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Polymorphism analysis in some Algerian *Opuntia* species using morphological and phenological UPOV descriptors

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Abstract

Background: In the present study, we have investigated the morphological variation in a set of five *Opuntia* species from the Algerian steppes using 49 UPOV descriptors.

Questions: Which of the 49 descriptors that can be used as powerful estimators of the phenotypic diversity within *Opuntia* species? How is the morphological diversity patterned in Algerian *Opuntia*?

Species study/ Mathematical model: *Opuntia ficus-indica*, *Opuntia amycleae*, *Opuntia streptacantha*, *Opuntia engelmannii*, *Opuntia robusta*. Principal Components Analysis (PCA) and Hierarchical Cluster Analysis were used.

Study site: Four counties were studied located in the Algerian steppes. The present research was carried out during 2014.

Methods: 49 descriptors adopted by the International Union for the Protection of New Varieties of Plants (UPOV) were employed in the present research, where cladode, flower and fruit traits were used to determine the overall degree of polymorphism among 5 *Opuntia* species.

Results: Principal Component Analysis and Hierarchical Cluster Analysis indicated a consistent differentiation between all studied species. The relative magnitude of the first two PCA eigenvectors showed that 8 descriptors out of 49 were identified as the most important descriptors for the classification of the species. The dendrogram performed on the calculated Euclidean distances between all species pairs allowed the identification of 3 groups, unlike the PCA that identified 4 groups. The species *Opuntia ficus-indica* and *Opuntia amycleae* were identified as very close morphologically.

Conclusions: The present outcome represents a paramount step towards the fast selection of interesting species and for their best management and conservation.

Key words: *Opuntia*, diversity, multivariate analyses, clustering, UPOV.

Resumen

Antecedentes: En el presente estudio, hemos investigado la variación morfológica en un conjunto de cinco especies de *Opuntia* de las estepas argelinas utilizando 49 descriptores UPOV.

Preguntas: ¿Cuáles de los 49 descriptores que pueden ser utilizados como estimadores poderosos de la diversidad fenotípica dentro de las especies de *Opuntia*?

¿Cómo se modela la diversidad morfológica en el *Opuntia* Argelina?

Especie en estudio/ Modelo matemático: *Opuntia ficus-indica*, *Opuntia amycleae*, *Opuntia streptacantha*, *Opuntia engelmannii*, *Opuntia robusta*. Análisis de Componentes Principales (PCA) y un Análisis de Agrupamiento Jerárquico fueron usados

Sitio de estudio y fechas: Se estudiaron cuatro condados situados en la estepa argelina. La presente investigación se realizó durante el año 2014.

Métodos: En la presente investigación se utilizaron 49 descriptores adoptados por la Unión Internacional para la Protección de las Obtenciones Vegetales (UPOV), en los que se utilizaron características de cladodio, flor y fruto para determinar el grado global de polimorfismo entre 5 especies de *Opuntia*.

Resultados: Se aplicó un análisis multivariado, constituido por un análisis de componentes principales (PCA) y un análisis de agrupamiento jerárquico, cuyo resultado indicó una diferenciación consistente entre las especies estudiadas. Con base en la magnitud relativa de los dos primeros eigen-vectores del PCA se seleccionaron 8 de los 49 descriptores morfológicos como los más relevantes para la clasificación de las especies siendo los que más contribuyeron a la variación morfológica detectada. Dichos descriptores pertenecen a la lista de descriptores primarios establecida por la UPOV para la caracterización de *Opuntia*. El dendrograma obtenido aplicando el método de Ward (1963) para el cálculo de las distancias euclídeas entre pares de poblaciones permitió identificar tres grupos distintos, mientras que el análisis PCA diferenció cuatro grupos. Por consiguiente, las especies *Opuntia ficus-indica* y *Opuntia amycleae* fueron identificadas como especies muy cercanas filogenéticamente.

Conclusiones: Estos resultados representan un avance crucial para la selección e identificación rápida de poblaciones de interés y una mejora para las estrategias de gestión y conservación.

Palabras clave: *Opuntia*, la diversidad, análisis multivariado, agrupación, UPOV.

T

he Cactaceae are dicotyledonous angiosperms, most plentiful in the arid and semi-arid regions between 35 N and 35 S composed of about 2,260 accepted species (Taylor 1997). Among these, the genus *Opuntia* L. Mill. has as many as 215 species.

Opuntia has been exploited since the pre-Columbian times and is mainly present in North and South America, but has also been introduced into the Mediterranean basin, Middle-East, South Africa, India, Thailand and Australia. The species reached the Mediterranean basin during the 16th century (Barbera 1995). Nevertheless, only towards the end of the 20th century have large plantations been established, where only rough estimates of the worldwide plantation area (Zoghlami *et al.* 2007) are allowed due to the absence of updated statistical data from countries in which this species is cultivated.

During the last decade, cacti (*Opuntia ficus-indica*) They have been used for almost 500 years as a fruit crop, a defensive hedge, and, more recently, as a fodder crop and as a standing buffer feed for drought periods; they can also play a key role in erosion control and land rehabilitation, particularly in arid and semi-arid zones, and as a shelter, refuge and feed resource for wildlife (Le Houérou 1996a).

Generally, the characterization of *Opuntia* genus is incomplete due to few morphological characters taken into account to classify them into the genus (Rebman & Pinkava 2001); also, the inadequate descriptors have led to misclassification (Caruso *et al.* 2010, Majure *et al.* 2012), they usually lack botanical references and other data, which makes it difficult to properly document and register different varieties under the basis of a reliable classification procedure (Gallegos-Vázquez 2012). Several authors have analyzed morphological diversity in several plants (Lamine *et al.* 2014, Arteaga *et al.* 2015). In *Opuntia*, morphological investigations have been carried out in Brazilian (Uchoa *et al.* 1998), Chilean (Wang *et al.* 1998), Mexican (Reyes-Agüero *et al.* 2005, Muñoz-Urias *et al.* 2008, Gallegos-Vázquez *et al.* 2011, Gallegos-Vázquez *et al.* 2012, Martínez-González *et al.* 2015) and Moroccan genetic resources (El Finti *et al.* 2013).

In Algeria, several ha are cultivated with *Opuntia*, where *Opuntia ficus-indica* (L.) Mill., is an important fodder crop, mainly for sheep, during low feed availability periods following drought and dry seasons. Large plantations have recently been established in the region of Souk-Ahras (commune of Sidi Fredj), also near the region of Tebessa, for the objective of improving and rehabilitating the steppe and the marginal areas (Neffar *et al.* 2011). Despite the importance of *Opuntia* in the country, only few studies have been undertaken to characterize the nutritional value of the fruits and cladodes as important food crop and fodder (Hadj Sadok *et al.* 2014), and the effect of the prickly pear plantation age on soil properties and vegetation cover (Neffar *et al.* 2011). Thus, studies that address the amount of morphological variation are still missing for Algerian *Opuntia* species.

Despite its great importance, some *Opuntia* species are threatened with extinction due to traditional agricultural practices unsuitable for their conservation and the negligence of some species. In addition, the morphological and genetic diversity of *Opuntia* have not been well assessed previously. The absence of such studies in Algeria motivated us to study the morphological and phenological polymorphism of these species and to determine the most discriminatory characteristics that allow separating this genetic richness.

In this study, we aim to assess the morphological diversity in five *Opuntia* species with potential agro-ecological values for human consumption present in the Algerian steppes, using 49 traits collected from cladodes, flowers and fruits following the International Union for the Protection of New Varieties of Plants (UPOV) guidelines. The phenotypic diversity among and within the species has been estimated to identify the traits that contribute most to the morphologic variation and classify the genotypes using PCA and hierarchical cluster analyses.

Materials and Methods

Plant material. Five *Opuntia* species of the subgenus *Platycopuntia* (Engelm.) Kreuz, *Opuntia ficus-indica*, *Opuntia amyacae* Ten., *Opuntia engelmannii* Salm-Dyck ex Engelm., *Opuntia streptacantha* Lem., and *Opuntia robusta* H.L.Wendl., were prospected from the Algerian steppes in Doukkara (Tébessa), Belaiba (M'sila), Mesrane (Djelfa) and Choucha (Laghouat) districts (Figure 1). In our study, we pre-considered *O. amyacae* as separate species from *O.*

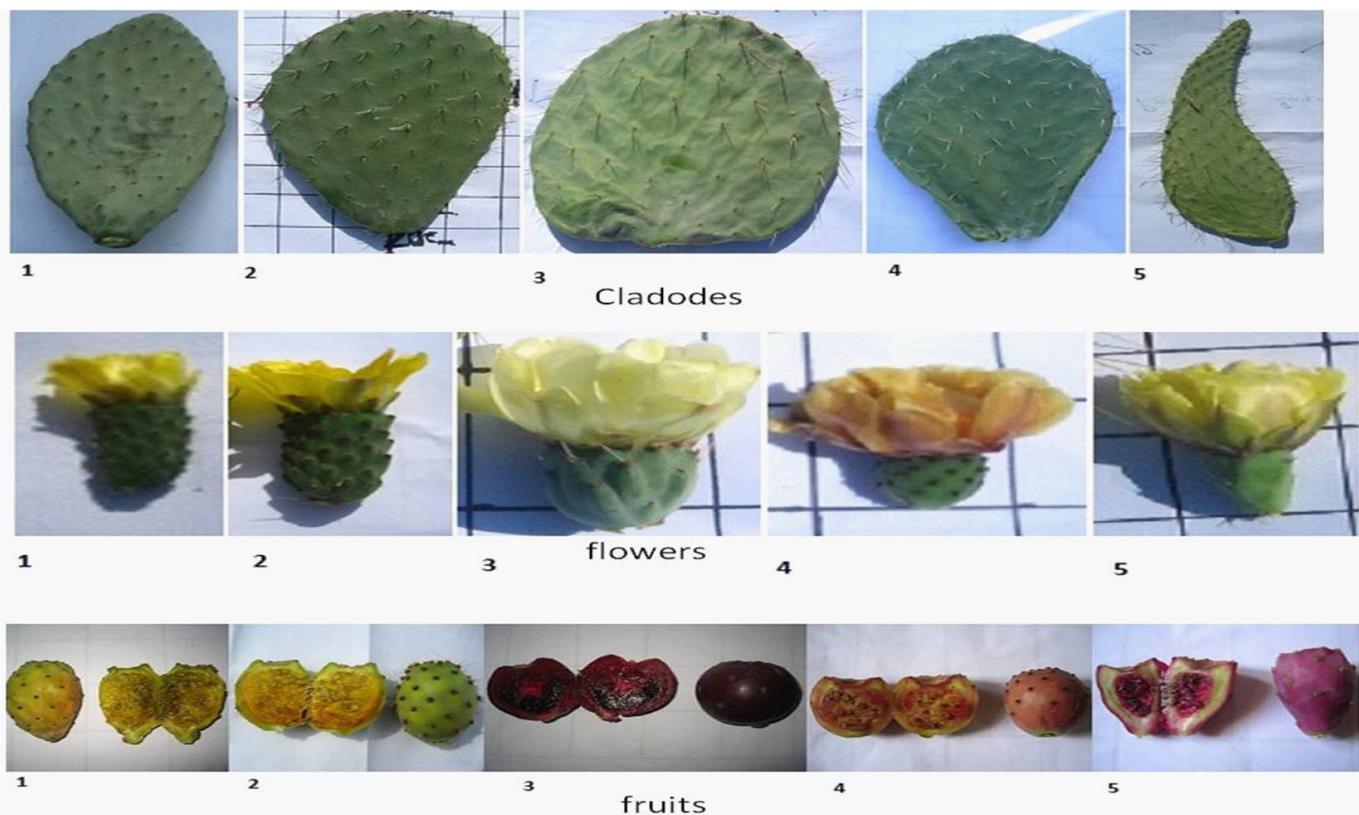


Figure 1. Algerian *Opuntia* species investigated in this study: 1: *Opuntia ficus-indica*; 2: *Opuntia amyacaea*; 3: *Opuntia robusta*; 4: *Opuntia streptacantha*; 5: *Opuntia engelmannii*.

ficus-indica just to check the morphological similarity. However, several authors consider *O. amyacaea* as synonyms of *O. ficus-indica*, and they divided this latter species into two botanical forms: a) *O. ficus-indica* f. *amyacaea* (Ten.) Schelle, with presence of spines and b) *O. ficus-indica* f. *inermis* (Web.) (Kiesling 1999), which is spineless. In the present work we adopted a random sampling method; the sampled species were then kept at the repository of the High Commissariat for Steppes Development (HCSD) and National Institute of Forestry Research (NIFR) in Algeria (Table 1).

Species analysis per species was applied to assess morphological variation, 10 individuals were scored, and from each individual 3 cladodes, 3 flowers and 3 fruits were investigated for their differences according to the 49 UPOV morphological descriptors (Table 2).

Morphological descriptors and data collection. Forty-nine major quantitative and qualitative traits, selected from the UPOV list (UPOV, 2006), comprising cladode, flower and fruit descriptors were measured and used to design a numbered-data matrix (Table 2). Ten individuals per species were evaluated for their morphological diversity across the 49 descriptors. Measurements were performed by the same two persons to avoid errors due to individual variations.

Statistical analysis based on the morphological diversity of cladode, flower and Opuntia fruits. Morphological and phenological data were analyzed by multivariate analyses and clustering using XLSTAT software (Addinsoft, www.xlstat.com). PCA was performed to identify species groups and to determine the axes and the characters significantly contributing to the variation. In this procedure, the similarity matrix was used to generate eigenvalues and scores for the species. The first two principal components, which accounted for the highest variation, were then used to plot two-dimensional scatter plots. Hierarchical cluster analyses (HCA) was carried out using Ward's minimum variance method as a clustering algorithm (Williams 1976) and squared Euclidean distances as a measure of dissimilarity (Ward 1963).

Table 1. List of the five Algerian *Opuntia* species evaluated in this study with their agro-ecological characteristics.

| Species | <i>O. ficus-indica</i> | <i>O. amycleae</i> | <i>O. streptacantha</i> | <i>O. robusta</i> | <i>O. engelmannii</i> |
|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Code | OFI | OA | OS | OR | OE |
| Mains use | Fruit | Fruit | fruit | fruit | fodder |
| Commercial importance | very high | very high | very high | medium | low |
| Locality | Belaiba (M'sila) | Doukkara (Tébessa) | Choucha (Laghouat) | Mesrane (Djelfa) | Mesrane (Djelfa) |
| Latitude | 35° 36' | 35° 58' | 34° 8' | 34° 36' | 34° 36' |
| Longitude | 05° 17' | 8° 14' | 3° 01' | 3° 03' | 3° 03' |
| Temp. min (C°) | 13,0 | 10,1 | 9,36 | 9,47 | 9,47 |
| Temp. Max (C°) | 24,3 | 22,3 | 23,0 | 23,3 | 23,3 |
| Precipitation (mm) | 238,2 | 406,7 | 236,4 | 229 | 229 |
| Soil type | sandy/muck land | muck land | sandy land | sandy land | sandy land |
| Soil pH | 7.69 | 7.52 | 7.53 | 8.55 | 8.55 |
| Bioclimatic Floor | semi-arid mild winter | semi-arid cold winter | semi-arid cold winter | semi-arid mild winter | semi-arid mild winter |

Results

Principal Component Analysis (PCA). The Principal Component Analysis (PCA) showed a high level of morphological variation between the five studied *Opuntia* species (Table 3). The first 2 axes of the PCA accounted for 67.217 % of the total variation with the first component (PC1) explaining 38.239 % of the total variation, while PC2 accounted for 28.978.

Out of 49 morphological descriptors, 8 were identified as the most discriminating and useful for the classification of the *Opuntia* species. The contribution of all parameters to the definition of the first two PCA axes is given in table 3. The traits that best contributed to the first component variability were the following: time of beginning of flowering (0.229), time of harvest

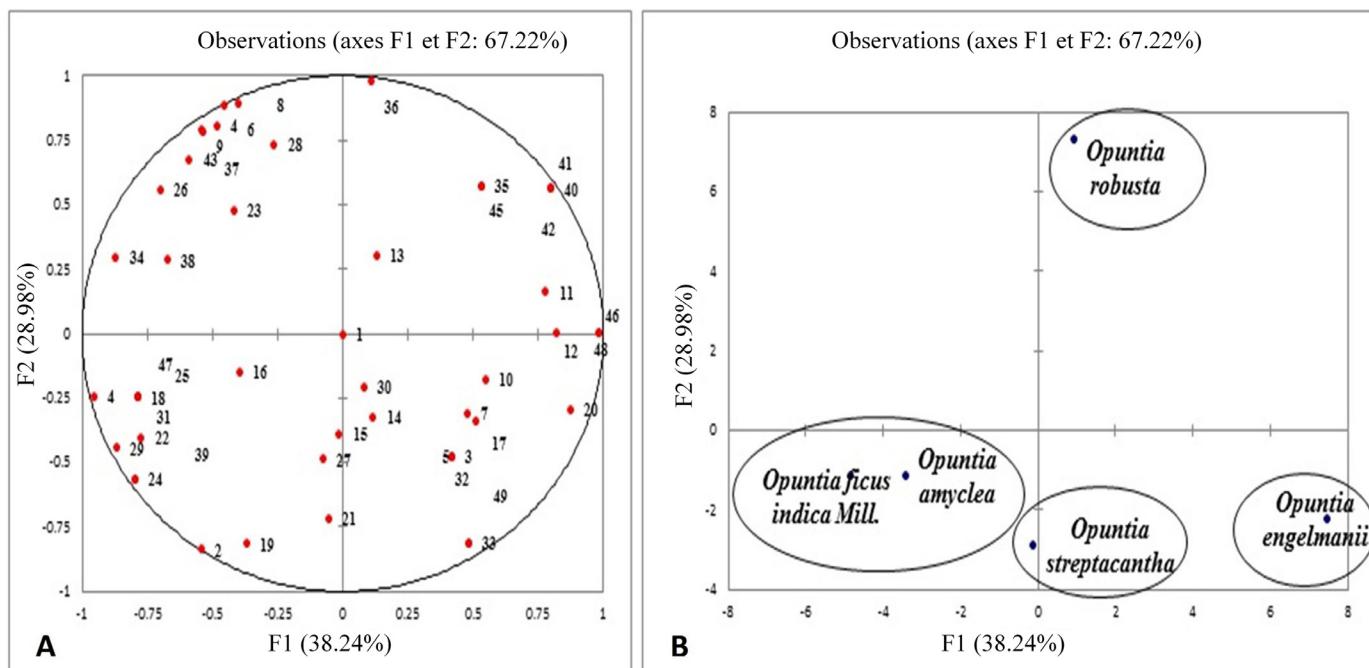


Figure 2. Principal Component Analyses showing: **A**) the contribution of the UPOV descriptors (from 1 to 49, see Table 2) to the obtained morphological variation; **B**) the distinction of all studied *Opuntia* species.

Table 2. Morphological and phonological descriptors used for the characterization of *Opuntia* species from Algeria (UPOV 2006).

| Code | Descriptor | Abbrev. | Category | Note | | | | |
|------|---|---------|---|------|----|----|----|----|
| | | | | OFI | OA | OS | OR | OE |
| 1 | Plant: growth habit | PGH | 1: upright; 2: spreading; 3: decumbent; 4: drooping | 1 | 1 | 1 | 1 | 1 |
| 2 | Plant: height | PH | 7: tall; 3: short; 5: medium | 7 | 7 | 7 | 3 | 5 |
| 3 | Cladode: length | CL | 5: medium; 3: short; 7: long | 5 | 5 | 3 | 3 | 7 |
| 4 | Cladode: width | CW | 5: medium; 7: broad; 3: narrow | 5 | 5 | 5 | 7 | 3 |
| 5 | Cladode: length/width ratio | CLW | 5: medium; 3: small; 7: large | 5 | 5 | 3 | 3 | 7 |
| 6 | Cladode: thickness | CT | 5: medium; 3: thin; 7: thick | 5 | 5 | 3 | 7 | 3 |
| 7 | Cladode: shape | CS | 3: elliptic; 7: obovate; 4: circular; 5: rhombic | 3 | 3 | 7 | 4 | 5 |
| 8 | Cladode: color | CC | 3: medium green; 2: light green; 5: bluish green; 1: yellow green | 3 | 3 | 2 | 5 | 1 |
| 9 | Cladode: color of areoles | CCA | 3: brown; 1: grey; 4: black | 3 | 3 | 1 | 4 | 1 |
| 10 | Cladode: number of spines per areole | CNSA | 1: none or very few; 7: many; 5: medium | 1 | 7 | 7 | 5 | 7 |
| 11 | Cladode: length of longest spine | CLLS | 5: medium; 3: short; 7: long | 3 | 5 | 3 | 5 | 7 |
| 12 | Spine: main color | SMC | 2: white; 3: yellow | 2 | 2 | 2 | 2 | 3 |
| 13 | Spine: number of colors | SNC | 2: tow; 1: one | 1 | 2 | 2 | 2 | 1 |
| 14 | Central spine: attitude | CSA | 1: erect; 5: horizontal | 1 | 1 | 5 | 1 | 1 |
| 15 | Central spine: Curvature (excluding base) | CSC | 1: absent; 9: present | 1 | 1 | 9 | 1 | 1 |
| 16 | Central spine: twisting | CST | 1: absent; 9: present | 1 | 9 | 1 | 1 | 1 |
| 17 | Cladode: presence of glochides | CPG | 1: absent or very few; 3: many; 2: few | 1 | 3 | 3 | 2 | 3 |
| 18 | Cladode: color of glochides | CCG | 2: brown; 1: yellow | 2 | 2 | 1 | 1 | 1 |
| 19 | Cladode: number of flowers | CNF | 7: many; 5: medium; 3: few | 7 | 5 | 7 | 3 | 5 |
| 20 | Flower: length | FL | 5: medium; 7: long | 5 | 5 | 5 | 5 | 7 |
| 21 | Flower: color of perianth | FCP | 2: yellow; 4: orange; 1: greenish yellow | 2 | 2 | 4 | 1 | 2 |
| 22 | Flower: color of style | FCS | 4: pink; 5: red; 2: white; 1: green | 4 | 4 | 5 | 2 | 1 |
| 23 | Flower: number of stigma lobes | FNSL | 5: medium; 7: many; 3: few | 5 | 5 | 7 | 7 | 3 |
| 24 | Flower: color of stigma lobe | FCSL | 2: green; 1: yellow | 2 | 2 | 2 | 1 | 1 |
| 25 | Fruit: length | FRL | 5: medium; 3: short | 5 | 5 | 3 | 3 | 3 |
| 26 | Fruit: maximum diameter | FMD | 5: medium; 3: broad | 5 | 5 | 3 | 5 | 3 |
| 27 | Fruit: ratio length/maximum diameter | FLD | 5: medium; 3: small | 5 | 5 | 3 | 3 | 5 |
| 28 | Fruit: shape | FS | 3: elliptic; 4: circular; 5: oblate; 2: narrow elliptic | 3 | 3 | 4 | 5 | 2 |
| 29 | Fruit: density of areoles | FDA | 7: dense; 5: medium; 3: sparse | 7 | 7 | 5 | 3 | 3 |
| 30 | Fruit: number of glochides | FNG | 3: few; 7: many; 5: medium | 3 | 7 | 7 | 5 | 5 |
| 31 | Fruit: color of glochides | FCG | 2: brown; 1: yellow | 2 | 2 | 1 | 1 | 1 |
| 32 | Fruit: length of stalk | FLS | 5: medium; 3: short; 7: long | 5 | 5 | 3 | 3 | 7 |
| 33 | Fruit: depression of receptacle scar | FDRS | 5: moderately depressed; 3: absent or slightly depressed; 7: strongly depressed | 5 | 5 | 5 | 3 | 7 |
| 34 | Fruit: diameter of receptacle scar | FDIR | 5: medium; 3: small | 5 | 5 | 5 | 5 | 3 |
| 35 | Fruit: thickness of peel | FTP | 5: medium; 3: thin; 7: thick | 5 | 5 | 3 | 7 | 7 |
| 36 | Fruit: weight of peel | FWP | 3: light; 5: medium | 3 | 3 | 3 | 5 | 3 |
| 37 | Fruit: weight of flesh | FWF | 5: medium; 7: heavy; 3: light; 9: very heavy; 1: very light | 5 | 7 | 3 | 9 | 1 |
| 38 | Fruit: ratio of weight of flesh/peel | FWFP | 5: medium; 7: large; 3: small | 5 | 7 | 3 | 5 | 3 |
| 39 | Fruit: evenness of color surface | FECS | 2: uneven; 1: even | 2 | 2 | 2 | 1 | 1 |
| 40 | Fruit: main color of surface | FMCS | 4: dark green; 8: medium red | 4 | 4 | 4 | 8 | 8 |
| 41 | Fruit: color of flesh | FCF | 3: yellow; 6: red | 3 | 3 | 3 | 6 | 6 |
| 42 | Fruit: firmness of flesh | FFF | 5: medium; 7: firm | 5 | 5 | 5 | 7 | 7 |
| 43 | Fruit: number of fully developed sedes | FNFD | 7: many; 5: medium; 3: few | 7 | 5 | 3 | 7 | 3 |
| 44 | Fruit: presence of abortive seeds | FPAS | 9: very many; 7: many; 5: medium; 3: few; 1: absent or very few | 9 | 7 | 5 | 3 | 1 |
| 45 | Seed: size | SS | 5: medium; 3: small; 7: large | 5 | 5 | 3 | 7 | 7 |
| 46 | Time of beginning of flowering | TBF | 3: early; 5: medium; 7: late | 3 | 3 | 5 | 5 | 7 |
| 47 | Flowering habit | FH | 2: twice flowering; 1: once flowering | 2 | 2 | 1 | 1 | 1 |
| 48 | Time of harvest maturity | THM | 3: early; 5: medium; 7: late | 3 | 3 | 5 | 5 | 7 |
| 49 | Duration of harvesting period of fruit | DHPF | 5: medium; 3: short; 7: long | 5 | 5 | 5 | 3 | 7 |

maturity (0.229), flower length (0.203), main spine color (0.191), main color of fruit surface (0.186), color of fruit flesh (0.186), firmness of fruit flesh (0.186), length of the longest spine per cladode (0.182). On the other hand, in the second PCA component, we obtained highly and positive correlation for the descriptions cladode width (0.217), cladode thickness (0.239), cladode color (0.238), color of areoles per cladode (0.210), fruit shape (0.197), peel weight per fruit (0.264), flesh weight per fruit (0.212), and number of fully developed seeds per fruit (0.181) (Table 3, Figure 2).

Table 3. Definition the first two components of the PCA based on 49 UPOV morphological descriptors in five *Opuntia* species from Algeria.

| Principal components | PC1 | PC2 | PC3 |
|-----------------------------------|-------------------|--------|--------|
| Eigenvalue | 18.354 | 13.910 | 11.314 |
| Variability (%) | 38.239 | 28.978 | 23.570 |
| % cumulative | 38.239 | 67.217 | 90.787 |
| Morphological descriptors* | Eigenvalue | | |
| 1 | 0.000 | 0.000 | 0.000 |
| 2 | -0.127 | -0.224 | 0.016 |
| 3 | 0.097 | -0.128 | -0.229 |
| 4 | -0.113 | 0.217 | 0.100 |
| 5 | 0.097 | -0.128 | -0.229 |
| 6 | -0.094 | 0.239 | -0.061 |
| 7 | 0.112 | -0.083 | 0.239 |
| 8 | -0.107 | 0.238 | 0.013 |
| 9 | -0.125 | 0.210 | -0.089 |
| 10 | 0.127 | -0.048 | 0.123 |
| 11 | 0.182 | 0.043 | -0.018 |
| 12 | 0.191 | 0.001 | 0.061 |
| 13 | 0.030 | 0.081 | 0.191 |
| 14 | 0.027 | -0.086 | 0.279 |
| 15 | -0.003 | -0.104 | 0.271 |
| 16 | -0.093 | -0.040 | -0.064 |
| 17 | 0.120 | -0.091 | 0.112 |
| 18 | -0.184 | -0.066 | -0.161 |
| 19 | -0.086 | -0.218 | 0.052 |
| 20 | 0.203 | -0.079 | -0.116 |
| 21 | -0.013 | -0.192 | 0.204 |
| 22 | -0.181 | -0.107 | 0.146 |
| 23 | -0.097 | 0.128 | 0.229 |
| 24 | -0.186 | -0.151 | 0.061 |
| 25 | -0.184 | -0.066 | -0.161 |
| 26 | -0.163 | 0.149 | -0.127 |
| 27 | -0.018 | -0.131 | -0.255 |
| 28 | -0.062 | 0.197 | 0.185 |
| 29 | -0.203 | -0.119 | -0.055 |
| 30 | 0.019 | -0.055 | 0.182 |
| 31 | -0.184 | -0.066 | -0.161 |
| 32 | 0.097 | -0.128 | -0.229 |
| 33 | 0.113 | -0.217 | -0.100 |
| 34 | -0.203 | 0.079 | 0.116 |
| 35 | 0.124 | 0.154 | -0.185 |
| 36 | 0.025 | 0.264 | 0.042 |
| 37 | -0.126 | 0.212 | -0.005 |
| 38 | -0.157 | 0.076 | -0.117 |
| 39 | -0.186 | -0.151 | 0.061 |
| 40 | 0.186 | 0.151 | -0.061 |
| 41 | 0.186 | 0.151 | -0.061 |
| 42 | 0.186 | 0.151 | -0.061 |
| 43 | -0.137 | 0.181 | -0.110 |
| 44 | -0.223 | -0.064 | -0.039 |
| 45 | 0.124 | 0.154 | -0.185 |
| 46 | 0.229 | 0.001 | 0.043 |
| 47 | -0.184 | -0.066 | -0.161 |
| 48 | 0.229 | 0.001 | 0.043 |
| 49 | 0.113 | -0.217 | -0.100 |

*See table 2.

The projection of the species on the plot delimited by the first two PCA axes, showed the classification of the species into four distinct groups (Figure 2). The first group (a) comprises *Opuntia engelmannii* a second group (b) includes two species, *Opuntia ficus-indica* and *Opuntia amycleae* situated at the negative side of the plot, *Opuntia robusta* constituted the third group (c) and *Opuntia streptacantha* constituted the fourth group (d).

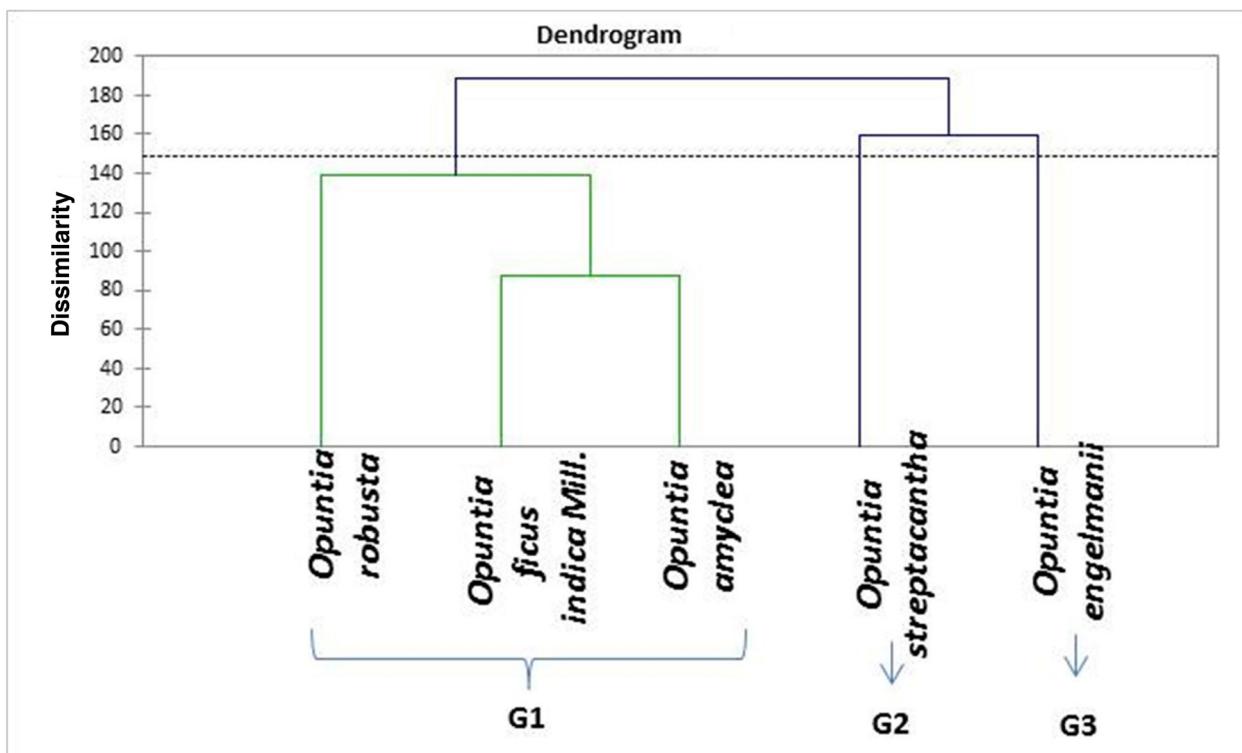


Figure 3. Dendrogram showing relationships between five *Opuntia* species in Algeria using 49 morphological descriptors (G1 and G2 stand for the identified groups).

Cluster Analysis. To understand how the 5 *Opuntia* species are structured a ward-derived dendrogram was constructed based on the data yielded by the 49 morphological descriptors (Figure 3). This allowed the identification of 3 groups, unlike the PCA and phenological that identified 4 groups (Figure 3). The first cluster (C1) was constituted by three species: *Opuntia robusta* (OR), *Opuntia ficus-indica* (OFI) and *Opuntia amyclea* (OA). Within this group, *Opuntia robusta* was very different and characterized by a high number of fully developed seeds per fruit, broad thick bluish green cladodes, with black areoles, oblate fruit shape, medium peel weight and heavy fruit flesh weight (Figure 3). *Opuntia ficus-indica* and *Opuntia amyclea* were the closest as both were characterized by brown glochides, medium flower length, pink flower style, green stigma lobes, a medium ratio of length/maximum diameter of fruit, dense areoles, medium diameter of the receptacle scar.

Opuntia streptacantha formed the second cluster (C2) having an obovate cladode shape, two types of color spines, a horizontal attitude of the central spine, orange colored perianth, high number of stigma lobes and glochides.

The third group (C3) was constituted by *Opuntia engelmannii* (OE) that is distinguishable particularly, by late time of beginning of flowering and harvest maturity, long flower length, yellow spine color, medium red fruit surface, red fruit firm flesh, high number of long spines per areole per cladode, and large amount of many glochides (Figure 3).

Discussion

The present study revealed the morphological and phenological diversity in the five dominant species of *Opuntia* grown in Algerian steppes using 49 morphological descriptors.

The set of the UPOV descriptors used here allowed, for the first time, the characterization of the morphological diversity in 5 Algerian *Opuntia* species. The number of the descriptors was high if compared to previous studies in *Cactaceae* with 24 descriptors: seven describing the cladode (length, width, thickness, ratio length/width, number of areoles in the central row, number of spines per areole, length of the longest spine), three for the flower (number of flowers per

cladode, length of the flower, number of stigma lobes) and 14 related to the fruit (length, maximum diameter, ratio length/maximum diameter, density of areoles, peduncle length, depression of receptacle scar, receptacle diameter, peel thickness, peel weight, pulp weight, ratio weight of peel/fruit weight, number of normal seeds, number of abortive seeds, total soluble solids (°Brix) in Gallegos-Vázquez *et al.* (2011), 27 descriptors from cladodes, flowers and fruits were measured in Gallegos-Vázquez *et al.* 2012 and 23 descriptors in Mejía *et al.* (2013).

According to the analysis performed, only few descriptors have shown an effective discriminating capacity. Two quantitative descriptors (length of flower and length of the longest spine per cladode,) and six qualitative descriptors (following: time of beginning of flowering, time of harvest maturity, spine main color, main color of fruit surface, color of fruit flesh, firmness of fruit flesh) have been identified as differential parameters in *Opuntia ficus-indica*, *Opuntia amyacaea*, *Opuntia streptacantha*, *Opuntia robusta* and *Opuntia engelmannii* (Table 3). These descriptors ranked within the primary list established by the UPOV for the characterization of *Opuntia* species (UPOV, 2006).

In comparison with other results, five variables were found to be significant to discriminate forty three accessions (*Opuntia ficus-indica*) fruit weight, peel weight, pulp weight, juice weight and peel thickness were considered as the most discriminative traits (Chalak *et al.* 2014). Three variables were selected as significant to discriminate twenty nine cactus pear varieties and four xoconostle varieties: peel thickness, pulp weight and to a lesser extent the diameter of the receptacle were the most discriminating of the UPOV guidelines traits (Gallegos-Vázquez *et al.* 2011). Moreover, spine number, margin hardness and areole distance were selected the three major variables to discriminate between three populations of *Hylocereus* Britton & Rose spp. (Mejía *et al.* 2013). In addition, ten morphological descriptors including; receptacular scar diameter, seed number, pulp weight, cladodes shape, spines, fruits number, fruits size, receptacular scar position, peel and pulp color were registered to be significant to discriminate *Opuntia ficus-indica* and *Opuntia amyacaea* (Erre & Chessa 2013).

It has been reported that phenotypic differences between populations can be linked to environmental and genetic variables (Muñoz-Urias *et al.* 2008). Additionally, Reyes-Agüero (2005), reported the elements of the syndrome of domestication in *Opuntia* and many traits are of primary interest for human utilization and have had a clear effect on domestication (Gallegos-Vázquez *et al.* 2012). The use of morphological descriptors comprising cladode, flower and fruit traits yielded a high number of morphotypes and allowed the discrimination of all of the studied species. In previous studies, the discrimination level was found to be as high as those reported by other studies using molecular markers for *Opuntia* spp (Labra *et al.* 2003, García-Zambrano *et al.* 2006, Zoghlami *et al.* 2007, García-Zambrano *et al.* 2009, Caruso *et al.* 2010, Majure *et al.* 2012, El-Banna *et al.* 2013, Valadez-Moctezuma *et al.* 2014, Ganopoulos *et al.* 2015, Samah *et al.* 2016).

Multivariate analyses based on morphological characters are continuously providing valuable information allowing the breeder to improve the species selected from specific geographical regions (Souza & Sorrells 1991). Previously, the multivariate techniques have been applied in *Opuntia* aiming at the morphological and the agronomic descriptions (Reyes-Agüero *et al.* 2005, Peña-Valdivia *et al.* 2008) and the industrial (Hammouch *et al.* 2013).

In our case, multivariate analyses have shown that the highest amount of variation was attained using fruits descriptors (Table 3). These were previously shown as the most important characters for the description of the species (Valdez-Cepeda *et al.* 2003, Reyes-Agüero *et al.* 2005, Gallegos-Vázquez *et al.* 2012).

Morphological descriptors were identified as powerful tools in providing meaningful data on the qualitative and the quantitative aspects in some *Opuntia* species. Morphological characterization is needed to provide the users with valuable information on individual accessions, relationship among traits, and the structure of collections.

In this study, we managed to provide insights on the level of polymorphism and the structuring of the diversity in five *Opuntia* species from the Algerian steppes using 49 UPOV morphological and phenological descriptors.

These results may contribute to the best management of the genetic diversity in *Opuntia* spe-

cies from Algeria throughout the establishment of a core-collection sheltering representatives from interesting species that best exhibit variation, and this would be a benefit for conservation and for future breeding programs.

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