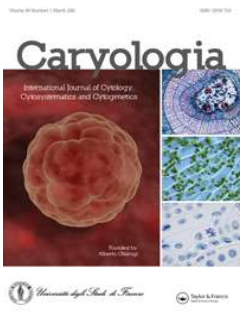

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Nuclear genome size, ploidy level and endopolyploidy pattern in six species of *Opuntia* (Cactaceae)

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ABSTRACT

This study describes the haploid chromosome number (*n*), recombination index (RI), ploidy level and DNA content by DNA flow cytometry of six *Opuntia* (prickly pear) species from Mexico. *Opuntia heliabravoana* was diploid ($2n = 2x = 22$, $x = 11$) with RI = 26; *O. jonocostle*, *O. matudae* and *O. oligacantha* were hexaploid ($2n = 6x = 66$) with RI = 74, 71, and 83, respectively; and *O. hyptiacantha* and *O. tomentosa* were octoploid ($2n = 8x = 88$) with RI = 99 and 103, respectively. The 2C DNA content was 3.81 pg in *Opuntia heliabravoana*. For hexaploid species 2C DNA content was: *O. jonocostle* = 5.84, *O. matudae* = 5.91 and *O. oligacantha* = 5.98 pg. The octoploid species *O. hyptiacantha* and *O. tomentosa* showed 2C DNA = 7.39 and 7.43 pg, respectively. The 1C*x* = 1.91 pg for diploid *O. heliabravoana* was 52% higher than the hexaploid species (mean 1C*x* = 0.99 pg) and 48% higher than the octoploid species (mean 1C*x* = 0.93 pg). These results suggest a loss of some DNA sequences after polyploidization in these *Opuntia* species. All accessions showed endopolyploidy, defined by the presence of nuclear populations of 2, 4 and 8C in cells of the stem parenchyma. The presence of endopolyploidy and polyploidy in prickly pear provide these plants with adaptive advantages in arid and semiarid environments. These results provide useful guidelines to set conservation strategies and breeding approaches for *Opuntia* species.

KEYWORDS

Opuntia; ploidy level; genome size; endopolyploidy

Introduction

Opuntia Miller is a genus that includes nearly 190 species (Guzmán et al. 2003) native to the Americas, extending from the Canadian High Plains to Argentine Patagonia. Mexico is the country with the highest richness of *Opuntia*, counting 83 species, more than 50 of which are endemic (Bravo-Hollis 1978; Pimienta-Barrios 1993; Guzmán et al. 2003; Hunt 2006).

The semiarid regions of Northern Mexico show the highest diversity of wild and cultivated species from the tribe Opuntioideae. These regions are considered as a diversity center of the genus *Opuntia* (Pimienta-Barrios 1990; Rebman and Pinkava 2001). The variability of *Opuntia* species in these regions has been associated with the mechanism of interspecific hybridization specifically, with an increment in polyploidy levels and geographic isolation (Pimienta-Barrios and Muñoz-Urías 1995; Scheinvar 1995; Majure et al. 2012).

Opuntia species, commonly known as prickly pear in the USA and *nopales* in Mexico, have been used as food, forage plants, and medicine. The tender stems (*nopalito*), the fruits (*tuna* and the more acidic *xoconostle*), and the seeds are consumed by humans (Bravo-Hollis and Sánchez-Mejorada 1991; Scheinvar et al. 2009).

Polyploidy, the possession of three or more complete sets of chromosomes representing the haploid genome (Ramsey and Schemske 1998; Soltis et al. 2003) has played significant roles in diversification and speciation processes in flowering plants (Stebbins 1971; Grant 1981; Leitch and Bennett 1997; Levin 2002; Coghlan et al. 2005; Leitch and Leitch 2008; Soltis et al. 2009; Soto-Trejo et al. 2013). Polyploidy is a mechanism that consists of a variation in the amount of nuclear DNA C value, with concomitant changes in chromosome number and structure (Murray et al. 2005).

Polyploidy is one of the major causes of speciation in the Cactaceae family, together with interspecific hybridization and cryptic chromosomal rearrangements (Grant and Grant 1980; Pinkava et al. 1985; Cota and Philbrick 1994; Pinkava 2002).

Cytological studies have shown that polyploidy is common in the Opuntioideae (Pinkava et al. 1985, 1998; Pinkava 2002; Parfitt and Gibson 2004; Rebman and Pinkava 2001). Of the 164 species of Opuntieae for which there are reported chromosome numbers, 26.2% are diploid, 60.4% are polyploid, and 13.4% are composed of diploid and polyploid cytotypes (Majure et al. 2012). The genus *Opuntia* has a base number $x = 11$, a

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Table 1. Provenance of *Opuntia* species studied.

Species	Origin of plants	Voucher information	Site of collection
O. heliabravoana	Wild	Scheinvar L & Fidardo S. 6524	Mexico, Hidalgo State, Pachuca. 20°07′21″ N, 98°44′10″W 2400 m asl
O. joconostle	Cultured	Scheinvar L & Domínguez D. 6502-H	Mexico. Mexico State, Otumba, Belen. 19°42′55″N, 98°49′48″W 2349.41 m asl
O. matudae	Cultured	Scheinvar L & Domínguez D. 6505-B	Mexico. Mexico State, Otumba, Belen. 19°42′55″N, 98°49′48″W 2349.41 m asl
O. oligacantha	Cultured	Scheinvar L. & Domínguez D. 6501-F	Mexico. Mexico State, Tepotzotlán. 19°42′58″ N, 99°13′25″W 2300 m asl
O. hyptiacantha	Cultured	Scheinvar L. 1405-A.	Mexico. Mexico State, Otumba, Belen. 19°42′55″N, 98°49′48″W 2349.41 m asl
O. tomentosa	Wild	Martínez J. 552.	Ecological Reserve of the Pedregal of San Angel, Mexico City. 19°20′22″ N, 99°8′26″W 2277 m asl

common number in all Cactaceae (Pinkava 2002). Levels of polyploidy in *Opuntia* vary from 3*x* to 30*x*, with tetraploids (4*x*) and hexaploids (6*x*) being the most frequent species (Pinkava et al. 1985; Majure et al 2012); nearly 6% of all polyploid species are 8*x* (Pinkava and Parfitt 1982). Meiotic and mitotic chromosome counting are the most common methods used to determine ploidy (Parfitt 1980; Ross 1981; Pinkava et al. 1985; Cid and Palomino 1996; Palomino et al. 1998; Palomino and Heras 2001; Del Angel et al. 2006; Negrón-Ortiz 2007; Soto-Trejo et al. 2013). Nuclear DNA content measurement using flow cytometry (FC) has also been an effective method for estimating ploidy levels (Mohanty et al. 1997; Das et al. 2000), for example in delineating infrageneric taxa (Ohri 2005), for identification of apomicts, and for dioecious sex determination in plant breeding (Dolezel et al. 2007a). Specific examples of prior DNA FC use in succulents include studies of *Opuntia microdasys* (Zonneveld et al. 2005) and several species of *Mammillaria* (Palomino et al. 1999; Del Angel et al. 2006).

Endopolyploidy is defined as the occurrence of different ploidy levels within an organism generated either by endoreduplication, predominantly in plants, or by endomitosis, which occurs primarily in animals (Barow 2006). Nearly 90% of angiosperms have tissues with different levels of ploidy, forming an endopolyploidy pattern (Joubès and Chevalier 2000; Barow 2006; Barow and Jovtchev 2007). The presence of an endopolyploidy pattern in different plant organs suggests that endoreduplication cycles are controlled by genes for differentiation and development of specialized functions (Cebolla et al. 1999). The presence of an endopolyploidy pattern is frequent in succulent desert plants with CAM metabolism (De Rocher et al. 1990; Cushman 2001). The endopolyploidy pattern could be an emergent trait in cacti and succulents that have a small genome size. This mechanism is hypothesized to provide succulents with the ability to generate larger cells with high levels of ploidy; these cells are able to store greater amounts of water, which is adaptive for these plants to survive and reproduce in arid environments (De Rocher et al. 1990).

This work describes variation for haploid chromosome number (*n*), nuclear DNA content, and ploidy level via FC among *Opuntia heliabravoana* Scheinvar, *O. joconostle* F.A.C. Weber. Ex Diguet, *O. matudae* Scheinvar, *O. oligacantha* C.F. Forst, *O. hyptiacantha* F.A.C. Weber, and *O. tomentosa* Salm-Dyck.

Materials and methods

Plant material

The accession and origin of adult plants of *Opuntia* species studied and their provenance are listed in Table 1. Voucher specimens were deposited in the MEXU herbarium (Herbario Nacional, Instituto de Biología, UNAM).

Haploid chromosome counts

Haploid chromosomes were prepared following Cid and Palomino (1996) and Martínez et al. (2000) with some modifications. Between 28 and 36 pollen mother cells (PMC) in metaphase I (MI) from three individual plants of each species of *O. heliabravoana*, *O. joconostle*, *O. matudae*, *O. oligacantha*, *O. hyptiacantha* and *O. tomentosa* were analyzed (Table 2). Ten anthers per bud were fixed in Farmer's solution (three parts absolute ethanol to one part glacial acetic acid) and then were stained in a solution of propionic-orcein at 1.8%. Metaphase I information was recorded, as follows: type of bivalents (IIs), type of quadrivalents (IVs), chiasma frequency per cell, and recombination index (RI) corresponding to the haploid number of chromosomes plus the average number of chiasmata per cell (Darlington 1937; White 1973). Hence, the value of RI was obtained by chromosome set. Slides were prepared and frozen with dry ice (Conger and Fairchield 1953) and mounted in Canada balsam. Photographs of the best PMCs in MI of each species were taken with a Zeiss photomicroscope II, using a Zeiss Axio Vision chamber (Hamburg, Germany).

Estimation of nuclear DNA content and ploidy level by FC

Ten to 13 young plants were used for the nuclear DNA content estimation, using flow cytometry in *O. heliabravoana*, *O. joconostle*, *O. matudae*, *O. oligacantha*,

Table 2. Type and frequency of bivalents, quadrivalents, chiasmata frequency and recombination index of *Opuntia* species.

Species	2*n*	Ploidy level	Bivalents II			Ring quadrivalents $\bar{X}$ ±SE				
			PMC	Ring $\bar{X}$±SE	Rod $\bar{X}$±SE		Chiasmata/cell $\bar{X}$±SE	Tukey*	RI $\bar{X}$±SE	Tukey*
O. heliabravoana	22	2*x*	36	3.00 ± 0.05	8.00 ± 0.07		15.25 ± 0.10	a	26.25 ± 0.14	a
O. matudae	66	6*x*	30	5.00 ± 0.07	28.00 ± 0.17		38.10 ± 0.20	c	71.10 ± 0.21	b
O. joconostle	66	6*x*	32	6.00 ± 0.70	25.00 ± 0.15	1.00 ± 0.03	40.77 ± 0.19	b	74.40 ± 0.26	c
O. oligacantha	66	6*x*	28	17.00 ± 0.14	16.00 ± 0.14		50.00 ± 0.25	d	83.00 ± 0.32	d
O. hyptiacantha	88	8*x*	30	9.00 ± 0.10	33.00 ± 0.14	1.00 ± 0.03	55.00 ± 0.24	e	99.00 ± 0.33	e
O. tomentosa	88	8*x*	31	12.00 ± 0.11	28.00 ± 0.17	2.00 ± 0.04	59.42 ± 0.24	f	103.42 ± 0.32	f

*The same letters indicate no statistical differences using α = 0.05, with ANOVA $p < 0.0001$.

O. hyptiacantha and *O. tomentosa*. Three replicates for each individual plant were analyzed. Internal standards were selected according to the ploidy level of each *Opuntia* species studied. *Solanum lycopersicum* cv. "Stupické polní rané", 2C DNA = 1.96 pg (Dolezel et al. 2007b) was used as internal standard for estimation of genome size in diploid *O. heliabravoana* (2*n* = 22). *Pisum sativum* L. cv. "Ctirad", 2C DNA = 9.09 pg (Dolezel et al. 2007b), was used as internal standard for estimation of genome size in *O. joconostle* (2*n* = 66), *O. matudae* (2*n* = 66), *O. oligacantha* (2*n* = 66), *O. hyptiacantha* (2*n* = 88) and *O. tomentosa* (2*n* = 88). Suspensions of intact nuclei were prepared according to Otto (1990) with some modifications as described by Dolezel et al. (2007b) and Palomino et al. (2003). Briefly, 500 mg of *Opuntia* stem parenchyma and 10 mg of young *Solanum lycopersicum* or *Pisum sativum* leaf tissue were simultaneously chopped with a razor blade in a Petri dish containing 1.5 ml of 0.1 M citric acid and 0.5% Tween 20. Chopped material was filtered through a 50 µm nylon mesh and incubated for 15 min at room temperature. The nuclei in the filtrate were then pelleted by centrifugation (90g for 3 min), suspended in 1 ml of the citric acid / Tween 20 solution, and incubated for 15 min at room temperature. Then, 2 ml of 0.4 M Na_2HPO_4 was added and the suspension was supplemented with 125 µl of propidium iodide and RNAse to a final concentration of 50 µg ml^{-1}.

The flow cytometric estimation of the nuclear DNA content was performed using a Partec CA II flow cytometer (FC), (Partec, Munster, Germany). Nuclei isolated from chicken red blood cells (Galbraith et al. 1998) were used to align the flow cytometer, checking its linearity by comparing the peak position of nuclei singles and clumps (doubles, triples, etc.). The gain record attributed to the instrument was adjusted so that the peak representing G_1 nuclei of *Solanum lycopersicum*, *Pisum sativum* or *Opuntia* species was positioned on channel 50 of the 250-channel linear scale. At least 10 thousand nuclei were analyzed in each sample. Peak means, areas and the coefficient of variation were calculated using DPAC software (Partec). Coefficients of variation were less than 5% assuring accurate measurements (Dolezel 1997). Nuclear genome size was then calculated according to Dolezel et al. (2007b), using the formula: A = (B/C) × D, where A = *Opuntia* 2C nuclear DNA content (pg); B = *Opuntia* G_0/G_1 peak mean; C = internal standard G_0/G_1 peak mean; and D = 2C DNA content internal standard (pg), 1 picogram (pg) = 978 megabase pairs (Dolezel et al. 2007b).

Endopolyploidy pattern

The endopolyploidy pattern was corroborated in the stem parenchyma in all *Opuntia* species. The number of nuclei was obtained by flow cytometry as described previously. As a measure of endopolyploidization, cycle value was calculated according to Barow and Meister (2003). The number of nuclei of each represented ploidy level was multiplied by the number of endoreduplication cycles necessary to reach the corresponding ploidy level. The sum of the resulting products was then divided by the total number of nuclei measured.

$$\text{Cycle value} = \left(0 \times n_{2C} + 1 \times n_{4C} + 2 \times n_{8C}\right) / \left(n_{2C} + n_{4C} + n_{8C}\right) \quad (1)$$

where $n_{2C} + n_{4C} + n_{8C}$ are the numbers of nuclei with the corresponding C-value (2C, 4C and 8C). An organ with a "cycle value" below 0.1 is considered not to be endopolyploid (Barow and Meister 2003).

Statistical analyses

Differences among the six *Opuntia* species in terms of the 2C DNA content in pg and Mbp of *Opuntia* species were evaluated according to an unbalanced one-way ANOVA, where species was considered as the main factor and the individual differences within each species were random effects; the number of individuals varied from 10 to 13.

Differences among six species of *Opuntia* in terms of chiasmata frequency per cell and RI were evaluated with an unbalanced one-way ANOVA, where the species was considered as the main factor and the individual differences within each species were the random effects. We analyzed 28 to 36 PMCs for each species. If results of these analyses were significant, a Tukey–Kramer HSD test for means differences was applied. The Shapiro–Wilk procedure was used to test normality for the standardized residuals in all statistical analyses. When needed, a Box–Cox transformation was applied

Table 3. Nuclear DNA content and genome size of *Opuntia* species.

Species	2*n*	2C nuclear DNA content (pg*) $\bar{X} \pm$ EE	1C*x* (pg*)	Tukey grouping	1C*x* (Mbp)
O. heliabravoana	22	3.81 ± 0.02	1.91	a	1863
O. joconostle	66	5.84 ± 0.03	0.97	b	952
O. matudae	66	5.91 ± 0.03	0.99	b	963
O. oligacantha	66	5.98 ± 0.04	1.00	b	975
O. hyptiacantha	88	7.39 ± 0.04	0.92	c	903
O. tomentosa	88	7.43 ± 0.04	0.93	c	908

1C*x* = value represents DNA content of one monoploid genome of chromosome number *x* (Greilhuber et al. 2005).
1 pg = 978 megabase pairs (Mbp) (Dolezel et al. 2007b).

to the standardized residuals. All statistical analyses were performed using the JMP version 11.0 software (SAS Institute, Cary, NC, USA).

Results

Haploid chromosome number

From the six species of *Opuntia* studied, *O. heliabravoana* was diploid (2*n* = 2*x* = 22), *O. joconostle*, *O. matudae* and *O. oligacantha* were hexaploid (2*n* = 6*x* = 66), and *O. hyptiacantha* and *O. tomentosa* were octoploid (2*n* = 8*x* = 88) (Tables 2, 3; Figures 1a-f).

Opuntia heliabravoana had 11 II; *O. joconostle* 31 II + 1 IV; *O. matudae* 33 II; *O. oligacantha* 33 II; *O. hyptiacantha* 42 II + 1 IV; and *O. tomentosa* 40 II + 2 IV. An RI value of 26.25 was recorded for *O. heliabravoana*, with RI values = 71.10, 74.40, 83.00 recorded for *O. matudae*, *O. joconostle*, and *O. oligacantha* (2*n* = 66), respectively. *Opuntia hyptiacantha* and *O. tomentosa* were octoploid with RI = 99.00 and 103.42, respectively (Table 2; Figure 1). Differences in the chiasmata frequency per cell and their RI among the six studied species of *Opuntia* were significantly different (ANOVA, $p < 0.0001$; Table 2).

Genome size and polyploidy levels

Combined data of all chromosome counts and DNA flow cytometry results confirm three ploidy levels in *Opuntia* studied species: diploid, hexaploid and octoploid (Tables 2, 3, 4). We obtained the smallest DNA content or genome size in *O. heliabravoana* diploid plants with 2C DNA = 3.81 pg (Table 3; Figure 2a), and the largest genome size corresponded to the octoploid *O. tomentosa* with 2C DNA = 7.43 pg.

There were significant differences in genome size among the diploid, hexaploid and octoploid species (Tukey–Kramer test; Table 3; Figure 2c).

The six species of *Opuntia* we studied showed an endopolyploid pattern composed of 2C, 4C, and 8C cells, with different percentages of nuclei in each one of the ploidy levels (Table 4; Figure 2a–d). The cycle values for the six *Opuntia* species are listed in Table 4. According to the threshold value established by Barow and Meister (2003), all the *Opuntia* species in this study had endopolyploidy patterns, since the cycle values were above 0.1. The lowest cycle value was found in *O. hyptiacantha* (0.44) and the highest cycle value was found in *O. heliabravoana* (1.07).

Discussion

This study confirms the base chromosome number *x* = 11 for *Opuntia* (Table 2; Figure 1), previously proposed by Pinkava et al. (1985), and by Pinkava (2002) for Cactaceae. Segura et al. (2007) also observed a diploid cytotype for *O. heliabravoana* (2*n* = 2*x* = 22). We observed a hexaploid cytotype for *O. joconostle* (2*n* = 6*x* = 66; Table 2; Figure 1b), whereas Segura et al. (2007) reported an octoploid cytotype for the same species (2*n* = 8*x* = 88). In the case of *O. matudae* and *O. oligacantha* we observed hexaploid cytotypes (2*n* = 66; Table 2; Figure 1c, d), thus confirming the results obtained previously by Segura et al. (2007). In *O. hyptiacantha* and *O. tomentosa* we observed octoploid cytotypes (2*n* = 88; Table 2; Figure 1e, f). Palomino and Heras (2001) and Segura et al. (2007) previously reported an octoploid cytotype (2*n* = 8*x* = 88) in *O. hyptiacantha*. The reports of Katagari (1952, 1953) and Yuasa et al. (1973) coincided with this observation (*n* = 44 and 2*n* = 88, respectively).

Meiotic chromosomal irregularities in the form of ring quadrivalents were recorded in polyploid *Opuntia* plants but were not observed in the diploid *O. heliabravoana*. This may represent increased genetic recombination in the polyploid plants, associations among multiple homologs in the case of autopolyploidy, or even homoeologous chromosome pairing. This behavior was observed in polyploid species of *Senecio* (Lawrence 1985). Results in this study showed that the RI increased incrementally with the level of polyploidy. Similar results had been found by Lawrence (1985) when studying several species of *Senecio*. On average, diploid species (*n* = 10) had RI = 22.7, tetraploid species (*n* = 20) had RI = 52.3, hexaploids (*n* = 30) had RI = 78.1, octoploids (*n* = 40) had RI = 95.70, and decaploid species (*n* = 50) had RI = 140.

The mean 1Cx value (1.91 pg; 1863 Mbp), which represents the mean DNA amount of the monoploid chromosome set in the diploid species *O. heliabroavana*, was 52% and 48% greater than those mean values showed by the hexaploid and octoploid species (1C*x* = 0.99 and 1Cx = 0.93 pg), respectively (Table 3). These results may

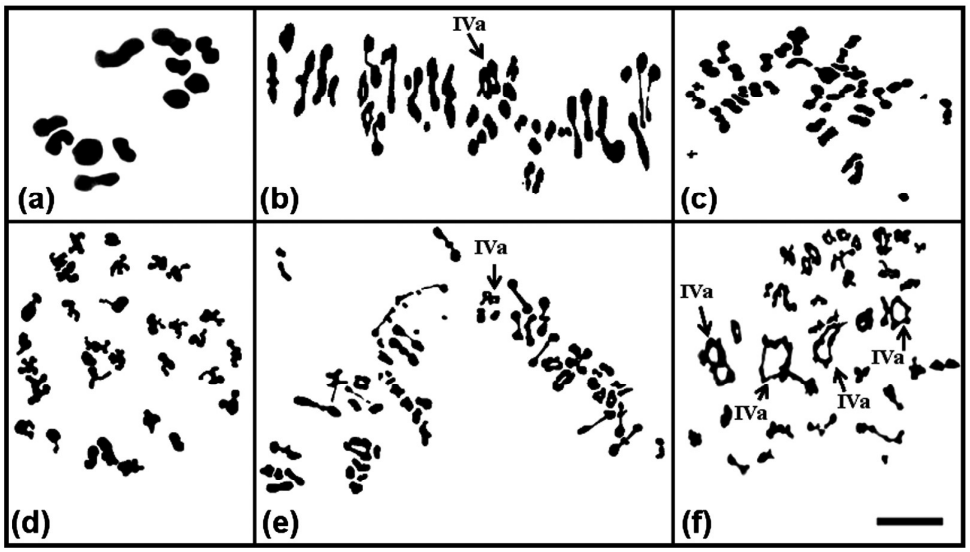

Figure 1. PMC in metaphase I of meiosis for different *Opuntia* species. (a) *Opuntia heliabravoana* $n = 11$, with 11 II (3 ring II + 8 rod II). (b) *Opuntia joconostle* $n = 33$, with 31 II (6 ring II + 25 rod II) + 1 ring IV. (c) *Opuntia matudae* $n = 33$, with 33 II (5 ring II + 28 rod II). (d) *Opuntia oligacantha* $n = 33$, with 33 II (17 ring II + 16 rod II). (e) *Opuntia hyptiacantha* $n = 44$, with 42 II (9 ring II + 33 rod II) + 1 ring IV. (f) *Opuntia tomentosa* $n = 44$, with 36 II (18 ring II + 18 rod II) + 4 ring IV. Note: Scale bar equals 10 µm.

Table 4. Pattern of endopolyploidy level and cycle value in stem parenchyma of *Opuntia* species.

		Percentage of nuclei populations			
Species	2*n*	2C	4C	8C	Cycle value
O. heliabravoana	22	24.31	44.82	30.87	1.07
O. joconostle	66	32.37	44.05	23.58	0.91
O. matudae	66	47.69	42.86	9.45	0.62
O. oligacantha	66	39.95	29.23	30.82	0.91
O. hyptiacantha	88	59.37	36.90	3.74	0.44
O. tomentosa	88	42.40	44.83	12.78	0.70

be indicative of the loss of some DNA sequences in the polyploid species. In other genera, the increase in ploidy level has also been linked to the loss of certain DNA sequences, as in *Fritillaria* (Bennett and Smith 1976) and *Helianthus* (Bennett and Smith 1991), *Bulnesia* (Poggio and Hunziker 1986), *Larrea* (Poggio et al. 1989), *Agave* (Cavallini et al. 1996; Palomino et al. 2003, 2005, 2012), *Passiflora* (Magalháes et al. 2004) and in several species of Asteraceae (Soto-Trejo et al. 2013).

The diploid level in *Opuntia heliabravoana* ($2n = 22$) has also been reported by Segura et al. (2007). These authors reported its genome size as 2C DNA = 6.53 pg. In this study we report a genome size of 2C DNA = 3.81 pg for this diploid species. The most probable explanation for this discrepancy is the use of different reference standards. Segura et al. (2007) used *Hordeum vulgare* cv. Sultan (2C DNA = 11.12 pg).

Plants of *O. joconostle* were hexaploid ($2n = 66$) and showed a genome size 2C DNA = 5.84 pg (Table 3). This value differs from what was reported for this species by Segura et al. (2007), who found the octoploid form ($8x$) for this species having 2C DNA = 4.70 pg. Segura et al. (2007) did an interpretation of ploidy level of the *Opuntia* species using the genome size estimated by DNA FC. We consider that chromosome counting should be determined together with estimation of DNA content by FC for determining ploidy level of a species.

According to our results, *O. matudae* and *O. oligacantha* were hexaploid ($2n = 66$, 2C DNA = 5.91 and 5.98 pg, respectively, Table 3). Segura et al. (2007) also reported hexaploid cytotypes for these species, but with a genome size of 2C DNA = 5.25 and 5.33 pg, respectively.

Plants of *O. hyptiacantha* were octoploid ($2n = 88$) and showed a genome size for 2C DNA = 7.39 pg (Table 3). Negrón-Ortiz (2007) reported a genome size of 2C DNA = 7.60 pg for *O. acaulis* ($8x$), which is a similar result to our observed values of 2C DNA = 7.39 pg and 7.43 pg, respectively, for octoploid *O. hyptiacantha* and *O. tomentosa* (Table 3).

The study species showed an endopolyploidy pattern according to cycle value results. The endopolyploidy pattern is considered a common metabolic adaptation of plants in arid and semiarid environments, as evidenced by plants of the Cactaceae and Crassulaceae families (Cushman 2001), and is considered an emergent trait in succulents and cacti with a small genome size that was favorable for increasing cellular water storage capacity (De Rocher et al. 1990). These authors

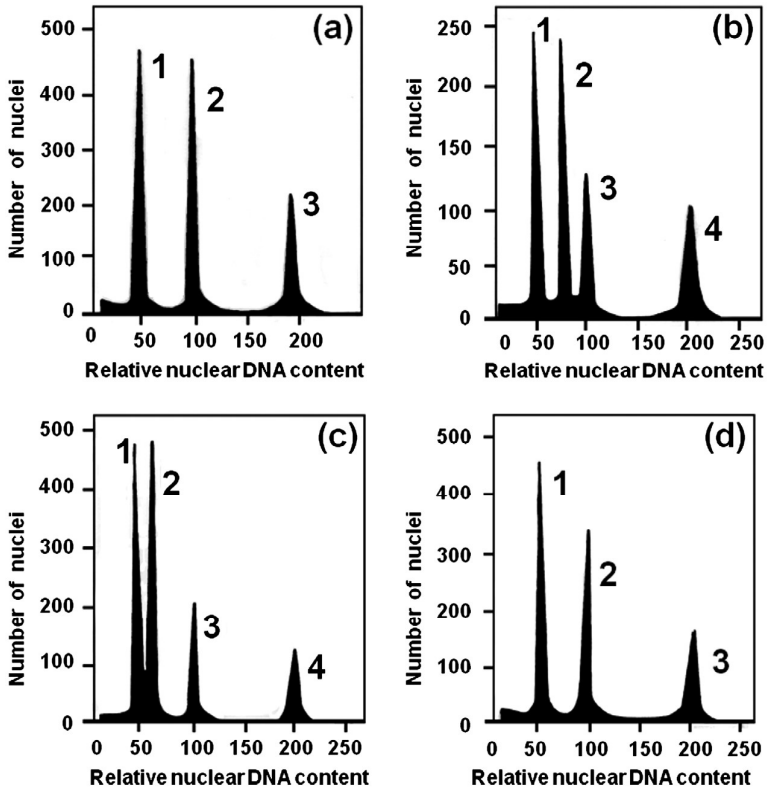

Figure 2. Estimation of nuclear DNA content in *Opuntia* species using flow cytometry. (a) Simultaneous analysis of nuclei isolated from *O. heliabravoana* (2*n* = 22) and *Solanum lycopersicum* L. "Stupické polní rané" used as internal standard. Peak 1 represents G_1 nuclei of *Solanum lycopersicum* L. Peaks 2 and 3 represent G_1 and G_2 nuclei of *O. heliabravoana*. (b) Simultaneous analysis of nuclei isolated from *O. oligacantha* (2*n* = 66) and *Pisum sativum* L. "Ctirad", used as internal standard. Peaks 1, 3, and 4 represent nuclei in G_1 (2C), G_2 (4C) and 8C in *O. oligacantha*. Peak 2 represents G_1 nuclei of *Pisum sativum*, used as internal standard. (c) Simultaneous analysis of nuclei isolated from *O. hyptiacantha* (2*n* = 88). Peaks 1, 3 and 4 represent nuclei in G_1 (2C), 4C and 8C in *O. hyptiacantha*. Peak 2 represent G_1 nuclei of *Pisum sativum*. (d) Distribution of nuclear DNA content of *O. joconostle* (2*n* = 66), showing a pattern of endopolyploidy. Peaks 1, 2 and 3 represent nuclei with 2C, 4C and 8C, respectively.

described a developmental gradient in ploidy levels (2C–64C) that was correlated with the age of leaves in *Mesembryanthenum crystillinum*. These plants showed six ploidy levels with the 2C nuclei comprising only a minor part of the total nuclei population. In *Pereskia grandiflora* the same authors observed endopolyploidy, defined by nuclei with 2, 4 and 8C. *Mammillaria san-angelensis* is a diploid species with an endopolyploidy pattern including 4C, 8C, 16C and 32 C DNA nuclei, with 2C nuclei being the most abundant (Palomino et al. 1999). A similar endopolyploidy pattern with values of 2, 4, 8 and 16 C DNA was found in seven diploid species of *Mammillaria* by Del Angel et al. (2006).

The common presence of an endopolyploidy pattern in cells from tissues of different organs suggests that the endoreduplication cycle in plants is coded by genes programmed for development, differentiation and specialized function (Cebolla et al. 1999). De Rocher et al. (1990) have associated the presence of endopolyploidy in succulents with CAM metabolism.

Cytogenetic analyses of the present study have allowed the characterization of the genomes of six species of *Opuntia*, including evaluation of their intra-specific genomic variation and interspecific divergence patterns. Results of this study can help to clarify taxonomic issues in *Opuntia* and also in planning *in situ* and *ex situ* conservation strategies for these species.

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