMA dendrogram (Fig. 5) grouped the 14 varieties into two main clusters. Group one consisted of individuals from the landraces OXC01, HEC02, HEC03, OXC04, JMM09 and JMM10. Group two consisted of individuals of the varieties OXC05, CHE06, PET07, PET08, PET11, CHE12, FCP13, and HAL14, and these were the most divergent of the accessions. This indicates that cowpea landraces grouped together share genetic similarities, whereas those categorized into separate groups may demonstrate increased diversity. The significant resemblance observed among cowpea varieties is likely a result of self-pollination, as suggested by Padulosi (1993). Thus, the landraces OXCO5 and CHE06 of group two were not included in the same group previously defined by the results of the ancestry analysis, since they were in the second group; the rest of the varieties were included in each group formed by the results of the ancestry analysis. Therefore, the more distant the varieties are from each other, the greater the possibility of higher genetic diversity (Igwe *et al*., 2017). The distribution of genetic diversity among the varieties observed in this study may indicate the existence of natural selection, founder effects, or the effects of genetic drift in different populations.

The dendrograms for the morphological and genotypic variables showed significant differences, indicating high diversity among the populations and the formation of two distinct groups from each analysis. However, the landraces were distributed across different groups, with only about 20% of the varieties sharing groups in both types of diversity analyses. The difference in results between diversity studies that used morphological and molecular markers may be due to the significant impact of the environment on the variable expression of morphological traits.

In a similar study, Ghalmi *et al*., (2010) did not find a significant correlation between genetic and morphological data when using RAPD markers, but there was a weak but significant correlation when using ISSR markers (R = 0.27). Piña-Escutia *et al*., (2010) conducted an analysis of morphological and genetic diversity in *Tigridia pavonia* using 21 morphological markers and five ISSR molecular markers. The dendrograms of the morphological and molecular markers were similar, indicating a positive correlation between the analyses.

These studies emphasize the significance of conducting analyses of morphological and genetic diversity in a complementary manner. Each approach provides valuable information on population variability. The correlation between genetic distance and environmental factors highlights the influence of the latter on the expression of morphological and genetic variability.

Table 6. Analysis of molecular variance (AMOVA) of 14 cowpea landraces grown in southeastern Mexico.

Source of variation	Degrees of freedom	Sum of squares	Mean square	Estimated variation	Percentage of variation
Between varieties	13	71.54	5.50	0.40	59.0 %
Within varieties	168	46.15	0.27	0.27	41.0 %
Total	181	117.70		0.67	100 %

Conclusions

The results obtained in this work contribute to our knowledge of genetic diversity in varieties of cowpea cultivated in the Yucatan Peninsula, Mexico, by including varieties collected in a region that had not been explored in previous studies. Most of the landraces had a similar magnitude of phenotypic diversity. Likewise, diversity among varieties was higher than diversity within varieties, and relatively high similarity was observed among cowpea genotypes for most morphological characteristics. The PCA grouped the populations studied into two main clusters, being similar both in ancestry analysis and in the dendrogram, although there was a low level of gene flow. The varieties FCP, PET07, and PET08 were the most genetically divergent and high yielding and thus should be prioritized for conservation in situ and given special attention for national conservation germplasm programs.

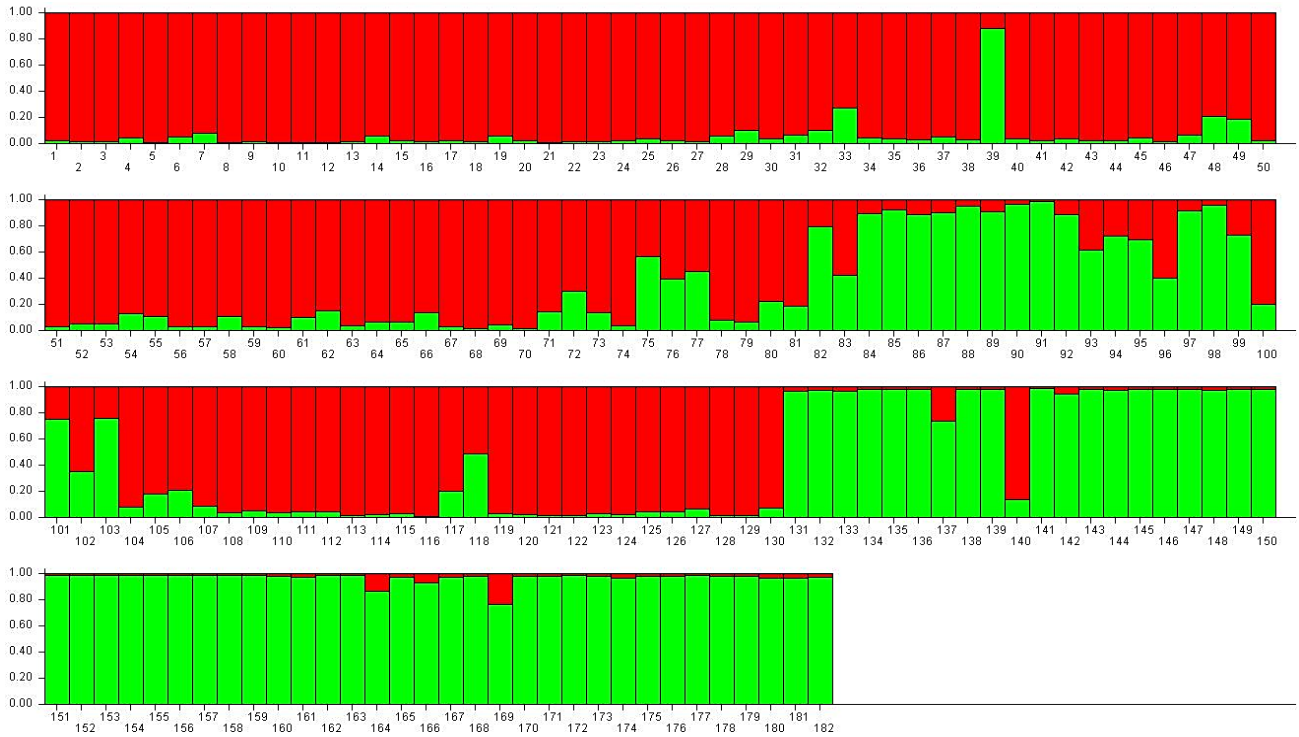

Fig. 4. Assignment analysis of individuals among 14 cowpea varieties showing the formation of two genetic groups and the levels of shared ancestry among the 182 individuals evaluated.

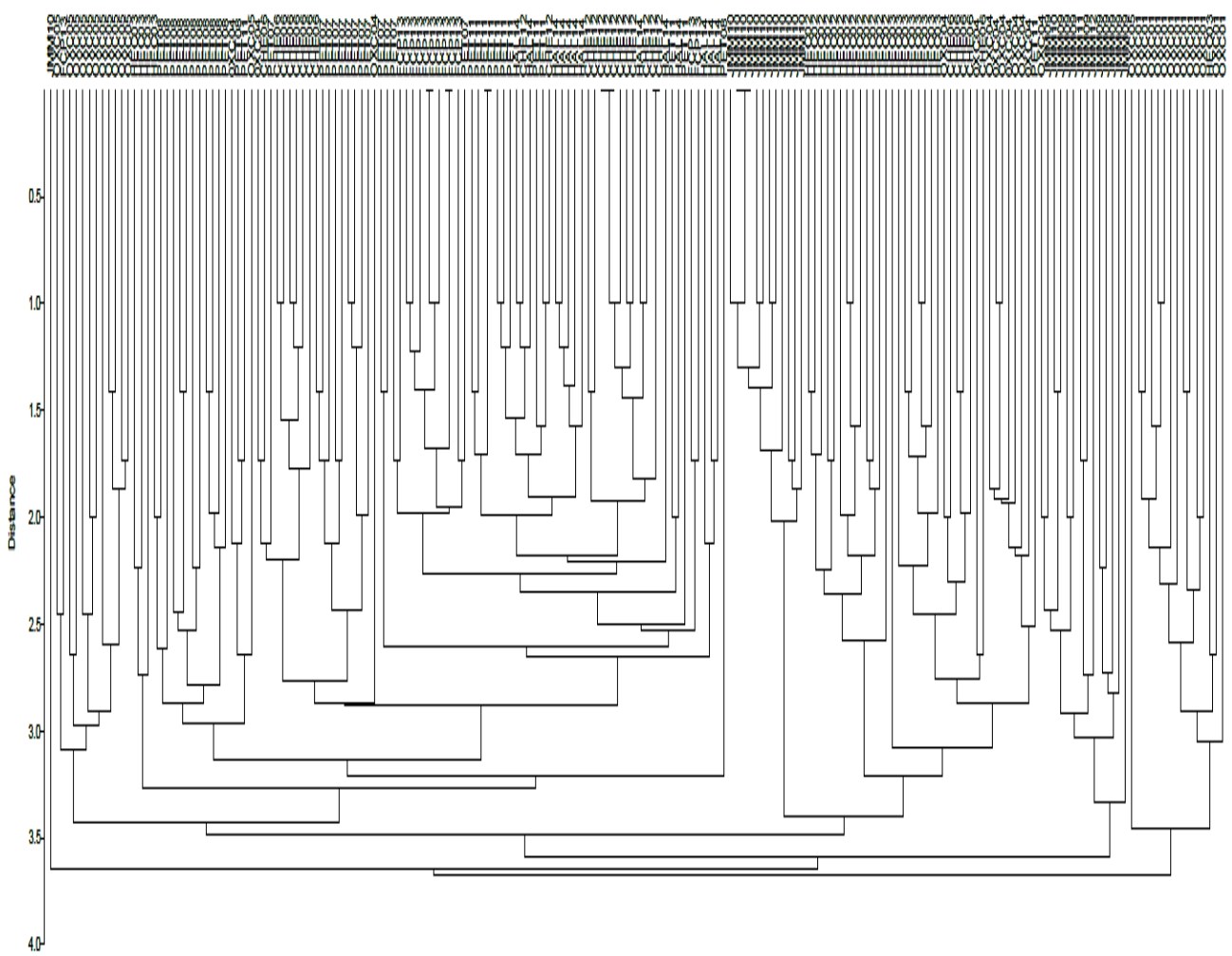

Fig. 5. UPGMA dendrogram of 14 cowpea varieties, obtained with Euclidean distance as the similarity measure.

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