CARYOLOGIA Vol. 59, no. 2: 177-186, 2006

Nuclear genome size and karyotype analysis in Mammillaria species (Cactaceae)

Del Angel Christian[1], Guadalupe Palomino[1*], Armando García[2] and Ignacio Méndez[3]

[1] Instituto de Biología, Jardín Botánico, Universidad Nacional Autónoma de México, México D.F., C.P. 04510, México.
[2] Instituto de Recursos Genéticos y Productividad, Colegio de Posgraduados, carretera México-Texcoco, km 36.5. Montecillos, Texcoco, Edo. de México, C.P. 56230, México.
[3] Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas, Universidad Nacional Autónoma de México, México D.F., 01000, México.

Abstract — Seven species of *Mammillaria* were studied, all diploid: 2*n*=2*x*=22, *x*=11. Genome size was determined by flow cytometry, varied from 2C DNA =3.20 pg, 1570 Mbp (1 C*x*) in *M. crucigera*, to 2C DNA = 3.04 pg, 1490 Mbp in *M. flavicentra*. The variation of these species in the nuclear content of DNA was 5% and was not significant (P = 0.3469). This indicates that they are small, very stable genomes, in which changes in DNA content are not very evident. The variation among species, however, was clearly evident in the relative length (L%) and arm ratio (r), the proportion of metacentrics and submetacentrics, and the position of satellites. The karyotype for *M. albilanata*, *M. dixanthocentron* and *M. flavicentra* was 11m, and in *M. huitzilopochtli*, 10m + 1 sm. Only one pair of chromosomes was observed with satellite in the four species. *M. dixanthocentron* and *M. flavicentra*, species considered synonymous, exhibited the satellite on different chromosomes. The interspecific variation observed among the species of *Mammillaria* is possibly due to spontaneous structural changes in their chromosomes. These mechanisms of restructuring in the genome of these species have not involved significant changes in nuclear DNA content. The *Mammillaria* species exhibited an endopolyploidy pattern with 2-16 C DNA content in the stem parenchyma, which may give them alternative strategies for adaptation in arid environments.

Key words: endopolyploidy, flow cytometry, genome size, karyotype, *Mammillaria*, nuclear DNA content, ploidy level.

INTRODUCTION

The genus *Mammillaria* Haworth (Cactaceae) comprises 166 species, of which 150 are endemic of Mexico (Hernández and Godinez 1994). This genus of the family Cactaceae is considered that of most recent evolution (Bravo-Hollis 1978). For the *Supertextae* series of the genus *Mammillaria*, Hunt (1987) recognizes seven species; Bravo-Hollis and Sánchez-Mejorada (1991) consider 20, while Lüthy (1995) recognizes eight. This indicates that there is synonymy for some species; for example, for Bravo-Hollis and Sánchez-Mejorada, *M. dixanthocentron* and *M. flavicentra* are independent species, while for the other two authors *M. flavicentra* is a synonym of *M. dixanthocentron*. Some species of the *Supertextae* series are endemic of Mexico and are included in official lists of threatened plants (Semarnat 2001; Cites 2005).

There are 159 reports of chromosomal number in species of *Mammillaria*, 146 refer to diploid species (2*n*=2*x*=22; *x*=11) and the rest report polyploids. Karyotypes of *Mammillaria* species, as in most cacti, are characterized by the presence of metacentric and submetacentric chromosomes (Remski 1954; Gill and Goyal 1984; Cota and Wallace 1996; Das *et al.* 1998a, 1999b, 1999c; Palomino *et al.* 1999; Briones *et al.* 2004). In some species of the *Supertextae* series, karyotype studies have been conducted in diploids (2*n*=2*x*=22), such as in *M. vaupelii* (Remski 1954), *M. lanata* (Gill and Goyal 1984), *M. san-angelensis* (Palomino *et al.* 1999), *M. supertexta*, *M. haageana* and *M. crucigera* (Briones *et al.* 2004), and in *M. ruestii*, tetraploid species (2*n*=4*x*=44) (Remski 1954). These authors observed interspecific variation in karyotypes of the

* Corresponding author: phone +52+55+5622-9045; fax +52+55+5622-9046; e-mail: palomino@ibiologia.unam.mx

species, in terms of proportion of metacentric and submetacentric chromosomes, as well as number and position of satellites, variations caused by chromosomal rearrangement.

Analysis of nuclear DNA content has revealed interspecific variation of the genome size in different groups of plants (BENNETT *et al.* 2000); in other species intraspecific variation has also been observed (OHRI 1998; PALOMINO and SOUSA 2000). Recent reviews have show that genome size has been determined in 4427 species of angiosperms (BENNETT and LEITCH 2005). In these plants genome size varies almost 1000 times, from 2C DNA = 0.2 pg in *Fragaria viridis* to 250 pg in *Fritillaria assyriaca* (BENNETT *et al.* 2000). Nuclear DNA content has been determined for two diploid species of the *Supertextae* series, *M. albilanata* with 2C DNA = 10.16 pg, obtained by Feulgen densitometry (MOHANTY *et al.* 1996) and *M. sanangelensis* 2C DNA = 3.20 pg, obtained by flow cytometry (PALOMINO *et al.* 1999).

Flow cytometry allows precise and rapid estimation of nuclear DNA content and ploidy levels; it is a method that has numerous applications in taxonomy and plant breeding (DOLEZEL and BARTOS 2005). This study reports somatic chromosomal number (2*n*), karyotype, and nuclear DNA content by flow cytometry in four species of *Mammillaria* of the *Supertextae* series: *M. albilanata*, *M. dixanthocentron*, *M. flavicentra* and *M. huitzilopochtli*. In *M. crucigera*, *M. haageana* and *M. supertexta*, only nuclear DNA content is reported; the karyotype of these species was obtained by BRIONES *et al.* (2004).

MATERIALS AND METHODS

Plant material - Plants of *Mammillaria albilanata*, *M. crucigera*, *M. dixanthocentron*, *M. flavicentra*, *M. haageana*, *M. huitzilopochtli* and *M. supertexta* were collected by G. Reyes from wild populations in the Mexican states of Puebla and Oaxaca (Table 1). Live plants were transplanted and maintained in a greenhouse at the Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México (JB-IBUNAM). Voucher specimens were deposited in the National Herbarium (MEXU) of the UNAM.

Mitotic chromosome analysis - For the observation of chromosome number (2*n*) and the karyotypes of the four species of *Mammillaria*, 2 to 6 mitotic cells at metaphase stage from 2 plants of each species were observed. Elongating secondary root tips were placed in a solution of 8-hydroxyquinoline 0.002 M for 6 hours at 18-20°C in darkness. Later the root tips were fixed in Farmer solution (3 parts absolute alcohol: 1 part acetic acid) for 24 hours. The root tips were hydrolyzed in hydrochloric acid (1N) for 11 minutes at 60° C and transferred to Feulgen reagent for 2 hours, following GARCÍA (1990) and CID and PALOMINO (1996) procedures. Slides were frozen with dry ice (CONGER and FAIRCHILD 1953) and mounted in Canada balsam. Three of the best cells were photographed with Technical Pan Film using Zeiss photomicroscope II.

Karyotype analysis - The negative film was used to draw and measure chromosome arms and total genome length. Centromere position was determined using a system of LEVAN *et al.* (1964); arm ratio (r = long arm/short arm) was calculated for each chromosome. Index of asymmetry (TF%) was obtained following GUPTA and GUPTA (1978) procedures.

Table 1 — Provenance and karyotype analysis of *Mammillaria* species, collected by G. Reyes.

Species and locality	2n	Range of chromosome length (μm)	Total haploid genome length (μm)	Karyotype formula	Number of Satellites	Index of asymmetry (TF%)
M. albilanata México. Oaxaca State. 3843	22	1.56 - 3.37	25.78	11 m	1	46.50
M. dixanthocentron México. Oaxaca State. 4823	22	1.54 - 2.53	22.98	11 m	1	44.38
M. flavicentra México. Oaxaca State. 4821	22	1.90 - 3.73	30.37	11 m	1	45.97
M. huitzilopochtli México. Oaxaca State. 4822	22	1.75 - 2.59	25.10	10m + 1sm	1	43.49

Meiotic chromosome analysis - Meiotic chromosome behavior was observed in pollen mother cells (PMC) from fresh anthers fixed in Farmer solution. Fresh anthers were squashed in 1.8% propionic-orcein solution. Slides were frozen with dry ice (CONGER and FAIRCHILD 1953). A total of 12 MI of PMC from a plant were analyzed. The following information was recorded: type of bivalents (IIs), chiasma frequency and recombination index (RI) (SÁEZ and CARDOSO 1978).

Pollen fertility - Estimates were made in samples of pollen stained with cotton blue in lactophenol of *M. albilanata*, *M. dixanthocentron*, *M. flavicentra* y *M. huitzilopochtli*. Percentages of well-filled stained grains were obtained from 3000 pollen grains collected from three plants from each species.

Estimation of nuclear DNA content - Five adult and young plants (1 to 5 years old) were used in the estimation of nuclear DNA content by flow cytometry. In all cases three replicates for each individual plant were analyzed. *Lycopersicon esculentum* cv. "Stupické polní rané", 2C DNA = 1.96 pg (DOLEZEL *et al*. 1992) was used as internal standard for estimation of genome size in *Mammillaria albilanata*, *M. crucigera*, *M. dixanthocentron*, *M. flavicentra*, *M. haageana*, *M. huitzilopochtli* and *M. supertexta*. Suspensions of intact nuclei were prepared according to OTTO (1990) and PALOMINO *et al*. (1999) with some modifications described by DOLEZEL and GÖHDE (1995). Briefly 550 mg of *Mammillaria* stem parenchyma and 3 mg of young *L. esculentum* leaf were simultaneously chopped with a razor blade in a petri dish containing 3 mL of 0.1 M citric acid and 0.5% Tween 20. The chopped material was filtered through 50 μm nylon mesh and incubated for 10 minutes at room temperature. The nuclei in the filtrate were then pelleted by centrifugation (1000 rpm per 3 min), suspended in 500 μl citric acid / Tween 20 solution, and incubated for 10 minutes at room temperature. Subsequent to this, 2 mL of 0.4 M Na_2HPO_4 was added and suspension was supplemented with 125 μL of propidium iodide and RNase to final concentration of 50 μg mL^{-1} and analyzed by flow cytometry.

Flow cytometric analysis - The flow cytometric estimation of nuclear DNA content was performed using Partec CA II flow cytometer (Partec GmbH Münster, Germany). Nuclei isolated from chicken red blood cells (GALBRAITH *et al*. 1998) were used to align the flow cytometer checking its linearity by comparing peak position of nuclei singles and clumps (doubles, triplets, etc). The instrument gain was adjusted so that the peak representing G_1 nuclei of *L. esculentum* was positioned on channel 50. At least ten thousand nuclei were analyzed in each sample. Peak means, areas and coefficients of variation were calculated using DPAC software (PARTEC). Nuclear genome size was then calculated according to DOLEZEL (1995) using the formula:

$$\textit{Mammillaria}\text{ species 2C DNA (pg)} = \frac{\textit{Mammillaria}\text{ species } G_1/G_0 \text{ peak mean}}{\textit{L. esculentum}\text{ plant } G_1/G_0 \text{ peak mean}} \times \text{2C DNA content standard plant (pg)}$$

Statistical analysis - Differences in 2C DNA content in pg and Mbp (1 pg = 980 Mbp; Bennett *et al*. 2000) for *Mammillaria* species were evaluated according to a complete nested unbalanced analysis of variance (ANOVA). The first level of analysis corresponded to the species, the second level to five individuals within species and the third level corresponded to replicates within individual plant (7 species x 5 individuals x 3-8 replicates). Additionally, restricted maximum likelihood estimation (REML) was applied to data analysis; in both cases variance homogeneity was tested. All statistical analysis was performed using the JMP version 5.01 software.

RESULTS AND DISCUSSION

The species *Mammillaria albilanata*, *M. dixanthocentron*, *M. flavicentra*, and *M. huitzilopochtli* exhibited a $2n=2x=22$ diploid chromosomal number (Table 1; Fig. 1 A-D). In *M. dixanthocentron* $n=11$ was observed. These reports are agree with observations on other species of the *Supertextae* series, such as *M. vaupelii* (REMSKI 1954), *M. lanata* (GILL and GOYAL 1984), *M. albilanata* (MOHANTY *et al*. 1996), *M. san-angelensis* (PALOMINO *et al*. 1999), *M crucigera*, *M. haageana* and *M. supertexta* (BRIONES *et al*. 2004), corroborating $x = 11$, reported for the genus *Mammillaria* and the Cactaceae family.

Including the chromosome counts in *M. dixanthocentron*, *M. flavicentra* and *M. huitzilopochtli*, reported in this paper, there are a total of 162 for species of *Mammillaria*, of these 149 (92%) are diploid (REMSKI 1954; GILL and GOYAL 1984; DAS *et al*. 1998a, 1999b, 1999c; PALOMINO *et al*. 1999; BRIONES *et al*. 2004), 5% are tetraploids and the remaining 3% comprise

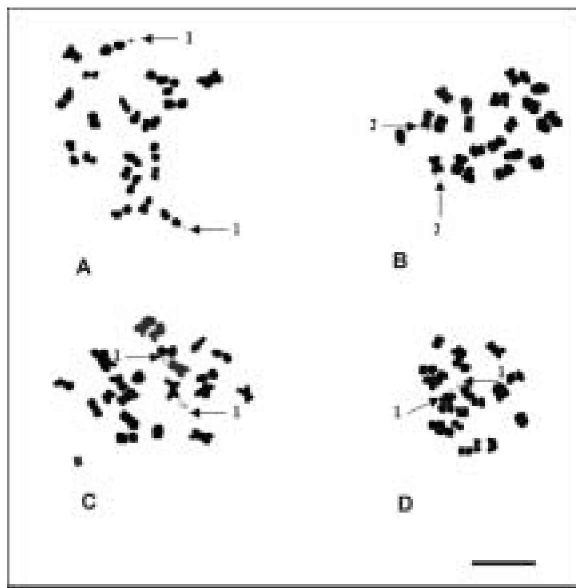

Fig. 1 — Somatic chromosomes, $2n=2x=22$, in A) *Mammillaria albilanata*, B) *M. dixanthocentron*, C) *M. flavicentra* and D) *M. huitzilopochtli*. Numbers show chromosomes with satellites. Scale equals 10 μm.

hexaploids, octoploids and 24*x*. In another 15 genera of the Subfamily Cactoideae, tetraploids have also been observed at low frequencies; this seems to indicate that polypoidy has not been determinant in speciation (GIBSON and NOBEL 1986).

Chromosome length varied in the four species studied; the greatest interval of chromosome length was found in *M. flavicentra* (1.90 – 3.73 μm) and the smallest in *M. dixanthocentron* (1.54 – 2.53 μm). The chromosomes of *M. albilanata* and *M. huitzilopochtli* were 1.56 – 3.37 and 1.75 – 2.59 μm, respectively (Tables 1 and 2; Figs. 1, 2 and 3). In the species of the *Supertextae* series, *M. crucigera*, *M. haageana* and in *M. supertexta* (BRIONES *et al.* 2004), interspecific variation was found in chromosome size, within the interval of 1.51 – 3.21 μm, and the lowest value for the interval in length of the chromosomes was 0.80 – 1.70 μm in *M. san-angelensis* (PALOMINO *et al.* 1999).

When chromosome size and total haploid genome length (GL) of the four species were compared with that reported for other species of the *Supertextae* series, it was observed that *M. san-angelensis* had shorter GL = 13.83 μm (PALOMINO *et al.* 1999) than *M. albilanata* (25.78 μm), *M. dixanthocentron* (22.98 μm), *M. flavicentra* (30.37 μm) and *M. huitzilopochtli* (25.10 μm) (Table 1). GL in *M. supertexta, M. crucigera* and *M. haageana* were 26.84, 23. 81 and 23.06 μm, respectively (BRIONES *et al.* 2004).

Comparison of relative length (L%) and the arm ratios (r) of the 11 chromosome pairs of the four species showed that for L%, pairs 1 and 10 are those that most differ among the species, while for the r value, they were pairs 9 and 11. When chromosome pairs of *M. flavicentra*, considered synonymous species of *M. dixanthocentron* (HUNT 1987), are compared, the greatest difference was observed in pairs 1 and 10 for L% and in 7 and 10 for r, although in both species all of their chromosomes were metacentric (Table 2; Fig. 2).

In *M. crucigera*, *M. haageana*, *M. supertexta* (BRIONES *et al.* 2004) and *M. san-angelensis* (PALOMINO *et al.* 1999), differences in L% and r were reported in 11 chromosome pairs; 1, 4 and 9 were those that differed most from their chromosome complements. In *M. haageana* and its synonymous species *M. san-angelensis* (HUNT 1987), greater differences for L% were observed in the chromosome pairs 1, 4 and 9; *M. san-angelensis* exhibited a karyotype formed by 11 metacentric pairs, while *M. haageana* had 2 submetacentric pairs, numbers 4 and 9 (BRIONES *et al.* 2004). This confirms that

Table 2 — Relative length (L%) and arm ratio (r) of somatic chromosomes of *Mammillaria albilanata*, *M. dixanthocentron*, *M. flavicentra* and *M. huitzilopochtli*.

Chromosome pair	*M. albilanata*		*M. dixanthocentron*		*M. flavicentra*		*M. huitzilopochtli*	
	L%	r	L%	r	L%	r	L%	r
1	13.07	1.02	11.00	1.12	12.28	1.14	10.31	1.00
2	11.28	1.03	10.92	1.26	11.52	1.12	10.31	1.33
3	10.55	1.19	10.09	1.25	10.50	1.21	10.27	1.00
4	10.04	1.33	9.61	1.23	9.51	1.29	9.92	1.07
5	9.30	1.16	9.48	1.18	9.28	1.27	9.56	1.16
6	9.30	1.16	9.13	1.23	8.72	1.28	8.84	1.00
7	8.96	1.08	8.83	1.20	8.62	1.31	8.84	1.00
8	7.99	1.14	8.39	1.32	8.26	1.18	8.80	1.18
9	6.98	1.16	8.13	1.20	8.10	1.23	8.08	1.70
10	6.43	1.00	7.65	1.25	6.81	1.43	8.04	1.19
11	6.05	1.00	6.70	1.26	6.25	1.50	6.97	1.10

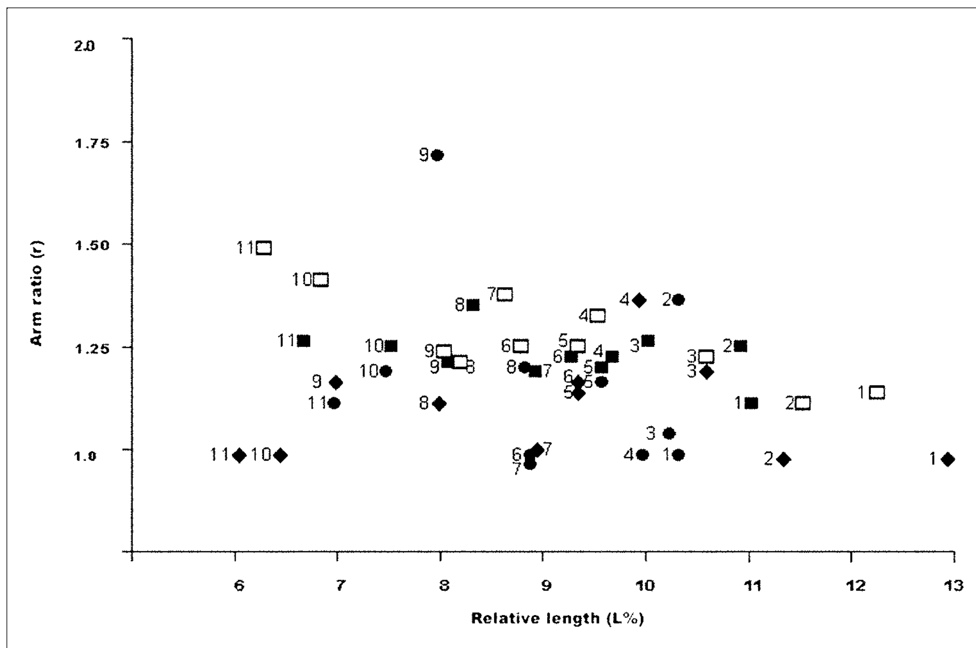

Fig. 2 — Graph of relative length (L%) and arm ratio (r) of chromosome measurement in table 2. *Mammillaria albilanata* (◆), *M. dixanthocentron* (■), *M. flavicentra* (□) and *M. huitzilopochtli* (●).

among the species of *Mammillaria* of the *Supertextae* series, there are interspecific differences in relative length and arm ratio (r) (Table 2; Fig 2).

The species studied, *M. albilanata*, *M. dixanthocentron* and *M. flavicentra,* exhibited 11 metacentric chromosome pairs like *M. san-angelensis,* also a species of the *Supertextae* series (Palomino *et al.* 1999). *M. huitzilopochtli* had a karyotype comprising 10 metacentric pairs and a submetacentric pair in pair 9; pairs 8 and 10 in *M. supertexta* and *M. crucigera* were submetacentrics and the 9 remaining pairs were metacentrics (Briones *et al.* 2004). It is evident that the species belonging to the *Supertextae* series vary in the proportion of metacentric and submetacentric chromosomes.

The karyotype comprising 11 metacentric pairs has been observed in other species such as *Mammillaria armillata* (Das *et al.* 1998a) and *M. brevispina* (Das *et al.* 1999b). The karyotype formed by 10 m + 1sm has also been reported for *M. boolii, M. hahniana, M. humboldtii, M. leucantha, M. occidentales, M. pectinifera* and *M. woodsii* (Das *et al.* 1998a; 1999b and 1999c).

The recent origin of the Cactaceae family is associated with processes of chromosomal mutations in their complements, and the existence of homogeneous karyotypes in members of the family can be explained by fusions or fissions in the centromeres of their chromosomes. These structural changes originate karyotypes with chromosomes that are predominantly metacentric and submetacentric (Palomino *et al.* 1988; Cota and Wallace 1996; Briones *et al.* 2004).

In the four species of *Mammillaria* studied a pair of satellites appeared; these were spherical, between 0.27 and 0.60 μm in length. In *Mammillaria albilanata*, *M. flavicentra* and *M. huitzilopochtli*, the presence of only one pair of satellites was observed in the short arm of the chromosomal pair 1, while in *M. dixanthocentron* they were observed in pair 2. The variation in the position of the satellites in *M. dixanthocentron* and its synonymous species *M. flavicentra*, makes it evident that, at the level of their karyotype, these species are different (Table 1; Fig. 3 A-D).

Comparing the karyotypes of *M. haageana* and its synonymous species *M. san-angelensis* (Hunt 1987), differences in the number of position of satellites were observed: *M haageana* had one pair of satellites (Briones *et al.* 2004), while *M. san-angelensis* had two (Palomino *et al.* 1999). The species *M. haageana* and *M. supertexta* had one pair of satellites in pair 2, and *M. crucigera* had two pairs in positions 1 and 4 (Briones *et al.* 2004). In species of *Mammillaria* one to three pairs of satellites in chromosomes have been reported, observing that in general their position is in the first pairs of the complement, with greater frequency in 1 and 2 (Das *et al.* 1998a; Das *et al.* 1999b, 1999c), as was observed in the four species analyzed here. Other species of cacti, of the genera *Melocactus* (Das *et al.* 1998b) and *Ferocactus* (Das *et al.* 1999

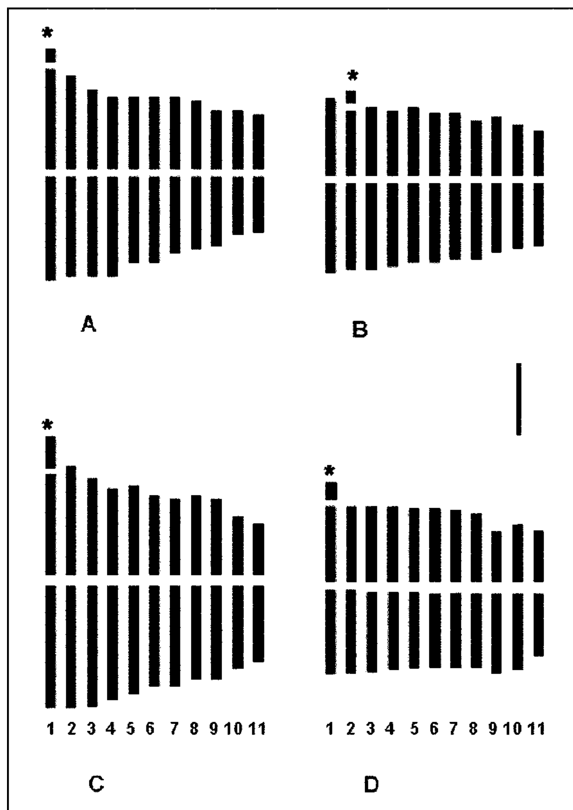

Fig. 3 — Idiograms of four species of *Mammillaria* with $2n$=22. The numbers indicate the homologous chromosome pairs: A) *M. albilanata*, 11m; B) *M. dixanthocentron*, 11m; C) *M. flavicentra*, 11m and D) *M. huitzilopochtli*, 10m+1sm. Asterisks show chromosome with satellites. Scale equals 1 µm.

d) also exhibit one to three chromosomal pairs with satellites. The differences in chromosome size and the karyotype structure in the different species of the same genus are considered evidence of the restructuring of their genomes, and it is attributed mainly to rearrangements in their chromosomes, such as deletions, duplications or translocations (Palomino *et al.* 1988; Cid and Palomino 1996; Cota and Wallace 1996; Das *et al.* 1998a, 1999b; Briones *et al.* 2004).

Values of the asymmetry index (TF%) of the studied species occurred within the interval of 43.49, *M. huitzilopochtli* with 10m + 1sm, to 46.50 *M. albilanata* with 11m, similar to the values obtained by Briones *et al.* (2004) for the species *M. crucigera* (TF% = 42.55), *M. haageana* (TF% = 42.71) and *M. supertexta* (TF% = 43.44). In other species of *Mammillaria*, the lowest TF% value was reported for *M. sempervivi* (TF% = 35.50) with a karyotypic formula of 9m + 2sm, while the highest was that reported for *M. armillata* (TF% = 45.71), with a karyotypic formula of 11m (Das *et al.* 1998a). In cacti, karyotypes tend to be symmetric (Cid and Palomino 1996; Cota and Wallace 1996; Das *et al.* 1999b).

Meiotic behavior in *M. dixanthocentron* was normal, having a total of 11 bivalents (n =11). Chiasma frequency per cell of 21.25 and 1.93 per bivalent was observed. The recombination index (RI) was 32.25. These values are higher than those reported for *M. albilanata* with a chiasma frequency of 20.25 and RI = 20.24 (Mohanty *et al.* 1996), *M. san-angelensis* with a chiasma frequency of 16.74 and RI = 27.74 (Palomino *et al.* 1999) and *M. haageana* with chiasma frequency of 13.86 and RI = 24.86 (Briones *et al.* 2004). In the species studied, high values of pollen stainability were observed; 91.74% in *M. huitzilopochtli,* 95.33 % in *M. albilanata,* 95.71 % in *M. dixanthocentron* and 97.76% in *M. flavicentra*, values that reflect stable behavior during meiosis. High values of pollen stability have also been reported for other species of the *Supertextae* series: *M. crucigera* (97.66 %), *M. haageana* (98.59 %) and *M. supertexta* (99.10 %) (Briones *et al.* 2004).

Analysis of relative nuclear DNA content of nuclei isolated simultaneously, of species of *Mammillaria* and *Lycopersicon esculentum* (internal standard), using flow cytometry is shown in Fig. 4. Most of the nuclei were in phase G_0/G_1, exhibiting nuclear 2C DNA content in histogram 1 (channel 50) for *L. esculentum* and in channel 75 for the species of *Mammillaria*. The histograms corresponding to nuclei G_2 (4C DNA content) were observed in channels 100 and 150, respectively. In the species of *Mammillaria*, histograms corresponding to nuclei with 8C and 16C were also apparent (Fig. 5).

Nuclear DNA content 2C estimated for seven diploid species of *Mammillaria* of the *Supertextae* series were *M. albilanata* = 3. 145 pg, *M. crucigera* = 3.205 pg, *M. dixanthocentron* = 3.184 pg, *M. flavicentra* = 3.040 pg, *M. haageana* = 3. 117 pg, *M. huitzilopochtli* = 3.121 pg and *M. supertexta* = 3.113 pg (Table 3; Fig. 4).

The difference between the highest value, 3.205 pg (1Cx=1570 Mbp) in *M. crucigera* and the lowest, 3.040 pg (1Cx=1489 Mbp) in *M. flavicentra* was 5%. The average value of the seven *Mammillaria* species studied was 2C DNA = 3.13 pg. Variation in the 2C nuclear DNA content of these species was not significant in the Anova (p = 0.347) and Reml test (p = 0.357). In six diploid species ($2n$=14) of the genus *Avena*, there was no significant difference in the 2C DNA content, where the lowest value occurred in *A. nudi-brevis*

Table 3 — Nuclear DNA content and genome size of diploid (2*n*=22) *Mammillaria* species.

Species	2C nuclear DNA content (pg)* ($\bar{X}$ ± S.E.)	$1C_x$ Genome size (Mbp)
M. albilanata	3.145 ± 0.05	1541
M. crucigera	3.205 ± 0.04	1570
M. dixanthocentron	3.184 ± 0.04	1560
M. flavicentra	3.040 ± 0.05	1489
M. haageana	3.117 ± 0.04	1527
M. huitzilopochtli	3.121 ± 0.04	1529
M. supertexta	3.113 ± 0.04	1535

* 1 pg = 980 Mbp (Bennett *et al.* 2000).
$1C_x$ = DNA content of one non-replicated monoploid genome with chromosome number *x* (Greilhuber *et al.* 2005).

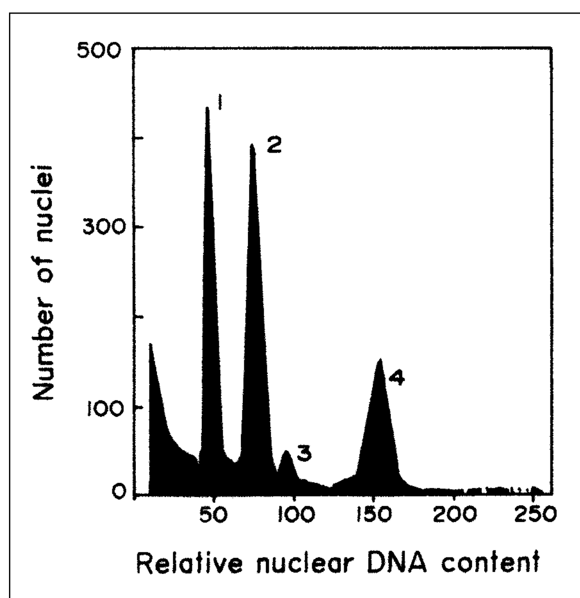

Fig. 4 — Distribution of nuclear DNA content obtained after flow cytometric analysis of propidium iodide-stained nuclei isolated from stem parenchyma of *Mammillaria* species. Peaks 2 and 4 represent nuclei with 2C (G_1) and 4C (G_2) DNA content of *Mammillaria huitzilopochtli*. Peaks 1 and 3 represent 2C and 4C nuclei of *Lycopersicon esculentum* as internal standard.

with 10.6 pg and the largest in *A. wiestii* with 12.4 pg (Bullen and Rees 1972). In 14 diploid species (2*n*=26) of the genus *Ficus*, the difference in DNA content among the species was not significant, within a range of 2C DNA values of 1.37 pg in *F. mysorensis* to 1.47 in *F. krishnae*. Stability in genome size in the species of *Ficus* was related to the fact that these plants form part of the climax vegetation and occupy a habitat of uniform climatic conditions (Ohri and Khoshoo 1987). In 16 bushy species of *Cistus* distributed in the Mediterranean, values within a range of 2C DNA = 3.92 pg in *C. crispus* to 5.88 pg in *C. monspeliensis* was estimated by Ellue *et al.* (2002). These authors observed that the differences in nuclear DNA content was not significant, and thus was related with chromosomal number stability (2*n*=20) and homogeneous karyotypes in these plants (Markova 1975). In the seven species of *Mammillaria* studied, the non-significant difference in 2C DNA content reflected stability in their genomes, which is related to the fact that all of them were diploid and exhibited very symmetric karyotypes with metacentric and submetacentric chromosomes.

In the species studied, the total length of the genome in μm (Table 1), estimated by summing the chromosomes length of each complement, showed no relationship to DNA content in pg (Table 3) because the chromosomes analyzed in the different species did not have the same degree of condensation; similar results have been reported for other species of *Mammillaria* (Mohanty *et al.* 1996; Das *et al.* 1999c).

The average nuclear DNA content of the *Mammillaria* species studied was 3.13 pg, indicating a small genome size according to the ranges defined for angiosperms of 1.4 – 3.5 pg. Species with small genome size are more evolutionarily flexible, allowing them to colonize new and more diverse environments (Leitch *et al.* 1998). The small genome size in *Mammillaria* species studied, added to the morphological and physiological adaptations of these cacti, has permitted them to adapt to arid and semiarid sites.

2C nuclear DNA content for the species of *Mammillaria* studied are found within the range of 3.040 pg in *M. flavicentra* to 3.205 pg in *M. crucigera* (Table 3), similar to values obtained by flow cytometry for *M. san-angelensis*, 2C DNA = 3.20 pg (Palomino *et al.* 1999), species of the *Supertextae* series and to other species of *Mammilaria* such as *M. woodsii*, 2C DNA = 3.10 pg and *M. bocasana*, 2C DNA = 4.10 pg, obtained by Feulgen densitometry (Barlow, cited in Bennett and Smith 1976). For 34 diploid species of *Mammillaria*, interspecific variation from 2C DNA = 7.80 pg in *M. pseudoperbella* to 2C DNA = 20.36 pg in *M. decipiens* (Mohanty *et al.* 1996, 1997a, 1997b; Das *et al.* 1999a) is reported. The 2C DNA = 7.80 pg in *M. pseudoperbella* is more than double the average values of 2C DNA = 3.13 pg obtained for the seven species of *Mammillaria* in this study. For *Mammillaria albilananta* diploid, species of the *Supertextae* series, nuclear 2C DNA content = 10.16 pg is reported (Mohanty *et al.* 1996). This

value is extremely high compared to that obtained in our work by flow cytometry for *M. albilanata,* 2C DNA = 3.14 pg. It has been proved that there is no significant difference between Feulgen densitometry and flow cytometry in the determination of genome size (Dolezel *et al.* 1998). The discrepancies in the C values reported for a single species by different authors probably reflects variations related with the use of different DNA reference standards that may result in different estimates of the same material (Dolezel *et al.* 1998). To determine the 2C DNA content in the species of *Mammillaria*, Mohanty *et al.* (1996, 1997a, 1997b) and Das *et al.* (1999a) used *Allium cepa* 2C DNA = 33.55 pg as an internal standard, while in our work *Lycopersicon esculentum* 2C DNA = 1.96 pg was used. Differences could also be due to an erroneous taxonomic identification of the material studied by those authors.

For other genera of cacti, there are seven reports of diploid species within a range of 2C DNA= 2.05 pg in *Pereskia grandifolia* (De Rocher *et al.* 1990) up to 2C DNA = 3.90 pg in *Trichocereus werdermannianus* (Barlow, cited in Bennett and Smith 1976). Our estimates of 2C DNA content in seven *Mammillaria* species (3.04 pg in *M. flavicentra* to 3.20 pg in *M. crucigera*) are agree with the range reported for these cacti.

The difference in genome size among the seven *Mammillaria* species studied was 5% (3.20 pg in *M. crucigera* to 3.04 in *M. flavicentra*), exhibiting small, very stable genomes, where changes in DNA content were not significant. Interspecific variations among the species, however, were more clearly observed in relative length (L%), arm ratio (r), proportion of metacentric and submetacentric chromosomes, and the position of satellites, indicating that speciation in these plants has involved structural rearrangements in their chromosomes. These genome restructuring mechanisms have been evidenced in other *Mammillaria* species and other genera of cacti.

Nearly 90% of angiosperms have cells of some of their tissues with somatic multiploidy, that is, with different levels of ploidy that form an endopolyploidy pattern, generated by processes of endoreduplication (D'Amato 1984; Joubes and Chevalier 2000).

In the seven species of *Mammillaria* studied, in nuclei of the stem parenchyma an endopolypoidy pattern was observed, with values of 2, 4, 8 and 16 C DNA; since the 2C DNA value was the lowest observed in the endopolypoidy pattern in these species, they are considered diploids (Table 4, Fig. 5). In stem parenchyma of *M. san-angelensis*, an endopolyploidy pattern was observed with cells containing 2C - 32 C DNA (Palomino *et al.* 1999). In *Pereskia grandiflora* the observed endopolyploidy pattern was defined by nuclei with 2, 4 and 8C; in *Mesembryanthemum crystallinum* multiple levels of ploidy (2C – 64C) were observed in different tissues with correlation between development stage and endopolyploidy pattern (De Rocher *et al.* 1990). In mature flowers of *Brassica oleracea*, the endopolyploidy pattern was 2C - 64 C (Kudo and Kimura 2001).

Constancy of an endopolyploidy pattern in tissues of different organs suggests that the endoreduplication cycles in plants are coded by genes programmed for development, differentiation and specialized functions (Cebolla *et al.* 1999). It has been observed that the presence of an en-

Table 4 — Pattern of endopolyploidy in stem parenchyma of *Mammillaria* species.

Species	Percentage of nuclei populations			
	2C	4C	8C	16C
M. albilanata	41.08	22.19	31.66	5.13
M. crucigera	17.64	25.47	37.54	19.33
M. dixanthocentron	32.59	29.43	29.08	8.88
M. flavicentra	24.44	29.02	28.24	18.30
M. haageana	22.93	34.25	31.96	10.84
M. huitzilopochtli	21.66	27.47	31.16	19.68
M. supertexta	26.49	34.48	19.67	19.34

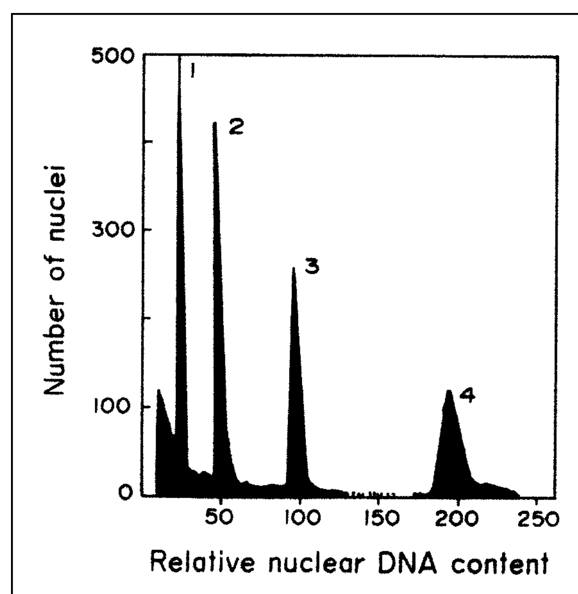

Fig. 5 — Distribution of nuclear DNA content of *Mammillaria flavicentra*, showing pattern of endopolyploidy. The peaks 1, 2, 3 and 4 represent nuclei with 2C, 4C, 8C and 16C, respectively.

dopolypolidy pattern is frequent in succulent plants that carry out CAM metabolism (De Rocher *et al.* 1990). This metabolic adaptation is typical of plants that live in arid and semiarid environments such as cacti (Cushman 2001). The endopolyploidy pattern could be an emergent trait in succulents and cacti that have small genome size, which provide them with the capacity to generate larger cells with high levels of ploidy and allow these plants to store greater amounts water for adaptation to arid environments (De Rocher *et al.* 1990).

Akcnowledgements — This study was supported by Jardín Botánico IBUNAM. Thanks to G. Reyes for collecting and identification the *Mammillaria* individual plants; J. Martínez for his the photographic work, J. Saldivar for computerized edition of figures, M. Laad for technical assistance and Felipe Villegas for art work.

REFERENCES

Bennett M.D., Bhandol P. and Leitch I.J., 2000 — *Nuclear DNA amounts in angiosperms*. Philosophical Transactions of the Royal Society of London, Series B 27: 227-274.

Bennett M.D., Parmjit B. and Leitch I.J., 2000 — *Nuclear DNA amounts in angiosperms and their modern uses-807 new estimates*. Annals of Botany 86: 859-909.

Bennett M.D. and Leitch I. J., 2005 — *Plant DNA C-values Database (release 4.0 October 2005).* http://www.rbgkew.org.uk/cval/homepage.html

Bravo-Hollis H., 1978 — *Las Cactáceas de México*. Vol. I. UNAM, México. 755 p.

Bravo-Hollis H. y Sánchez-Mejorada H., 1991 — *Las Cactáceas de México*. Vol III. Unam México. 643 p.

Briones S.F., Palomino G. and García A., 2004 — *Chromosome analysis of Mammillaria supertexta, M. crucigera and M. haageana and their comparison with M. san-angelensis (Cactaceae)*. Caryologia 3: 211-218.

Bullen R. and Rees H., 1972 — *Nuclear variation within Avenae*. Chromosoma 39: 93-100.

Cebolla A., Vinardell J., Kiss E., Oláh B., Roudier F., Kondorosi A. and Kondorosi E., 1999 — *The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants*. The European Molecular Biology Organization 18: 4476-4484.

Cid R. and Palomino G., 1996 — *Cytotypes and meiotic behavior in mexican populations of Myrtillocactus geometrizans var. geometrizans (Cactaceae)*. Cytologia 61: 343-348.

Cites., 2005 — *Apéndices I, II y III de la CITES. Convención sobre el Comercio Internacional de especies Amenazadas de Flora y Fauna Silvestres (CITES).* http://www.cites.org/esp/app/index.shtml

Conger A.D. and Fairchild L.M., 1953 — *A quick-freeze method for making smear slides permanent*. Stain Technology 28: 281-283.

Cota J. H. and Wallace R. S., 1996 — *La citología y la sistemática molecular en la familia Cactaceae*. Cactáceas y Suculentas Mexicanas 2: 27-44.

Cushman J. C., 2001 — *Crassulacean acid metabolism. A plastic photosynthetic adaptation to arid environments*. Plant Physiology 127: 1439-1448.

D'amato F., 1984 — *Role of polyploidy in reproductive organs and tissues*. In B.M. Johri, (Eds). "Embryology of Angiosperms", p. 519-566. Springer-Verlag, Berlin.

Das A.B., Mohanty S. and Das P., 1998a — *Interspecific variation of nuclear DNA and structural changes in meiotic and mitotic chromosome in some species of Mammillaria (Cactaceae)*. Caryologia 3-4: 289-301.

Das A. B., Mohanty S. and Das P., 1998b — *Variation in karyotype and 4C DNA content in six species of Melocactus of the family Cactaceae*. Cytologia 63: 239-247.

Das A. B., Mohanty S. and Das P., 1999a — *Studies on chiasma frequency and nuclear 4C DNA amount in seven species of Mammillaria (Cactaceae)*. Cytobios 97: 95-101.

Das A. B., Mohanty S. and Das P., 1999b — *Karyotype diversity in thirteen species of Mammillaria in the family Cactaceae*. Cytobios 97: 117-125.

Das, A. B. Mohanty S., Marrs R. and Das P., 1999c — *Somatic chromosome number and karyotype diversity in fifteen species of Mammillaria of the family Cactaeae*. Cytobios 97: 141-151.

Das A. B., Mohanty S. and Das P., 1999d — *4C DNA variation and karyotype diversity in nine species of Ferocactus*. Cytologia 64: 17-24.

De Rocher E. J., Harkins K., Galbraith D. and Bohnert H., 1990 — *Developmentally regulated systemic endopolyploidy in succulents with small genomes*. Science 250: 99-101.

Dolezel J., Sgorbati S. and Lucretti S., 1992 — *Comparison of three fluorochromes for flow cytometric estimation of nuclear DNA content in plants*. Physiologia Plantarum 85: 625-631.

Dolezel J. and Göhde W., 1995 — *Sex determination in dioecious plants Melandrium album and M. rubrum using high-resolution flow-cytometry*. *Cytometry* 19: 103-106.

Dolezel J., 1995 — *Flow cytometry principles and applications in mutation breeding*. In Fourteenth IAEA/FAO (Eds), "Interregional training course on advances in plant mutation techniques", p. 1-25. Vienna, Austria (C7-INT-5.135).

Dolezel J., Greilhuber J., Lucretti S., Meister A., Lysak M.A., Nardi L. and Obermayers R., 1998 — *Plant genome size estimation by flow cytometry: Inter-laboratory comparison*. Annals of Botany (Suppl A) 82: 17-26.

DOLEZEL J. and BARTOS J., 2005 — *Plant DNA flow cytometry and estimation of nuclear genome size*. Annals of Botany 95: 99-110.

ELLUE P., BOSCAIU M., VICENTE O., MORENO V. and ROSSELLO J. A, 2002 — *Intra and inter-specific variation in DNA content in Cistus (Cistaceae)*. Annals of Botany 90: 345-351.

GALBRAITH D.W., LAMBERT G.M., MACAS J. and DOLEZEL J., 1998 — *Analysis of nuclear DNA content and ploidy in higher plants*. In: J.P. Robinson, Z. Darzynkiewicz, P.N. Dean, L.G. Dressler, A. Orfao, P.S. Rabinovitch, C.C. Stewart, H.J. Tanke, L.L. Wheeless (Eds.), "*Current protocols in cytometry*". New York: John Wiley & Sons, 7.6.1-7.6.22.

GARCÍA V.A., 1990 — *Técnicas y procedimientos de citogenética vegetal*. Colegio de Posgraduados, México. 144 p.

GIBSON C.A. and NOBEL P.S., 1986 — *The cactus primer*. Harvard University Press, Cambridge. 286 p.

GILL B.S. and GOYAL V., 1984 — *Cytology of some members of Cactaceae*. Cell Chromosome Review 7: 58-60.

GREILHUBER J., DOLEZEL J., LYSAK M. and BENNETT M., 2005 — *The origin, evolution and proposed stabilization of the terms ' genome size' and 'C-value' to describe nuclear DNA contents*. Annals of Botany 95: 255-260.

GUPTA R. and GUPTA P.K., 1978 — *Karyotypic studies in the genus Crotalaria Linn*. Cytologia 43: 357-369.

HERNÁNDEZ M. H. y GODÍNEZ A. H., 1994 — *Contribución al conocimiento de las cactáceas mexicanas amenazadas*. Acta Botánica Mexicana 26: 33-52.

HUNT D., 1987 — *A new review of Mammillaria*. Bradleya 5: 45-48.

JOUBES J. and CHEVALIER C., 2000 — *Endoreduplication in higher plants*. Plant Molecular Biology 43: 735-745.

KUDO N. and KIMURA Y., 2001 — *Flow cytometric evidence for endopolyploidization in cabbage (Brassica oleracea L.) flowers*. Sex Plant Reproduction 13: 279-283.

LEITCH I.J., CHASE M. and BENNETT M.D., 1998 — *Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants*. Annals of Botany (Suppl A) 82: 85-88.

LEVAN A., FREDGA K. and SANDBERG A., 1964 — *Nomenclature for centromeric position on chromosome*. Hereditas 52: 201-220.

LÜTHY J., 1995 — Taxonomische untersuchung der gattung *Mammillaria* Haw. (Cactaceae). Ph.D. dissertation, Universität Bern, Bern, Switzerland.

MARKOVA M., 1975 — *Karyosystematische untersuchungen an den Cistaceae Bulgariens*. Plant Systematics and Evolution 123: 283-315.

MOHANTY S., DAS A.B. and DAS P., 1996 — *Analysis of chiasma frequency and nuclear DNA variation in some species of Mammillaria*. Cytobios 88: 173-181.

MOHANTY S., DAS A.B. and DAS P., 1997a — *Studies on nuclear DNA and meiotic chromosome in 8 species on Mammillaria*. Cytologia 62: 331-336.

MOHANTY S., DAS A.B. and DAS P., 1997b — *Estimation of 4C DNA content and determination of chiasma frequency in eigth species of Mammillaria*. Cytobios 91: 15-23.

OHRI D. and KHOSHOO T., 1987 — *Nuclear DNA contents in the genus Ficus (Moraceae)*. Plant Systematics and Evolution 156: 1-4.

OHRI D., 1998 — *Genome size variation and plant systematics*. Annals of Botany 82:75-83.

OTTO F., 1990 — *DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA*. In: Z. Darzynkiewicz and H. A. Crissman (Eds), "Methods in Cell Biology" Vol. 33, p. 110-115. Academic Press Inc., New York.

PALOMINO G., ZULETA S. and SCHEINVAR L., 1988 — *Estudios citogenéticos de dos especies y una variedad del género Nyctocereus (Cactaceae)*. Boletín de la Sociedad Botánica de México 48: 75-79.

PALOMINO G., DOLEZEL J., CID R., BRUNNER I., MÉNDEZ I. and RUBLUO A., 1999 — *Nuclear genome stability of Mammillaria san-angelensis (Cactaceae) regenerants induced by auxins in long term in vitro culture*. Plant Science 141:191-200.

PALOMINO G. and SOUSA M., 2000 — *Variation of nuclear DNA content in biflorus species of Lonchocarpus (Leguminosae)*. Annals of Botany 85: 69-76.

REMSKI M.F., 1954 — *Cytological investigations in Mammillaria and some associated genera*. Botanical Gazette 116: 163-171.

SÁEZ F. and CARDOSO H., 1978 — *Citogenética básica y biología de los cromosomas*. OEA. Whashington, D.C. 117 p.

SEMARNAT., 2001 — *Norma Oficial Mexicana NOM-059-ECOL-2001, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo*. Diario Oficial. México.

Received 02.02.2006; accepted 20.05.2006