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CLADODE MORPHOLOGY AND AFLP PATTERNS CAN HELP IN THE IDENTIFICATION OF MEXICAN CACTUS GENOTYPES (*OPUNTIA* SPP.)

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Abstract

Fifty genotypes of prickly pear (*Opuntia* spp.) native to Mexico were analyzed based on cladode morphology and AFLP patterns. Genotypes grouped by cladode morphology were categorized as "spiny" and "few-spiny." An image catalog was also generated, and length/width ratios were calculated as the primary identifier. Molecular analysis revealed species-specific relationships by size and degree of domestication. Species such as *O. albicarpa* and *O. megacantha* were located at the extremes, while *O. ficus-indica* occupied a central position in the dendrogram. Two large groups were formed: Group I, which included six genotypes (three *O. albicarpa* and three *O. ficus-indica*) with similar fruit color (yellow). Group II included 44 genotypes subdivided into two groups. Subdivision 1 included only three genotypes of *O. albicarpa* and *O. ficus-indica* that matched the cladode's broad elliptical shape, large size, and light green color. Subdivision 2 included genotypes with diverse shapes, from elliptical and oval to rhomboid, but characterized by the presence of medium to large spines. The most closely related *O. ficus-indica* genotypes had values greater than 0.8, suggesting an intention to generate species with fewer spines. In summary, this work could be used as a reference to relate the morphological characteristics of cladodes with AFLP for better identification of *Opuntia* genotypes.

Key words: Characterization; Germplasm; DNA; Morphology; *Opuntia*

Introduction

In Mexico, approximately 60% of the areas correspond to arid and semi-arid regions, where few plant species tolerant to water stress coexist, producing food such as fruits and vegetables, which should be characterized and exploited in a sustainable manner (Pontifes *et al*., 2018). From these, outstanding species should be established in germplasm banks to ensure permanence and sustainable utilization (Nguyen & Norton, 2020). In this context, establishing lines of research for the conservation and later use of Mexico´s plant resources, but rich in biological diversity, is considered a fundamental task in food production programs (Falcón-Brindis *et al*., 2021; Silos-Espino *et al*., 2023). There are descriptions of commercial cultivars of nopal and outstanding genotypes, including those frost-tolerant (Muñoz *et al*., 1995; Mondragón & Pérez, 1996; Reyes Agüero *et al*., 2005; Gallegos Vázquez *et al*., 2012; Valdéz *et al*., 1997; Parish & Felker, 1998). Their identification has been based on morphology, which can vary due to environmental effects, and errors of appreciation in the descriptors are frequent, especially in relation to qualitative characters, so it is necessary to support the species identification with additional techniques To assist in the identification process, there are molecular markers such as isoenzymes (Chessa *et al*., 1997, Uzun, 1997), RAPDs (Wang *et al*., 1998), RAPDs & ISSR (Luna-Páez *et al*., 2007; Valadez-Moctezuma *et al*., 2014; Ganopoulos *et al*., 2015) and AFLPs (Labra *et al*., 2003). The authors working with molecular techniques proposed that *O. ficus-indica* arose from *O. megacantha*. For example, García-Zambrano *et al*., (2018) analyzed 100 genotypes of *Opuntia* and did not find a convincing relationship with the known taxonomy. Contributing to the above, Espinoza-Sánchez *et al*., (2014) analyzed 85 *Opuntia* genotypes (both wild and cultivated) and found a distribution gradient in their species dendrogram for their best attributes (cladode, fruit size, and yellow to red fruit color). From the listed molecular techniques, the AFLP is recognized for its reliability and consistency and has the ability to identify several polymorphic loci across the genome, making it useful for genetic fingerprinting or mapping (Besse, 2021). Therefore, the objective of this project was to analyze 50 cactus genotypes of 5 species (*O. megacantha*, *O. albicarpa*, *O. ficus-indica*, *O. tezontepecana,* and *O. chaveña*) to estimate genetic diversity within the Germplasm Bank of the Instituto Tecnológico El Llano Aguascalientes (ITEL).

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Material and Methods

Cactus species: Fifty cactus pears genotypes were analyzed: *O. ficus-indica* (21), *O. albicarpa* (17), *O. megacantha* (10), *O. tezontepecana* (1), and *O. chaveña* (1) established three years ago in the cactus germplasm bank of ITEL (21°49′07″N; 102°05′53″W). Additionally, these species are registered by the Servicio Nacional de Inspección y Certificación de Semillas (SNICS) of the Secretaría de Agricultura y Desarrollo Rural (SADER) in Mexico.

Qualitative and quantitative characteristics of the cladodes: Ten descriptors (Table 1) were evaluated exclusively on cladodes (length, width, length/width ratio, shape, thickness, color), presence of pubescence, number of areoles in the center, color of areoles, density of areoles per cladode, number and size of spines, as well as the size and number of spines per areole according to the graphic descriptor for the varieties of cactus pear cactus and xoconostle (*Opuntia* spp.) proposed by Gallegos-Vázquez *et al*., (2008). The studied genotypes were adult plants established in open field conditions at the Germplasm Bank of ITEL.

DNA extraction: DNA extraction was performed following the UltraQuick-SOYA protocol, recommended for a wide range of plant species. The concentration of DNA obtained was quantified with a Nanodrop 2000 spectrophotometer (Thermo Scientific UV-Vis) at an absorbance of 260/280 nm, adjusting the concentration to 10 ng/ µL.

Fragment amplification conditions: AFLP analyses were performed in the DNA and Genomics laboratory of the CNRG of INIFAP in Tepatitlán de Morelos, Jalisco, according to the methodology of Vos *et al*., (1995) and LI-COR (2010).

Nine primer combinations were used: Mse I-CAA/Eco RI-AAC, Mse I- CAA/Eco RI-AGC, Mse I-CTC/Eco RI-AAG, Mse I-CAC/Eco RI-ACA, Mse I-CAC/Eco RI-AAG, Mse I-CAG/Eco RI-AAG, Mse I-CAA/Eco RI-ACC, Mse I-CTA/Eco RI-AAG, Mse I-CAG/Eco RI-ACC. Digestion was performed in a reaction tube containing 6.82 µL of nuclease-free water, 2 µL of CutSmart 1X Buffer, 0.125 µL of EcoRI (2.5 U), 0.05 µL of MseI (2.5 U), 1 µL of NaCl (50mM), 10 µL of genomic template DNA, to complete a final volume of 20 µL, at 37ºC for 3 h and 72ºC for 10 min. Ligation was performed in a reaction tube containing 4 µL of nuclease-free water, 1.5 µLof T4 DNA Ligase 1X Buffer, 1 µL of MseI Adapter (50 µM), 1 µL of EcoRI Adapter (µM), 0.01 µL of T4 DNA Ligase (100 U/ µL), 7.5 µL of Digestion Product, to complete a final volume of 15 µL, at 37ºC for 3 h and 72ºC for 15 min.

Preamplification was performed in a reaction tube containing 1.76 µL of nuclease-free water, 10 µL of RedTaq 2X, 1.12 µL of MseI+C (10 µM), 1.12 µL of EcoRI+A (10 µM), 6 µL of Ligation Product, to complete a final volume of 20 µL with one cycle of 94ºC for 2 min, 20 cycles of 94ºC for 2 min, 72ºC for 1 min and 7 min at 72ºC; samples were diluted using a dilution factor of 0.01 (1 µL with 99 µL of ultrapure water). Selective amplification was performed in a reaction tube containing 6.5 µL of RedTaq 2X, 0.32 µL of MseI (CAA, CAC, CTA, CTC, CAG) 10 µM, 0.32 µL of EcoRI (AAG, ACC, AGC, AAC, ACA) 10 µL, 6.1 µL of preamplification product, to complete a final volume of 13 µL, cycled at 94ºC for 5 min and 30 cycles of 94ºC for 20 sec and 30 cycles for 1 min at the same temperature, 72ºC for 90 sec and 30 min at 72ºC. Subsequently, they were run on polyacrylamide gels at 250 Volts for 1:20 min and stained with silver nitrate.

Morphological and AFLPs data analysis: Based on the morphological data, averages of quantitative and qualitative traits were estimated, and a matrix was constructed in Microsoft Excel. This matrix was imported into Past 4.13 to perform multivariate analyses, including Principal Component Analysis (PCA), and to generate the corresponding dendrogram. For the AFLPs data, the total number of amplified bands was counted, and a binary matrix was created in Excel. These data were then used to construct dendrograms by applying the Dice coefficient with the UPGMA method using NTSYS software.

Results and Discussion

Interpretation of cladode morphological data: The dendrogram was constructed from the analysis of morphological characters of cladodes in Opuntia spp. clearly shows two main clusters: Group I, composed of spiny genotypes, and Group II, which groups those with few spines (Fig. 1). This clear distinction aligns with previous findings that identify spine presence as a key variable in the morphological characterization of the genus (Peña-Valdivia *et al*., 2008).

Within Group I, four subgroups are identified: subgroups *a* and *b* include genotypes such as *O. albicarpa* and *O. megacantha*, characterized by medium to large cladodes with elliptical or oval shapes and intermediate green coloration. Subgroup *c* consists of *O. ficus-indica* genotypes with few spines, primarily used as forage, having large, light green cladodes of elliptical or rhomboidal shape, consistent with descriptors used in morphological diversity studies conducted in Portugal and South Africa. Finally, subgroup *d* groups the few wild genotypes (*O. tezontepecana* and *O. chaveña*) along with some medium-sized spiny types, suggesting distinct genetic linages highly relevant for conservation.

Group II also presents subdivisions reflecting variability in cladode size, shape, and color, linked to ecological adaptations and agronomic uses. Overall, these patterns are consistent with those observed in Morocco by El Kharrassi *et al*., (2017), who analyzed 124 accessions using 10 morphological descriptors and observed clusters that did not strictly correspond to species or geographic origin, suggesting the influence of domestication and human selection. Additionally, complementary analyses such as PCA have demonstrated coherence between morphological and genetic clustering in other *Opuntia* contexts, as reported by Louati *et al*., (2019).

Studies conducted in various parts of the world, including Mexico, Tunisia, Morocco, Portugal, and South Africa, have documented similar morphological clustering patterns in *Opuntia* genotypes, supporting the consistency of these classification criteria across diverse geographic contexts.

In conclusion, the dendrogram confirms that the presence or absence of spines is a determining morphological characteristic in *Opuntia*. Moreover, the identified subgroups reflect real variation in cladode shape, size, and color, associated with domestication, regional adaptations, and agricultural use.

Table 1. Descriptors and parameters used for the description of 50 species of cactus pear (*Opuntia*).

Descriptor	Measurements
Cladode length	Centimeters in mature stalks
Cladode width	Centimeters in mature stalks
Length/width ratio	Length/width from 1 to 9, where: (<1.38=very small) (>2.43=very large)
Cladode shape	Narrow elliptic, medium elliptic, broad elliptic, circular, rhomboid, narrow ovate, and broad ovate
Cladode thickness	Millimeters in the central part
Cladode color	From 1 to 5, where: 1=yellow green, 2= light green, 3= medium green, 4= dark green, and 5= bluish green
Number of areoles in the center	From 1 a 9, where: (<5.88=very few) (>9.73=very abundant)
Color of areoles	From 1 to 4, where: 1=gray, 2= yellow brown, 3= brown, and 4=black
Number of spines per areole	1 to 9 where: (<1.28=none or very few) (>5.22=very abundant)
Spine size	Centimeters of the largest areolar spine

Cladode

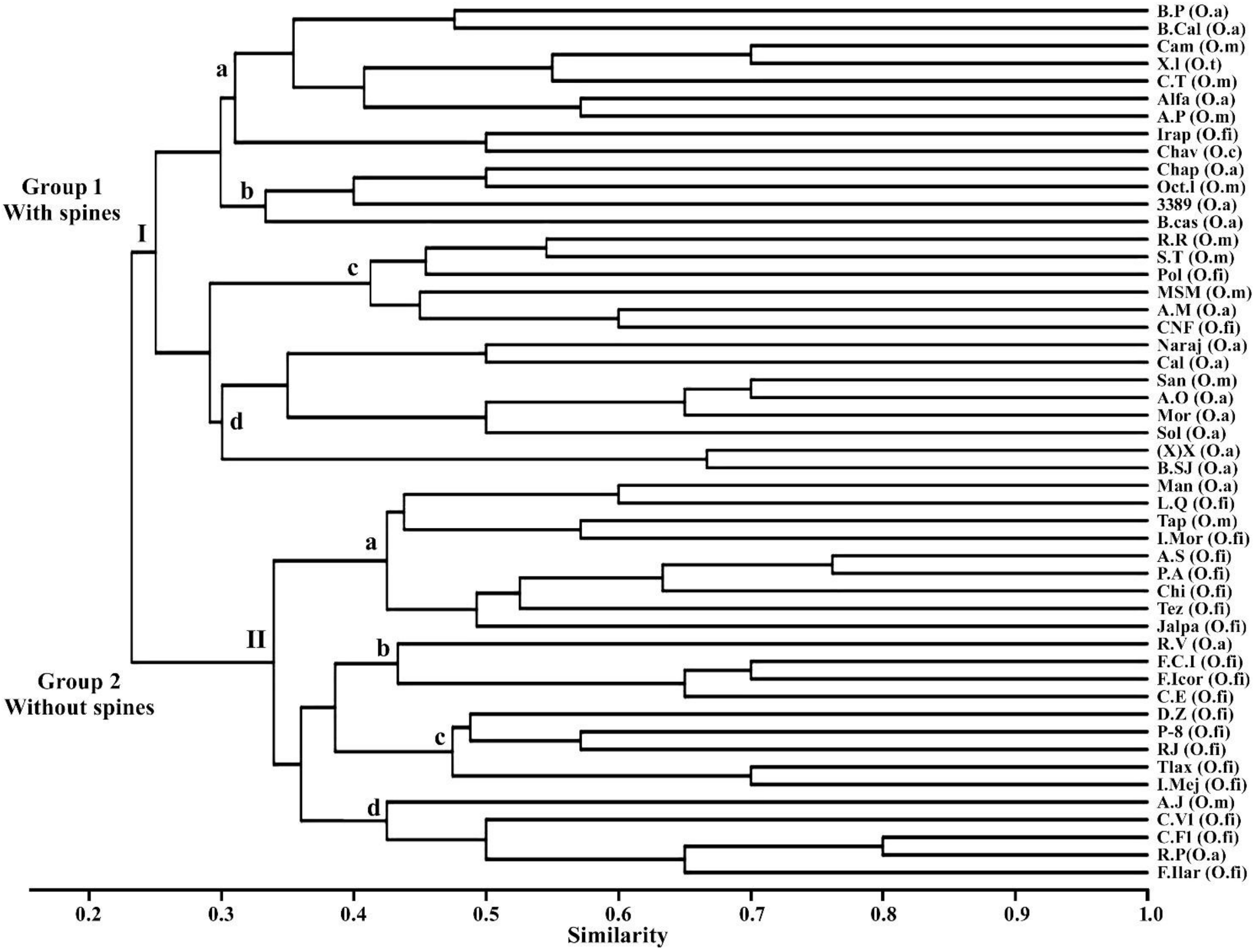

Fig. 1. Dendrogram generated from the measurement of qualitative and quantitative morphological descriptors of the cladode in *Opuntia* spp. using the Dice coefficient and UPGMA grouping.

Principal Component Analysis (PCA): In the scree plot, a pronounced drop in explained variance was observed between the first two principal components (PC1 = 36% and PC2 = 20%). From PC3 to PC11, each accounted for less than 10% of the variance, delineating a clear inflection point (Fig. 2). This trend indicates that the first two components together capture most of the morphological variability (approximately 56-57%), whereas the subsequent components describe the main morphological differences among *Opuntia* genotypes.

The traits with the highest loadings on these components were cladode size and shape, spine presence or absence, and cladode color and thickness. These variables emerged as key features for discriminating genotypes within the genus *Opuntia*. In line with these results, Mondragón-Jacobo *et al*., (2001) reported that in a morphological study of *O. ficus-indica*, the first two principal components explained between 55 and 60% of the variability, with fruit size, cladode thickness, and number of areoles being the most influential traits.

Our analysis revealed two primary patterns of differentiation. The number of spines per areole and the length of the longest spine showed strong positive loadings on Components 1 and 2, emphasizing their key role in distinguishing highly spiny genotypes. Conversely, the cladode length-to-width ratio had a strong negative influence on Component 2, indicating an independent contribution to overall morphological variability (Fig. 3).

These results aligned with Peña-Valdivia *et al*., (2008), who used PCA to discriminate *Opuntia* accessions based on spine presence and cladode size and shape, identifying morphological combinations that formed coherent clusters. Likewise, Dev *et al*., (2024) reported that over 77% of the variance in 30 morphological and forage quality traits of *Opuntia* and *Nopalea* was captured by the first ten components, with cladode size and structural traits in genotypes differentiation and demonstrated the power of multivariate analysis for identifying key morphological features critical for *Opuntia* spp. characterization.

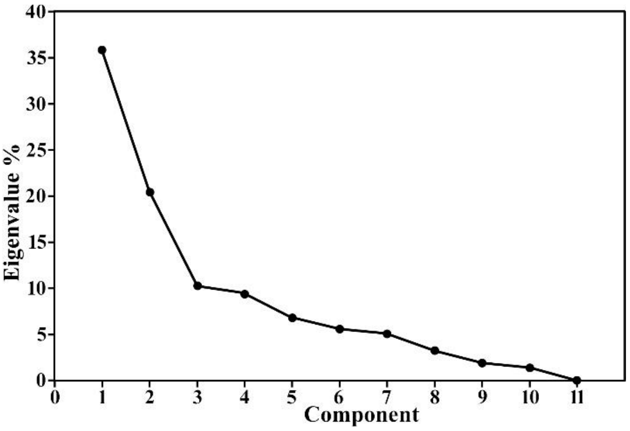

Fig. 2. Scree plot showing the principal components (PCs) on the X-axis and the percentage of variance explained (%) on the Y-axis, facilitating the identification of the most relevant PCs for multivariate analysis.

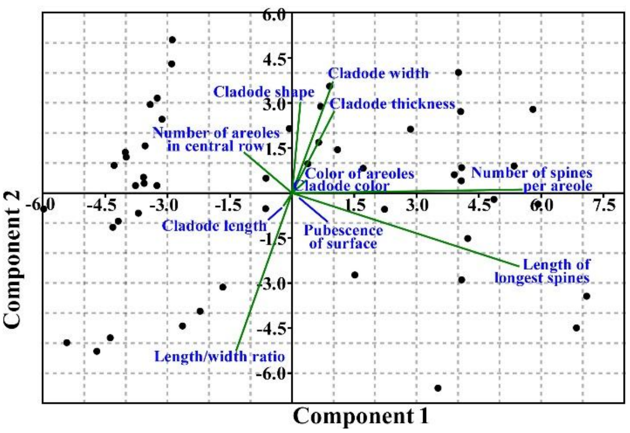

Fig. 3. Principal Component Analyses (PCA) of cladode morphological traits in *Opuntia* genotypes. Arrow represents the contribution and direction of the evaluated morphological variables (cladode width, thickness, shape, color, areole color and number, length/width ratio, number of spines per areole, and length of the longest spine) on the first two principal components. Dots represent the evaluated genotypes.

General characteristics of the species according to their cladodes: Table 2 presents the genotypes classified as "spiny", while Table 3 includes those with "few spines". A total of 20 genotypes with few or small spines (<11.69 mm) were identified, among which the varieties Rojo Vigor and Rojo Pelón (*O. albicarpa*), as well as Amarilla Salinas and La Quemada (*O. ficus-indica*), stood out. On the other hand, 30 genotypes exhibited spines, with seven of them notable for having abundant and large spines (31-35 mm), including Blanca Pepina and Blanca San José (*O. albicarpa*).

Regarding qualitative variables, cladode shape was the most dominant trait: elliptical and rhomboidal forms were the most frequent (Fig. 4). This characteristic has been associated with selection preferences in cultivars intended for consumption as "nopalito", where thinner cladodes are favored due to their superior culinary quality (Fig. 5).

In fact, the genotype Chicle (*O. ficus-indica*) exhibited the thinnest cladode in the collection, with an average thickness of 16 mm; similar values were observed in Alfajayucan and Copena F1. These findings are consistent with previous studies that highlight thickness as an indicator of food quality and ease of handling in nopal cultivars (Majure & Ervin, 2007). Moreover, no pubescence was detected in any of the genotypes, which aligns with other analyses where this trait is rarely observed in cladodes used for consumption or forage purposes (Bougdaoua *et al*., 2022).

AFLPs profiles of cactus pear (*Opuntia* spp.): The number of fragments generated by the different primer combinations in the 50 genotypes analyzed ranged from 13 to 221, with the lowest and highest values corresponding to the genotypes Amarilla Salinas and P-8 (*O. ficus-indica*), respectively. All genotypes reacted with the five AFLP primer pairs, generating fragments ranging from 12 to 221 bp, with a total of 155 polymorphic fragments detected. The bands with the highest resolution were concentrated in the range of 100 to 1000 bp.

The M-CAC/E-AAG combination provided the best resolution pattern for all genotypes (Fig. 6) being the most informative among those evaluated (Key II in Table 6). The variability observed in amplification may be related to the amount of mucilage present in each genotype, which in *Opuntia* is a heterogeneous polysaccharide with high viscosity and compounds that inhibit enzymatic reactions (Lorenzo *et al*., 2017; Van Rooyen *et al*., 2004).

For instance, the lanes corresponding to the genotypes Amarilla Oro (*O. albicarpa*) and Amarilla Salinas showed low resolution with the indicated combination; however, other combinations allowed for fragment detection (Tables 4 and 5), suggesting that the presence of mucilage or secondary metabolites may have affected amplification.

In studies involving DNA extraction from tissues rich in mucilage, it has been documented that high viscosity and the presence of polysaccharides interfere with DNA purity and concentration, requiring specialized protocols that include washing steps with NaCl to reduce viscosity (Choudhary *et al*., 2016).

Tables 4 and 5 show the number of fragments amplified by each primer combination in different *Opuntia* genotypes. On average, 42.4 fragments were amplified per combination, a value lower than that reported by Espinoza-Sánchez *et al*., (2014) in studies with Mexican nopal accessions, but higher than that found by Labra *et al*., (2003), who recorded 169 total bands, of which 131 (77.5%) were polymorphic when using AFLP in Mediterranean cacti.

García-Zambrano *et al*., (2018) also indicated that the M-CAC/E-AAG primer combination produced a high number of potentially polymorphic fragments in *Opuntia* spp., whereas the M-CAG/E-ACG combination was the least efficient in terms of total bands obtained.

In contrast, in the present study, the M-CAA/E-ACC combination generated the highest number of amplified fragments, both in spiny genotypes (1,172 bands) and in those with few spines (681 bands). Conversely, the lowest amplification was observed with the M-CTA/E-AAG combination, with 724 and 416 fragments, respectively.

These results highlight the variability in the number of amplified fragments depending on the primer combination used, emphasizing the importance of carefully selecting primer pairs in genetic diversity studies using AFLP. The M-CAA/E-ACC combination proved to be the most efficient in detecting polymorphism in *Opuntia* spp., making it a valuable tool for future genetic and taxonomic analyses of this species.

Table 2. Species of cactus pear (*Opuntia* spp.) "with spines" and grouped from largest to smallest cladode size.

Genotype	Species	L/A Ratio	Geometric shape	Color
X(X)	*O. albicarpa*	9	Narrow ovate	1
Italiano Morado	*O. ficus-indica*	7	Broad elliptical	3
Blanca San José	*O. albicarpa*	6	Narrow ovate	1
Morada San Martín	*O. megacantha*	6	Broad elliptical	3
Amarilla 3389	*O. albicarpa*	6	Broad elliptical	3
Blanca Pepina	*O. albicarpa*	5	Broad elliptical	3
Blanca de Castilla	*O. albicarpa*	5	Rhomboid	3
Tapona	*O. megacantha*	5	Broad elliptical	1
Blanca Calera	*O. albicarpa*	5	Broad elliptical	3
Rubí Reyna	*O. megacantha*	4	Narrow ovate	3
Sangre de Toro	*O. megacantha*	4	Medium elliptic	3
Copena Torreoja	*O. megacantha*	4	Broad elliptical	3
Sandía	*O. megacantha*	4	Narrow ovate	1
Chapeada	*O. albicarpa*	4	Rhomboid	3
Morada	*O. albicarpa*	4	Narrow ovate	3
Calabaza	*O. albicarpa*	3	Broad elliptical	1
Xoconostle de invierno	*O. tezontepecana*	2	Broad elliptical	3
Alfajayucan	*O. albicarpa*	2	Broad ovate	3
Amarilla Oro	*O. albicarpa*	2	Narrow ovate	1
Naranjona	*O. albicarpa*	2	Broad elliptical	1
Camueza	*O. megacantha*	2	Broad elliptical	3
Amarilla Plátano	*O. megacantha*	2	Broad ovate	3
Polotitlán	*O. ficus-indica*	2	Broad ovate	1
Amarilla Montesa	*O. albicarpa*	2	Rhomboid	3
Solferino	*O. albicarpa*	2	Narrow ovate	1
Mango	*O. albicarpa*	2	Rhomboid	1
Octubreña ITA 20	*O. megacantha*	2	Rhomboid	3
CNF	*O. ficus-indica*	2	Broad elliptical	3
Irapuato	*O. ficus-indica*	1	Narrow ovate	3
Chaveña	*O. chavena*	1	Broad ovate	3

Table 3. Species of cactus pear (*Opuntia* spp.) "with few spines" and grouped from largest to smallest cladode size.

Genotype	Species	L/A Ratio	Geometric shape	Color
Copena V1	*O. ficus-indica*	9	Narrow elliptical	3
Rojo Pelón	*O. albicarpa*	9	Medium elliptical	3
Copena F-1	*O. ficus-indica*	9	Medium elliptical	3
Diabétes Zacatecas	*O. ficus-indica*	5	Rhomboid	3
Jalpa	*O. ficus-indica*	5	Broad elliptical	1
Roja Jalpa	*O. ficus-indica*	4	Rhomboid	3
Chicle	*O. ficus-indica*	4	Narrow ovate	1
P-8	*O. ficus-indica*	3	Rhomboid	3
Rojo Vigor	*O. albicarpa*	3	Broad elliptical	1
Forrajero Chapingo ITA 20	*O. ficus-indica*	3	Narrow ovate	3
Forrajero ITA 20 (Corto)	*O. ficus-indica*	3	Broad elliptical	3
Cero espinas	*O. ficus-indica*	3	Broad elliptical	3
Pabellón Amarilla	*O. ficus-indica*	2	Broad elliptical	1
La Quemada	*O. ficus-indica*	2	Rhomboid	1
Tlaxcalcingo	*O. ficus-indica*	2	Rhomboid	2
Italiano Mejorado	*O. ficus-indica*	2	Broad ovate	3
Forrajero ITA 20 (Largo)	*O. ficus-indica*	2	Broad ovate	3
Amarilla Salinas	*O. ficus-indica*	2	Broad ovate	1
Amarilla El Jarro	*O. megacantha*	1	Broad ovate	3
Tezontepec	*O. ficus-indica*	1	Narrow ovate	1

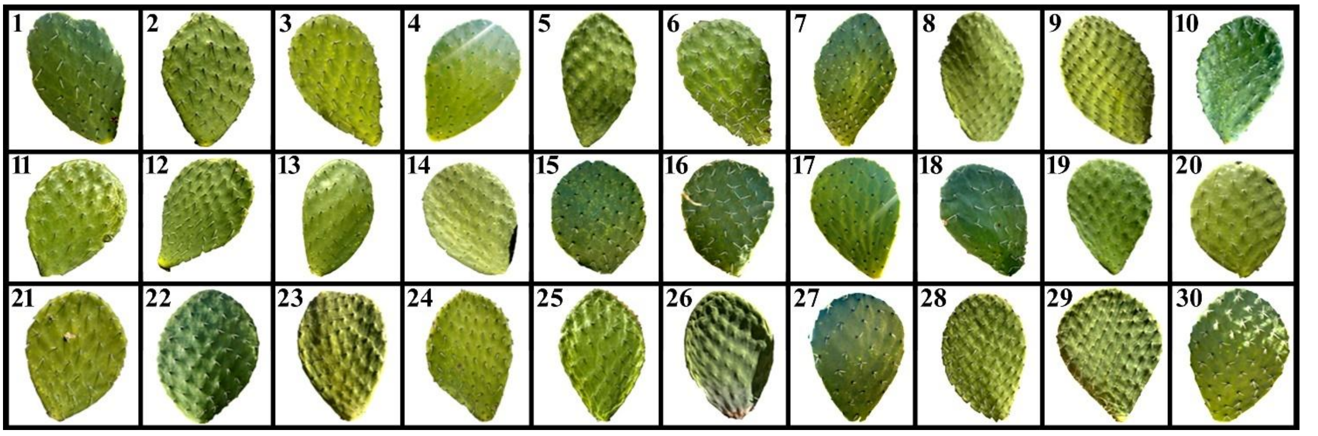

Fig. 4. Cactus pear species (*Opuntia*) identified "with spines" in the cladodes: 1, XX (*Opuntia albicarpa*); 2, Italiano Morado (*O. ficus-indica*); 3, Blanca San José (*O. albicarpa*); 4, Morada San Martin (*O. megacantha*); 5, Amarilla 3389 (*O. albicarpa*); 6, Blanca Pepina (*O. albicarpa*); 7, Blanca de Castilla (*O. albicarpa*); 8, Rubi Reyana (*O. megacantha*); 9, Sangre de Toro (*O. megacantha*); 10, Copena Torreoja (*O. megacantha*); 11, Sandia (*O. megacantha*); 12, Chapeada (*O. albicarpa*); 13, Morada (*O. albicarpa*); 14, Calabaza (*O. albicarpa*); 15, Xoconostle (*O. tezontepecana*); 16, Alfajayucan (*O. albicarpa*); 17, Amarilla Oro (*O. albicarpa*); 18, Naranjona (*O. albicarpa*); 19, Camuesa (*O. megacantha*); 20, Amarilla Plátano (*O. megacantha*); 21, Polotitlán (*O. albicarpa*); 22, Amarilla Montesa (*O. albicarpa*); 23, Solferino (*O. albicarpa*); 24, Mango (*O. albicarpa*); 25, Octubreña ITA20 (*O. megacantha*); 26, CNF (*O. ficus-indica*); 27, Irapuato (*O. ficus-indica*); 28, Chaveña (*O. chaveña*); 29, Tapona (*O. megacantha*); 30, Blanca Calera (*O. albicarpa*).

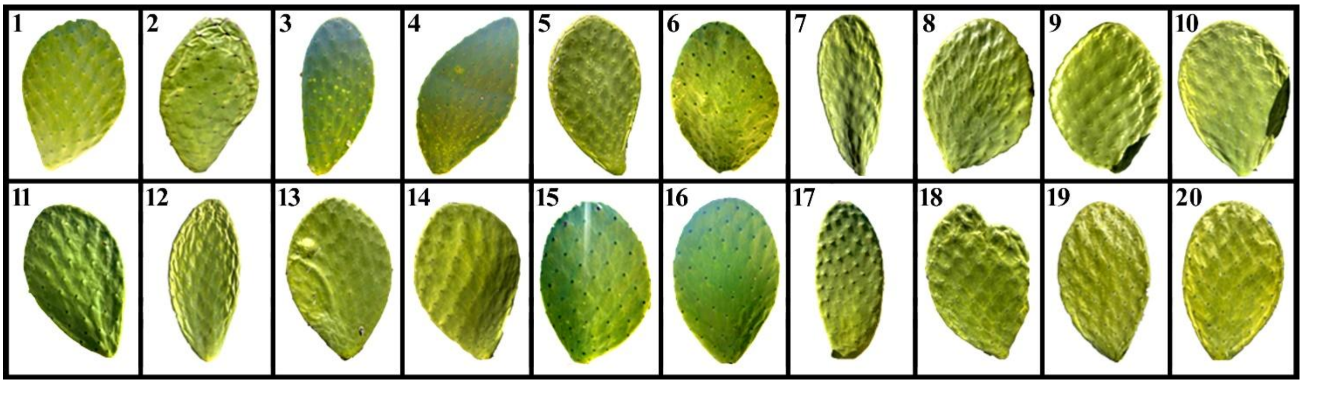

Fig. 5. Cactus pear species (*Opuntia* spp.) identified as "few spines" in the cladodes: : 1, Copena V1 (*Opuntia ficus-indica*); 2, Rojo Pelón (*O. albicarpa*); 3, Copena F1 (*O. ficus-indica*); 4, Diabetes Zacatecas (*O. ficus-indica*); 5, Jalpa (*O. ficus-indica*); 6, Roja Jalpa (*O. ficus-indica*); 7, Chicle (*O. ficus-indica*); 8, P-8 (*O. ficus-indica*); 9, Rojo Vigor (*O. albicarpa*); 10, Forrajero C ITA 20 (*O. ficus-indica*); 11, Forrajero Corta ITA 20 (*O. ficus-indica*); 12, Cero Espinal (*O. ficus-indica*); 13, Pabellón Amarilla (*O. ficus-indica*); 14, La Quemada (*O. ficus-indica*); 15, Tlaxcalalcingo (*O. ficus-indica*); 16, Italiano Mejorado (*O. ficus-indica*); 17, Forrajero L ITA 20 (*O. ficus-indica*); 18, Amarilla Salinas (*O. ficus-indica*); 19, Amarilla El Jarro (*O. megacantha*); 20, Tezontepec (*O. ficus-indica*).

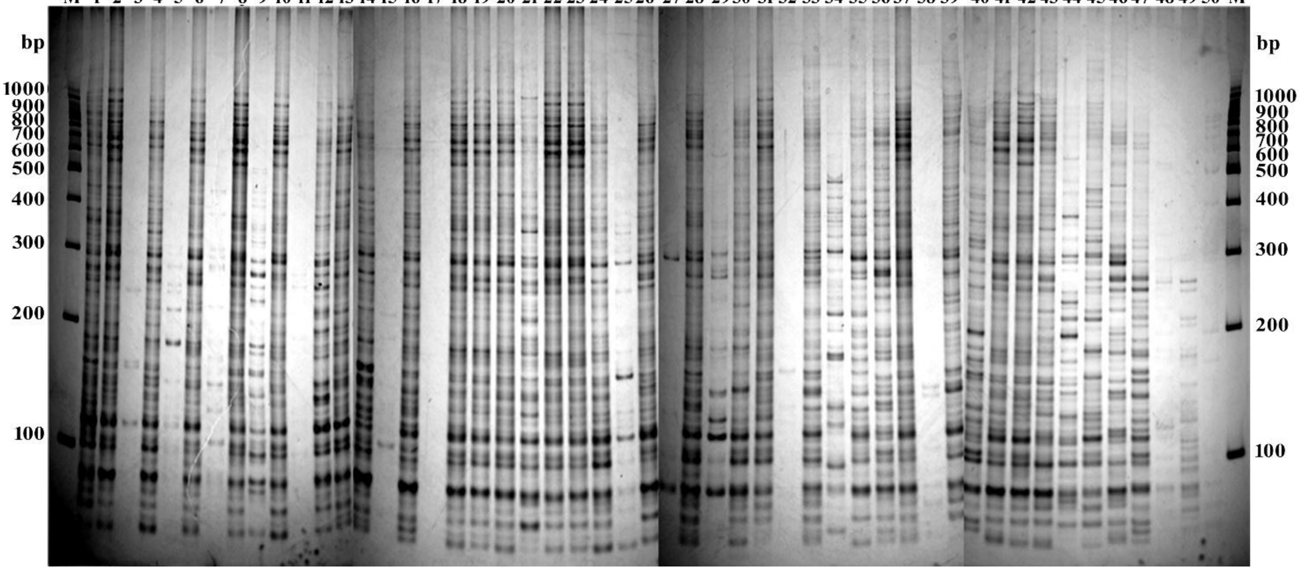

Fig. 6. Banding profiles of one the five AFLP combinations (M-CAC/E-AAG) in the 50 *Opuntia* spp. DNA samples.

Table 4. DNA fragments amplified by AFLPs in cactus pear (*Opuntia* spp.) were identified as "with spines".

Genotype	Primers					
	A	B	C	D	E	Total
X (X)	41	39	46	33	39	198
Italiano Morado	15	23	8	4	2	52
Blanca San José	41	43	46	33	33	196
Morada San Martín	48	44	50	34	35	211
Amarillo 3389	44	46	47	21	30	188
Blanca Pepina	42	38	44	35	40	199
Blanca de Castilla	48	45	47	35	34	209
Rubí Reyna	13	19	24	8	16	80
Sangre de Toro	40	42	46	32	39	199
Copena Torreoja	36	40	48	33	43	200
Sandía	23	39	37	25	39	163
Chapeada	47	40	51	30	40	208
Morada	39	40	48	31	42	200
Calabaza	13	9	3	0	3	28
X. de Invierno	42	36	54	35	44	211
Alfajayucan	43	36	47	30	31	187
Amarilla Oro	6	22	33	15	30	106
Naranjona	19	21	30	9	19	98
Camueza	41	41	50	32	36	200
Amarilla Plátano	44	42	50	31	34	201
Polotitlán	33	20	30	14	21	118
Amarilla Montesa	42	45	41	30	39	197
Solferino	27	30	38	22	26	143
Mango	3	15	1	0	2	21
Octubreña ITA 20	32	37	50	25	41	185
CNF	32	40	51	31	39	193
Irapuato	45	44	48	30	41	208
Chaveña	46	44	52	34	39	215
Tapona	34	37	49	27	41	188
Blanca Caldera	5	17	3	5	7	37
	984	1034	1172	724	925	4832

Combinations: A. M-CAC/E-AAG, B. M-CAG/E-AAG, C. M-CAA/E-ACC, D. M-CTA/E-AAG, E. M-CAG/E-ACC

Table 5. DNA fragments amplified by AFLPs of cactus pear (*Opuntia* spp.) identified as "with few spines".

Genotype	Primers					
	A	B	C	D	E	Total
Copena V-1	31	34	46	27	36	174
Rojo Pelón	12	21	27	5	17	82
Copena F-1	38	42	38	22	35	175
Diabetes Zacatecas	50	42	52	35	36	215
Jalpa	39	40	54	26	41	200
Roja Jalpa	4	15	4	2	6	31
Chicle	29	32	38	28	35	162
P-8	48	41	55	37	40	221
Rojo Vigor	38	39	48	33	35	193
Forrajero Chapingo ITA 20	39	41	40	33	41	194
Forrajero ITA 20 (Corta)	42	42	36	25	35	180
Cero espinas	13	22	3	1	4	43
Pabellón Amarillo	43	37	52	30	39	201
La Quemada	27	37	35	12	25	136
Tlaxcalalcingo	40	41	37	25	30	173
Italiano Mejorado	40	41	38	25	35	179
Forrajero ITA 20 (Larga)	29	31	39	20	22	141
Amarilla Salinas	0	13	0	0	0	13
Amarilla Jarro	29	39	36	27	35	166
Tezontepec	1	18	3	3	13	38
	592	668	681	416	560	2917

Combinations: A. M-CAC/E-AAG, B. M-CAG/E-AAG, C. M-CAA/E-ACC, D. M-CTA/E-AAG, E. M-CAG/E-ACC

Table 6. Common names and keys for species cactus pear identification within the dendrogram (Key I) and AFLPs banding profile (Key II).

Key I	Key II	Commun Name	Specie	Key I	Key II	Commun Name	Specie
BSJ	1	Blanca San José	*O. albicarpa*	P8	26	P-8	*O. ficus-indica*
X(X)	2	X(X)	*O. albicarpa*	RJ	27	Roja Jalpa	*O. ficus-indica*
Man	3	Mango	*O. albicarpa*	FHI	28	Forrajero Chapingo ITA 20	*O. ficus-indica*
BP	4	Blanca Pepina	*O. albicarpa*	LQ	29	La Quemada	*O. ficus-indica*
RR	5	Rubí Reyna	*O. megacantha*	CF1	30	Copena F-1	*O. ficus-indica*
ST	6	Sangre de Toro	*O. megacantha*	AM	31	Amarilla Montesa	*O. albicarpa*
Nar	7	Naranjona	*O. albicarpa*	Tez	32	Tezontepec	*O. ficus-indica*
Cam	8	Camueza	*O. megacantha*	Tlax	33	Tlaxcalcingo	*O. ficus-indica*
San	9	Sandía	*O. megacantha*	Sol	34	Solferino	*O. albicarpa*
RV	10	Rojo Vigor	*O. albicarpa*	IMej	35	Italiano Mejorado	*O. ficus-indica*
AO	11	Amarilla Oro	*O. albicarpa*	FIcort	36	Forrajero ITA 20 (penca corta)	*O. ficus-indica*
CT	12	Copena Torreoja	*O. megacantha*	Irap	37	Irapuato	*O. ficus-indica*
Mor	13	Morada	*O. albicarpa*	BC	38	Blanca Caldera	*O. albicarpa*
XI	14	Xoconostle de invierno	*O. tezontepecana*	3389	39	Amarilla 3389 (con espinas)	*O. albicarpa*
Cal	15	Calabaza	*O. albicarpa*	Jalpa	40	Jalpa	*O. ficus-indica*
Chav	16	Chaveña	*O. chavena*	Tap	41	Tapona	*O. megacantha*
AS	17	Amarilla Salinas	*O. ficus-indica*	Oct	42	Octubreña ITA 20	*O. megacantha*
MSM	18	Morada San Martín	*O. megacantha*	CNF	43	CNF	*O. ficus-indica*
BCas	19	Blanca de Castilla	*O. albicarpa*	FIlarg	44	Forrajero ITA 20 (penca larga)	*O. ficus-indica*
DZ	20	Diabétes Zacatecas	*O. ficus-indica*	CV1	45	Copena V1	*O. ficus-indica*
Alfa	21	Alfajayucan	*O. albicarpa*	AJ	46	Amarilla El Jarro	*O. megacantha*
Chap	22	Chapeada	*O. albicarpa*	Chi	47	Chicle	*O. ficus-indica*
PA	23	Pabellón Amarilla	*O. ficus-indica*	RP	48	Rojo Pelón	*O. albicarpa*
AP	24	Amarilla Plátano	*O. megacantha*	CE	49	Cero espinales	*O. ficus-indica*
Pol	25	Polotitlán	*O. ficus-indica*	IMor	50	Italiano Morado	*O. ficus-indica*

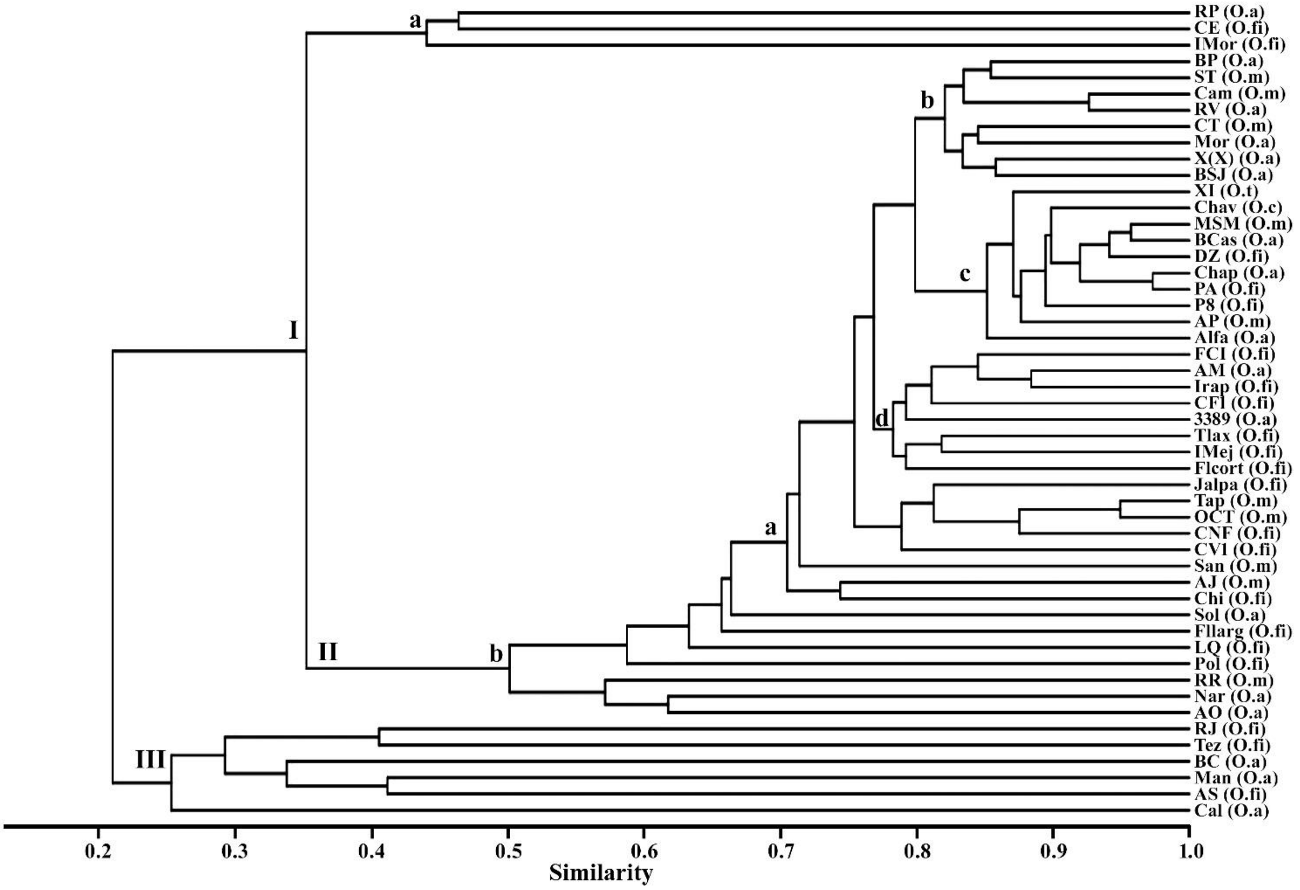

Fig. 7. Dendrogram generated with AFLPs products in 50 cactus pear species (*Opuntia*) from genetic distance calculations using the Dice coefficient and UPGMA clustering.

Dendrogram generated with AFLPs for *Opuntia* spp.: The dendrogram revealed two main clustering patterns. First, the genotypes were organized according to their species: *Opuntia albicarpa* and *Opuntia megacantha* were located at opposite ends of the dendrogram, while *O. ficus-indica* occupied an intermediate position. This distribution suggests that *O. ficus-indica* genotypes may have originated through domestication or hybridization processes involving the other two species, which is consistent with previous reports on its evolutionary origin. Studies based on AFLP and cpSSR markers have also demonstrated close genetic relationships between *O. ficus-indica* and *O. megacantha*, supporting the hypothesis of an evolutionary relationship through domestication or hybridization between these species (Labra *et al*., 2003; Valadez-Moctezuma *et al*., 2014).

Secondly, two main groups were identified, with Group I being the most diverse, subdivided into five subgroups. Subgroups *a* and *b* mainly included spiny species such as *O. albicarpa*, *O. megacantha,* and some genotypes of *O. ficus-indica*. These are characterized by green, medium to large cladodes with broad elliptical shapes. Subgroup *c* comprised *O. ficus-indica* genotypes with few spines, generally intended for forage use, with medium-sized, light green, rhomboid-shaped cladodes. Subgroup *d* grouped the only two wild genotypes analyzed, *O. tezontepecana* and *O. chaveña*, along with some medium-sized spiny genotypes. Finally, subgroup e stood out for its morphological diversity, including cladodes of different sizes and shapes, a mix of spiny and low-spine genotypes, and fruits ranging in color from yellow to red.

Similar results have been reported by Reis *et al*., (2018) and Modise *et al*., (2024) using ISSR and SSR markers, where spineless genotypes tended to cluster separately. Furthermore, clustering did not always correspond to agricultural use (fruit or forage) but rather to evolutionary and origin patterns. Akroud *et al*., (2022) also identified well-defined clusters between local and improved materials in Morocco, with comparable branching patterns.

Group II was composed mainly of *O. ficus-indica* and *O. albicarpa* genotypes, including both spiny and low-spine specimens. These genotypes have yellow-green cladodes with variable shapes, ranging from rhomboid to broad elliptical.

The dendrogram allowed the establishment of relationships between morphological traits of cladodes and fruits, and in some cases, concordance with the classical taxonomy of *Opuntia* spp., was observed. Previous studies have demonstrated that dendrograms can be useful tools for associating clustering patterns with visual and physicochemical traits such as fruit color, cladode size, Brix, or pH (Espinoza-Sánchez *et al*., 2014). However, accession clustering did not always reflect traditional taxonomic classification, highlighting the need to review and redefine species boundaries within *Opuntia* (Samah *et al*., 2016).

A high genetic similarity (similarity index between 0.90 and 0.95) was observed among some fruit-producing spiny genotypes, except for Pabellón Amarillo. Among the pairs with the highest similarity were Tapona with Octubreña, Morada San Martín with Blanca de Castilla and Chapeada with Pabellón Amarillo (Table 6).

The genetic diversity index (GD) calculated for the 50 genotypes was 0.588, a value similar to that reported by García-Zambrano *et al*., (2018) in xoconostle, indicating considerable genetic variability in the analyzed population. Furthermore, the dendrogram did not reveal duplicate genotypes, as none had a genetic distance of zero (Fig. 7), indicating a genetically differentiated collection.

Overall, this analysis suggests that although consistent patterns exist between the morphological and genetic traits of the studied genotypes, the traditional taxonomic classification of *Opuntia* spp., does not always accurately reflect the molecular relationships observed, reinforcing the need for a systematic revision of this genus.

Conclusions

The analysis of cladode morphological parameters allowed us to form and distinguish two groups based on the "with spines" or "fewer spines". The use of AFLP molecular markers constitutes an effective tool to distinguish the different genotypes of *Opuntia* spp. This study provided key information to confirm that all species in the genebank are distinct, with no repeats in the collections, and that their genetic profiles can serve as evidence for their molecular identification and future breeding program according to the desired trait.

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