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CYTOLOGY AND MATING SYSTEMS IN THE CLIMBING CACTI *HYLOCEREUS* AND *SELENICEREUS*[1]

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Chromosome numbers and meiotic behavior are reported for the climbing cacti species *Hylocereus undatus, Hylocereus polyrhizus,* and *Selenicereus megalanthus.* The *Hylocereus* spp. are diploid ($2n = 22$), while *S. megalanthus* is a tetraploid ($2n = 44$). Irregular chromosome disjunction at anaphase I in pollen mother cells of *S. megalanthus* is probably the major cause of its reduced pollen viability and may contribute to low seed set, low number of viable seeds and, consequently, low fruit mass. A pollination study confirmed self-incompatibility in *H. polyrhizus* and a weakened incompatibility reaction in *H. undatus* and *S. megalanthus.* Major crossability barriers do not exist between the *Hylocereus* spp. investigated. Reciprocal intergeneric crosses were successful between *Hylocereus* spp. and *S. megalanthus,* suggesting that an *Hylocereus sp.* might be one of the diploid progenitors of the tetraploid *S. megalanthus.* The implications of the results on cacti nomenclature and systematics are briefly discussed.

Key words: cacti; polyploidy; *Hylocereus*; *Selenicereus*; self-incompatibility; semi-sterility; systematics.

Night-blooming climbing cacti of the genera *Hylocereus* (Berger) Br. & R. and *Selenicereus* (Berger) Br. & R. have received increased attention during the last decade for their potential as new exotic fruit crops. The species of these genera have been studied mainly from the physiological aspect, and a review of their reproductive biology has recently been published (Nerd and Mizrahi, 1997).

These genera belong to the Cactaceae subfamily Cactoideae, tribe Hylocereeae (Br. & R.) Buxbaum (Barthlott and Hunt, 1993). About 16 *Hylocereus* species are dispersed along Central America and Northern South America. The genus *Selenicereus* comprises 20 species distributed through tropical America and the Caribbean region (Barthlott and Hunt, 1993).

Commercial plantations of *H. undatus* (Harworth) Br. & R. exist in Colombia (Cacioppo, 1990), Nicaragua (INRA, 1994), and Vietnam (Mizrahi, Nerd, and Nobel, 1997); and of *S. megalanthus* (Schum. ex Vaupel) M. in Colombia (Cacioppo, 1990; Mizrahi, Nerd, and Nobel, 1997). Small-scale production of these species and of *H. polyrhizus* (Weber) Br. & R. is emerging in Israel.

Fruit size in these genera determines their economic value. Fruit of *S. megalanthus* (80–300 g) are much lighter than *Hylocereus* spp. (200–800 g). Weiss, Nerd, and Mizrahi (1994) found a positive correlation between fruit fresh mass and total seed number, and a positive relationship seems to exist between the seed set data and pollen viability in these species. Pollen viability is high (>90% stainability) in the *Hylocereus* group but low in *S. megalanthus,* 25% for a Colombian clone and 40% for an Ecuadorian clone (Weiss, Nerd, and Mizrahi, 1994). Lichtenzveig (1996) observed that the reduced pollen stainability values of Colombian *S. megalanthus* clones occurred only at the early- and late-season flower buds as compared to 70–78% stainability during flowering peak. A high rate of ovule failure was reported in *S. megalanthus* (>77%) as compared to only 10% for *H. undatus* (Weiss, Nerd, and Mizrahi, 1994).

Studies on Cactaceae cytology deal with chromosome numbers but not with structural chromosome aberrations (Beard, 1937; Banerji and Sen, 1955; Spencer, 1955; Ross, 1981). To the best of our knowledge there are no reports relating pollen viability to chromosomal aberrations in the Cactaceae.

Both self-fruitful and self-unfruitful (i.e., self-incompatible) species have been observed in *Hylocereus. Hylocereus undatus* was reported as self-compatible, but with partial self-fruitfulness (50–80% fruit set), while *H. polyrhizus,* which bears attractive fruit of high commercial potential, was reported as self-incompatible (Weiss, Nerd, and Mizrahi, 1994). The largest fruits of each of the *Hylocereus* spp. were obtained by interspecific crossings. Fruit set occurs both after self- and cross-pollination (interclonal) in *S. megalanthus* (Weiss, Nerd, and Mizrahi, 1994).

In the framework of a developmental project to introduce climbing cacti as crops for the Negev Desert of Israel, we studied the reproductive biology and cytology of *H. undatus, H. polyrhizus,* and *S. megalanthus.* The objective of the study reported here was to determine whether the reduced pollen viability and high rate of ovule failure of *S. megalanthus* occur due to chromosomal aberrations and to identify the fac-

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TABLE 1. Source of plant material (introduction origin, original code, and locality data), fruit characteristics, and chromosome numbers.

Species	Local code	Fruit characteristics	Source	Chromosome no. (2*n*)
Hylocereus undatus (Harworth) Britton & Rose	88-024	Fruit oblong; light-red peel with large scales; white pulp	Huntington Botanical Garden Code: 39872, Virgin Islands USA.	22
Hylocereus polyrhizus (Weber) Britton & Rose	89-027	Fruit round; dark red peel with large scales; scarlet pulp	Huntington Botanical Garden Code: 15886, Nicaragua.	22
	89-028	Fruit oblong; dark red peel with large scales; scarlet pulp	Huntington Botanical Garden Code: 15885, Nicaragua.	
Selenicereus megalanthus (Schum. ex Vaupel) Moran syn. *Mediocactus megalanthus* Britton and Rose	90-001	Fruit oblong; yellow peel with tubercles and spines; white pulp	Plantation, Colombia.	44
	90-002	Fruit oblong; yellow peel with tubercles and spines; white pulp	Mr. H. Preiva Plantation, Colombia.	44
	90-003	Fruit oblong; yellow peel with tubercles and spines; white pulp	Mr. Burga Plantation, Colombia.	44
	88-023	Fruit oblong; yellow peel with tubercles and spines; white pulp	Volcani Center, Wild, Ecuador.	44
	96-666	Fruit oblong; yellow peel with tubercles and spines; white pulp	Tel Aviv University, Dr. Friedman; Wild, Ecuador.	44

tor(s) that prevent fruit set following self-pollination in *H. polyrhizus.*

MATERIALS AND METHODS

Plant materials and growing conditions—Three species were selected for this study: *H. undatus* (one clone), *H. polyrhizus* (two clones), and *S. megalanthus* (five clones) as shown in Table 1. The clones were originally introduced as cuttings to Israel either from the Huntington Botanical Garden in California or from three different commercial plantations in Colombia, or as seeds from the jungles in Ecuador (Table 1). The study was carried out at the Ben-Gurion University of the Negev campus in Beer-Sheva, during 1995 and 1996. The experiments were performed on 3–4 yr-old-plants grown on a trellis in a net-house. The plants were irrigated once a week with 2 L per plant during the cold wet season (November–April) and twice a week with 2.5 L per plant during the hot season (May–October); water contained 70 ppm N, 9 ppm P, and 70 ppm K. The average max/min greenhouse temperatures were 28°/6°C in the coldest month (January) and 35°/18°C in the hottest month (August). The maximum temperature was 45°C during the summer, and the minimum temperature was 2°C during the winter.

Cytology—Chromosome numbers were determined at pollen mother cells (PMCs) for the following clones: *H. undatus* (89–024), *H. polyrhizus* (89–027), and *S. megalanthus* (90–001, 90–002, 90–003, 88–023, and 96–666). Meiotic chromosome behavior was studied in *H. undatus* (89–024), *H. polyrhizus* (89–027), and *S. megalanthus* (90–003 and 88–023). Flower buds of 5 cm long, were collected between 1100 and 1300, dissected, and fixed in 3:1 ethanol:acetic acid. After 48 h flower buds were transferred to 70% ethanol. Squash preparations of PMCs were stained with 2% aceto-carmine. Photomicrographs were taken with a Zeiss Axioplan microscope using Kodak technical pan film.

Pollination techniques, seed set determination, and fruit mass—Two *Hylocereus* species (one *H. undatus* clone and two *H. polyrhizus* clones) and *S. megalanthus* were self-pollinated and intercrossed during the night. A full diallel was not achieved because the species did not flower synchronously. In selfing, pollen from the same flower was applied to the stigma; in interclonal, interspecific, or intergeneric crosses, pollen from two or more flowers was combined. The stigma was covered with parafilm 1–2 h before and immediately after hand-pollination to avoid contamination. For all pollinations, fresh pollen was collected from flowers at anthesis. Pollen abortion was evaluated under a light microscope throughout the flowering season using Alexander's stain (Alexander, 1969). The percentage of pollen abortion was scored from a minimum of 400 grains pooled from at least two flowers per month for each species or clone.

Fruits were harvested at maturity, based on skin color, at seed maturation. Fruit mass and seed number were determined. Brown- and black-coated seeds were observed in some of the fruits. Brown-coated seeds were found nonviable. Seed viability was determined by germinating the black-coated seeds on moist filter paper, in covered petri dishes at 20° ± 2°C under permanent cool-white fluorescent lamps of 40 W. Germination percentage was determined 21 d after seed imbibition.

Pollen germination and pollen tube growth in vivo—*Hylocereus polyrhizus* clone 89–028 was either selfed or crossed reciprocally with *H. undatus* clone 89–024. Reciprocal crosses were made between *H. undatus* clone 89–024 and *S. megalanthus* clones as well as selfing of parental lines. From each described combination three to five flowers were pollinated. Samples of stigmatic lobes were removed 10–12 h after pollination, while pistils were separated from the flowers 2–4 d after pollination (DAP) and were immediately fixed with a 1:8:1 formalin: 80% ethanol:acetic acid solution and stored. The pistils were cut into six sections and a cross slice was sampled from each section for fluorescence microscopy. Both stigmatic lobes and pistil samples were soaked in a 1% sodium carbonate solution for 1 h, washed with a 0.1% solution of aniline blue in 0.1 mol/L K_3PO_4 for at least 4 h. Squash preparations of the samples were observed under an epifluorescence Zeiss microscope equipped for UV excitation. Percentage of pollen germination was calculated from counts of at least 100 pollen grains in each sample; a pollen grain was considered germinated when the pollen tube length exceeded the grain diameter.

RESULTS AND DISCUSSION

Polyploidy in Hylocereeae—Chromosome numbers and meiotic behavior are reported for the first time for *S. megalanthus* and *H. polyrhizus,* and previously published chromosome counts are confirmed for *H. undatus.* According to the base number of $x = 11$ (Beard, 1937; Ross, 1981), both diploids and tetraploids were observed. In pollen mother cells (PMCs) of *H. undatus* and *H. polyrhizus* $2n = 22$ chromosomes were observed. In *S. megalanthus,* chromosome counts in PMCs of three clones from Columbia (90–001, 90–002, and 90–003), as well as in two clones from Ecuador (88–023 and 96–666)

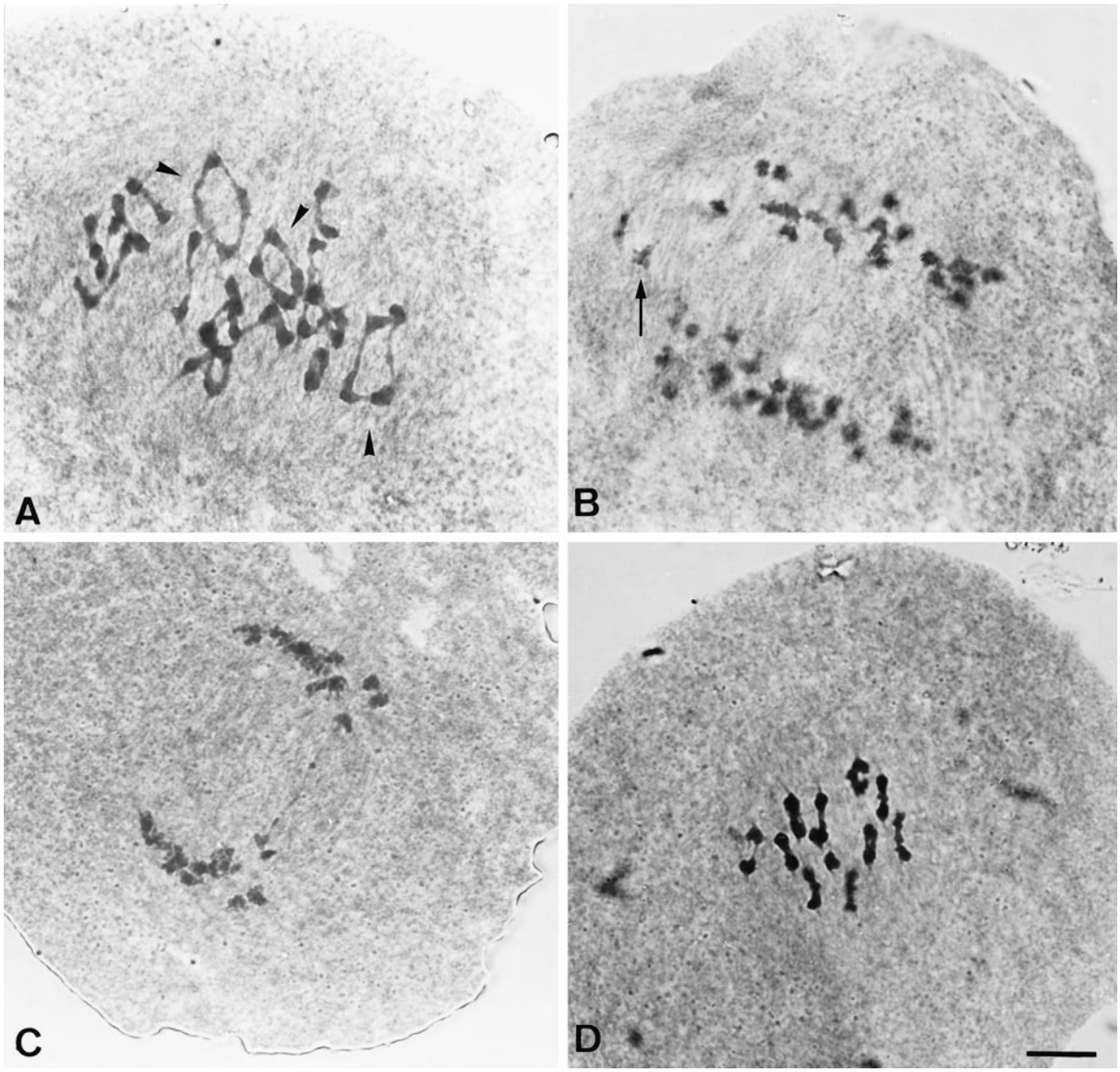

Fig. 1. Meiotic chromosomes of pollen mother cells (PMCs) from the cacti species *Selenicereus megalanthus* (90–003), *Hylocereus undatus,* and *Hylocereus polyrhizus*. (A) *S. megalanthus,* metaphase I. (B) *S. megalanthus,* anaphase I. (C) *H. undatus,* anaphase I. (D) *H. polyrhizus,* metaphase I. Bar scale = 9 μm (all micrographs were taken at the same magnification). *Abbreviations:* arrowhead = ring quadrivalent; arrow = univalent in ''mitotic-like'' disjunction.

were $2n = 44$, indicating that *S. megalanthus* is a tetraploid. Meiotic chromosomes in the seven clones studied were of similar size and morphologically indistinguishable from one another.

Chromosome number variation, especially polyploidy, is believed to be one of the major phylogenetic processes in Cactaceae evolution (Beard, 1937; Pinkava and McLeod, 1971; Ross, 1981; Cota and Philbrick, 1994). Polyploidy is more common in the subfamilies Opuntiodieae, e.g., *Opuntia* (Pinkava and McLeod, 1971; Pinkava, McGill, and Brown, 1973; Baker and Pinkava, 1987), and Cactoideae, e.g., *Mammillaria* and *Echinocereus* (Ross, 1981; Cota and Philbrick, 1994). Cytological studies on Hylocereeae have been reported for four genera: seven *Hylocereus* spp. (Beard, 1937; Spencer, 1955), five *Selenicereus* spp. (Beard, 1937; Spencer, 1955), one *Weberocereus* Br. & R. sp. (Beard, 1937), and one *Mediocactus* Br. & R. sp. (Beard, 1937). Except for *Mediocactus coccineus* (Salm-Dyck) Br. & R., thus far the only tetraploid ($2n = 44$) known in this tribe, all Hylocereeae species were reported as diploids ($2n = 22$).

The genus *Mediocactus* is no longer considered an independent taxon, but rather a synonym of the genus *Selenicereus* (Barthlott and Hunt, 1993; Weiss, Scheinvar, and Mizrahi, 1995). Two species were included in this genus: *M. coccineus* and *M. megalanthus* syn.: *S. megalanthus* (Weiss, Scheinvar, and Mizrahi, 1995). *Mediocactus* was first classified by Britton and Rose (1963) and described as follows, ''in habit and flowers this plant much resembles *Hylocereus,* but differs from it in its tubercular ovary and in the felted and spine-bearing areoles of the fruit, which resemble those of *Selenicereus* . . . its

TABLE 2. Meiotic configurations at metaphase I of PMCs of *S. megalanthus* ($2n = 44$). Average and range of the different configurations per cell (15 PMCs from clone 90-003 and 19 from clone 88-023) are shown.

Configuration	No. and range per cell	
	Clone 90-003	Clone 88-023
Univalent	2 (0–4)	0.57 (0–2)
Bivalent		
Rod	9.8 (3–15)	8.5 (1–14)
Ring	4.1 (3–9)	8.9 (2–17)
Multivalent		
III	1.6 (0–4)	0.57 (0–2)
IV	1.7 (0–4)	1.6 (0–6)
V	0.4 (0–2)	0
VI	0.1 (0–1)	0.10 (0–1)

name implies intermediate characters . . . .'' These morphological features might imply that the polyploidy observed in *S. megalanthus* originated from an intergeneric hybridization between diploid species of the genera *Hylocereus* and *Selenicereus.* If this is the case, *S. megalanthus* should be considered an allopolyploid.

Meiotic chromosomes behavior of Hylocereus spp. and Selenicereus megalanthus—Multivalent pairing was observed in most PMCs tested in *S. megalanthus* clones (Fig. 1A, B). Frequencies of the different meiotic configurations observed in the two clones are provided in Table 2. Bivalent formation and regular disjunction were observed during the first metaphase of meiosis in PMCs from the species *H. undatus* and *H. polyrhizus* (Fig. 1C, D).

Examination of over 20 meiocytes at diakinesis and at first metaphase showed no irregularities in the meiotic process in the tested *Hylocereus* spp. In a few PMCs, however, late disjunction of bivalents was observed (Fig. 1C), which did not disturb regular chromosomal disjunction since 11 chromosomes were regularly observed at both poles at anaphase. These observations agree with prior cytological studies on *H. undatus* (Banerji and Sen, 1955). In *S. megalanthus* the presence of multivalents and univalents was typical of metaphase I. An example of an univalent in ''mitotic disjunction'' at anaphase I in *S. megalanthus* 90–003 is presented in Fig. 1B.

Occasional multivalents in an organism with regular bivalent pairing may be a consequence of heterozygosity of translocations. In perennials with vegetative propagation, such translocations can easily be fixed and maintained for many generations. In such cases one would expect ~50% pollen viability in clones heterozygous for a single translocation and further reduction in viability with more than one translocation. This, however, is not the case in the *S. megalanthus* clones tested. That is, despite the observed frequencies of multivalent pairing (3.8 per cell) (Table 2), pollen viability in *S. megalanthus* was above 70% during peak flowering. Thus, reciprocal translocations were ruled out as the cause for these multivalent associations in metaphase I.

In polyploids with multivalent pairing, chiasmata failure may result in unbalanced anaphase I disjunction due to presence of unpaired univalents. Having observed univalents in metaphase I cells, we were interested in determining the degree of unbalanced anaphase I disjunctions. Three different anaphase separations were observed for the *S. megalanthus* clones (90–003 and 88–023) at anaphase I; 22–22, 23–21, and 24–20 (Table 3). Some degree of aneuploidy could be tolerated, both under the assumption of autotetraploidy or allotetraploidy (with some degree of homoeology between parental genomes). For example, we have counted $2n = 28$ in root tip cells of a hybrid resulting from the cross *H. polyrhizus* × *S. megalanthus.* The deviation from the expected triploid number ($2n = 3x = 33$) may be explained by fusion of a normal haploid gamete ($n = 11$) with an unbalanced, but still viable, $n = 17$ ($2x - 5$) gamete from the tetraploid pollen parent. Chromosome deficiency for both copies of a particular chromosome, in an autotetraploid, or of both partial homologues (homoeologues) in an allotetraploid, is likely to result in a nonviable gamete. Alternatively, a gamete deficient for one homologue from each of several nonhomologous chromosomes might still be viable. It is impossible to determine what portion of the 20-chromosome situations in anaphase I (Table 3) represents such a case. Clearly, the chromosome disjunction data at anaphase I (Table 3) explain the reduced pollen viability counts (70–78%) of *S. megalanthus.* While it is possible that some of the aneuploid pollen might be viable, it is likely that pollen grains from 24 to 20 disjunction events would be nonviable, hence the reduced stainability counts.

TABLE 3. Chromosome distribution at anaphase I of PMCs of *S. megalanthus* ($2n = 44$). Frequency of 17 and 25 PMCs of clones 90-003 and 88-023, respectively, is shown.

Segregation class	Frequency (%)	
	Clone 90-003	Clone 88-023
22–22	53	56
23–21	35	28
24–20	12	16

Most polyploid Cactaceae species exhibit bivalent pairing at metaphase I of meiosis (Pinkava and McLeod, 1971; Ross, 1981). The exceptions are *Rebutia spegazziana, Rebutia* cv. Nivea, *Mammillaria compressa,* and *M. prolifera,* each of which form three to five quadrivalents (Ross, 1981). The frequency of multivalent pairing in *S. megalanthus* may assist in determining its status as an autotetrapolyploid or as an allotetraploid. Exclusive bivalent formation in metaphase I could serve as an indication for allopolyploidy. Quadrivalent formation, in certain frequencies, could indicate an autoploid origin (Jackson and Casey, 1982). The observed frequencies of quadrivalent + trivalent in PMCs of both studied *S. megalanthus* clones are by far below the expected frequencies predicted by the model suggested by Jackson and Casey (1982). Therefore, it is unlikely that *S. megalanthus* is an autotetraploid species. If this is the case, the occurrence of an average number of 3.8 multivalents per PMC could reflect some chromosome homoeology. In such a case *S. megalanthus* might be a segmental allopolyploid indicating the homoeology between some of the chromosomes of its parental complements.

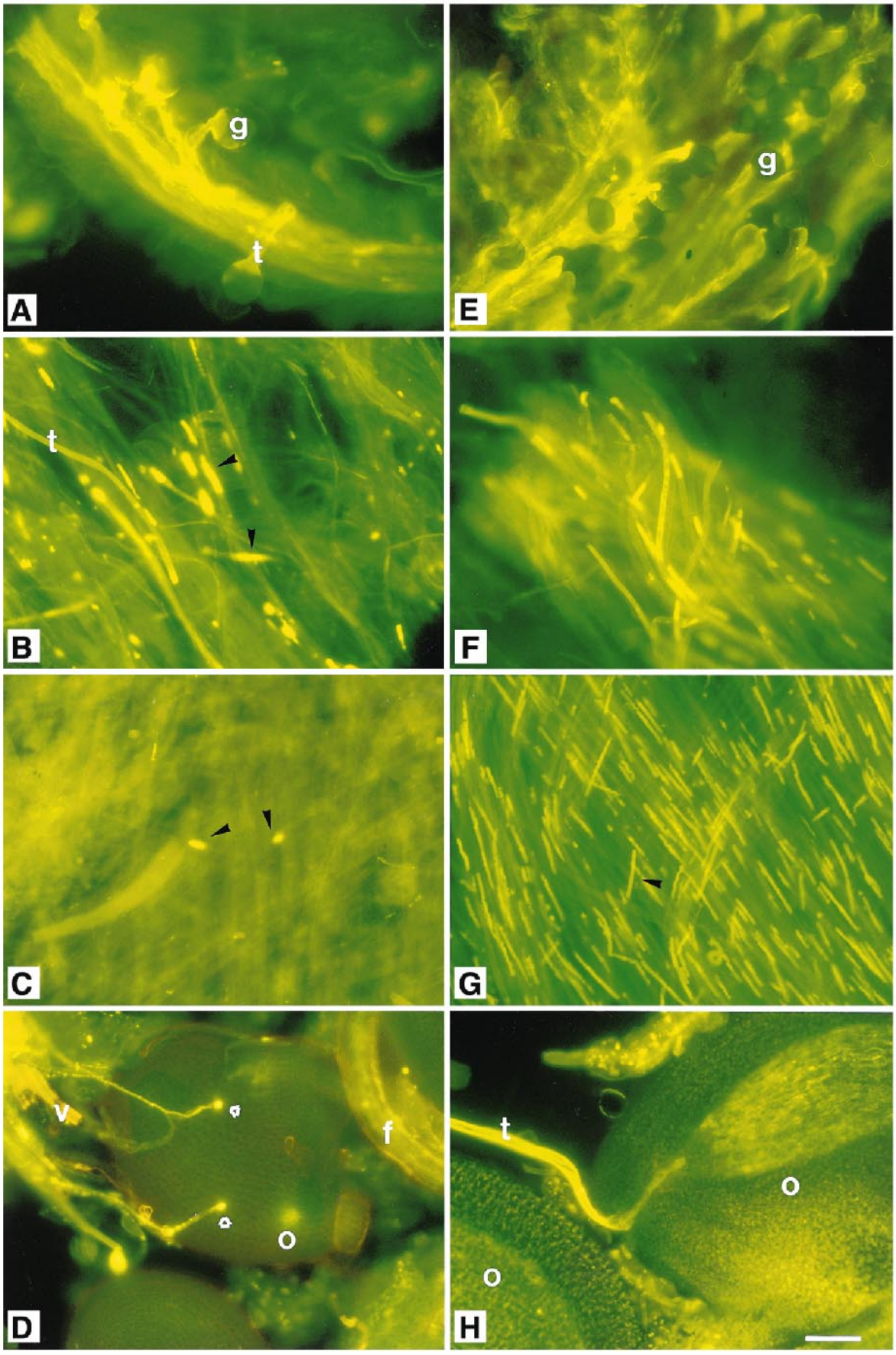
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TABLE 4. Results of self- and cross-pollination of *S. megalanthus.*

Pollination	Pollen parent	Fruit set (%)	Fresh mass (g)[a]	No. of seeds[a]	Germination rate of black coated seeds (%)	Rate of brown (nonviable) seeds (% of total)
Self-[b]		60	108 ± 20	508 ± 85	41	17
Cross-Interclonal[c]	*S. megalanthus* 88-023	100	240 ± 30	891 ± 188	49	19
Intergeneric[d]	*H. polyrhizus*	100	169 ± 15	1126 ± 146	37	54
	H. undatus	100	143 ± 6	1407 ± 95	19	60

[a] Values are mean ± 1 SE of 5–19 replications. Data collected at full skin color change.
[b] *S. megalanthus* clones 90-001, 90-002, and 90-003 were grouped since no significant female influence was found. Data do not include clone 88-023.
[c] *S. megalanthus* clones 90-001, 90-002, and 90-003 were grouped since no significant female influence was found. Data do not include clone 88-023 as a female parent.
[d] The female clones were *S. megalanthus* clones 90-003 and 88-023.

Possible cause of low fruit mass in S. megalanthus—Weiss, Nerd, and Mizrahi (1994) reported a positive correlation between fruit fresh mass and total seed number in *S. megalanthus* ($r = 0.46$, which differ significantly from zero at $P < 0.05$). We observed a higher correlation between fruit mass and viable seed number; the number of viable seeds per fruit accounted for 42% of the variation in fruit mass ($r = 0.65$, which differ significantly from zero at $P < 0.05$). *Selenicereus megalanthus* ovaries contain in average 1969 ± 176 ovules (Weiss, Nerd, and Mizrahi, 1994). A low percentage of these ovules developed into viable seeds: 8.7% after self-pollinations, 5.4 and 9.7% after intergeneric crosses with *H. undatus* and *H. polyrhizus,* respectively, and 17.9% after interclonal crosses, where clones 90–001, 90–002, and 90–003 were the female parent and clone 88–023 was the male (Table 4). Undeveloped brown seeds and viable black seeds were also observed in fruits of the columnar cactus *Cereus peruvianus* (L.) Mill. (Weiss, 1995), which have similar characteristics to fruit of *Selenicereus. Cereus peruvianus* fruit have white juicy pulp, which develops from the feniculi, but the pulp originates only from the feniculi of the black seeds (Weiss, 1995). Such might be the situation in *S. megalanthus* fruits and could be the reason for the correlation between fruit mass and viable seeds.

Chiasmata failure resulting in unbalanced gametes is a likely cause of reduced pollen and megaspore viability and, thus, seed set. Pollen viability values of 75–80% in *S. megalanthus,* compared with ~95% in the diploid species, do not explain a reduction of nearly 50% in percentage of seed set (above) and fruit mass (Tables 4 and 5). Chromosome counts of embryo and endosperm cells in developing seeds would help to determine whether *S. megalanthus* seed abortion is due to chromosomal irregularities. An anatomical study of the developing seeds is required to clarify the developmental differences between the viable (black) seed and the nonviable (brown) seed of *S. megalanthus.*

Mating systems of the genera Hylocereus and Selenicereus—In the self-fruitful *Hylocereus* clones, fruits obtained by self-pollination were significantly smaller than those obtained by interspecific cross-pollination (Table 5). These results are in line with data reported by Weiss, Nerd, and Mizrahi (1994). Self-pollinations in *H. polyrhizus* resulted in inhibition of pollen tube growth at the ovary (Fig. 2A–D) and low percentage of light mass fruit set (Table 5). *Hylocereus polyrhizus* clones were reciprocally cross-compatible; the percentage of fruit set and the fruit mass were almost as high as after interspecific pollination (Table 5).

By 12 h after pollination, pollen grains had germinated and their tubes had penetrated the stigmatic surface on selfed and outcrossed pistils (Table 6; Fig. 2A, B, E, and F). However, there were differences in the percentages of pollen grains germinating on the stigma after the distinct pollination treatments (Table 6). At 2 d after pollination (DAP), a few pollen tubes extended through the base of the style on selfed pistils of *H. polyrhizus* (Table 6; Fig. 2K). By that time, numerous pollen tubes were present at the base of the style on outcrossed pistils of *H. polyrhizus* (Table 6). At 4 DAP, only a few pollen tubes had entered the ovary cavity on *H. polyrhizus* selfed pistils. No ovule penetration was observed even 5 DAP in selfed *H. polyrhizus* ovaries (Fig. 2D). At 4 DAP, large numbers of pollen tubes had entered the ovary cavity and penetrated in ovules of *H. undatus* following self or reciprocal interspecific pollination between *H. undatus* and *H. polyrhizus* (Table 6; Fig. 2H). Pollen tube growth after self-pollinations in *H. undatus* and *S. megalanthus* was similar to the growth in interspecific or intergeneric pollinations (Table 6). Interclonal and interspecific pollinations within and between *Hylocereus* spp. led to 100% fully developed seed, in which 80–100% germinated in contrast to the low seed germination (20–25%) from selfing. Low seed viability might be the major cause for the differences observed in fruit fresh mass (Tables 4 and 5).

Both fruit set percentage and fruit mass were low after

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Fig. 2. Fluorescence microscopy of pollen tube growth in *H. polyrhizus* pistil after self- and cross-pollination. Pollen tubes were stained with aniline blue. (A–D) Pollen tubes after self-pollination. (E–H) Pollen tubes after crossing with compatible pollen (*H. undatus*). (A, B, E, F) Pollen germination on stigma 48 h after pollination. (C, G) Pollen tubes at section 5, the style base. (D, H) Pollen tubes at ovary cavity 72 h after pollination. Bar scale = 80 μm (all micrographs were taken at the same magnification). *Figure abbreviations:* arrowheads = callose plugs; open arrowhead = burst tips of pollen tubes; f = fenicula; g = pollen grain; o = ovule; t = pollen tube; v = vascular bundle.

TABLE 5. Percentage of fruit set (FS), mean fruit fresh mass (FM), and mean number of seeds (S) per fruit following selfing and crossing of four *Hylocereus* cultivars, using *Hylocereus* spp. and *Selenicereus megalanthus* as pollen donors.

Seed parent	Variable	Pollen parent: *H. polyrhizus* 89-028	*H. polyrhizus* 89-027	*H. undatus* 88-024	*S. megalanthus*[c]
H. polyrhizus	FS (%)[a]	22.2	100	100	100
89-028	FM (g)[b]	74	399.8 ± 25	437.7 ± 18	268.2 ± 60
	S (no.)[b]	803.7	7489 ± 342	8603 ± 615	1285 ± 328
H. polyrhizus	FS (%)	100	30	100	ND[d]
89-027	FM (g)	219.64 ± 15	175.07	289.6 ± 108	
	S (no.)	6658 ± 418	566	7800 ± 2008	
H. undatus	FS (%)	100	100	100	90
88-024	FM (g)	585.9 ± 62	328.7 ± 30	174.7 ± 37	132.5 ± 14
	S (no.)	11 125 ± 944	11 125 ± 944	2871 ± 804	849 ± 113

[a] (No. of fruit at full skin color change/total flowers pollinated) × 100.
[b] Values are mean ± 1 SE of 4–16 replications.
[c] *S. megalanthus* clones from both Colombia and Ecuador used as pollen donors.
[d] ND = not detected.

selfing the *S. megalanthus* clones 90–001, 90–002, and 90–003 (Table 4). However, pollen tubes were observed at selfed ovary cavity 4 DAP, and no SI reaction (i.e., burst pollen tube tips) was detected at any pistil section. No major barriers seem to limit interspecific crossings between *Hylocereus* spp. Numerous ovules were fertilized by pollen tubes 3 DAP and fruits contained mostly viable seeds (*H. undatus*, 83% of viable seeds per fruit; *H. polyrhizus*, 94%). Intergeneric pollinations (between the species *H. undatus* and *H. polyrhizus*, and *S. megalanthus* clones 90–003 and 88–023) resulted in high fruit set but relatively small size fruit (Tables 4 and 5) containing a high percentage of nonviable seeds. In cases where an *Hylocereus* sp. was the female parent, abortive seeds were mainly black-coated and empty (just 29% of viable seeds per fruit); when the *S. megalanthus* was the female parent, fruit contained brown seeds that failed to germinate (Table 4). In both cases pollen germination at the stigma was particularly high (Table 6), and pollen tubes were observed in the ovary cavities 4 or 5 DAP. Since abortion seems to occur some time after seed development has started, the low seed viability after reciprocal intergeneric pollination could be related to postzygotic abortion events (probably, in part, due to ploidy level differences between the gametes). The fact that several *Hylocereus* spp. clones are cross-compatible with our tetraploid *Selenicereus megalanthus* (syn. *Mediocactus megalanthus*) is in line with the assumption that an *Hylocereus* sp. is one of the diploid progenitors of *S. megalanthus*. Different *Hylocereus* species—*H. undatus* and *H. polyrhizus*, employed in this study, and *H. costaricensis* (Weber) Br. & R. (Weiss, Nerd, and Mizrahi, 1994)—are cross-compatible with each other despite their classification as independent botanical taxons. This means that the above species may be considered as members of the same gene pool (biological species), after Mayr (1970).

The experimental results confirm that self-incompatibility (SI) occurs in *H. polyrhizus*. All species tested in this study possess hollow styles that, according to de Nettancourt (1977), probably restrain the contact between pollen tubes and the stylar tissue. Inhibition of incompatible pollen tubes was not confined to a particular stylar region for any of the pollination types, hence, in-

TABLE 6. Pollen germination (%) in vivo and relative growth rate of pollen tube after self- and cross-pollination in *Hylocereus* and *Selenicereus*. Three to five flowers were analyzed for each pollination treatment. In crosses, the female (seed parent) is listed first.

Pollination type			Pollen germination on stigma (%)[a]	Pollen tube growth through pistil[b]: 48 h	72 h	96 h
Self-						
H. undatus	89-024		49	5–6	5–6	5–6
H. polyrhizus	89-028		18	2–5	5	5–6
S. megalanthus	90-003		43	ND[c]	5	5–6
Interspecific						
H. undatus	89-024 × *H. polyrhizus*	89-028	30	5–6	6	6
H. polyrhizus	89-028 × *H. undatus*	89-024	45	5	6	6
Intergeneric						
H. undatus	89-024 × *S. megalanthus*[d]		45	ND	5	6
S. megalanthus[e]	× *H. undatus*	89-024	53	ND	5	5

[a] (No. pollen grain germinated/pollen grain on stigma) × 100. Data collected 10–12 h after pollinations.
[b] Section number (1 = stigma; 2–5 = style; 6 = ovary) to which the pollen tube reached.
[c] ND = not detected.
[d] *S. megalanthus* clones from Colombia and Ecuador used as pollen donors.
[e] *S. megalanthus* clones 90-001, 90-002, and 90-003.

hibition of self-pollen tubes probably occurs at the ovary before fertilization. The term ''late-acting self-incompatibility'' (Sedgley, 1994) seems appropriate to describe the SI mechanism in *H. polyrhizus.* The pollen tube growth behavior suggests that *H. polyrhizus* presents a gametophytic SI system, however, genetic studies are required to ascertain whether the gametophyte or sporophyte determines the incompatible phenotype of the pollen. Further observations are needed to determine the incompatibility reaction site in *H. polyrhizus* ovary; although postzygotic embryo abortion seems less possible, it cannot be ruled out yet.

The modern European (fresh) fruit markets are the main targets of the emerging Israeli climbing cacti industry. These markets have certain demands in terms of desired fruit mass as well as stable product delivery during the season. In Israel, climbing cacti cropping is a high input investment, mainly due to the required trellis, irrigation, and shedding facilities and labor cost. Hence, high fruit set of marketable size (mass) is a prerequisite for a lucrative and viable industry. In the absence of natural pollinators (native to Latin America), optimizing artificial pollination techniques including pollen storage protocols are of significant economic implications. In addition, development of self-compatible types with valuable fruit sizes is an important long-term goal in climbing cacti breeding. Such types will require less manual labor for pollen collection and pollination, thereby reducing farming costs.

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