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Chromosome Doubling in Vine Cacti Hybrids

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Abstract

We performed reciprocal crosses between the tetraploid *Selenicereus megalanthus* and the diploid *Hylocereus* species, *H. undatus* and *H. polyrhizus*. *S. megalanthus* × *H. undatus* gave rise to viable hexaploids and 6*x*-aneuploid hybrids rather than to the expected triploids. No genuine hybrids were obtained in the reciprocal cross. The pollen diameter of the tetraploid *S. megalanthus* varied widely, indicating the occurrence of unreduced gametes, while that of *H. undatus* pollen was very uniform, indicating an extremely low frequency of unreduced gametes. This finding suggests that the hexaploids were formed by chromosome doubling after the formation of the hybrid triploid zygote rather than by fusion of unreduced gametes of the two species.

During the past 15 years, a number of cactus species have been introduced into Israel from northern South America, Central America, and Mexico (Nerd and Mizrahi 1997). Among these are the vine cacti of the genera *Hylocereus* (Berger) Br. and R. and *Selenicereus* (Berger) Br. and R., which have a considerable economic potential as new exotic fruit crops because of their attractive edible fruits (Mizrahi and Nerd 1999). The two species of *Hylocereus* bear large (250–800 g) attractive red-purple fruits, whereas *Selenicereus megalanthus* bears spiny yellow fruits that are sweeter than those of *Hylocereus*, but relatively small (180–250 g). From a breeding and horticultural point of view, a combination of the size and attractiveness of *Hylocereus* spp. fruit and the taste features of the *S. megalanthus* fruit would be ideal. Intergeneric and interspecific crosses in this group of cacti are known to yield viable hybrids (Lichtenzveig et al. 2000). Therefore selection for high fruit quality and superior horticultural characteristics among hybrid progeny may be relatively simple. The main obstacle to rapid progress in such a breeding program may be the difference in ploidy levels between the two genera, as *Hylocereus* species have $2n = 2x = 22$ and *S. megalanthus*, $2n = 4x = 44$. Other difficulties may be the long juvenile period (3–4 years) and the large acreage required for screening the hybrid progeny.

A large crossing scheme was initiated in Beer-Sheva, located in the northern Negev of Israel, with the aim of producing commercial cultivars of vine cacti. In this framework, a number of interspecific and intergeneric hybrids were grown and tested for fertility and fruit quality. Among the F_1 hybrids, three plants from the cross between *S. megalanthus* and *Hylocereus undatus* were found to be hexaploid rather than the expected triploid. Published data on polyploid formation suggest that a considerable number of polyploid plants originated by fusion of 2*n* (unreduced) gametes (Bretagnolle and Thompson 1995; Darlington 1937, 1956; deWet 1980; Harlan and deWet 1975; Karpechenko 1927; Lewis 1980). Other mechanisms leading to polyploidy have been considered negligible (Bretagnolle and Thompson 1995; deWet 1980; Harlan and deWet 1975; Lewis 1980). Herein we report on the occurrence of hexaploid vine cactus hybrids derived from zygote (or postzygote) chromosome doubling. The cytology of the parental lines and the polyploid hybrids is described.

Materials and Methods

Plant Material and Growth Conditions

The taxa used in this study were *Selenicereus megalanthus* ($2n = 4x = 44$), *Hylocereus undatus* ($2n = 2x = 22$), and *Hylocereus polyrhizus* ($2n = 2x = 22$). Plant husbandry details were the same as those described by Lichtenzveig et al. (2000).

Artificial pollination was performed manually as the flowers opened. The stigma was then covered to prevent self-pollination. However, this measure was not fully

effective, and chromosome counting and characterization of F_1 fruit morphology were required.

Chromosome Counts

Chromosome counts of pollen mother cell excised from flower buds (5–6 cm long) of 3- to 6-year-old plants were performed as described by Lichtenzveig et al. (2000). Photomicrographs were taken with a Zeiss Axioplan microscope using a Kodak technical pan film.

Pollen Diameter

Pollen grains were collected from 7-year-old plants at anthesis and stained with 2% acetocarmine. Staining with Alexander's reagent and FDA gave similar values to those obtained from acetocarmine stainability. We opted to use acetocarmine because it can be used with stored pollen. The diameters of about 300 pollen grains per flower were measured for each species by means of a calibrated micrometer under a Zeiss AxiosKop 2 light microscope at 100× magnification. Photomicrographs were taken with a Zeiss AxioCam, program AxioVision, version 3.0.6 SP2.

Fluorescent In Situ Hybridization (FISH)

Anthers containing pollen mother cells excised from flower buds of 3- to 6-year-old hybrids were fixed in ethanol:glacial acetic acid (3:1 v/v) overnight and then stored in 70% ethanol at 4°C. Anthers were squashed on the slide with a drop of 45% acetic acid. After immersion in liquid nitrogen and removal of the coverslips, the slides were dehydrated by consecutive immersion in 75%, 95%, and 100% ethanol (5 min for each immersion) and air dried. The preparations were stored at −20°C until use.

Fluorescent in situ hybridization was performed according to Reader et al. (1994). For probing chromosomal sites of rDNA loci, the entire wheat rDNA repeat unit (pTa71 clone) was used (Gerlach and Bedbrook 1979). The rDNA probe was labeled with fluorescein-11-dUTP according to the method of Simpson et al. (1988). The preparations were counterstained with DAPI (4′,6-diamidino-2-phenylindole) and mounted in antifade solution (Vector Laboratories). The slides were examined under a Zeiss AxiosKop 2 fluorescence microscope, and photomicrographs were taken using Fujicolor (800 ASA) film with a 4 s exposure. The images obtained were scanned and manipulated with an HP Image Editor by changing the brightness and contrast uniformly across the image. At least 10 metaphase I or anaphase I cells were examined per F_1 hybrid or parent.

Results

The pollen grains of the diploid species were found to be viable and quite uniform in size, a diameter of 70–80 μm being typical for *H. undatus* (Figure 1A) and 70–90 μm for *H. polyrhizus*. In the tetraploid *S. megalanthus*, only 70–80% of the grains stained normally, their diameter varying widely (i.e., between 90 and 190 μm) (Figure 1B). The diameter of about 83% of the stainable pollen grains ranged between 110 and 140 μm; 5% had a smaller diameter and 12% a larger diameter (Figure 2).

The vast majority of the plants had the morphological features of the maternal parent and therefore could not be considered as putative hybrids. Seventy-seven putative hybrids from the cross *S. megalanthus* × *H. undatus* were planted. Of the 11 plants studied, 5 were confirmed as hybrids by chromosome counts (Table 1), their chromosome number being higher than the expected triploid ($2n + n = 33$). One hybrid was found to be hexaploid (hybrid J-42, $2n = 6x = 66$, as seen in Figure 3) and one $6x$ aneuploid (58 chromosomes). For the other three hybrids, chromosome counting was difficult; the respective numbers recorded ranged between 51 and 59, 51 and 56 (possibly $5n$ or $5x$ aneuploid), and 64 and 67 (either $6n$ or $6x$ aneuploid). In the reciprocal cross, no genuine hybrids were found among the 13 plants planted. Based on fruit morphology and chromosome number ($2n = 2x = 22$), they were most likely products of self-fertilization of *H. undatus*.

In the cross *S. megalanthus* × *H. polyrhizus*, 84 putative hybrids were planted. Of the seven plants studied, one pentaploid hybrid was identified. In the reciprocal cross, 48 putative hybrids were planted. Of the eight plants studied, seven were confirmed triploids or $3x$ aneuploids (Table 1). Morphological comparison of the progeny was in accordance with the results obtained by chromosomal counts.

In situ hybridization with an rDNA probe exposed two hybridization sites in the diploid parental clones and four sites in the tetraploid clones (data not shown). FISH analysis of the hybrid plants was in accordance with the respective chromosome counts. Three hybridization sites were observed in the $3n$ hybrids, five sites in the putative $5n$ hybrid, and six sites in the hexaploid hybrid J-42 (Figure 4).

Discussion

The genomic in situ hybridization (GISH) technique is routinely used to identify the source of chromosome complements, individual chromosomes or chromosome arms in artificial hybrids, and natural allopolyploids (e.g., Morgan et al. 2001; Sánches-Morán et al. 1999, and the citations therein). The possibility of applying GISH to identify the chromosome complement donors in the studied hybrids was explored. However, even with the strict precautions employed (posthybridization high-stringency washing and a low ratio of tester DNA:block DNA), no differential fluorescent signals were observed. The full details of our attempts to optimize the GISH technique in this group are provided elsewhere (Tel-Zur N et al., submitted for publication).

Chromosome counting in polyploids is generally difficult and time consuming. In cases of uncertain chromosome counts, FISH with a ribosomal probe may be applied to verify the ploidy determined cytologically (Weiss and Maluszynska 2001). Indeed, the number of labeled rDNA

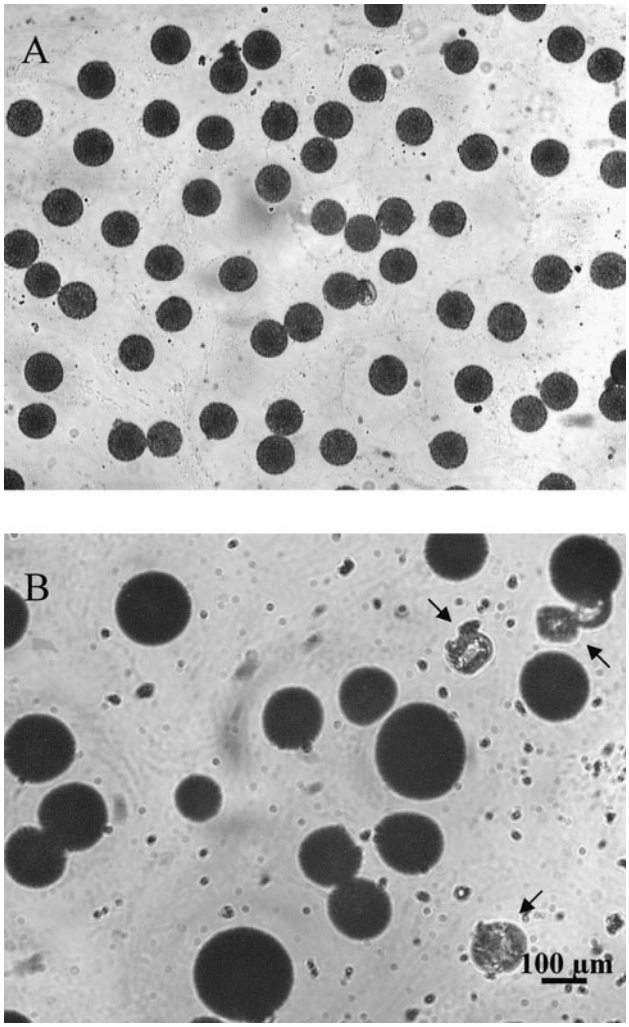

Figure 1. Photomicrographs of pollen grains of the diploid *H. undatus* **(A)** and the tetraploid *S. megalanthus* **(B)** stained with 2% acetocarmine. Arrows indicate aborted pollen grains. Both micrographs were taken at the same magnification.

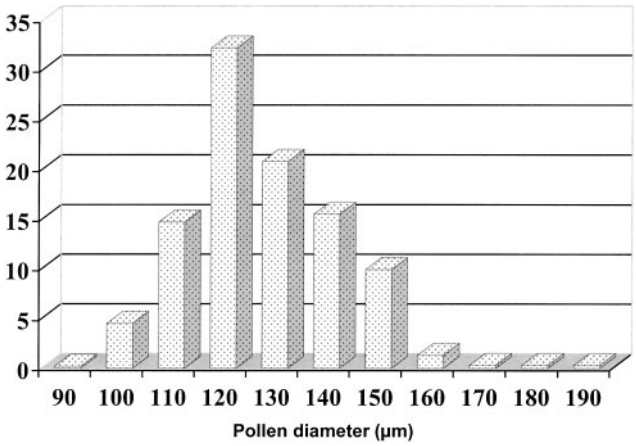

Figure 2. Distribution of pollen grain diameter in *S. megalanthus*. Results are means of three populations of about 300 viable pollen grains each.

sites, observed in the male meiocytes, were in accordance with the assumed ploidy level of the hybrids.

The intergeneric crosses between *S. megalanthus* and the two species of *Hylocereus* yielded not only the expected triploid hybrids (in one type of cross), but also pentaploid, hexaploid, and aneuploid hybrids. Triploid hybrids were found only in the cross between *H. polyrhizus*, as the female parent, and *S. megalanthus*, as the male parent, but not in the reciprocal cross or in the two reciprocal crosses involving *H. undatus*. This fact seems to indicate that *S. megalanthus* is closer genetically to *H. polyrhizus* than to *H. undatus*. The reciprocal cross, *S. megalanthus* × *H. polyrhizus*, yielded a pentaploid hybrid, which is probably the result of fertilization of an unreduced ($4n$) female gamete by a haploid (n) male gamete. This suggestion is supported by the abundance of large pollen grains in *S. megalanthus*, assuming that similar meiotic irregularities occur during female gametogenesis. It has long been recognized that pollen size correlates with DNA content, thus the presence of large pollen grains may serve to indicate unreduced gametes (Den Nijs and Peloquin 1977; Mendiburu and Peloquin 1976). We consider the small pollen grains (5%) produced by *S. megalanthus* as haploid ($n = 11$) because their diameter is close to that of the pollen grains produced by the diploid species. The majority of the pollen grains of *S. megalanthus* have an intermediate diameter and are most likely the product of regular meiosis, carrying $n = 2x = 22$ chromosomes. The large grains (12%) are probably unreduced ($2n = 4x = 44$) gametes. It is noteworthy that the pentaploid hybrids were produced only when the female parent was the tetraploid *S. megalanthus*. Apparently, despite their regular appearance (stainability), the male unreduced ($4n$) gametes are either nonfunctional or fail to compete with

Table 1. Cross combination and ploidy of F_1 plants

Cross combination				
{female}	{male}	No. progeny studied	No. confirmed hybrids[a]	Chromosome no. of confirmed hybrids
S. megalanthus × *H. undatus*		11	5	51–56, 51–59, 58, 66, 64–67
H. undatus × *S. megalanthus*		2	0	—
S. megalanthus × *H. undatus*		7	1	55
H. undatus × *S. megalanthus*		8	7	33 (4 plants), 32, 34, 35

[a] The rest of the plants had the chromosome number and the fruit morphology of the female parent.

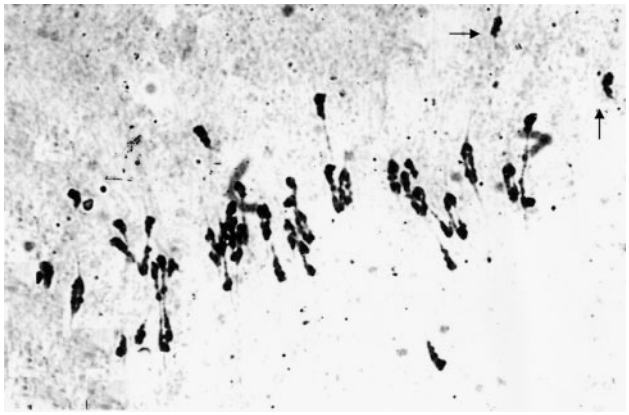

Figure 3. Photomicrograph showing meiotic metaphase I in a pollen mother cell of the hexaploid hybrid J-42 from the cross *S. megalanthus* × *H. undatus*. Arrows indicate univalent chromosomes.

the reduced ($2n$) gametes. Also worth noting is the fact that pentaploid hybrids were formed only in the *S. megalanthus* × *H. polyrhizus* cross, while the *S. megalanthus* × *H. undatus* combination produced $6n$, $5n$, or $5x$, $6x$ aneuploids.

The mechanism of the hexaploid hybrid formation from the cross *S. megalanthus* × *H. undatus* is questionable. The literature suggests that the production of $2n$ gametes is the dominant process underlying the origin of polyploid plants (Bretagnolle and Thompson 1995; deWet 1980; Harlan and deWet 1975). However, from pollen size data for the diploid species *H. undatus* and *H. polyrhizus*, we found no evidence for the occurrence of $2n = 2x = 22$ gametes that could have fused with $2n = 4x = 44$ gametes from *S. megalanthus*. The relatively uniform pollen diameter is in full accord with the regular chromosome disjunction at anaphase reported for these diploid taxa (Banerji and Sen 1955; Lichtenzveig et al. 2000). Furthermore, tested interspecific *Hylocereus* × *Hylocereus* crosses were found to be diploids, showing normal meiosis with regular pairing in the pollen mother cells analyzed, which suggests the negligible occurrence of unreduced gametes in this species. In addition, the occurrence of three $6n$ and $6x$ aneuploid plants among 28 plants studied (out of more than 200 F_1 plants planted from the crosses of *S. megalanthus* and *Hylocereus* spp.) is much higher than the expected value based on the frequency of large-size pollen grains produced by the diploid species. In addition, the absence of tetraploid hybrids from the $4n \times 2n$ crosses also refutes the proposed mechanism for hexaploid production through unreduced gametes from the diploid parent.

An alternative mechanism for the formation of hexaploid hybrids is zygote or postzygote somatic chromosome doubling. The available data do not indicate the time of the doubling event. However, the absence of chimeric plants may suggest that chromosome doubling occurred immediately after zygote formation or shortly afterward. Only very few well-documented cases of somatic doubling have been

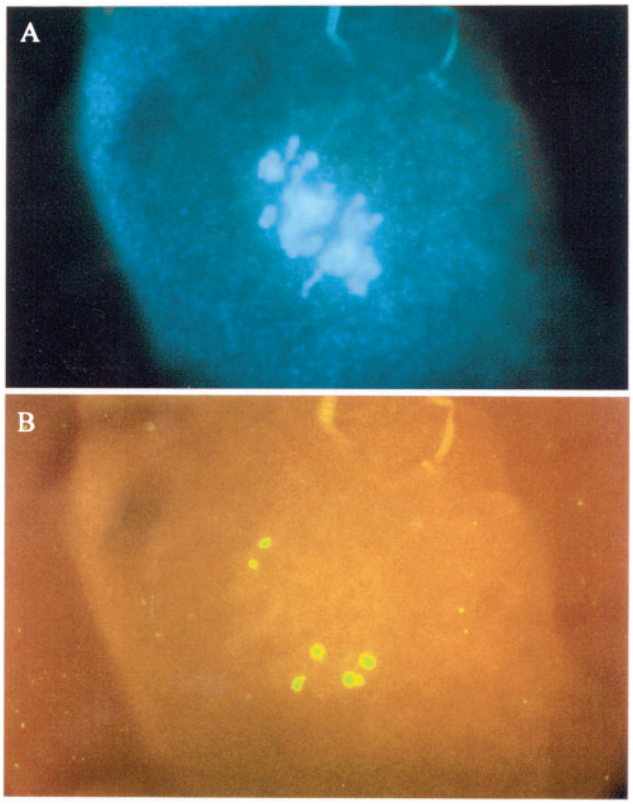

Figure 4. Photomicrographs showing meiotic metaphase I in the hexaploid hybrid J-42. **(A)** DAPI counterstaining after FISH analysis. **(B)** The same cell showing six hybridization sites with a pTa71 probe (yellow points).

reported; for example, the spontaneous tetraploid, *gigas*, of *Oenothera lamarckiana* Ser. (Gates 1924) and the amphidiploid hybrid between *Nicotiana glutinosa* L. and *N. tabacum* L. (Clausen and Goodspeed 1925). At this stage the limited number of intergeneric hybrids analyzed prevents us from drawing any general conclusions regarding the frequency of the phenomenon or its role in the evolution of this group. Still our hexaploid hybrids constitute additional important experimental evidence for polyploidization by means of spontaneous somatic chromosome doubling in higher plants.

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