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Self-compatible and -incompatible Reactions in Asiatic Hybrid *Lilium* X 'Enchantment': Influence of Pistil Age on Seed Set

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Summary

Cross-pollination (*L*. hybrid 'Enchantment' × *L. maculatum* Thunb.) was made before or after anthesis. The receptivity of pistils was observed from 6 days before anthesis (day −6) to 6 days after flowering (day +6). On crossing made on day 0, the number of mature seeds was 185 per capsule, whereas those on day +2 yielded 236 seeds per capsule. Flowers self-pollinated on day 0 yielded no seeds, and the ratio of pollen-tube length / style-length in the pistil was 0.85. Numerous abnormal pollen tubes with swollen or deformed bulbous tips with delayed generative nuclear division were observed compared with flowers selfed on day +3. Self-pollinated flowers on day −3, −2, −1, +1, +2, +4, and +6 developed capsules. Capsules from day +6 flowers produced 46 mature seeds, whereas the others yielded only 13 to 27 seeds per capsule. These findings show that *L*. X 'Enchantment' is a relatively weak self-incompatible cultivar. When seeds resulting from flowers self- or cross-pollinated at different stages were planted, differences in germination time and final germination percentage were observed. Of the cross-pollinated mature seeds made on day 0, 75% germinated; of the self-pollinated seeds made on day +2, 72% germinated, whereas those made on day +6, only 44% germinated.

Introduction

Since J. de Graaff (1947) bred Asiatic hybrid *Lilium* X 'Enchantment'(Shimizu, 1971), the hybrid has been cultivated as a popular garden plant, primarily because they bear several large, nasturtium-red, upright chalice-shaped flowers. The hybrid has also been used as a parent in the breeding of Asiatic hybrid lilies (Shimizu, 1971) because it bears bulbils which simplify propagation of *Lilium* sp. and has a moderate resistance to *Fusarium*, a disease which infects the bulbs and roots (Straathof, et al. 1993). These characteristics are advantageous to lily breeders; the lily is heterozygous and breeders are striving to a homozygous line with desirable traits through self-pollination. Unfortunately, *L*. X 'Enchantment' produces no mature viable seeds by self-pollination at anthesis (Cheng and Mattson 1972; van Tuyl et al., 1982; Li and Niimi, 1995). By cut-style pollination (Li and Niimi, 1995) and cultivating mother plants in a greenhouse at 26 °C (van Tuyl et al., 1982) several mature seeds can be obtained in this lily. This study was made to determine the most effective pollination time in obtaining as many viable seeds as possible by self- and cross-pollination in this variety. Thus, pollination was made at different stages of flowering to investigate pollen germination on the stigma, tube growth in the style, and time of sperm cell division in pollen tubes.

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Materials and Methods

Bulbs of Asiatic hybrid *L*. X 'Enchantment' and *L. maculatum* were planted in clay pots in the fall and allowed to overwinter. After the bulbs sprouted, the pots were transferred to a glasshouse, where the night/day temperatures, 10～20 °C / 15～25 °C, respectively, fluctuated depending on the weather during pollination.

Experiment 1. Self- and Cross-pollination at different stages of pistils

Anthers in flower buds of the *L*. X 'Enchant-

ment' were removed before pollination. The pistils were hand-self- or cross-pollinated (*L*. X 'Enchantment' × *L. maculatum* Thunb.) with freshly collected pollen.

Self- and cross-pollination were made at several stages from 7 days before (day −7) to 8 days after flowering (day +8). The stages of pistils pollinated before anthesis were then determined based on day 0. For each stage, 3 to 11 flowers were crossed (Table 1) or 4 to 23 flowers were self-pollinated (Table 2) on different plants. Enlarged capsules were harvested 10 weeks after pollination, and the number of mature and empty seeds was identified by the following method: seeds were spread on a transparent glass, and fully developed seeds with an embryo and endosperm were discerned from empty seeds through the transmitted light.

Germination tests of mature seeds were made as follows: 5 seeds were sown in soil (vermiculite and sand = 1 : 1 v/v) in a 10.5 cm × 10.5 cm plastic pot; and 5 pots were used for each treatment. The pots were kept in a growth chamber at 23/15 °C (day/night) under a 16-hr photoperiod. The frequency of seed germination was recorded every 5 days for 40 days.

Experiment 2. Observations of pollen germination on stigma, pollen-tube growth, and sperm-cell division in pistils self-pollinated on day 0 and day +3

1) Pollen germination on stigma

Three to 9 flowers self-pollinated on day 0 and day +3 were collected 1, 6, and 24 hr after pollination. Stigma with style tissue, about 1 cm long, were fixed in a modified Karnovsky's solution (Takeoka and Wada, 1985) at 4 °C for 12 hr. After rinsing the specimen in phosphate buffer solution (pH 7.2), dehydrating in a graded ethanol and 3-methylbutyl acetate, and critical-point drying with CO_2 in an ABT critical point dryer CP-5A, they were coated with gold and observed in a Model ABT-55 scanning electron microscopy (Akashi beam technology Co.) at 15 kV.

2) Pollen-tube growth and sperm-cell division in self-pollinated styles

Three to 9 flowers were collected 12, 24, 32, 48, 72, 96, 120, and 144 hr after pollination. Styles with stigmatic tissue attached were dissected from flowers and fixed in FAA (ethyl alcohol 70% solution : formaldehyde 37% solution : acetic acid 99.7% solution = 90 : 5 : 5 v/v) and stored at room temperature until microscopically examined. The entire length of fixed styles from the top of the stigmatic tissue to the base of a style ranged from 40 to 51 mm; the average style length was about 45 mm.

The fixed styles were washed in tap water for 1 hr, followed by immersion for about 40 min in a 1 N solution of NaOH at 60 °C and washed with tap water. To observe the growth of pollen tubes and their forms with special reference to their tips, the hollow styles were dissected using methods described in a previous paper (Niimi, 1991): (1) a sharp razor blade inserted into the style at the base of stigma and incised circularly; (2) the stylar tissue was longitudinally cut through from the top to the base; and (3) the bundles of pollen tubes including those attached to the surface layer of the stylar canal were removed from the stylar tissue by picking up the stigma with a pair of forceps. The bundles, stained by methods described below, were observed under fluorescence microscopy.

(1) Determination of pollen-tube length and frequency of deformed pollen tubes.

The softened styles were stained for 12 hr in aniline blue solution (Kho and Baër, 1968). The pollen tubes were straightened as much as possible with the aid of a streaming 45% glycerin (v/v) solution for fluorescence microscopy (Merck). After mounting the bundle with a micro-cover glass, both the length of the ten longest pollen tubes and the frequency of abnormal ones with swollen or deformed bulbous tips were determined using a stage micrometer. Three to 7 styles were measured for each treatment. Based on the average length of pollen tubes observed in each style, the ratio of tube length to style-length was calculated.

(2) Observation of sperm-cell division in growing pollen tubes

Three to 7 softened styles per treatment were washed in running water for 48 hr and then rinsed 6 times with distilled water. A bundle of pollen tubes removed from the stylar tissue was transferred onto a slide-glass in humidified petri dishes and stained with a citrate buffer solution

(pH 4) containing 1 mg·liter^{-1} DAPI (4' 6-diamidino-2-phenylindole·2HCl) and 1% polyoxyethylene (10) octylphenyl ether. The bundles were kept for 4 hr in an incubator held at 37 °C and DAPI was dripped onto them hourly.

Results

(1) Cross-pollination

1) Seed yield

Pistils of flowers cross-pollinated at day −6 to day +6 yielded mature seeds (Table 1); 6 mature seeds per capsule were obtained from flowers crossed on day −6; the yield per capsule increased gradually in the pistils toward day 0. The total number of mature and empty seeds per capsule was about 300. The highest number of mature seeds (236 seeds per capsule) was obtained from flowers crossed on day +2.

2) Seed germination

The most rapid germination was observed in seeds collected from flowers crossed on day 0, and the germination rate 40 days after sowing was 75%. Flowers cross-pollinated on day +4 developed more seeds per capsule than did those of day 0, but the germination rate was only 40% (Fig. 1).

(2) Self-pollination

1) Seed yield

Self-pollination was made between day −7 to day +8 (Table 2). Flowers pollinated at day 0 resulted in no capsules. About 48 to 100% of flowers selfed on day −3 to +6 developed capsules; the maximum number of mature seeds (46 seeds

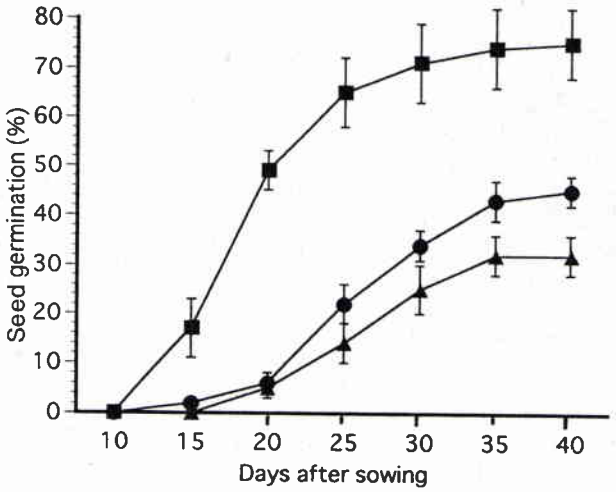

Fig. 1. Cumulative percentage germination of seeds yielded from cross-pollinated flowers of *L.* X 'Enchantment' × *L. maculatum* on day 0 (■), day +4 (●), and day +6 (▲). The vertical bars indicate standard errors.

Table 1. Number of capsules and seed set from flowers of *L.* X 'Enchantment' cross-pollinated at different floral stages.

Floral stages	No. pollinated pistils	capsules (%)	No. maturre seeds per capsule	No. empty seeds per capsule
Day − 7	3	0	0	0
Day − 6	6	67	6 ± 2	134 ± 31
Day − 5	5	80	21 ± 8	228 ± 17
Day − 4	7	100	52 ± 10	215 ± 10
Day − 3	5	100	137 ± 29	151 ± 22
Day − 2	10	90	175 ± 10	109 ± 7
Day − 1	11	100	180 ± 9	120 ± 10
Day 0	5	100	185 ± 14	137 ± 12
Day + 1	8	100	229 ± 11	80 ± 8
Day + 2	5	100	236 ± 10	71 ± 3
Day + 4	5	100	206 ± 11	102 ± 10
Day + 6	4	100	149 ± 8	188 ± 14
Day + 8	8	0	0	0

± indicates standard error.

Table 2. Number of capsules and seed set from flowers of *L.* X 'Enchantment' self-pollinated at different floral stages.

Floral stages	No. pollinated pistils	capsules (%)	No. maturre seeds per capsule	No. empty seeds per capsule
Day − 7	4	0	0	0
Day − 6	5	0	0	0
Day − 5	5	0	0	0
Day − 4	5	0	0	0
Day − 3	11	55	20 ± 6	145 ± 22
Day − 2	7	57	27 ± 7	190 ± 11
Day − 1	23	48	13 ± 9	157 ± 23
Day 0	5	0	0	0
Day + 1	6	100	15 ± 3	176 ± 15
Day + 2	5	100	15 ± 4	192 ± 11
Day + 4	5	100	19 ± 11	225 ± 11
Day + 6	6	100	46 ± 13	234 ± 15
Day + 8	8	0	0	0

± indicates standard error.

per capsule) was yielded from pistils selfed on day +6, but the capsules in each treatment had many empty seeds.

2) Seed germination

About 72% of the mature seeds obtained from the pistils selfed on day −2 germinated. However, in the pistils selfed on day +6, only 44% of the mature seeds germinated (Fig. 2).

(3) Comparison of pollen-tube growth in the styles self-pollinated at day 0 and day +3

1) Pollen germination on stigma

Almost all stigmatic papillae swelled well on day 0 (Fig. 3 A), whereas those of day +3 had wilted (Fig. 3 D). Pollen germination on the stigma on day 0 began late compared with that which germinated on the stigma on day +3; the pollen germination and the tube growth of day 0 flowers were inferior to the latter when observed 6 (Figs. 3 B, E) and 24 hr (Figs. 3 C, F) after pollination.

2) Tube growth in the style

Whether pollinated at anthesis or 3 days later, pollen tubes in the styles showed little difference in length when observed 24 hr after pollination. Thereafter the growth of tubes in the former styles was gradually delayed and almost stopped 48 hr after pollination (Fig. 4 A). Pollen tubes in the styles pollinated on day +3 continuously grew well and reached the base of the styles 48 hr after pollination (Fig. 4 B).

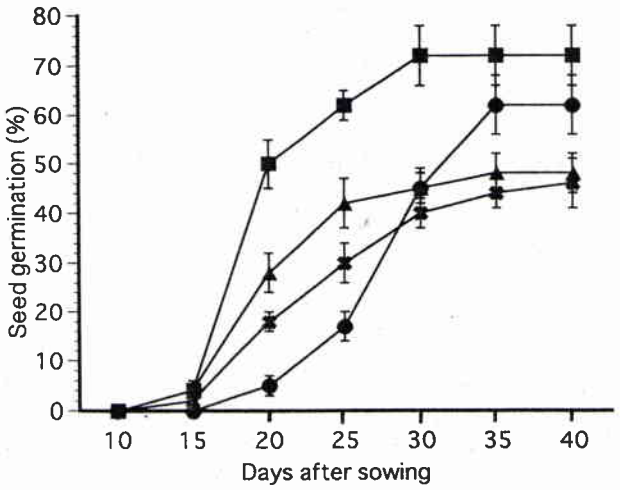

Fig. 2. Cumulative percentage germination of seeds yielded from flowers of *L*. X 'Enchantment' self-pollinated on day −2 (■), day +2 (●), day +4 (▲), and day +6 (×). The vertical bars indicate standard errors.

3) Abnormal pollen-tubes

In the pistils pollinated at anthesis, abnormal tubes with swollen or deformed bulbous tips were frequently observed in the styles at different times after pollination (Fig. 5). The number of abnormal tubes gradually increased with time, eventually reaching 44%, 72 hr after pollination, whereas only 15% of the pollen of day +3 had abnormal pollen tubes (Fig. 6).

4) Division of generative cell

Generative cell division was first observed 12 hr after pollination (Table 3). In pistils pollinated on day +3, all pollen tubes had two sperm cells and one vegetative cell ('2S+V') 36 hr after pollination, whereas in the pistils pollinated at anthesis, the generative cell division was delayed significantly so that only 10% had '2S+V' 36 hr after pollination; their number increased to 94% 144 hr after pollination.

Discussion

Receptivity

Seed yield in plants depends on pollen quality and female receptivity. Times favorable to pollination seem to be easily determined by the number of seeds obtained by pollinating female flowers at different times. Our results show that the female receptivity of the *L*. X 'Enchantment' began at a relatively early stages of pistil development and remained so for about 10 days. Additionally, the production of mature seeds in the flowers of day +1, day +2 and day +4 was found to be higher than that in the flowers of day 0 (Table 1). This indicates that the pistils of *L*. X 'Enchanment' reach optimum receptivity during a period following anthesis. Cross-pollination in *L. longiflorum* 'Ace' was effected from day −1 to day +9 (Ascher and Peloquin, 1966).

Leopold and Kriedemann (1975) cited that in some species the receptive condition of the ovary is indicated by the exudation of a viscous material on the stigma. Janson et al. (1994) reported that in *L. longiflorum*, the exudate covers the entire stig-

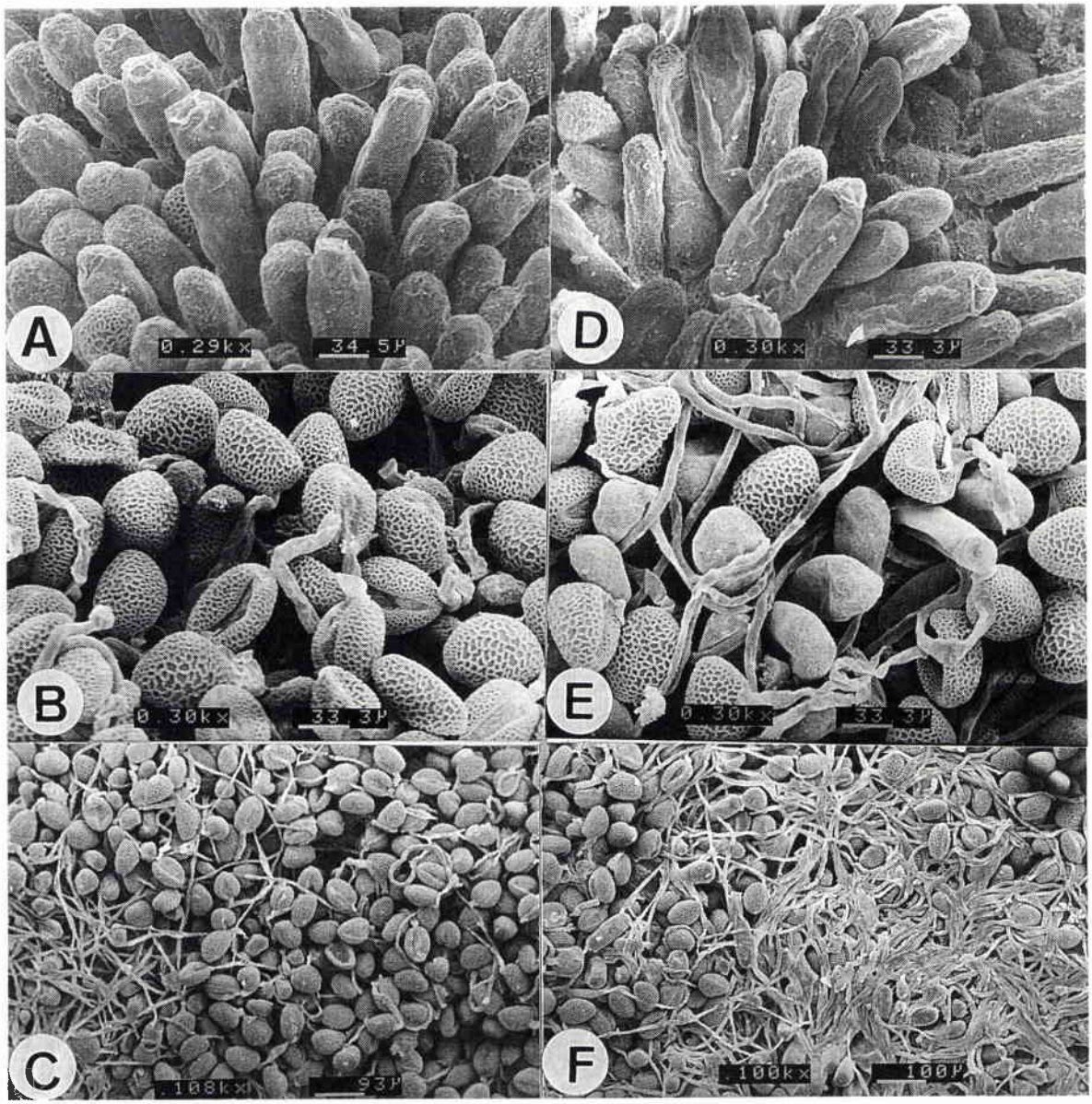

Fig. 3. Scanning electron micrographs of stigmas self-pollinated at the stages of day 0 (A, B, C) and day +3 (D, E, F). Observations were made 1 hr (A, D), 6 hr (B, E) and 24 hr (C, F) after self-pollination, respectively.

ma on day +2; Ichimura and Yamamoto (1992) found that the exudate continuously increases in the style until day +7. According to our macroscopic observations, exudates rarely appeared on the stigma of *L*. X 'Enchantment' prior to or at anthesis and the top of stigma began to be covered by the exudates from day +1 to day +2 (unpublished data).

Based on our results and those of Ascher and Peloquin (1966), Ichimura and Yamamoto (1992), and Janson et al. (1994), pollination of *Lilium* spp. at post-bloom stages is more favorable for the production of viable seeds than pollination at anthesis.

Self-incompatibility

Our results show that on pollination made on day 0, pollen germination and tube growth were retarded and that no viable seeds were obtained, compared with pollination made on day +3 (Fig. 3, Table 2). These findings agree with those of Cheng and Mattson (1972), van Tuyl et al. (1982), and Li and Niimi (1995). Furthermore, in self-incompatible plants *L. longiflorum* 'Georgia' and

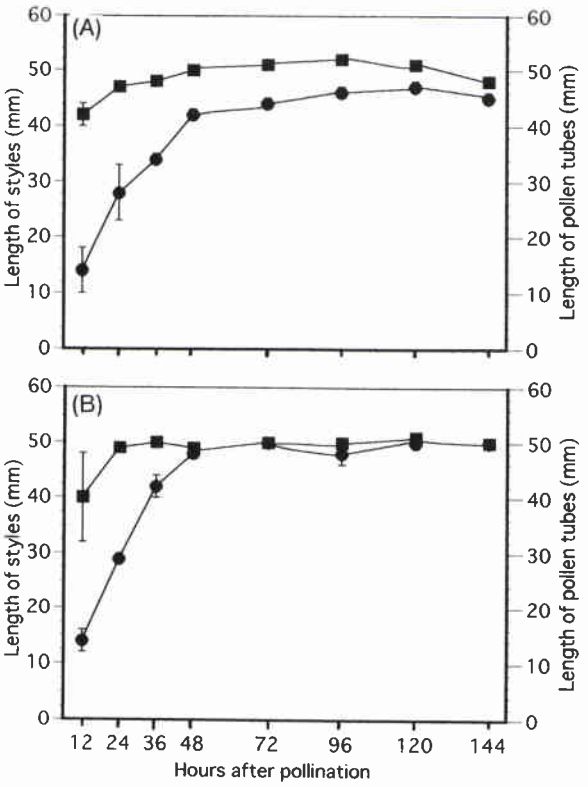

Fig. 4. Pollen tube growth in the styles of *L*. X 'Enchantment' flowers self-pollinated at anthesis (A) and on day + 3 (B). (■): style length. (●): pollen tube length. The vertical bars indicate standard errors.

'Hinomoto' (Li and Niimi, 1995) and *Petunia* spp. (Ünal, 1986; Yasuda, 1929), the percentage of abnormal pollen tubes was higher in self-pollinated pistils than in cross-pollinated ones. Finally, generative cell division was delayed in pistils pollinated at anthesis compared with in those on day + 3. Ünal (1986) observed that in the pollen tubes

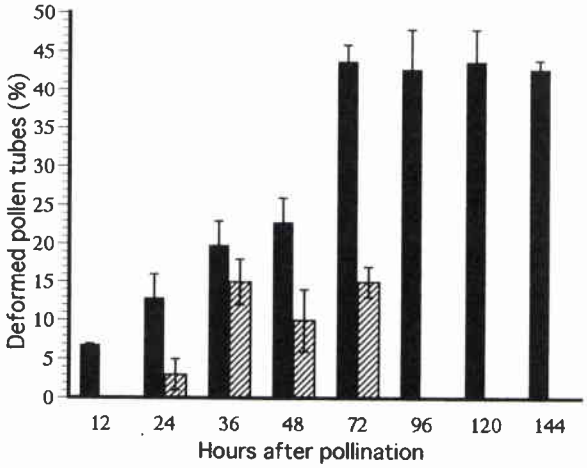

Fig. 6. Percentage of abnormal pollen tubes of *L*. X 'Enchantment' observed at different times after being self-pollinated at anthesis (■) and on day + 3 (▨). In the latter no tests were made on and after 96 hr. The vertical bars indicate standard errors.

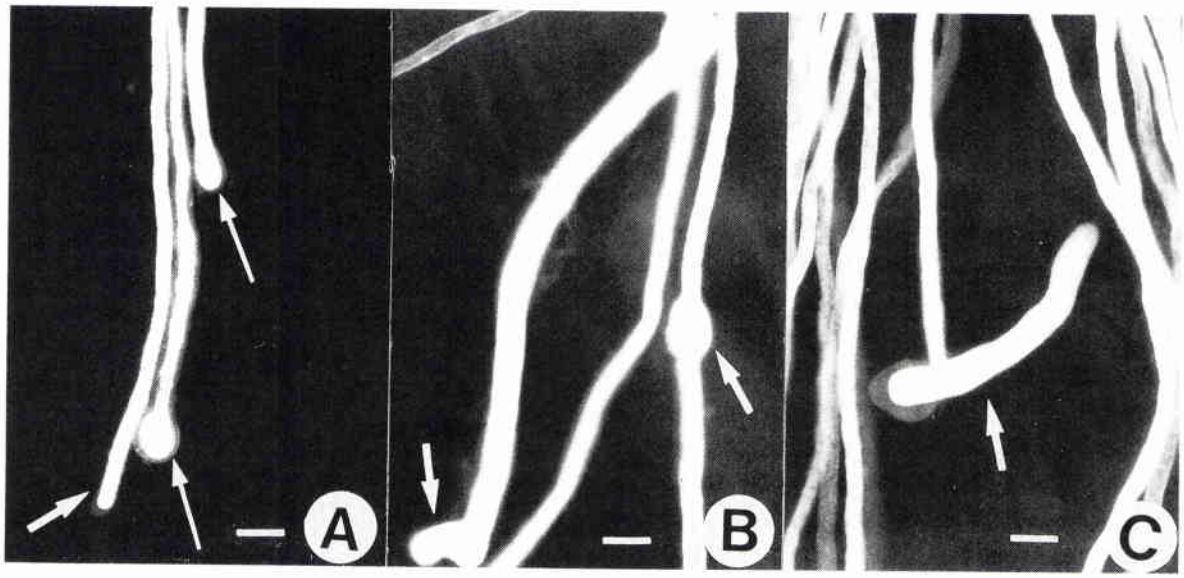

Fig. 5. Fluorescent photomicrographs of deformed pollen tubes in the styles of *L*. X 'Enchantment' flowers self-pollinated at anthesis. Observations were made at different time after pollination: (A) 24 hr, a normal pollen tube (left arrow) and two pollen tubes with a swollen tip; (B) 48 hr, pollen tubes with a protuberant tip (left arrow) or protuberance formed at a region away from a pollen tube tip (right arrow); and (C) 72 hr, a pollen tube with a fishhook-like tip. Bars represent 50 μm.

Table 3. Relative pollen tube length (RPTL) and pollen tubes having one generative cell and one vegetative cell (G + V) and 2 sperm cells and one vegetative cell (2 S + V) in the styles of *L.* X 'Enchantment' flowers self-pollinated at anthesis and on day + 3.

Hours after pollination	Styles pollinated at anthesis				Styles pollinated on day + 3			
	No. styles observed	RPTL (%)	Pollen tubes with		No. styles observed	RPTL (%)	Pollen tubes with	
			G + V (%)	2S + V (%)			G + V (%)	2S + V (%)
12	3	34 ± 9	93 ± 5	7 ± 5	3	36 ± 5	100	0
24	7	59 ± 1	94 ± 3	6 ± 3	6	59 ± 3	78 ± 10	22 ± 10
36	3	70 ± 3	90 ± 8	10 ± 8	3	82 ± 4	0	100
48	7	84 ± 2	23 ± 13	77 ± 13	4	98 ± 1	0	100
72	7	86 ± 3	13 ± 8	87 ± 8	5	98 ± 1	0	100
96	5	88 ± 2	14 ± 5	86 ± 6	5	96 ± 2	*	*
120	7	92 ± 1	7 ± 4	93 ± 4	5	99 ± 1	*	*
144	5	92 ± 2	6 ± 4	94 ± 4	7	100	*	*

RPTL : (pollen-tube length/entire style-length) × 100.
* : no tests were made.

of *P. hybrida*, mitosis occurred in incompatible tubes later than in compatible ones. Our observations give no clear evidence that some relationships exist between the delay of generative cell division in the pollen tubes and the self-incompatibility reaction. Lafleur and Mascarenhas (1978) showed that the RNA synthesis inhibitor, actinomycin D, prevents the division of generative cells in the *Tradescantia* pollen tubes. Therefore, in the pistils of *L.* X 'Enchantment' pollinated at anthesis, RNA and/or protein synthesis which are required for generative cell division may not progress smoothly.

We conclude from these findings that *L.* X 'Enchantment' is a weakly self-incompatible cultivar.

Seed viability

Differences in mature seeds among those from selfed and crossed flowers at different stages were quite evident. Seeds from pistils which had been self-pollinated on day − 2 or cross-pollinated on day 0 had the highest percent viability. The differences in seed viability among those pistils pollinated at different floral stages may be partly attributable to the selection of pollen tubes. Mulcahy and Mulcahy (1975) mentioned statistically significant differences in germination time and seedling weight when pollination was made either on the tip or the basal portion of the stigmatic surface in *Dianthus chinensis*, and they suggested that the quality of the F_1 generation can be modified by competition among pollen tubes. We postulated that when self- and cross-pollination are made a few days after anthesis or under conditions favorable for tube growth, pollen tubes bearing undesirable characteristics succeed in fertilizing the egg because competition among pollen tubes is relatively weak. Consequently, some ovules filled slightly were weak and rarely germinated. Further studies are needed to fully support our postulation.

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アジアテイックハイブリッド'エンチャントメント'の受容性と自家不和合性反応. 種子形成に及ぼす花齢の影響

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摘　要

本研究では，アジアテイックハイブリッド品種'エンチャントメント'の受容性と自家不和合性反応を開花7日前から開花8日後までの蕾または花に受粉して調査した．

'エンチャントメント'×イワユリ（*L. maculatum*）の交雑受粉の結果，開花6日前から開花6日後までの雌ずいは健全な有胚種子を形成し，開花前日，開花1，2，4日後に受粉した場合，完熟種子数は開花当日に受粉した場合より多く得られ，開花2日後の雌ずいは，さく果あたり236粒の種子を生じた．

'エンチャントメント'の自家受粉では，開花当日では種子は形成されず，花粉管は花柱長の約85%しか伸びなかった．そして開花3日後に受粉した場合と比較して，花粉管の先端が異常に肥大したり，奇形となった花粉管の割合が高く，また生殖核の分裂が遅れた．開花3日前から開花6日後までに受粉された雌ずいは，開花当日を除いてすべての処理区でさく果を形成し，開花6日後の雌ずいは46粒の完熟種子を，他のものは10数粒から20数粒の完熟種子を形成した．

自家受粉あるいは交雑受粉で得られた完熟種子を播種してそれらの発芽力を調べたところ，その発芽開始日および播種45日後の発芽率に違いがあった．交雑受粉では開花当日に受粉した雌ずいから得られた種子の発芽力がすぐれ，最終発芽率75%となった．一方，自家受粉では開花2日前の受粉によって得られた種子は72%の発芽を示し，種子が最も多く得られた開花6日後の雌ずいの種子の発芽率は44%であった．