

Morphological Characterization of *Opuntia* spp.: A Multivariate Analysis[♦]

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ABSTRACT

The *Opuntia* genus is a valuable source of ancestral Mexican food. Different plant parts are used, including the stems (cladodes), fruits (“tunas”), and flowers. Additionally, the *Opuntia* genus is important because of its species richness, chemical composition, and adaptability for growing in very dry environments. A morphological characterization of 46 accessions of *Opuntia* spp. growing in Bermejillo, Durango, Mexico was carried out. Sixty-five vegetative characteristics of six plants of each *Opuntia* accession were quantified. Analysis of variance, multiple comparisons of means, and multivariate analysis by cluster and principal components were used. The multivariate analysis clearly separated the 46 *Opuntia* accessions in two groups by presence or absence of spines on the cladodes. Only width and length of cladodes, areole width, number of areoles per cladode face, and leaf length were significantly different between the two groups. The results suggest that the *Opuntia* genus in this plantation showed morphology uniformity and the reason could be associated with the persistent farmer selection, which takes into account agronomic practices and consumers preferences.

Key words: *Opuntia*, classification, principal components, Mexico

INTRODUCTION

The *Opuntia* genus (*sensu stricto*) is endemic to America. Approximately 181 species have been recognized (Anderson 2002). Among them, 76 have been found wild in Mexico (Guzmán *et al.* 2003). In the wilderness, *Opuntia* grows in arid and semiarid regions as well as in temperate or tropical environments. Some examples of *Opuntia* production systems in Mexico are wild communities, orchard gardens, house fences, agricultural fields, and commercial plantations. The wide *Opuntia* richness shows a gradient of domestication: from typical wild

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types to the further domesticated plants. Currently, some *Opuntia* species and cultivars are cultivated in several world regions in America, Africa, Asia, Europe, and Oceania (Felker and Inglese 2003).

Opuntia plants are a source of food for human and animals. The young, soft, flattened, cladode, known as “nopalito” in Spanish, is consumed as a vegetable, the cactus pear are consumed as fresh fruit (“tuna” in Spanish), flowers and mature cladodes (“nopales” in Spanish) low lignified are used as forage (Corrales-García *et al.* 2004; Felker *et al.* 2006). Also, *Opuntia* plants are a source for pharmaceutical and cosmetic products (Felker and Inglese 2003). Several positive physiological effects of soluble, insoluble, and total alimentary *Opuntia* spp. fiber on humans have been documented. Also, consumption of the nopal plant gives protection against some diseases (diabetes, atherosclerosis, hypercholesterolemia, coronary heart disease, obesity, colon cancer, gastric ulcers, among others) (Wolfram *et al.* 2002; Galati *et al.* 2003; Corrales-García *et al.* 2004).

The preservation of *Opuntia* genetic resources is obligatory, and requires the morphological, physiological, biochemical, genetics, and taxonomic research to expand the information and knowledge about the species diversity, effects of environmental changes, and modifications resulting during the nopal domestication. The significance of cladode morphology to characterize the *Opuntia* types has been stressed in some studies (Valdéz-Cepeda and Blanco-Macías 2002; Reyes-Agüero *et al.* 2005a, b) and the multivariate analysis for *Opuntia* fruit characterization (Gutiérrez-Acosta *et al.* 2002; Valdéz-Cepeda *et al.* 2003). Morphology of nopal types has been used:

- a) to know *Opuntia* reaction to cultivation and potential uses,
- b) to make clear, support or improve *Opuntia* taxonomy (Pimienta-Barrios 1990; Sudzuki-Hillis 1995);
- c) to quantify *Opuntia* genetic variability; and
- d) to characterize intra- and inter-specific hybridization processes in *Opuntia* (Gibson and Nobel 1986; Pimienta-Barrios and Muñoz-Urias 1995; Mondragón-Jacobo and Pérez-González 2001; Mondragón-Jacobo and Pérez-González 2002.).

Nowadays, different Mexican institutions maintain *Opuntia* germplasm genotypes from wild communities, orchards, fences, gardens or agricultural fields, and commercial plantations, in order to analyze phenotypic and genetic variation of *Opuntia* germplasm to contribute to its preservation and develop improved cultivars (Gutiérrez-Acosta *et al.* 2002; Reyes-Agüero *et al.* 2005a).

The aim of this research was the morphological characterization of vegetative organs of 46 *Opuntia* accessions to contribute to nopal variability knowledge, and to identify the main characteristics contributing to discriminate nopal accessions. It was hypothesized that reiterative selection by farmers, consumers, and continuous cultivation reduce morphological variability (induce homogeneity) between *Opuntia* types, but the morphometric analysis of vegetative characteristics allows group separation and highlights the distinctive characteristics in a nopal collection.

MATERIALS AND METHODS

This research was carried out in the *Opuntia* experimental plantation at the Regional Arid Zones Unit (URUZA), Autonomous Chapingo University (UACH), in Durango, Mexico, 1,119 m above

sea level, at latitude 25°52'28" north and longitude 103°37'07" west; with an average mean annual temperature of 21°C, mean annual rainfall of 200 mm, and more than 50% precipitation during summer (García 1988). The URUZA-UACH plantation includes 55 *Opuntia* accessions obtained from several Mexican research centers and commercial plantations (Flores 1994). The plantation is kept without irrigation, fertilization, or any sanitary control, but hand weeding is used. Pruning controls nopal plants development; separation between furrows and plants is 1.20 and 1.50 m, respectively. Forty-six *Opuntia* spp. accessions were selected for this study; among them, there were types commonly used for nopalitos or fruit production, or both (Table 1).

Ten plants of each accession were selected and 65 morphological attributes were evaluated, including whole plant and plants' structures, i.e., branches and cladodes (nopal and nopalito). The evaluation was carried out according to the descriptor list for *Opuntia* previously utilized (Reyes-Agüero *et al.* 2005b). One representative plant of each *Opuntia* accession was used for recording 12 characteristics of plant growth habit and branches. Also, six healthy, two three-year-old nonlignified cladodes, without any malformation or evidence of buds or sprouts production, and six nopalitos (young cladodes, still with true leaves) located on different plants (89% of the cases) were evaluated. But when six plants with at least one useful cladode were not available, two or more cladodes were sampled from the same plant.

The number of areole rows, areoles per cladode, areoles with or without spines, and spines per cladode were quantified only on one cladode face. The areole per cladode area was measured on 25 cm² of the cladode central area. Also, the color of the branch cortex, areole, spine, glochid, leaves, and stem was evaluated by using the Munsell color chart (Anonymous 1977).

Eight cladode characteristics (*i.e.* cladode margin, cladode texture, areole color, number of radial spines with angles between 0 and 20° in relation to cladode surface, number of spines longer than 3 cm, primary and secondary color of spines, and spines texture) which were constants in all *Opuntia* accessions and the 12 characteristics recorded in only one representative plant of each *Opuntia* accession were not included in the statistical analysis.

The cluster and principal components (PC) multivariate tests were carried out and only 47 morphological characteristics, on rows, and 46 accessions, on columns were selected (Table 2). Because a preliminary analysis showed that 18 variables had no effect on the variability among nopal accessions. Multivariate analysis was carried out including the mean value of continuous variables and discontinuous variables, with values between 0 and 5. In order to reduce the effect of the variable magnitude, the data matrix was standardized by rows (procedure STAND) using means of the linear transformation algorithm in NTSYS (Numerical Taxonomy System software for PC); as a result, the average and the standard deviation of each characteristic were used like division and subtraction options, respectively. The cluster analysis included a matrix of similarity (SIMINT) among *Opuntia* accessions using the coefficient of correlation (Sneath and Sokal 1973). This matrix of similarity among accessions was used for carrying out the hierarchic grouping (SHAN) using the unweighted pair-group arithmetic averages method (UPGMA) and then the grouping was represented in a dendrogram (TREE).

A correlation matrix obtained from a standardized data matrix (STAND) was used to carry out eigen analysis (EIGEN) in order to know the variance proportion in each PC and the discriminatory value of the characters with the matrix of eigenvectors. Finally, the matrix of eigenvectors was multiplied by the standardized matrix to project the accessions (PROJ) in the space of the characteristics.

RESULTS AND DISCUSSION

A uniform tendency of growth habit was observed in *Opuntia* accessions. Bushy shape was common for all *Opuntia* plants. It seems, partially, a result of the continuous pruning to control plant size (182 ±19 cm mean height and between 110 ±24 cm and 137 ±27 cm width for canopy). Similarly, branching patterns were quite homogeneous, including a similar number of straight branches (3.8 ±1.5), with only one exception (accession 26, 'Amarilla'), number of diffuse branches (1.7 ±1.4 beside plants did not shown neither straight nor inclined branches, and the basal branch was 10 ±2.6 cm over the ground, with only one exception (accession 02, 'Copena 15'). A similar decrease in morphological variability has been reported in *Opuntia* populations growing in the wild (Felker *et al.* 2002) and fruit producer cultivars, which were mostly bushy type (Pimienta-Barrios 1990). It could be assumed that plant morphological patterns (including branches length, shape, orientation, and number, as well as whole-plant dimensions) in the *Opuntia* plantation mainly are the result of controlled pruning and agricultural handling.

Qualitative morphological characteristics of cladodes

The cladodes margin was entire in all *Opuntia* accessions and cladode color was similar among accessions. This was confirmed when the hue and its components, value and chroma, were quantified with the Munsell color chart (Anonymous 1977) (Table 3). In contrast, Pimienta-Barrios (1990) reported qualitative differences in cladode color between accessions of a nopal group. Nobel (2001) stressed that some *Opuntia* types stem are pale green. As in many other species, the color of photosynthetic organs (cladodes in *Opuntia*) mainly depends on the chlorophyll content and environmental factors, such as irradiance, water availability, and macro- and micro-nutriments (Irizar-Garza and Peña-Valdivia 2000; Nobel 2001). Also, texture, color, and composition of the cuticle might also affect value and chroma (Gibson and Nobel 1986). Likewise, some factors determining the plant color might be altered by agricultural practices (i.e., distance between plants, cladode orientation, irrigation, fertilization, including macro- and micro-nutriments, among others). This is the typical case of cladode (*O. ficus-indica*) green color when plants are cultivated on land fertilized with 840 t ha⁻¹ of manure (García and Grajeda 1982). But, when *Opuntia* plants grown for fruits are cultivated on 4 t ha⁻¹ of manure, they have green-yellow cladodes (Savás 1995).

Spine distribution and orientation were significantly different among spiny accessions. Apastillada, Cascarón, Chapeada, and Copena 17 (Accessions 29, 25, 24 and 15 in Table 1) lacked radial spines with slope between 0 to 20°. Average spine length in this group was 3 cm and had similar primary and secondary color and texture. According to Gibson and Nobel (1986) the young spines color might vary, but the spines color homogeneity is possibly due to bleaching during ripening (Gibson and Nobel 1986). The morphological variability among accessions included in the present study, when they are growing in their native place, is unknown. But the results indicated the possibility that environmental factors and cropping in the experimental field probably promoted the homogeneity in some *Opuntia* morphological characteristics. It should be stressed that experimental evidences indicated that spinelessness is relatively simply inherited and it has been suggested that it is possible to obtain spineless individuals if the parents yield fertile progeny and if one of the parents were spineless (Felker *et al.* 2006).

Quantitative morphological characteristics of cladodes

Only a small number of quantitative cladode characteristics were significantly different ($P < 0.05$) between spiny and spineless accessions (i.e., cladode width and area, and areole length and width, and areole number per face, beside the average leaf length of nopalitos) (Table 3). According to Muñoz-Urías *et al.* (1995), the cladode dimensions might be a ploidy indicator, since higher size fruits, cladodes and stomata are common in polyploid ($6n$, $8n$) than diploid *Opuntia* plants. Sudzuki-Hillis (1995) suggested that the areole dimensions could be used as taxonomical character. The significant differences between both *Opuntia* groups, in the present study, were relatively inconspicuous. Spiny *Opuntia* cladodes averaged 10.4% wider and had only 16.3% more area than the nonspiny group. The nonspiny accessions group had 27% longer and 20.7% wider areoles, and 9.9 % fewer areoles per cladode face than the spiny group. Also, nopalito leaf length of the nonspiny accessions was only 10 mm higher than spiny accessions, and this difference (2.1%) was statistically significant (Table 3), which suggests that the nopalito leaf length is potentially useful as a morphological marker of *Opuntia*, because minimal differences of this characteristic are significant.

Results indicate that spiny accessions had significantly more and wider areoles than the spineless accessions (Table 3). Experimental observation suggests that the commercial *Opuntia* nopalito type has cladodes with lower number and size of areoles in comparison with fruit types (Reyes *et al.* 2005a). It is possible that these differences resulted after selection of nopalito type plants and reiterate nopalitos harvest before fruit development. Felker *et al.* (2006) hypothesized that human intervention is perhaps responsible for the transition from spiny to spineless varieties, and called attention to the facts that spineless *Opuntias* are extremely palatable to wildlife and domestic stock and nowhere do spineless *Opuntias* exist without protection from humans. Reduction of spiny number is considered a result of domestication for some species (Hawkes 1983). In the studied collection there were not found accessions with radial spines, which might be important because, according to Bravo (1978), *O. streptacantha* is different from the other species because their higher quantity of radial spines and this species has been an important element during the domestication process of *Opuntia*. It is opportune to indicate that in the Cacti family, areoles have two types of meristems; one produces vegetative structures and the other reproductive structures (Gibson and Nobel 1986). In *Opuntia* accessions for nopalito production, the natural phenological expression primarily conduces to development of vegetative structures (cladode), but when the people cut the nopalito the possibility of reproductive meristem development for fruit production is eliminated or reduced and the vegetative growth is favored. Spine characteristics significantly contributed to the differentiation of nopal accessions (Tables 3 and 4). The taxonomical importance of *Opuntia* spine morphology was stressed by Robinson (1974) and Gibson and Nobel (1986) who pointed out that the environment frequently affects the spine morphology during growth.

Cluster analysis

The cluster analysis showed two main groups, one constituted by accessions in position 1 to 35 on the dendrogram, and characterized by cladodes having white, brittle, longitudinal striated spines. A second group included the accessions located in the position 36 to 46 on the dendrogram, with spineless cladodes (Figure 1). Mondragón-Jacobo (2003) obtained a similar result in a cluster analysis including 32 spine and spineless *Opuntia* accessions. Felker *et al.* (2006) suggested that spinelessness should not be considered as a character for taxonomical classification of *Opuntia* species because this characteristic seems to be simply inherited and controlled by one gene. In the present study the spinelessness was used for describing

morphologic variation among *Opuntia* accessions instead of a formal taxonomic treatment of the *Opuntia* group.

The spiny accessions were grouped in four sets (Figure 1). Seven accessions with grayish-green (2.5GY 7/2 Value/Chroma ratio) cladodes, yellow glochids, few areoles with 1 or 2 hard spines, without any particular coloration around the areoles, and nopalitos with longer leaves were in the first set. The second set included accessions with wider cladodes (area between 474 and 781 cm²) and shape-diversity cladodes, grayish-green color (2.5GY, 6/2 to 6/4 Value/Chroma ratio) cladodes with subulate spines, and a high number of areoles in relation to the other sets. The third set included accessions with short (25.7 to 36.2 cm length) cladodes, low number of areole rows (7-9) per face, and few areoles (28-46) with the fewest (1-2) spines than the first two sets. The fourth set included accessions without short spines (<0.99 cm), but with 1.0- to 2.9-cm spines length, and high number of spines (20-30) on the nopalitos.

The second group included the spineless accessions (Figure 1) with white and 0.20-mm-wide areoles (except one accession) and four areoles per 25 cm² in the cladode face. This group was subdivided into three sets; one included accessions with low number but thicker areole rows on the cladodes. The second set included oblong cladodes and areoles, with small leaves on young cladodes; and the third set included cladodes with 0.31 to 0.34-cm areole length, higher total number of areoles and areole rows per cladode face, purple color around the areole and longer leaves on nopalitos. This second main group includes one morphologically different accession (Lisa Blanca) than the previous subsets, with longer and wider cladodes and low areole density, as well as less canopy width.

Up to now, the physiological functions of spines are partially known. According to Gibson and Nobel (1986), spines reduce plant transpiration and, together with cladode pubescence, protect stems from harmful electromagnetic waves and excessive solar radiations. Nobel (1988) suggested that spines affect plant growth because they seem to reduce plant interception of photosynthetic-active radiation and CO₂ capture. It also has been suggested that spineless cultivars might be an *Opuntia* product of the domestication process because plants with spineless cladodes do not flourish in the wild (Colunga *et al.* 1986; Mondragón-Jacobo and Pérez-González 2001).

It seems that cladode size is a specie-dependent character and, in the wild, a large gradient of cladode size among species exists (Bravo 1978). According to Pimienta-Barrios and Muñoz-Urias (1995) and Muñoz-Urias *et al.* (1995) cladode size among domesticated types is a character associated with ploidy; large cladodes correspond to domesticated polyploidy types. Mondragón-Jacobo and Pérez-González (2001) presume that intra-varietal differences in cladode size might correlate with soil nutrient. According to Nobel (1988), cladode thickness, color, arrangement and number of spines per areole depend on the environment and *Opuntia* genotype. Likewise, the genotype-environment interaction might have had some effect on the cladode traits and determined the accessions grouping in this study (Figure 1).

Morphological similitude among the main *Opuntia* groups included: cladodes with and without spines, oblanceolate cladodes, and nopalito areole color. Nevertheless, using the cladode shape, Valdéz-Cepeda and Blanco-Macías (2002) separated nopal accessions. Morphological similitude, including cladode size, spine number (in spiny accessions), and areole number (in accessions with or without spines), might be explained and interpreted in different ways. *Opuntia* morphology might result of intra- and inter-specific hybridization. Gibson and Nobel (1986) pointed out that hybridization is a contributing factor for morphological variation in *Opuntia*. Also, Mondragón-Jacobo and Pérez-González (2001 and 2002) indicated that partial

or total crossing is common between cultivated *Opuntia* types. Besides, Pimienta-Barrios and Muñoz-Urias (1995) stated that Mexican cultivated nopal types were originated by hybridization between *Opuntia ficus-indica* with other wild *Opuntia* species. This could help to explain some morphological similitude among *Opuntia* accessions included in the present study; simultaneously, our results showed that cluster analysis is valuable for characterizing *Opuntia* diversity.

Principal components analysis

The first three principal components (PC) explained 46% of the total variability, with 29.4, 8.6 and 8.0%, respectively. When PC1 was plotted against PC2 and PC3, two groups and some isolated accessions were clearly defined (Figures 2 and 3). The first PC divided the 46 *Opuntia* accessions into two groups and three isolated accessions. One group included 35 spiny accessions and was located on the negative side of PC1, while the second group included accessions without spines and was located on the positive side of this component. PC2 tended to separate the Apastillada and Lisa Blanca accessions from the spiny and nonspiny groups, respectively (Figure 2). The relation of first and third PC indicated that the spiny Copena 1; Copena 12, L2, L3, and R1; as well as the nonspiny SN accessions were partially different than the whole group (Figure 3). In concordance with the present study, Colunga *et al.* (1986) separated the spiny and spineless (cultivation adapted) accessions from a 55 *Opuntia* group, which included cultivated and spontaneous types. The eigenvectors indicated that the two main *Opuntia* groups were ordered on the PC1 in a negative way by eight spine attributes: number per areole, maximum and minimum mean longitude, main and secondary color, texture, shape and consistency (Table 5). According to Gibson and Nobel (1986) and Nobel (1988) several spiny characteristics, such as longitude, thickness, inclination, color and arrangement as well as number per areole are partially dependent on the environment and nutrient availability. It is likely that some differences among spiny accessions in the present study are associated with plants' locations in the URUZA experimental field.

The PC2 was mainly and negatively related with areole color and cladode chroma, and positively related with the cladode size such as longitude, width, and area (Table 5). This result partially contrasted with the multiple mean comparisons, which did not detect significant differences in the cladode color between both groups. In Figures 2 and 3, the Blanca, A2, Zarca, Copena 14, Copena 16, and Copena 17 accessions were close together because they have longer and wider cladodes, in contrast to Apastillada, Blanca, Copena 1 and Copena 15, with small cladodes. These differences might be related to ploidy because polyploidy nopal types (6n, 8n) have large fruits, cladodes, and stomata than diploids (Muñoz-Urias *et al.* 1995). It is also possible that these differences are related with nutrients availability (Mondragón-Jacobo and Pérez-González 2001), or both factors.

CONCLUSIONS

The Mexican richness of the *Opuntia* species is evident in experimental fields of national institutions such as URUZA, where accessions from different localities and variable level of domestication are located. In order to document the *Opuntia* richness, the morphological characterization of *Opuntia* species is indispensable because this could help us to recognize physiological and biochemical mechanisms that confer resistance to extreme environments. The phenetic analysis carried out seems to be an adequate procedure to recognize morphological differences among *Opuntia* accessions. Only a few morphological quantitative characteristics

are significantly different among the nopal groups, with or without spines. Leave length seems to be a reliable characteristic to be used as a morphological marker in the characterization of *Opuntia* accessions, because small differences of this characteristic are statistically significant. In general, the *Opuntia* accessions used to produce “nopalitos” have less and small areoles than those for “tuna” production. The most relevant morphological characteristics among URUZA accessions belong to areoles, like spine presence or absence. The agriculture practices and environmental conditions at URUZA promote morphological homogeneity among *Opuntia* accessions. Besides, it seems that the repeated selection of types has contributed to the morphological homogeneity between accessions independently of the place where they were collected.

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Table 1. Description of *Opuntia* spp. accessions from the Regional Unit of Arid Zones (URUZA), from the Autonomous Chapingo University, Durango, México, used for the morphological characterization of cladodes.

Accession	Local Name	Scientific Name	Use	Mexican State
01	L3	<i>Opuntia</i> sp.	T and N	Zacatecas
02	Copena 15	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
03	Copena 1	<i>Opuntia megacantha</i>	T and N	Zacatecas
04	R 1	<i>Opuntia</i> sp.	T and N	Zacatecas
05	Copena 14	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
06	SN	<i>Opuntia</i> sp.	T and N	Zacatecas
07	Copena 12	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
08	Copena 13	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
09	Ficus indica	<i>Opuntia ficus-indica</i>	N	Zacatecas
10	L2	<i>Opuntia</i> sp.	T and N	Zacatecas
11	Copena 18	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
12	Pabellón	<i>Opuntia</i> sp.	T and N	Zacatecas
13	A 2	<i>Opuntia</i> sp.	T and N	Zacatecas
14	Copena 16	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
15	Copena 17	<i>Opuntia ficus-indica</i>	T and N	Zacatecas
16	L 1	<i>Opuntia</i> sp.	T and N	Zacatecas
17	Blanca	<i>Opuntia</i> sp.	T and N	Aguascalientes
18	Zarca	<i>Opuntia</i> sp.	T and N	Aguascalientes
19	Lisa Blanca	<i>Opuntia</i> sp.	T and N	Aguascalientes
20	L 2	<i>Opuntia</i> sp.	T and N	Zacatecas
21	R 1	<i>Opuntia</i> sp.	T and N	Zacatecas
22	L 1	<i>Opuntia</i> sp.	T and N	Zacatecas
23	Blanca	<i>Opuntia</i> sp.	T and N	Coahuila
24	Chapeada	<i>Opuntia ficus-indica</i>	T and N	San Luis Potosí
25	Cascarón	<i>Opuntia</i> spp.	T and N	Aguascalientes
26	Amarilla	<i>Opuntia streptacantha</i>	T and N	San Luis Potosí
27	Preseño	<i>Opuntia</i> sp.	T and N	San Luis Potosí
28	Charola	<i>Opuntia</i> sp.	T and N	San Luis Potosí
29	Apastillada	<i>Opuntia</i> sp.	T and N	San Luis Potosí

Table 1. Continuation

Accession	Local Name	Scientific Name	Use	Mexican State
30	Blanca	<i>Opuntia</i> sp.	T and N	San Luis Potosí
31	Amarilla	<i>Opuntia megacantha</i>	T and N	San Luis Potosí
32	Colorada	<i>Opuntia</i> sp.	T and N	Coahuila
33	Rocío	<i>Opuntia</i> sp.	T and N	Coahuila
34	Amarilla	<i>Opuntia</i> sp.	T and N	Coahuila
35	Verde Limón	<i>Opuntia</i> sp.	T and N	Coahuila
36	Apastillada	<i>Opuntia</i> sp.	T and N	San Luis Potosí
37	Blanca	<i>Opuntia</i> sp.	T and N	Aguascalientes
38	Nopalito	<i>Opuntia</i> sp.	N	Aguascalientes
39	Nopalito	<i>Opuntia</i> sp.	N	Aguascalientes
40	Palilla	<i>Opuntia megacantha</i>	T and N	Aguascalientes
41	Pachón	<i>Opuntia</i> sp.	T and N	Aguascalientes
42	Colorada	<i>Opuntia</i> sp.	T and N	Aguascalientes
43	Nopalito	<i>Opuntia</i> sp.	N	Nuevo León
44	SN	<i>Opuntia albicarpa</i>	T and N	Aguascalientes
45	SN	<i>Opuntia crassa</i>	T and N	Zacatecas
46	SN	<i>Opuntia</i> sp.	T and N	Edo. de México

N: nopalito; T: tuna; SN: without typical local name. Local name could be the same in different localities although they are not the same item and many of them have not been classified taxonomically.

Table 2. Morphological characteristics of the accessions of *Opuntia* from the Regional Unit of Arid Zones, Autonomous Chapingo University, Durango, México.

Stem and branches

- 1) Height of the plant (cm).
- 2) Width one of plant canopy (cm).
- 3) Width two of plant canopy (cm).
- 4) Height of the first branch (cm).
- 5) Main color (1=yellow, 2=brown, 3=green, 4=other).
- 6) Secondary color (1=yellow, 2=brown, 3=dark brown, 4=green, 5=other).
- 7) Number of straight branches between 75 and 90°.
- 8) Number of inclined branches between 21 and 74°.

Cladodes

- 9) Shape (1=rounded, 2=oval, 3=obovate, 4=oblanceolate, 5=oblong).
- 10) Munsell color or matiz (2.5GY –5GY).
- 11) Munsell value.
- 12) Munsell chroma.
- 13) Length (cm).
- 14) Width (cm).
- 15) Thickness (cm).
- 16) Area (cm²).

Areoles

- 17) Length (cm).
- 18) Width (cm).
- 19) Distance between areoles (cm).
- 20) Number of rows in a face.
- 21) Number in the margins.

- 22) Number in a face.
- 23) Density in 25 cm².
- 24) Number of areoles with spines.
- 25) Shape (1=rounded, 2=ovate, 3=obovate, 4=oblanceolate, 5=oblong).

Table 2. Continuation

26) Color (1=white, 2=gray, 3=brown).

27) Color around the areole (0=without any special color, 1=purple).

Spines

28) Number in the cladode

29) Number per areole.

30) Number of diffuse spines, from 21 to 74°.

31) Number of straight spines, from 75 to 100°.

32) Maximum longitude (cm).

33) Minimum longitude (cm).

34) Number of spines lower than 0.99 cm.

35) Number of spines from 1.0 to 2.9 cm.

36) Main color (0=there are not spines, 1=white).

37) Secondary color (0=there are not spines, 1=yellow).

38) Texture (0=there are not spines, 1=longitudinal striations).

39) Shape (0=there are not spines, 1=aciculars, 2=subulates, 3=cylindricals).

40) Shape of mean transversal section (0= there are not spines, 1=rounded, 2=ovate, 3=flat).

41) Consistency (0= there are not spines, 1=hard, 2=breakable).

42) Distribution in the cladode (0=there are not spines, 1=third superior, 2=homogeneous, 3=two third superior, 4=irregular).

Nopalito (young cladode)

43) Quantity of glochids (0=without glochid, 1=few, 2=partly abundant, 3=abundant).

44) Color of glochids (1=white, 2=yellow, 3=reddish).

45) Podarium (1=flat, 2=a little prominent, 3=prominent).

46) Longitude of the leave (cm).

47) Number of spines.

Table 3. Quantitative morphological characteristics of 35 accessions of *Opuntia* with spines and 11 accessions without spines in the cladodes of the experimental field of the Regional Unit of Arid Zones, Autonomous Chapingo University, Durango, Mexico.

Quantitative Characteristics	Accessions with Spines	Accessions without Spines
Cladode		
Length (cm)	32.65 a	31.48 a
Width (cm)	18.92 a	16.95 b
Thickness (cm)	1.28 a	1.28 a
Area (cm ²)	497.73 a	416.66 b
Color	4.57 a	4.72 a
Value	6.22 a	6.10 a
Chroma	6.16 a	5.64 a
Areoles		
Length (cm)	0.45 a	0.33 b
Width (cm)	0.29 a	0.23 b
Distance among them (cm)	3.05 a	2.96 a
Rows/face (Num.)	9.44 a	9.84 a
In the margin (Num.)	24.52 a	23.73 a
In a face (Num.)	49.03 b	53.86 a
Density (Num/25 cm ²)	3.91 a	3.93 a
With spines (Num.)	34.72 a	0.00 b
Spines by areole		
Quantity (Num.)	2.02 a	0.00 b
Diffuses, with angle from 21-74° (Num.)	0.81 a	0.00 b
Straight, with angles from 75-100° (Num.)	1.16 a	0.00 b
Average of maximum longitude (cm)	1.67	-
Average of minimum longitude (cm)	1.04	-
Lower of 1 cm (Num.)	0.53 a	0.00 b
From 1 to 3 cm (Num.)	1.46 a	0.00 b
Nopalito		
Longitude of the leave (cm)	0.47 b	0.48 a
Spines (Num.)	13.59 a	0.00 b

Values followed with the same letter are statistically similar between rows, with a probability level of $\leq 5\%$.

Table 4. Distinctive qualitative morphological characteristics between 35 *Opuntia* spp. accessions with spines and 11 without spines in the cladodes from the experimental field of the Regional Unit of Arid Zones, Autonomous Chapingo University.

Qualitative Characteristics	Accessions with Spines	Accessions without Spines
Cladode		
Shape	Oblanceolate	Oblanceolate
Areoles		
Shape	Oblanceolate- obovates	Oblanceolate
Color	White	White
Secondary color of the indumentum	White	-
Spines		
Shape	Curve-straight	-
Shape of the transversal section	Rounded	-
Consistency	Hard-breakable	-
Distribution in the cladode	Two superior divisions	-
Abundance of glochid	Few to partly abundant	Few
Color of glochid	White-yellow	White-yellow
Nopalito		
Podarium	Flat	Less prominent

Table 5. Eigenvectors for the first three principal components (PC) generated from morphological characteristics of 46 *Opuntia* spp. accessions.

Characteristics	PC1	PC2	PC3	Characteristics	PC1	PC2	PC3
Cladode				Spines			
				Spines per areole	-0.90	-0.02	0.04
Color	-0.10	0.18	0.75	Average of			
				maximum longitude	-0.94	-0.06	0.06
Value	-0.01	-0.19	-0.76	Average of			
				minimum longitude	-0.94	-0.03	0.01
Chroma	0.26	-0.63	-0.21	Longitude between	-0.85	-0.03	0.24
				1 and 3 cm			
Length	-0.19	0.65	0.04	Main color	-0.97	-0.07	-0.15
Width	-0.39	0.62	-0.06	Secondary color	-0.97	-0.07	-0.15
Area	-0.22	0.74	0.06	Texture	-0.97	-0.07	-0.15
Areoles				Shape	-0.89	0.01	-0.23
Length	-0.86	0.15	0.08	Shape of the			
				transversal section	-0.86	-0.16	-0.10
With spines	-0.87	-0.01	0.19	Consistency	-0.92	-0.08	-0.09
Color	-0.20	-0.64	0.36	Nopalito			
Density (25cm ²)	-0.05	-0.52	0.34	Color of glochid	0.01	-0.19	0.44

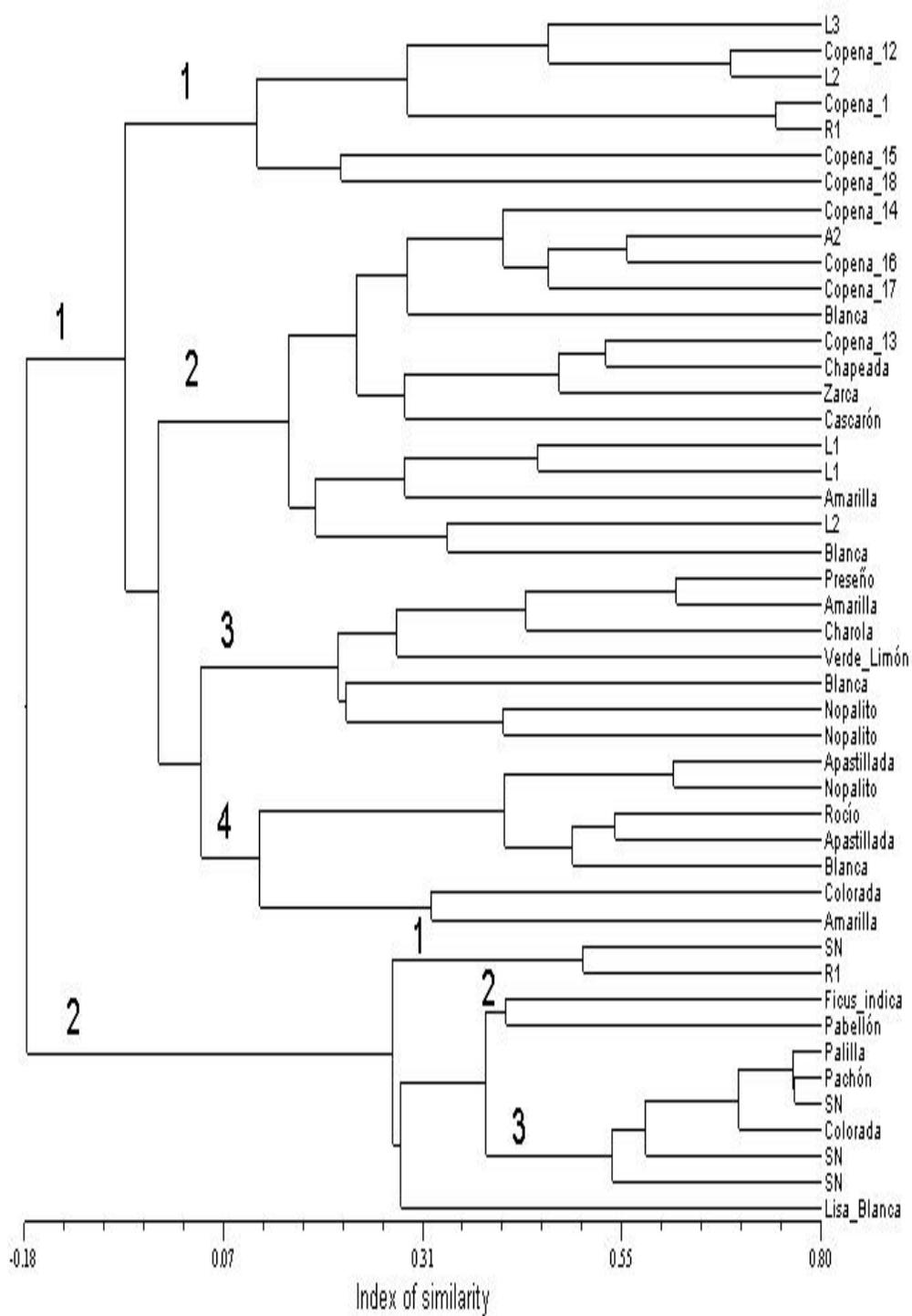


Figure 1. Dendrogram classification of the accessions of nopal (*Opuntia* spp.)

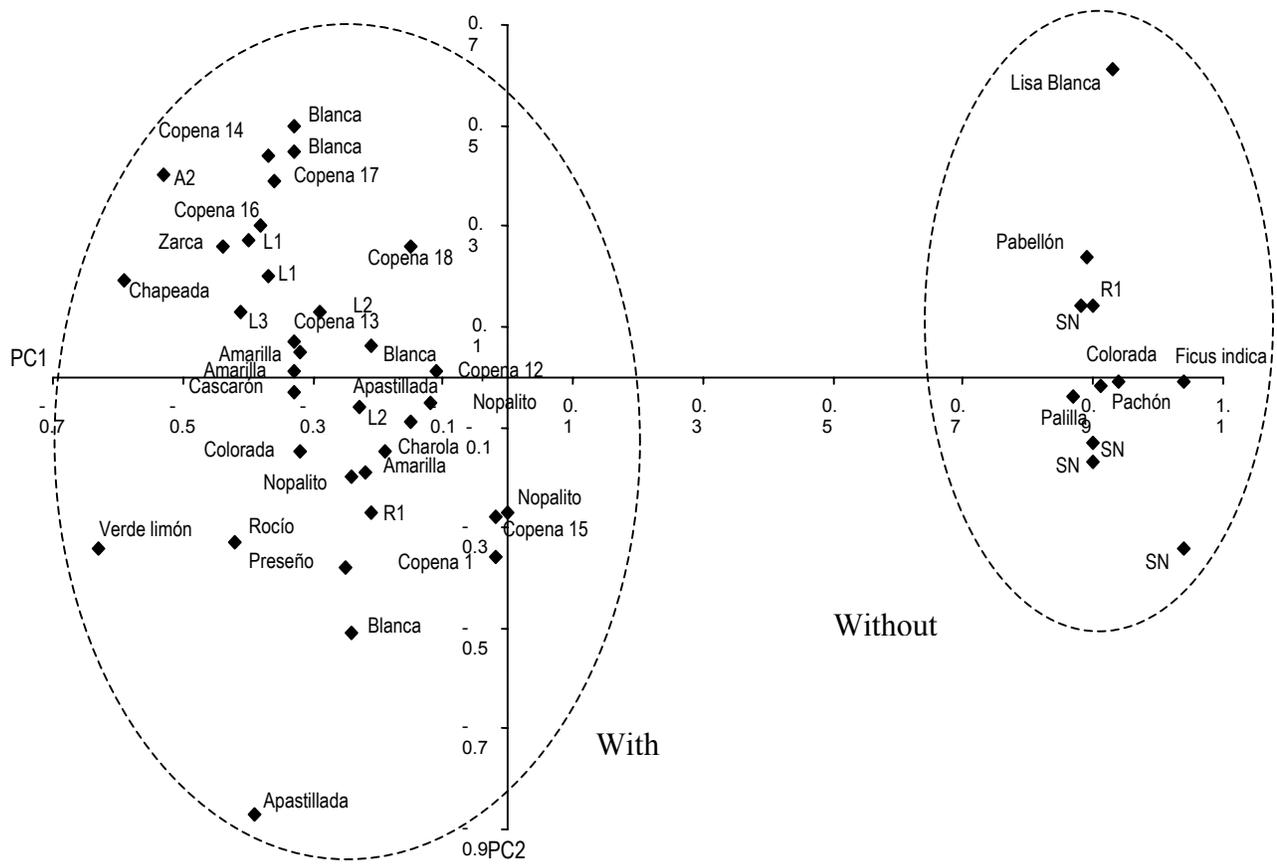


Figure 2. Order of the nopal accessions in axes 1 and 2 of the principal component analysis. Accessions with spines (■), accessions without spines (◆). SN= accessions without name.

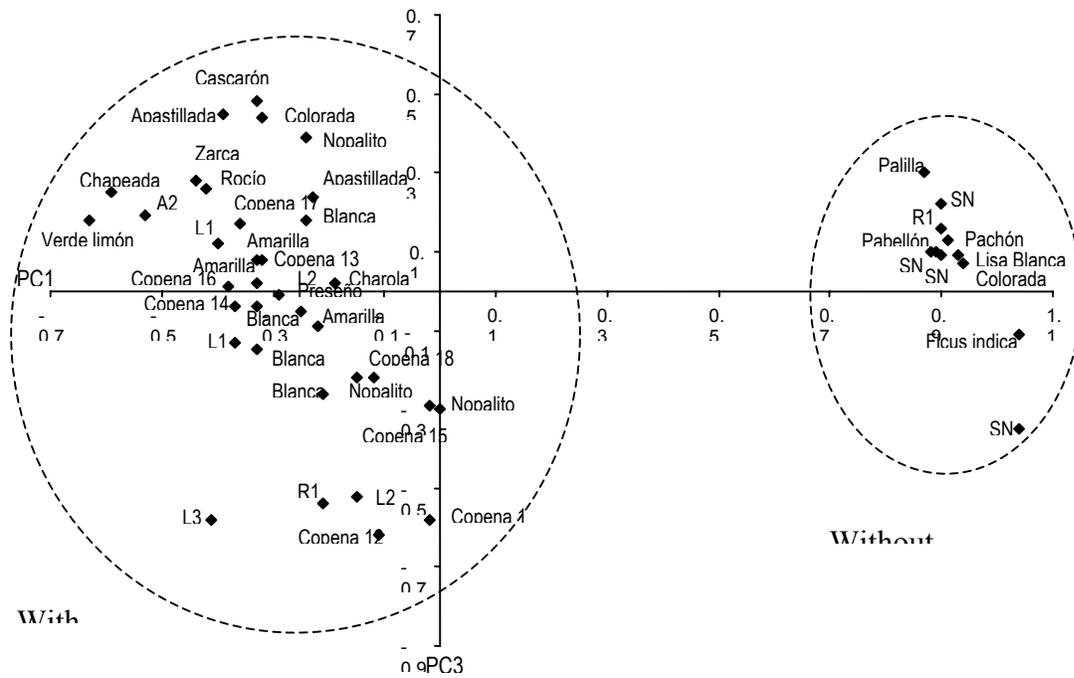


Figure 3. Order of the nopal accessions in axes 1 and 3 of the principal component analysis. Accessions with spines (■), accessions without spines (◆). SN= accessions without name.