Evidence of Heterozygous Chromosome Interchange and Chromatid Exchange in Autotetraploid Cytotype of *Gibasis schiedeana* (Tradescantieae-Commelinaceae)

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Gibasis (Kunth) D. Hunt (tribe Tradescantieae: Commelinaceae) contains 16 species most of which are tuberous perennial herbs, distributed in tropical and subtropical regions from Central America and Mexico (Jones 1978, Kenton 1982, Kenton *et al.* 1987, 1988). Diploid plants with $2n=10$ and tetraploids with $2n=16$, and basic numbers of $x=5$ and 4, respectively, have been described in *G. schiedeana* (Jones 1974). In order to show the origin of tetraploid plants ($2n=16$) starting from the diploid ones ($2n=10$) in *G. schiedeana*, Jones (1974) carried out the hybridization of both, obtaining F1 triploid hybrid plants $2n=13$. These plants showed bivalents (IIs) trivalents (IIIs) and quadrivalents (IVs) in metaphase-I involving acrocentrics and metacentrics of the parents. From this study, Jones (1974) concluded that the tetraploid ($x=4$) has arisen from the diploid ones ($x=5$) by Robertsonian changes followed by chromosome doubling to produce a new basic number of $x=4$ in the genus.

Jones (1974), Jones *et al.* (1975), and Kenton (1984), studying several collections of *G. schiedeana* distributed in the states of Chiapas, Tabasco and San Luis Potosi, Mexico, defined six autotetraploid cytotypes of this species. These cytotypes showed different frequencies of multivalents indicating heterozygous chromosome interchange. Additionally, three of them showed 1 to 6 B chromosomes. The main mechanism involved in the variation and evolution of the karyotypes of this species is heterozygous interchange (Jones *et al.* 1975).

In the present study, the cytotype of *Gibasis schiedeana* from the Ecological Reserve of the Pedregal de San Angel in Mexico, D.F. (ERPSA-Mexico) is analyzed and compared to other cytotypes of *G. schiedeana* previously reported from different populations.

Material and methods

Plant material. The plants of *Gibasis schiedeana* (Kunth) D. Hunt studied belong to the collection of the Botanical Garden, Institute of Biology, UNAM. Voucher specimens were deposited at the National Herbarium (MEXU, Appendix 1). Live plants, from ERPSA-Mexico were transplanted from the field and placed in pots containing a mixture of sand and organic soil and maintained in a greenhouse.

Mitotic, meiotic chromosome and pollen fertility analyses. For the present study 15–25 plants were analyzed. Elongating secondary root tips were placed in a saturated solution of paradichlorobenzene for 6 hr at 4°C. They were stained following Feulgen technique (García 1990). Preparations from 75 mitotic cells were made for karyotype analysis. Chromosomes were classified according to Levan *et al.* (1964). Index of asymmetry (TF%) was obtained following Gupta and Gupta (1978).

To study meiotic behavior, fresh anthers from young buds were squashed in 1.8% aceto-orcein, and 1103 pollen mother cells (PMC) in metaphase-I (M-I) and 4006 PMCs, in

anaphase-I (A-I) were analyzed.

Pollen fertility was estimated by stained samples with cotton blue in lactophenol. Percentage of well-filled stained grains were obtained. This was carried out in 1162 pollen grains.

Results

The plants of *Gibasis schiedeana* analyzed were tetraploid with $2n=16$, showing a karyotype of 12 metacentrics, and four acrocentrics with a satellite in the short arm (12M+4A, Table 1). Based on the mean length of the chromosomes, they were assembled in three groups of metacentrics and 1 of acrocentrics (acrocentrics=subtelocentrics, according to Levan *et al.*

Table 1. Cytotype of *Gibasis schiedeana* ($2n=16$) of the ERPSA-Mexico

Karyotype formula	Secondary constrictions	Range of chromosome length (μm)	Total haploid chromatin length (μm) $\bar{X} \pm \text{EE}$	TF (%) $\bar{X} \pm \text{EE}$
12M+4A	4A	6.7–13.9	153.6 ± 0.59	41.34 ± 0.22

Fig. 1. Cytotype of *Gibasis schiedeana* from ERPSA-Mexico. (A). Somatic chromosomes, $2n=16$. (B). Idiogram with 12 metacentrics and four acrocentrics with a satellite. Arrows and numbers indicate satellites. Asterisks show heteromorphic chromosomes. Scale equals $10 \mu\text{m}$.

Fig. 2. PMC's showing MI and irregular AI in *Gibasis schiedeana*. (A). MI with 8 IIs. (B). MI with 5 II+IV+2 Is. (C). MI with 2 IIs+III+IV+5 Is. (D). MI with 4 IIs+V+3 Is. (E). MI with 3 II+IV+VI. (F). AI with two bridges (b). (G). AI with bridge (b) and fragment (f). (H). AI with fragment (f). (I). AI with two lagging chromosomes (I). Numbers indicate: 1. heteromorphic IIs. 2. heteromorphic IIIs. 3. heteromorphic IVs. 4. heteromorphic Vs. 5. heteromorphic VI. Scale equals 10 μ m.

Table 2. Type and frequency of heteromorphic bivalents (IIs), and multivalents: trivalents (IIIs), quadrivalents (IVs), pentavalents (Vs) and hexavalents (VIs) of *Gibasis schiedeana*

	Total of cells with bivalents and multivalents		Homomorphic bivalents and multivalents		Heteromorphic bivalents and multivalents	
	No.	%	No.	%	No.	%
II	653	59.20	6589	76.40	2035	23.60
III	10	0.91	7	70.00	3	30.00
IV	418	37.90	591	73.14	217	26.86
V	13	1.18	10	76.92	3	23.03
VI	9	0.82	8	88.89	1	11.11

Table 3. Normal and irregular anaphase-1 (A-1) of *Gibasis schiedeana*

PMC's in A-I	Disjunction at A-I	PMC's	
		Total	%
Number of PMC's		4006	100.00
Regular	8:8	3309	82.60
Cells with one lagging chromosome	8:1:7	124	3.10
Cells with two lagging chromosomes	8:2:6	102	2.55
Cells with three lagging chromosomes	8:3:5	68	1.70
Cells with one bridge		185	4.62
Cells with one bridge and fragment		164	4.10
Cells with acentric fragment		54	1.35
Total of irregular PMC's		697	17.40

1964). The karyotype showed 8 heteromorphic chromosomes. Within the first group they were observed in chromosomes 3 and 4; in the second group, they were observed in chromosomes 5, 6, 7 and 8; and, in the fourth group, in chromosomes 15 and 16 (Figs. 1A, B).

The analysis of PMC's in metaphase-I showed 59.20% of the cells with bivalents only (IIs), from which 23.60% were heteromorphic (Figs. 2A, B, Table 2). 40.81% of the PMC's showed IIs and multivalent in variable combinations. The highest frequency corresponded to IIs+IVs (37.90%), of which 26.86% were heteromorphic (Fig. 2C, Table 2). Among those observed in lower frequencies are IIs+IIIs (0.91%), with 30.00% of them heteromorphic, IIs+V (1.18%) with 23.03% of them heteromorphic (Fig. 2D, Table 2) and IIs+VI (0.82%), with 11.11% of them heteromorphic (Fig. 2E, Table 2). In the PMC's where IIs and multivalent were present, 1 to 4 univalent in a frequency of 5.35% were observed.

The analysis of PMC's in anaphase-I showed 17.40% of irregular anaphase. 4.62% corresponded to cells with side arm bridges (SAB) without an acentric fragment and 4.10% cells with U-type bridge associated with an acentric fragment (Figs. 2F, G, Table 3). 1.35% of irregular anaphase showed an acentric fragment (Fig. 2H, Table 3). Additionally, cells with 1, 2 and 3 lagging chromosomes in frequencies of 3.10%, 2.55% and 1.70% respectively were observed (Fig. 2I, Table 3).

Discussion

The plants of *Gibasis schiedeana* from the ERPSA-Mexico were autotetraploid with $2n = 16$, $x=4$ and a karyotype of $12M+4A$, which included three groups of metacentrics and one of acrocentrics with satellite, indicating their polyploid origin. The tetraploid origin of the species was also evidenced by the high frequency of quadrivalents (37.90%) observed. These results agree with those of Jones (1974), Jones *et al.* (1975) and Kenton (1984) for 6 collections of *G. schiedeana* from the states of Chiapas, Tabasco, Morelos and San Luis Potosí, Mexico.

The high frequency of cells with IIs (59.20%), as opposed to the low frequency in cells with IVs (37.90%) in *G. schiedeana* from the ERPSA-Mexico, is evidence of a diploidization process. This process could be due to accumulation of heterozygous chromosome interchange that produce a gradual differentiation in its genome and alteration in gene regulation of meiosis, as Gilles and Randolph (1951) showed in *Zea mays*. These authors observed 88.70% of cells with IVs in the first generation of autotetraploid plants of *Zea mays*, diminishing to a frequency of 52% and increase of IIs, in the tenth generation. Similar diploidization mechanisms have been observed in autotetraploid plants of *Nicotiana* sp. (Riley and Law 1965), *Avena* sp. (Ladizinsky 1973), *Zea perennis* (Kato 1984) and *Festuca* sp. (Jauhar 1975, 1993).

In the plants of *Gibasis schiedeana* from the ERPSA-Mexico, multivalent associations of the V (1.18%) and VI (0.82%) types were observed, some of them heteromorphic (Table 2), which indicated heterozygous structural interchange in their chromosomes, originating 8 heteromorphic chromosomes.

Evidence of the nature of plant heterozygosity for chromosome interchange in *G. schiedeana* has been well documented by Jones *et al.* (1975), where upon analyzing 6 populations of this species, they found cells with high frequencies of multivalents (V=3.3–6%, VI=1–24%, VII=2–3.3% and VIII=6–16%) and in lower frequencies combinations of 2 multivalents. Univalents were common in three of these cytotypes. Jones *et al.* (1975) observed them in frequencies of 10–30–60% of the cells, these plants were distinguished in having 1–6 B chromosomes. The *G. schiedeana* cytotype from the ERPSA-Mexico showed a very low frequency of univalents (5.35%) and no B chromosomes were observed.

The divergence in the frequency of V (1.18%) and VI (0.82%) of the cytotype of *G. schiedeana* from the ERPSA-Mexico, in relationship to the high frequencies of multivalents observed by Jones *et al.* (1975), indicates the variation of the cytotypes found for the several populations of *Gibasis schiedeana* in different locations of Mexico. The frequency of multivalents evidenced in the plants of *G. schiedeana* from the ERPSA-Mexico, and those observed by Jones *et al.* (1975) for other populations, indicates that *G. schiedeana* has a highly variable genotype, and that structural interchange in its chromosomes is the principal mechanism in its evolution.

In addition to the heterozygous chromosome interchange, the plants of *G. schiedeana* from the ERPSA-Mexico showed irregular anaphase-I (17.40%), which included U-type (4.10%) and SAB (4.62%) chromatid exchange, originated from an abnormal function at the places where the chiasmata are formed, causing asymmetry in their karyotypes (Jones 1968, Brandham 1969, 1970, 1974, Brandham and Johnson 1977). Acentric fragments were also observed (1.35%), probably resulting from sister chromatid reunion, as Kenton (1984) has observed in individual plants of *G. linearis*. Also, in *G. schiedeana* from the ERPSA-Mexico, the presence of 1 to 3 lagging chromosomes (7.35%) were observed, caused by an abnormal disjunction in some of their chromosomes. The spontaneous chromatid exchange found in the present study has not been reported in previous studies of cytotypes of *G. schiedeana*. In other species of the genus, such as *Gibasis consobrina*, *G. pulchella*, *G. linearis*, *G. venustula* and *G. heterophylla*, these rearrangements have been evidenced in several frequencies (Jones *et al.* 1975, Kenton 1981, 1984). The heterozygous chromosome interchange and chromatid exchange observed in *G. schiedeana* from the ERPSA-Mexico, caused the formation of abnormal tetrads that originated 8.52% of non-viable pollen. In plants of *Gibasis pulchella* heterozygous for chromosome interchange, Jones *et al.* (1975), Kenton (1981) and Kenton *et al.* (1987), have found 50–60% reduction in pollen viability.

In summary, the presence of heterozygosity for major interchanges have played an important role in the evolution of *Gibasis schiedeana*. The chromatid exchanges is a mechanism also involved in the evolution of these plants.

Appendix 1

G. schiedeana. Mexico, D.F. Ecological Reserve of the Pedregal de San Angel. J. Martínez and G. Palomino 517 (MEXU).

Summary

Gibasis schiedeana from the ERPSA-Mexico, showed an autotetraploid cytotype with

$2n=16$, $x=4$ and had a karyotype $12M+4A$, four acrocentrics had satellites. In the cytotype from the ERPSA-Mexico, heterozygous chromosome interchange was observed, evidenced in M-I by multivalent: III (0.91%), IV (37.90%), V (1.18%) and VI (0.82%), some of them heteromorphic. In addition, the analysis in A-I showed cells with bridge and fragment (U-type chromatid exchange) and cells with bridge without fragment (sub-chromatid aberrations, SAB) in frequencies of 4.62% and 4.10% respectively. Heterozygous chromosome interchange and chromatid exchange were also evidenced in 8 heteromorphic chromosomes. Lagging chromosomes were also recorded in A-I (7.35%). The chromatid exchange and lagging chromosomes have not been reported in other cytotypes of *G. schiedeana*.

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