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A Study on the Storage of *Lilium* Pollen

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Summary

Pollen grains of *Lilium* species, cultivars and hybrids were stored at 4°C and tested for their viability by in vitro pollen germination and in vivo seed formation. Gelatin capsules were superior to paraffin paper pockets as receptacles to retain the germination capacity of pollen grains of *L.* X 'Enchantment'; 60~65% relative humidities (RH) were found to be optimum for their storage. Pollen grains of 12 *Lilium* plants were enclosed in gelatin capsules and stored in desiccators with 60~65% RH at 4°C for periods of 9 to 12 months. Eight of them retained the germination capacity in vitro, but the rate of germination and the length of pollen tubes varied with *Lilium* species and cultivars. Stored pollen grains of *L. maculatum* and *L. auratum* formed as many seeds as freshly harvested pollen. Pollen grains of other *Lilium* plants germinated well in vitro, but they produced only a few seeds per pod. These results revealed that stored pollen grains may have the in vitro germination capacity, but only those from a few species and cultivars have the ability to produce seed.

Introduction

Long-term pollen storage is a basic need of plant breeders and horticulturists, particularly when crosses are required between selected parent plants that flower at different times. Vasil (1962) reported that −5° to 8°C and 20~50% relative humidities (RH) are suitable for long-term storage of most pollen grains. Pfeiffer (1938) recommended storage of *Lilium* species pollen either at −5° or 5°C under reduced pressure or at −10°C after the pollen grains are enclosed in a gelatin capsule or a paper pocket. Storage below freeze-drying temperatures (Nath and Anderson, 1975) or at low temperatures under low humidity (Saxena and Saini, 1979) have been reported to be successful in preserving *Lilium* species pollen. Based on these reports we attempted to lengthen the viability of pollen of *L. rubellum* and *L. maculatum* with an integrated method: The pollen grains enclosed in small paper pockets, were placed in capped vials with 30~40% relative humidity controlled by silica gel (SiO_2) and stored at −20°, −10° and 3°C; however the pollen viability deteriorated rapidly, 80 to 30% in 10 days, and was 0% in 30 days (unpublished data). Therefore, we initiated research to develop a simple and practical method suitable for preserving pollen of *Lilium* plants for at least one year. The viability was tested by in vitro pollen germination and in vivo pollination.

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Materials and Methods

Experiment I.

The effect of relative humidity (RH) in desiccators on the longevity of pollen grains of *Lilium* X 'Enchantment' was investigated on gathering anthers on the day of anthesis. Pollen grains were collected by sweeping the anthers with a soft brush. About 20 mg of the pollen grains were put in paraffin paper pockets and placed in desiccators with 30~40%, 60~65% and 90~95% RH at 4°C in a refrigerator, controlled by SiO_2, $NaNO_2$ and water, respectively.

Methods of collecting and storing pollen grains were also tested. Anthers of *L.* X 'Enchantment' were gathered at anthesis and the pollen grains were collected by two methods: (1) anthers were swept with a soft brush and (2) anthers were dipped and shaken in 5 ml of acetone in a 25 ml beaker for about 10 min. Pollen grains collected in acetone were filtered and dried at room tempera-

ture. About 20 mg of collected pollen grains were enclosed in a paper pocket or a lidded gelatin capsule with two small holes made with a needle. These receptacles were placed in desiccators with 30~40% or 60~65% RH at 4°C, controlled by SiO_2 and $NaNO_2$, respectively.

The respective containers of pollen grains were removed from the desiccators either weekly or every four weeks. The paper pockets and the opened gelatin capsules were left at the room temperature for 1 hr before culturing the pollen in vitro. The stored pollen grains were cultured on a liquid medium consisting of 10% sucrose and 100 mg·liter^{-1} boric acid, adjusted to pH 5.5~6.0. About 4 mg of the pollen was transferred to a 10 ml-Erlenmeyer flask containing 3 ml of the medium, which was then shaken on a reciprocating shaker (90 rpm) for 3 hr at 25°C. Pollen grains were considered to be germinated when their pollen tubes elongated at least twice the grain diameter. Percentage of pollen germination was determined by projecting pollen on video monitor through a CCTV camera (kp-140, Hitachi Denshi) attached to a light microscope. Pollen tube lengths were measured on the video monitor with the aid of a digital curve-meter (Uchida). The tests were run replicated 10 times with 50~100 pollen grains each.

Experiment II.

Pollen grains of 7 species, 4 cultivars and 1 hybrid (Table 1) were collected at anthesis and enclosed in: (1) a lidded gelatin capsule with two small holes, or (2) an open gelatin capsule plugged with absorbent cotton. Both types of capsules were stored in desiccators with 60~65% RH at 4°C. Between 9 to 12 months of storage, the stored pollen grains were cultured in vitro and assayed as described in *Experiment I.*

Experiment III.

The pollen grains used in *Experiment II* were transferred to stigmata in vivo to test if they could form seeds. These stored pollen grains were left in opened capsules for about 1 hr under the room temperature before in vivo pollination.

All flowers were emasculated one day before anthesis to avoid contamination and pollinated with either the stored pollen grains or with fresh ones. The number of pods and seeds containing embryo and endosperm was determined 3 months after pollination.

Results and Discussion

Experiment I. Influence of relative humidity in desiccators and kinds of receptacles on pollen viability

Pollen viability of *L.*X 'Enchantment' stored in paper pockets was influenced by relative humidities in desiccators. Pollen viability stored in 30% or 90% RH was reduced after 1 week, with no germination after 12 weeks (Fig. 1). However, germination of pollen grains stored in 60% RH, declined from 90% to 60% after 1 week and to 30% after 16 weeks. Excessive loss of water at 30% RH and the high humidity at 90% were not beneficial for the longevity of the pollen.

Pollen storage receptacle types affected the viability of pollen grains of *L.*X 'Enchantment' (Fig. 2). Pollen grains collected with a soft brush or by dipping them in acetone retained high viability for a long time provided they were stored in gelatin capsules. However, acetone-dipped pollen grains

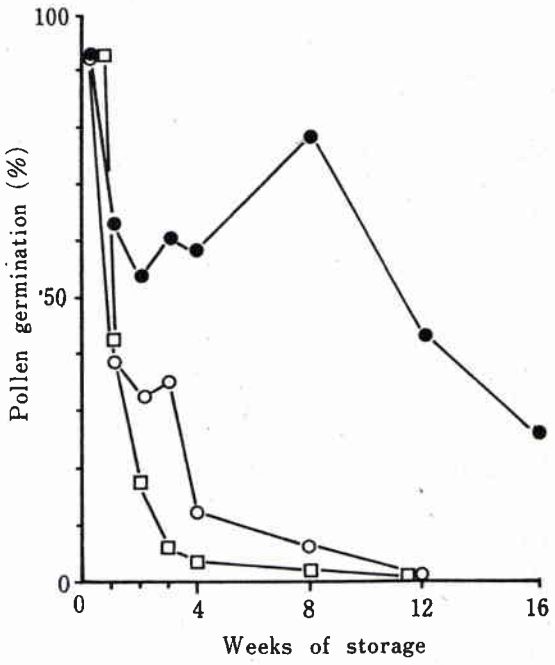

Fig. 1. Germination of pollen grains of *L.*X 'Enchantment' stored in desiccators with 30~40% (□), 60~65% (●) or 90~95% (○) RH at 4°C. Pollen grains were enclosed in paper pockets and then placed in each of desiccators.

Table 1. In vitro germination % and tube length of pollen stored in gelatin capsules and kept at 60% RH at 4°C.

Lilium species and cultivars	Time of pollen collection[z]	Pollen germination at anthesis (%)	Stored pollen		
			Duration (months)	Germination (%)	Tube length (μm)
L. auratum	Middle July	25	11	0	0
L. formosanum	Early Sep.	no test	10	39	no test
L. japonicum	Middle June	no test	12	0	0
L. maculatum	Early June	85	12	35	567±31
L. nobilissimum	Late July	46	10	0	0
L. rubellum	Late May	85	11	77	587±26
L. speciosum	Middle Aug.	61	9	63	659±26
*L.*X 'Casa Blanca'	Late July	86	11	32	449±26
*L.*X 'Enchantment'	Middle June	80	12	28	no test
*L.*X 'Le Reve'	Late June	49	12	0	0
*L.*X 'Star Gazar'	Middle July	67	12	16	562±39
L. hybrid[y]	Middle June	no test	12	7	197±23

[z] Each of early, middle and late means 1st to 10th, 11th to 20th and 21st to 30th (or 31st) of each month, respectively.
[y] *L. auratum* × *L. rubellum*

Table 2. Fertility of pollen stored under the conditions of 60% RH at 4°C for 0, 9, 11, 12 or 13 months.

Combination	Duration of pollen storage (months)	Number of flowers pollinated	Number of pods	Number of pods with seeds	Total number of seeds
L. maculatum × *L. maculatum*	0	5	5	5	1220
	12	5	5	5	885
L. rubellum × *L. rubellum*	0	5	5	5	295
	12	4	4	1	3
L. maculatum × *L.*X 'Enchantment'	11	4	4	0	0
L. rubellum × *L. auratum*	11	5	4	4	272
L. rubellum × *L.* hybrid	12	5	5	2	8
L. rubellum × *L. japonicum*	11	4	4	1	1
*L.*X 'Casa Blanca × *L. japonicum*	13	2	2	1	4
L. rubellum × *L. speciosum*	9	6	4	1	1

stored in paper pockets lost their viability after 1 week. It appeared that excessive drying in the paper pockets may have occurred during the storage as lipids on the pollen surface were removed with acetone. Pfeiffer (1938) also reported that pollen grains of *L. auratum* wrapped in paraffin paper gave less satisfactory result, when viability was compared to pollen stored in gelatin capsules. We concluded that gelatin capsules were superior to paper pockets for pollen storage because excessive drying of pollen during the storage were prevented.

Experiment II. In vitro germination capacity of Lilium pollen after long-term storage

Pollen from *L. auratum, L. japonicum, L. nobilissimum* and *L.*X 'Le Reve' failed to germinate in vitro 11, 12, 10 and 12 months of storage, respectively. Pollen from the other *Lilium* plants germinated after a year in storage. The germination rate ranged from 7% in *L.* hybrid (*L. auratum* × *L. rubellum*) to 77% in *L. rubellum*. The pollen tube length also varied from 197 to 659 μm (Table 1).

We cannot fully explain the loss of pollen viability in the four *Lilium* plants, *L. auratum, L. japonicum, L. nobilissimum* and *L.*X 'Le Reve'. The poor viability may be partly due to the inherent properties of pollen. The other speculation is that the pollen grains were collected during the hot rainy season in Japan when temperature and humidity were high. Their germination rates were less than 50% at the time of the start of storage. Thus, they

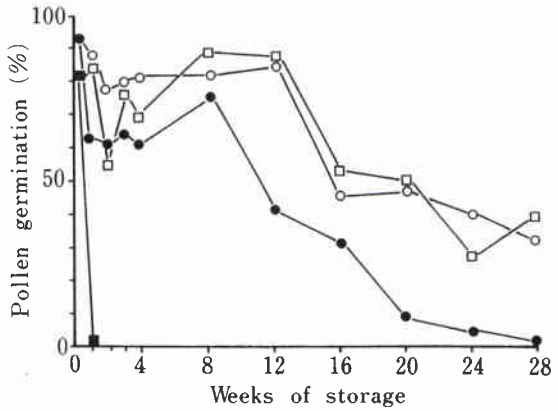

Fig. 2. Germination of pollen grains of *L.*X 'Enchantment' collected by a soft brush or dipping the anthers in acetone. Pollen grains were stored in desiccators with 60~65% RH at 4°C after enclosing them in paper pockets or gelatin capsules. ● a brush and a paper pocket; ○ a brush and a gelatin capsule; ■ acetone and a paper pocket; □ acetone and a gelatin capsule.

might have lost their germination capacity by the end of the storage. Stanley and Linskens (1974) have also reported that pollen grains in general are poorly protected against high temperature and humidity conditions. Therefore, it seems to be most desirable that pollen grains for breeding of *Lilium* plants are collected from flowers growing when moderate temperatures and low humidities are prevailing.

Experiment III. The ability of stored pollen to form seeds

In vivo pollination was performed to test the pollen fertility of 8 *Lilium* plants with the same pollen as shown in Table 1 (Table 2).

The stored pollen grains of *L. maculatum* had similar fertility levels as the fresh ones. Many ma-

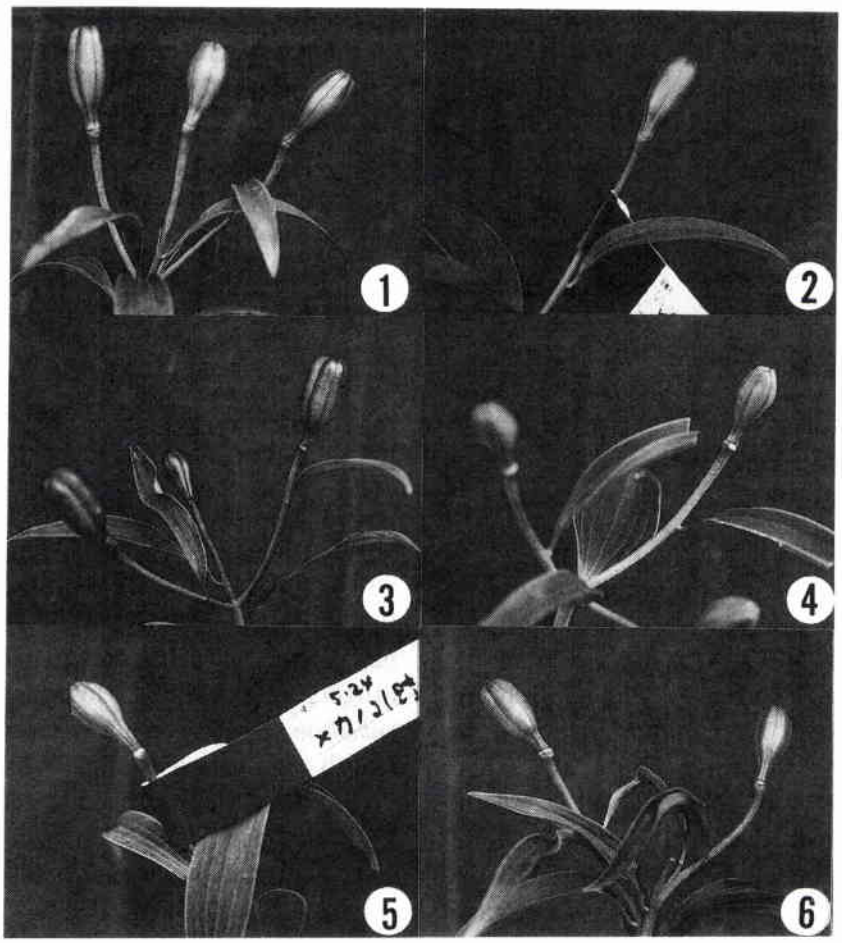

Fig. 3. Growth of ovaries of *L. rubellum* pollinated with fresh pollen grains as control (1) or with stored pollen grains of *L. rubellum* (2), *L. auratum* (3), *L. japonicum* (4), *L. speciosum* (5) and *L.*x 'Le Reve' (6). The same stored pollen grains as shown in Table 1 were used.

ture seeds were obtained from the *L. rubellum* × *L. auratum* cross although the stored pollen grains of *L. auratum* seldom germinated in vitro (Table 1). This disagreement between data cannot be explained. When other *Lilium* plants were pollinated with the same lot of pollen grains, nearly all ovaries enlarged (Fig. 3), but a few mature seeds formed in each combination. No seeds developed in the combination of *L. maculatum* × *L.*X 'Enchantment' (Table 2), indicating that even if stored pollen grains germinate in vitro, they do not necessarily have the ability to form seed. Similarly, Stanley (1962) reported that pine pollen grains, germinated in vitro, were unable to form normal pollen tubes in vivo, because they fail to penetrate the female tissue, or to form normal zygote.

Stored pollen grain behavior after pollination was not analyzed in detail. Thus, the causes of in vitro germination or pollination failures are still obscure. Additional experiments are under way.

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ユリ花粉の貯蔵と受精能力

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摘　要

４℃で数か月から１年にわたって貯蔵したユリの花粉の発芽能力および種子形成能力を人工培地での発芽試験および植物体上での交配実験により調べた．毛筆を用いてやくから集めたユリ'エンチャントメント'の花粉をパラフィン紙に包み，４℃，相対湿度30～40%，60～65%および90～95%に調節したデシケータに貯蔵すると，60～65%で貯蔵した花粉が発芽力を長く維持した．

一方，やくをアセトンに浸漬して集めた花粉はパラフィン紙に入れて貯蔵すると発芽力は貯蔵１週間後には無くなり，ゼラチンカプセルに入れて貯蔵すれば花粉の発芽能力は長期間維持された．これらの結果に基づきゼラチンカプセルに入れて，４℃，相対湿度60～65%で９～12か月貯蔵した12種類のユリ花粉を人工培地で培養したところ，４種類はまったく発芽せず，８種類は発芽したがその発芽率および花粉管長は品種間で大きな差がみられた．また，これらの貯蔵花粉の種子形成能力を交配実験によって調べたところ，イワユリおよびヤマユリは新鮮花粉とほぼ同程度の種子形成能力を持っていたが，人工培地で比較的よく発芽したヒメサユリや他のユリの花粉はほとんど種子を形成せず，人工培地での発芽能力と植物体上での種子形成能力は必ずしも一致しないことが明らかになった．