Cladode traits variation and chromosome number of three species of the genus *Opuntia* in Algeria

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Abstract

Morphological measurements of the cladodes of *O. ficus-indica*, *O. megacantha*, and *O. streptacantha* were subjected to statistical and numerical analyses. Chromosome counts were also performed to determine ploidy levels. Analysis of variance revealed significant differentiation among the populations. The most discriminating traits were the length of the longest spine, the number of spines per areole, and the length and density of the areoles. Principal Component Analysis, including the main parameters of the Mediterranean bioclimate, revealed intra- and inter-population variability, which appeared to be associated with environmental factors. Three main groups of individuals were clearly differentiated. The first phenotypic group comprised all the samples of *O. streptacantha* and some of *O. megacantha* from arid areas, easily distinguished by their very broad cladode bases. The second group, composed exclusively of *O. ficus-indica* individuals from subhumid and semi-arid bioclimates, was characterized by the longest cladodes, a high length/width ratio, and greater areoles spacing and density. The remaining *O. megacantha* individuals, originating from humid bioclimates, formed a third group distinguished by their long spines and high number of spines per areole. The chromosome numbers, reported here for the first time for Algerian material, indicated two ploidy levels, 6*x* and 8*x*, with a base number of *x* = 11, i.e., hexaploid (2n = 6x= 66) for *O. streptacantha* and octoploid (2n = 8x= 88) for both *O. ficus-indica* and *O. megacantha*. The metaphases revealed very small chromosomes of metacentric appearance, measuring from 0.83 to 2.28 microns in total length.

Keywords: Cladode traits, variability, chromosome number, polyploidy, Algeria

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Introduction

The genus *Opuntia* Mill., 1754, is one of the largest genera of the family Cactaceae, subfamily Opuntioideae. It includes more than 180 species native to Mexico but widespread in Central America and Southern USA and introduced in Europe, Africa and Asia (Majure et al., 2012; Samah et al., 2016; Porras-Flórez et al., 2017). All the species of *Opuntia* are well adapted to drought

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and high temperatures (Gallegos-Vazquez et al., 2012). In the Mediterranean basin, many species were introduced, which grow in grasslands and woodlands (Barbera et al., 1994; Kiesling, 1998).

Plants of this genus are perennial and arborescent cacti showing a high morphological diversity, especially in the size and color of the fruits, shape of the cladodes and the presence or absence of spines (Pimienta-Barrios and Muñoz-Urias, 1995). This diversity is probably linked to changes of the local ecological environment but also to the occurrence of hybridization and polyploidy (Majure et al., 2012). The taxonomic classification within genus *Opuntia* has been considered as complex, and several series were recognized (Britton and Rose, 1919). The series Ficus indicae containing *O. ficus indica* (L) Mill., 1768, is taxonomically closely linked to the series Streptacanthae which includes 12 species of which *O. megacantha* Salm-Dyck, 1834, and *O. streptacantha* Lem., 1839 (Britton and Rose, 1919). Other species such as *O. ficus indica*, *O. robusta*, *O. megacantha*, *O. streptacantha*, *O. albicarpa*, and *O. amyclaea* have been cultivated for their economic and ecological values (Kiesling, 1998). They were highly valued in agri-food, cosmetic, and pharmaceutical products (Ribeiro et al., 2015; Santos-Diaz et al., 2017). The most important species is undoubtedly *O. ficus-indica*, known as prickly-pear, and representing the oldest domesticated and the most widespread cactus crop (Griffith, 2004; Reyes-Agüero et al., 2005a; Majure and Puente, 2014; Sinicropi et al., 2022). Prickly-pear is diversly used for its fruits, and as fodder and hedges. It is well-known as an excellent source of energy due to its high carbohydrate content, and also for its richness in vitamins, amino acids, proteins, and minerals (Ahumada and Trillo, 2017).

Domestication process in genus *Opuntia* was directed towards producing plants with spineless cladodes and large and sweet fruits. Spines considered as the main diagnostic characters, some taxa have been consequently assumed as putative ancestors of *O. ficus-indica* including *O. streptacantha* and *O. megacantha* (Kiesling, 1998). The latter was sometimes considered synonymous with *O. ficus-indica* (Benson and Walkington, 1965) and that the cultivated forms would derive from *O. streptacantha* only (Scheinvar, 1995). Kiesling (1998), suggested that the spiny and spineless specimens were only forms of *O. ficus-indica*. Although, *O. ficus-indica* and *O. megacantha* were taxonomically and morphologically distinct from each other (Reyes-Agüero et al., 2005b), molecular phylogenies revealed a strong genetic similarity between these two taxa which led Labra et al. (2003) and Verloove et al. (2024) to assume *O. ficus-indica* as a domesticated form of *O. megacantha* and *O. streptacantha*. All these species show a close phylogenetic relationship (Caruso et al., 2010; Majure et al., 2012; Samah et al., 2016).

Moreover, in the Cactaceae, the basic chromosome number is x = 11 (Las Peñas et al., 2017). Within the subfamily Opuntioideae, polyploidy is very common and range from diploid (2n = 2x = 22) and triploid (2n = 3x= 33) to octoploid (2n = 8x = 88) (Majure et al., 2012). The octoploid level occurs only in genus *Opuntia* particularly among members of series Streptacanthae and Ficus-indicae (Kiesling, 1998). However, di-, penta-, hexa-, and heptaploid levels were also reported in *Opuntia* spp, depending on the origin of populations (Majure et al., 2012).

In Algeria, species of genus *Opuntia* have been introduced and cultivated for centuries as hedges around fields exclusively for the consumption and sale of fruits in local markets. In recent years, the plantation of these species in arid and semi-arid areas have been developed considerably as part of the fight against desertification, erosion, soil protection, and as novel source of forage in Algerian forestry services and High Commission for Steppe Development (HCDS). This approach can allow the restoration of degraded lands and the economic development of marginal areas and above all, improvement in the income level of the rural population (Neffar et al., 2011).

These planting efforts for the agroeconomic valorization of *Opuntia* species in our country should be supported by taxonomic and genetic characterizations which, until today, have not been addressed in depth. The aim of this study is to assess by morphological and karyological criteria, the differentiation between the three

Table 1
Origin and biogeographic information of the nine sampling sites of *Opuntia* in Algeria

Localities	Symbols of populations	Longitude	Latitude	Bioclim	P	m	M	Alt.	Species collected
Nador	OMN	02°,49'E	36°,57'N	SH	616	8.9	28.7	90	*O. megacantha*
Fergoug	OFF	00°,03'E	35°,51'N	SA	357	6.3	35.0	200	*O. ficus indica*
Arris	OFA	06°,32'E	35°,25'N	SA	578	-0.2	29.6	1300	*O. ficus indica*
Zeboudja	OFZ	01°,43'E	36°,31'N	SA	458	5.0	35.9	178	*O. ficus indica*
El-Khenig	OMK	08°,23'E	35°,05'N	SA	404	0.7	34.1	970	*O. megacantha*
Choucha	OS	03°,01'E	34°,13'N	A	270	0.0	34.7	916	*O. streptacantha*
Beni Snous	OMBS	-1°,53'E	34°,66'N	SA	433	1.7	33.3	765	*O. megacantha*
Ben Azouz	OMB	07°,29'E	36°,86'N	H	767	5.3	33.4	49	*O. megacantha*
Sidi Fredj	OFS	08°,25'E	35°,96'N	SA	622	2.1	32.4	487	*O. ficus indica*

Bioclimate stages are from Quézel and Santa (1962) and Stewart (1974); H, Humid; SH, Subhumid; SA, Semiarid; A, Arid; Alt: altitude in meters; P: the annual rainfall in millimeters; M and m: the average in °C of the maximum temperature of the hottest month in summer and the minimum of the coldest month in winter, respectively for the period 1991-2021

Table 2
Morphological parameters used in the statistical analysis of the three species of the genus *Opuntia* from Algeria

	Abbreviations	Parameters	Quantitative values (measurement units)
Cladodes	LC	Length	cm
	WC	Width	
	L/W	Length / width ratio	
	CC	Circumference of the base of cladode	
Areoles	LA	Length	cm
	WA	Width	
	D	Distance between areoles	
	DE	Density per 25 cm^2	
Spines	NS	Number of spines per areole	
	LS	Length of longest spine	cm

main species exploited in Algeria, namely *O. ficus-indica*, *O. megacantha* and *O. streptacantha*. The analyses involved several populations sampled in various bioclimatic conditions along the east-west biogeographic gradient of northern Algeria. The morphological analysis focused on size, shape, and spines of the cladodes, which constitute the main vegetative organ of responses to environmental pressures as well as the predominant mode of propagation. Chromosome counting in somatic metaphases was performed to assess the ploidy levels for all population and species.

Material and Methods

Plant Material

Samples of nine populations belonging to *O. ficus indica*, *O. mégacantha*, and *O. streptacantha*, were collected in some localities representative of the bioclimatic diversity of northern Algeria (Table 1). The collection of cladodes was carried out at the same vegetative stage during early spring. About twenty-seven healthy and non-lignified cladodes, without buds or sprout, were selected per population. To avoid the effect of cloning, the cladodes were collected from individuals at least 5 m apart from each other and then planted in pots in the experimental garden of the National Forestry Research Institute (INRF, Algiers) in order to set up a living collection. Taxonomic determination was made on the basis of the main specialized floras (Britton and Rose, 1919; Bravo-Hollis and Sánchez-Mejorada, 1978).

Morphological analyses

Ten diagnostic characters were selected according to the UPOV List (UPOV, 2004) and from the main descriptive criteria from Britton and Rose (1919), and Bravo-Hollis and Sánchez-Mejorada (1978) (Table 2). Twenty-seven individuals per population were examined for the morphological variability of cladodes. The global data was subjected to analysis of variance (ANOVA) using GenStat Software Edition 12.0 (VSN International, 2009). A

Fig. I. Cladodes, flowers and fruits of populations sampled of *Opuntia*. (a-h) *O. ficus indica*; (i-l) *O. megacantha*; (m-o) *O. streptacntha*

Principal Components Analysis (PCA) was also performed using XLStat to all populations, including the three main parameters of the Mediterranean bioclimate, P the average annual rainfall, M and m the average temperatures in Celsius of the hottest and the coldest month, respectively, and Alt, the altitude.

Chromosomal counting

Karyological examinations were performed using the Feulgen staining technique as described in Boubetra et al. (2017). The cladodes were washed then planted at room temperature in pots with vermiculite as substrate. Root tips were collected and pretreated with 2 mMol 8-hydroxyquinoline for 4 h at 4 °C and then washed before being fixed in ethanol-acetic acid (3:1) for 48 h and hydrolyzed in 1N HCl at 60 °C for 12 min. Staining was done in Schiff 's reagent for 2 h in the dark at room temperature. Root meristems were squashed in a drop of 2% acetocarmine. Mitotic metaphase plates were observed and photographed with a Leica-microscope.

Results

Taxonomy and description

Taxonomical and chorology criteria are described below for each taxon.

***Opuntia ficus-indica* (L.) Mill., 1768**

Shrubby or arborescent reached up to 6 m in height with a lignified, dark-brown, cylindrical, and well-defined primary stem. It was observed that the root-system spreads horizontally. The cladodes are very thick, succulent, generally spineless, circular, obovate, oblong to spatulate, 20 − 52 cm long (x) 14 − 28 cm width (Fig. I. a). Usually, the cladodes are pale to dark green with spiral areole series spaced 3.15 − 5.25 cm with density varies between 2 − 9 per 25 cm^2, 0.1 − 0.6 cm long (x) 0.10 − 0.3 cm width. The spines are usually absent, but sometimes a few areoles have short, acicular, and depressed spines. The glochids usually abundant in the proximal areoles of the cladode, are sometimes absent. The flowers are large, hermaphroditic, and rich in showy petals of intense yellow or yellow-orange color, without aroma (Figs. I., b, c & d). Fruits are greenish, yellow-orange, or reddish, either oval or cylindrical (Fig. I., e, f, g & h).

***Opuntia megacantha* Salm-Dyck, 1834**

Tall plant succulent shrub or tree grows up to 5 m tall, often with a clear stem, much branched with

Table 3
Mean values per populations of the ten morphological characters of cladodes in the three species of the genus *Opuntia* in Algeria

Populations/Traits	LC	WC	L/W	CC	LA	WA	D	DE	NS	LS
OFA	39.68±3.70a	22.81±2.03a	1.74±0.16d	13.69±1.96d	0.20±0.019f	0.10±0.000e	4.34±0.41b	6.48±1.06b	0.00c	0.00c
OFF	33.70±4.25c	17.59±1.63d	2.66±0.19bc	11.19±1.31e	0.19±0.009f	0.10±0.000e	4.38±0.80ab	7.22±0.99a	0.00c	0.00c
OFS	35.09±6.12bc	19.91±3.47bc	1.77±0.20d	16.48±2.49bc	0.29±0.068d	0.18±0.052d	3.94±0.55cd	2.66±0.69d	0.00c	0.00c
OFZ	39.52±5.57a	19.47±2.45	2.04±0.26ab	13.15±1.92d	0.22±0.045ef	0.11±0.040e	4.28±0.45bc	6.18±1.14b	0.00c	0.00c
OMB	37.35±6.04ab	20.32±2.62bc	1.84±0.22cd	15.67±2.65cd	0.36±0.068bc	0.21±0.032c	4.72±0.66a	6.66±0.83b	4.18±0.80a	2.84±0.50a
OMBS	30.53±4.26d	15.52±1.88e	1.97±0.29b	10.77±1.51e	0.24±0.058e	0.15±0.049d	3.72±0.65d	7.44±1.26a	2.68±0.46b	2.07±0.63b
OMK	37.30±6.17ab	24.07±4.47a	1.56±0.22e	18.31±2.59a	0.39±0.086ab	0.25±0.077a	3.80±0.68d	2.59±0.70d	3.77±1.20b	2.74±0.51a
OMN	36.07±4.12bc	16.89±2.03de	2.14±0.20a	15.74±3.12bc	0.41±0.069a	0.24±0.064ab	4.07±0.85bcd	7.48±1.45a	4.16±0.64a	2.52±0.52b
OS	27.07±3.06e	21.19±2.04b	1.27±0.08f	16.94±2.86b	0.35±0.048c	0.22±0.056bc	3.06±0.34e	3.52±0.64c	2.29±0.68b	1.71±0.28b
General Mean	35.15	19.75	1.81	14.66	0.299	0177	4.03	5.58	1.90	1.32
Lsd	2.676	1.520	0.114	1.264	0.032	0.026	0.372	0.538	0.326	0.200
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Lsd: Least significant difference,

drooping upper branches. The cladodes are large and very thorny, usually, green narrowly elliptical to oval, 19 − 51.5 cm long (x) 13 − 40 cm width (Fig. I. i). Areoles are arranged in series spaced between of 2.65 − 8.4 cm, and measure 0.1 − 0.6 cm long (x) 0.1 − 0.4 cm width with a density of 2 − 10 per 25 cm^2. Spines are white, elongated, conical rigid, and yellowish apex; the higher varies between 2 to 3 cm, generally 2 to 7 per areole. The flowers are large and yellow. The fruits are yellow and white, fleshy, conical to obovoid, and persistent on the plant (Figs. I., I, j, k & l).

Opuntia streptacantha Lem., 1839

Treelike plant very branched, reached up to 5 m high with a well-defined stem. The cladodes are green, thick, oval, and are less long than the two previous species, 24 − 35 cm long (x) 18 − 26 cm width (Figs. I., m, n & o). Areoles arranged in series, small, 0.15 − 0.4 cm long (x) 0.15 − 0.4 cm width and very closely together separated 2.5 − 3.85 cm with a density of 2 − 4 per 25 cm^2. The spines are less typical, flexible, acicular, white, and more or less small compared to the *O. megacantha,* unequal, the longest reached 2.7 cm, with number of 2 − 4 per areole. The flowers are yellow to orange, and the fruits are globular and dark red, and sometimes yellowish.

Morphological variability of the cladodes

Analysis of variance (ANOVA) showed significant differences between the populations for all the variables (Table 3). The majority of high values for cladode traits are shared between *O. ficus indica* and *O. megacantha*. The samples of *O. ficus indica* collected in Arris and Zeboudja have the highest cladodes, reaching up to nearly 40 cm. Those of *O. megacantha* from El Khenig present the greatest widths of the cladodes and the areoles as well as the base of the cladode (24.07 cm, 0.25 cm, and 18.3 cm, respectively). The samples from Ben Azouz present the highest distance between the areoles (4.72 cm), the average number of spines per areole (4.18), and length of the longest spine (2.84 cm). Population of Nador, showed the highest length and density of areoles with 0.41cm and 7.48, respectively. In contrast, *O. streptacantha* from Choucha, the southernmost population, stands out with the lowest values for these characteristics, compared to *O*. *ficus indica* and *O. megacantha*.

The PCA carried out on the basis of the average values of the characters per population includes the main bioclimatic parameters m, M, P, and Alt. In Fig. II, the relative contributions of the variables are plotted simultaneously with the distribution of the populations. The first two principal components, PC1 and PC2, explain 64.64% of the overall variance, i.e., 37.58% and 27.06%, respectively. The length of areoles (LA), the width of areoles (WA), the width of cladodes (WC), and the circumference of the base of the cladodes (CC) are positively correlated to PC1. In contrast, the distance between areoles (D), the density of areoles per 25 cm^2 (DE), the length of cladodes (LC), and the length/width ratio of cladodes (L/W) are located in the negative part of this axis. PC2 displays on positive side, the correlation between number of spines per areole (NS), the length of the longest spine (LS), the annual rainfall (P), and the minimum average of winter temperatures (m °C), while on the negative side, it displays the correlation with altitude (Alt) and maximum average of summer temperatures (M °C).

The distribution of the populations shows three main groups (Fig. II). Relative to PC1, two groups of populations are clearly distinguished. Populations from El-Khenig and Choucha belonging to *O. megacantha* and *O. streptacantha*, respectively, are located in the positive side of PC1. This group is characterized by the high values of width of cladodes and circumference of the base of cladode. On the opposite side, a second phenotypic group concerns only the populations of *O. ficus indica* from Fergoug, Zeboudja, and Arris, which are distinguished by the longest cladode with high length/width ratio, and high distance and density of areoles. PC2 highlights the third group of two populations of *O. megacantha* (Nador and Ben Azouz), characterized by a high number of spines per areole and a high length of longest spine. Two populations of *O. ficus indica* and *O. megacantha* (Sidi Fredj and Beni Snous) are distributed in an intermediate position between the two previous groups.

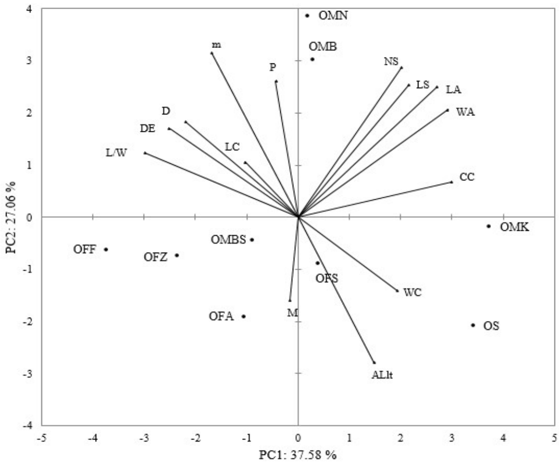

Fig. II. Principal components analysis of populations of three *Opuntia* species from Algeria based on morphological traits of cladodes and bioclimatic parameters.

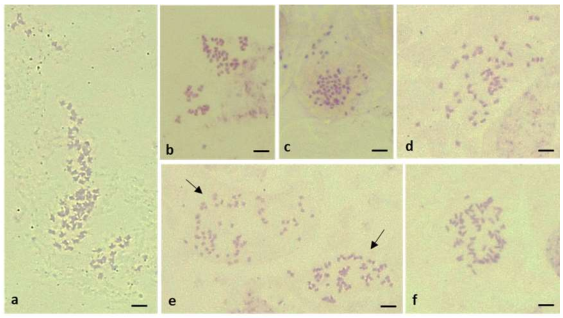

Fig. III. Mitotic metaphases plates: *O. ficus indica* 2n = 88 (a, b); *O. megacantha* 2n = 88 (c); *O. streptacantha* 2n = 66 (d,e,f); Scale bars = 5μm

Chromosome counts

The chromosome count revealed two ploidy levels for the populations studied, hexaploid (2n = 6x= 66) for *O. streptacantha* and octoploid (2n = 8x= 88) for both *O. ficus indica* and *O. megacantha* (Fig. III). All metaphases examined had very small metacentric chromosomes and mostly homogeneous in form, ranging from 0.83 to 2.28 µm in length. The smallest chromosomes were observed in *O. ficus indica* and *O. megacantha* varies between 0.83 – 1.69 µm and 0.91 – 1.87 µm, respectively. Metaphases in *O. streptacantha* showed the presence of larger chromosomes ranging from 1.93 to 2.28 µm.

Discussion

Taxonomical and biogeographical remarks

Several species of *Opuntia* have been introduced in various regions of the world, which have subsequently undergone a long process of naturalization and inbreeding (Erre et al., 2009). In Algeria, these species are found in various ecological conditions but are more widespread in areas where they are well-adapted to conditions of drought, irregular rainfall, and poor soils. Some species, such as *O. stricta* and *O. tomentosa*, became invasive and are often found associated with the xerophytic species in degraded stages of forests (Githae, 2018). Two of the studied species, *O. ficus-indica* and *O. megacantha*, are widespread and grow in various biotopes, while *O. streptacantha* is less common and is only found in Sidi Makhlouf, Sidi Taifour, and in Choucha located in the arid region of the south of Djelfa. In the Mediterranean basin, *O. streptacantha* has only been reported in Italy (Galasso et al., 2018) and in Spain in the province of Valencia (Verloove et al., 2024). *O. ficus-indica* can be easily differentiated from the two other species by the presence of large cladodes, totally or almost devoid of spines.

Plants of *O. streptacantha* were distinguished from *O. megacantha* by their small cladodes, being more roundish, having reduced and less typical spines, and being appressed and partly deflexed. Kiesling (1998), had recognized within *O. ficus-indica*, two botanical forms, *O. ficus-indica f. amyclae* with the presence of spines as main characteristic, and *O. ficus-indica f. ficus-indica* which is spineless. *O. megacantha* and *O. streptacantha* were considered by Kiesling (op. cited) synonymous of the first form. Some authors had assumed that *O. ficus-indica* is a distinct species (Reyes-Aguero et al., 2005b), while others suggested that *O. ficus-indica* is a domesticated taxon from the two species *O. megacantha* and *O. streptacantha* (Labra et al., 2003; Verloove et al., 2024).

Morphological traits of cladodes

The present study revealed a high morphological variability of the cladodes both within and between populations of *O. ficus indica*, *O. megacantha*, and *O. streptacantha*. According to results of PCA, the length of the longest spine, the number of spines per areole, the density and size of the areoles, are the most discriminating variables between the populations. Some variables, such as the length of the cladodes, the length, and the density of the areoles allow for better differentiation between *O. ficus indica* and *O. megacantha* compared to *O. streptacantha*. In addition, *O. streptacantha* revealed a lower number of spines than *O. megacantha*. The reduction of the number of spines is considered a consequence of domestication in some species of *Opuntia* (Hawkes, 1983). The spiny characteristics and the dimensions of the areoles are significant in species differentiation in *Opuntia* (Peña-Valdivia et al., 2008).

Hadjkouider et al. (2017) report that quantitative traits, such as flower length and the longest spine, or qualitative characteristics such as spine color, fruit color, flowering period, and timing of harvest maturity are relevant in the differentiating *O. ficus-indica*, *O. amyclaea*, *O. streptacantha*, *O. robusta*, and *O. engelmannii* from Algeria. In fact, species of genus *Opuntia* exhibit significant morphological variation, which makes their taxonomy difficult and confusing (Reyes-Agüero et al., 2005a; Muñoz-Urias et al., 2008). According to Peña-Valdivia et al. (2008), morphological analysis appears to be an effective method for detecting species differences within the genus *Opuntia*. In general, the shape and size of the cladodes and spines are the most distinctive characteristics, particularly within the *O. ficus indica* complex (Valdéz-Cepeda et al., 2003; Reyes-Agüero et al., 2005a, 2005b). The number of spines and the distances between the areoles are also among the major identification criteria (Mejía et al., 2013). Some authors have linked cactus diversity to environmental conditions, particularly precipitation and temperature (Mourelle and Ezcurra, 1996; 1997). Robinson (1974), reported that the characteristics of the spines, such as thickness, inclination, color, and arrangement, as well as the number per areole are also partially influenced by the environment during growth. A gradual variation of spines and areoles due to the bioclimatic effect has been observed in some populations of *O. ficus indica* from Algeria (Abli et al., 2017). According to the region of origin, the variation in the number of areoles and spines was also highlighted by Nefzaoui et al. (2019). Nieddu and Chessa (1996) had already shown that the presence and number of spines per areole are determined by the expression of genes under changing environment.

Chromosome number and polyploidy

The number of chromosomes of *O. ficus indica*, *O. megacantha* and *O. streptacantha* occurring in Algeria are reported here for the first time. The results allowed us to highlight two ploidy levels with base number x = 11, consistent with the genus *Opuntia* and most genera of Cactaceae (Las Peñas et al., 2017). The hexaploid level (2n = 6x = 66) was observed in *O. streptacantha*, and the octoploid level (2n = 8x = 88) was found in all samples *of O. ficus-indica* and *O. megacantha*.

The octoploid number is in accordance with previous numerations in some members of the series Ficus-indicae and Streptacanthae (Segura et al., 2007), and other species such as *O. hyptiacantha*, *O. tomentosa*, *O. cochinera,* and *O. hyptiacantha* (Palomino and Heras, 2001; Palomino et al., 2016).

The most quoted number of chromosomes for *O. ficus indica* is 2n = 88 (Palomino and Heras, 2001; Segura et al., 2007; Ahumada et al., 2019). The diploid (2n = 22) and tetraploid (2n = 44) cytotypes of *O. ficus-indica* would be the putative ancestors of the 6*x* and 8*x* polyploid cultivated varieties (Pimienta and Munoz-Urias, 1995). The origin and evolution of polyploidy from 2*x* to 8*x* depending on their origin, played an important role in the domestication of *O. ficus-indica* (Segura et al., 2007; Majure et al., 2012; Ahumada et al., 2019).

In the case of *O. streptacantha*, the hexaploid number found in our study does not correspond to either the diploid number (2n = 2x= 22) observed by Yuaza et al. (1973) or the octoploid number (2n =8x = 88) reported by Pinkava and Parfitt (1982) and Palomino and Heras (2001), respectively. The hexaploid number seems to be less frequent and has only been observed in few species such as *O. stricta*, *O. matudae*, and *O. oligacantha*.

The three genera of *Opuntia* studied have small chromosomes ranging in size from 0.83 to 2.28 µm. Compared to other species of *Opuntia*, such as *O. monacantha*, *O. megapotamica*, and *O. elata*, the mean chromosome length was 2.37 µm (1.43 – 2.94 µm) (Las Peñas et al., 2017). Studies by Palomino and Heras (2001), revealed that the chromosomes of *O. hyptiacantha* and *O. cochinera* were small, varying from 1.07 to 2.50 µm and 1.19 to 2.72 µm, respectively. In *O. streptacantha* from Algeria, the chromosomes were smaller (1.93 – 2.28 µm) than those observed by Palomino and Heras (2001), which were larger (1.55 – 3.57 µm). Others as *O. microdasys var. lutea*, *O. stricta var dillenii,* and *O. cylindrica*, including *O. ficus indica* from different localities in Eastern India showed also small chromosomes varying from 0.97 to 3.46 um (Bandyopadhyay and Sharma, 2000).

The diploid species are less frequent than the polyploids (Majure et al., 2012). The occurance of polyploidy is favored by natural hybridizations, which have played a major role in the evolution of the subfamily Opuntioideae (Las Peñas et al., 2009, 2017).

High ploidy levels are expressed phenotypically as an increase in vegetative parts (such as cladode size) and reproductive vigor (Scheinvar et al., 1995; Ahumada et al., 2019). Previously, Muñoz-Urias et al. (1995) had shown that the size and shape of the cladode might be a ploidy indicator. Therefore, polyploidy occurring in *Opuntia* would provide adaptive advantages in arid and semi-arid environments (Palomino et al., 2016).

Similarly, Pinkava (2002), highlighted a relationship between ploidy levels and the geographical distribution of certain *Opuntia* species growing in different regions. The lower genetic diversity observed within and between these regions suggest that the polyploidy in *Opuntia* has an autopolyploid origin (Omar et al., 2021).

Conclusion

Our study on *O. ficus-indica*, *O. megacantha*, and *O. streptacantha* emphasized the importance of morphological traits of cladodes (size and shape, number and length of spines, and areoles), which are likely linked to environmental conditions. New data on chromosome number and polyploidy have been acquired for the first time for these three *Opuntia* species in Algeria. These findings underline the morphological variability and the taxonomic complexity within the genus *Opuntia*. Further studies combining morphometric, genetic, and ecological data from a wider range of populations and species are needed to clarify taxonomic relationships and polyploid distribution patterns. This would improve our understanding of the diversification and evolution of this taxonomic group.

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