The Relationship of Storage and Viability of Lily Pollen

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Abstract

The study was conducted to estimate the pollen viability of eight lily genotypes and optimum storage conditions. Pollen viability was assessed by in vitro germination, FCR (Fluorochromatic raction) test and fruit set test. Pollen grains were stored in the dark chamber where the temperature was maintained at 4 °C, -20 °C, and –70 °C and ambient room temperature (control) for one year. The highest percentage of germination in *L. henryi*, *L. lancifolium*, *L.* 'Casa Blanca', *L. formolongi* 'F1 August' and *L.* 'Raizan' was observed when pollen grains were stored less than –20 °C from FCR test and in vitro germination test. On the other hand, the lowest percentage was found in pollen of *L.* 'Connecticut King', *L.* 'Barbaresco', and *L.* 'Kissproof '. These cultivars had shown less than 10 percent germination under four storage conditions. The best storage temperature should be lower than –20 °C. Pollen grains of *L.* 'Raizan', *L.* 'Casa Blanca', and *L. lancifolium* still had sufficient capacity for fruit set after cross-pollinations after 1-year storage (except for room temperature). Highly significant correlations were found among the FCR test, in vitro germination, and fruit set test.

INTRODUCTION

Pollen storage is a valuable tool in a breeding program because it makes recurrent and breeding lines available on an as-needed basis regardless of flowering response and planting date. Rapid exchange and use of germplasm between scientists nationally and internationally can be facilitated by pollen storage (Hanna, 1990). When the production of intervarietal and interspecific hybrids is difficult due to asynchronous flowering, the use of stored viable pollen can facilitate crossing. Furthermore, the maintenance of pollen viability during long-term storage is important to establish a pollen gene bank (Zebrowska, 1995).

One essential requirement in the study of pollen storage is to have a convenient method for pollen viability test with a reasonable level of accuracy. Many breeders had investigated the pollen viability of other species. Long-term storage at low temperatures had successfully retained pollen viability in *Pistacia* (Polito and Luza, 1988), *Pennisetum* (Bezdickova, 1989), *Litchi* (Carstens, 1989), *Capsicum* (Shi and Tian, 1989), *Fragaria* and *Potentilla* (MacFarlane Smith et al., 1989), *Secale* (Hanna, 1990), and *Citrus* (Weng et al., 1990).

Pollen viability is generally considered as an indication of the ability of pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination. Assessment of pollen viability on the basis of its function is cumbersome, time-consuming, and not always feasible (Heslop-Harrison et al., 1984). Many short-cut methods that reflect the competence of the pollen to perform its normal function in the pistil had been devised. Of these, the in vitro germination test and the fluorochromatic reaction (FCR) test had been found to be satisfactory. However, in vitro germination test could not be applied to many systems, particularly three-celled systems in which optimal in vitro germination was difficult to achieve. The FCR test worked with

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both two-celled and three-celled systems, and now being used routinely to assess pollen viability (Shivanna et al., 1991).

Cultivars derived from *L. formolongi* were propagated by seed and were subjected to daylength. These cultivars did not match the flowering time of Asiatic and Oriental hybrids. Therefore, to breed interspecific hybrids by using *L. formolongi*, the use of stored viable pollen is a prerequisite. In this paper, the research was conducted to search for adequate storage condition and to sustain the viability of the pollen stored before pollination in lily.

MATERIALS AND METHODS

Pollen Materials and Storage

Lilium lancifolium, L. 'Connecticut King' (Asiatic Group), *L.* 'Casa Blanca' (Oriental Group), *L.* 'Barbaresco' and *L.* 'Kissproof', *L. henryi, L. formolongi* 'Raizan' and 'F1 August' were used to study lily pollen storage and viability.

Lily plants grown under the greenhouse were used as pollen source in this study. Temperatures varied from 20-25 °C at night to 35- 45 °C in daytime. Pollen grains were collected from freshly dehisced anthers and prepared on the same day. Pollen grains without anther wall were put in 1.5 mL effendorf tube. These tubes were preserved in bottles with dry silica gel and stored at –70 °C, -2 °C, 4 °C, and room temperature for 1 year. Then, the quality of stored pollen was compared with fresh pollen by using three methods, i.e., fluorescein diacetate (FDA) test, in vitro germination test on a boric acid medium, and fruit set test.

Fluorescein Diacetate (FDA) Test

Pollen viability was assessed on basis of the fluorochromatic reaction with fluorescein diacetate (FDA; 2 $ug \cdot mL^{-1}$) (Heslop-Harrison et al., 1984). Viable pollen is fluorescent after excitation with UV microscope. At least 200 pollen grains were counted in each determination.

In Vitro Germination Test

Pollen germination test was performed in a medium containing 100 g sucrose, 5 g agar, 20 mg boric acid per liter. Pollen grains were spreaded on the surface of the medium in a petri dish. The dish was incubated at 25 °C. Percentage of germinated pollen grains was observed 12 hours after incubation under a light microscope. Each determination was made by randomly counting the germinated pollen grains at three locations in a petri dish. At least 200 pollen grains were counted for each sample.

Fruit Set Test

The 1-year old pollen grains and fresh pollen grains were used in stigmatic pollination in order to assess their capacity for seed setting. Pollinations were conducted under the greenhouse conditions. Eighty days after pollination, matured seeds were harvested and the number of seeds per pod was compared. The experiments were completely randomized (especially FDA test and in vitro germination), with at least three replications for each treatment. Simple linear regressions were generated among the fluorescing percentages, in vitro germination percentages of the pollens, and fruit-setting percentages.

RESULTS AND DISCUSSION

The viability of the pollen stored for 1 year was investigated by FDA, in vitro germination, and in vivo assay (Figs. 1, 2, 3, 4, and Table 1). Under the UV microscope, the color of viable pollen turned to yellow while non-viable pollen did not (Fig. 1). The reason that the color of pollen turned to yellow is that fluorescein accumulates. Pollen grains that lack enzyme activity and intact plasmalemma did not show FCR (Heslop-Harrison, 1970). The color change of pollen during storage was monitored to determine the pollen viability. Once the viability of pollen was diminished, the color of pollen

changed to dark gray color. This may be a criterion, which can distinguish the viability of pollen.

The percentage of pollen viability diminished as storage temperature increased in most of species and cultivars by FDA test and in vitro germination. Fresh pollen viability for the tested taxa ranged from 95% to 42% by FDA and from 90% to 50% by in vitro germination test. When pollen was stored at lower than –20 °C, the percentage of pollen viability in the majority of cultivars and species maintained more than 50%. However, the percentage of pollen viability in three cultivars (*L.* 'Connecticut King', *L.* 'Barbaresco', and *L.* 'Kissproof') was drastically reduced to less than 10% in FDA and 0% in germination test. Unlike these cultivars, the germination percentage of *L.* 'Casa Blanca' showed 75% in fresh pollen, 65% in pollen stored at –70 °C, 60% at –20 °C, 50% at 4 °C, and 0% at room temperature (Figs. 2 and 3). These results were also supported by Yoshiji and Shiokawa (1992).

Pollen of Oriental group, *L. auratum, L. japonicum, L. nobilissimum*, and *L.* 'Le Reve', failed to germinate after 1-year storage at 4 °C. However, pollen grains of other Oriental lily, *L. speciosum*, *L.* 'Casa Blanca', and *L.* 'Star Gazer' germinated 63%, 32%, and 16%, respectively. These results could not be explained with the limited data. It is possible that the poor viability might be partly due to the inherent properties of pollen. Pollen viability of all lily taxa stored at room temperature was not maintained. Pollen viability by FDA was significantly ($p < 0.05$) correlated with the percentage of germination and seed maturity (Fig. 4). The capacity for fruit set after stigmatic pollination with pollen grains stored for 1 year and fresh pollen were presented in Table 1. These results suggested that *L.* 'Casa Blanca' and *L.* 'Raizan' were suitable for fruit set when they were stored at lower than 4 °C, *L. lancifolium* at lower than –20 °C, and *L.* 'Connecticut King' at –70 °C.

In conclusion, these results indicated that the FDA (fluoroscein diacetate) test was a convenient and reliable evaluation method of stored pollen viability. Also, we can estimate the viability deterioration by color change in lily pollen. Since pollen viability of Oriental hybrids was very low for stored pollen, fresh pollen should be used in lily breeding program. For the majority of lily taxa, the pollen could be stored in freezer at –20 °C or lower temperature.

ACKNOWLEDGEMENTS

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Tables

Table 1. Effects of pollen storage temperatures on seed production of lilies.

Geno-type	Cross combinations	Condi-tions[z]	No. of flowers pollinated	No. of seed-sets matured	Avg. no. of seeds per pod
A×A	Gisl × *L. lancifolum*	Fresh[y]	3	3	119.0
		-70°C	3	3	78.7
		-20°C	3	3	36.7
		4°C	3	0	-
		RT[x]	3	0	-
O×O	Alliance × Casa Blanca	Fresh	3	3	33.7
		-70°C	3	3	17.0
		-20°C	3	3	16.7
		4°C	3	3	9.0
		RT	3	0	-
F×F	Raizan × Raizan	Fresh	2	2	410.0
		-70°C	2	2	423.5
		-20°C	2	2	232.0
		4°C	2	2	188.5
		RT	2	0	-

[z] Stored for 1 year except fresh pollen.
[y] Fresh pollen just harvested without storage.
[x] Room temperature.

Figures

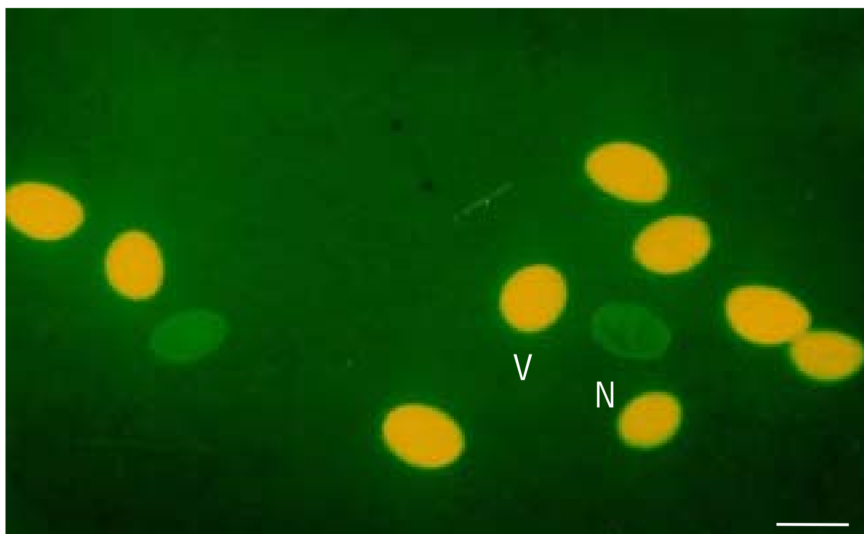

Fig. 1. Lily pollen viability observed in 20% sucrose solution containing 0,002% FDA under fluorescence microscope. N: Non viable pollen grains, V: Viable pollen grains. Bar = 50 µm.

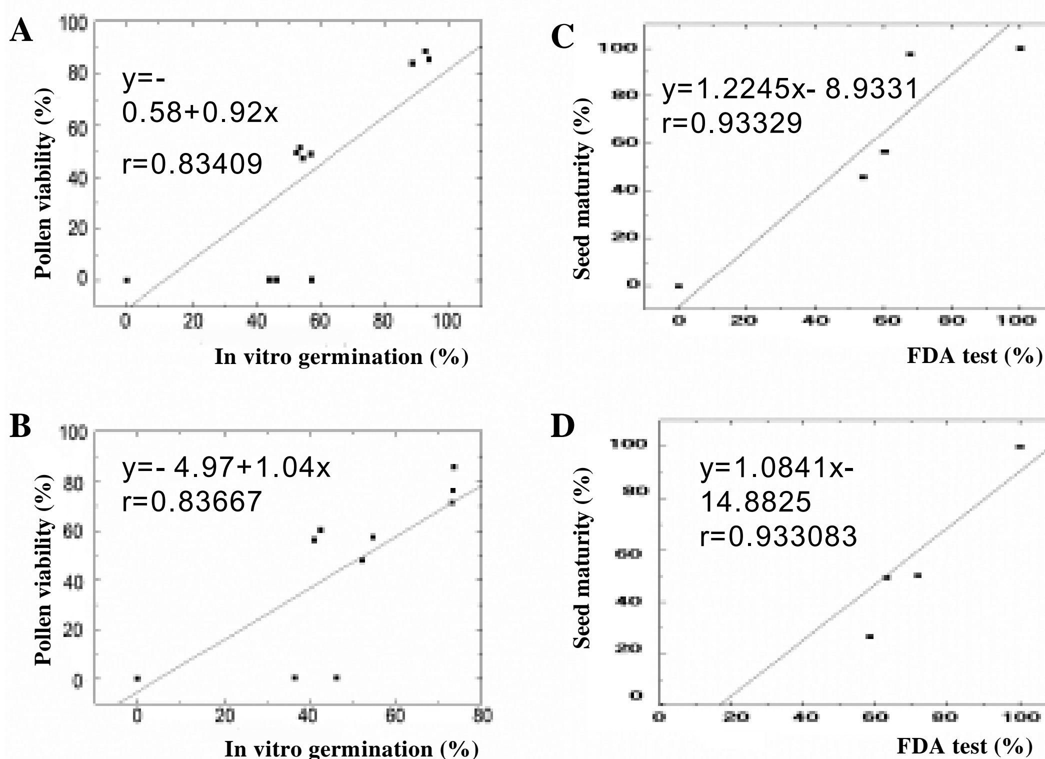

Fig. 4. Relationships among pollen viability observed by FDA test, seed maturity and in vitro germinaton rate. (A, C) *L. formolongi* 'Raizan' (B, D) *L.* Oriental 'Casa Blanca'.

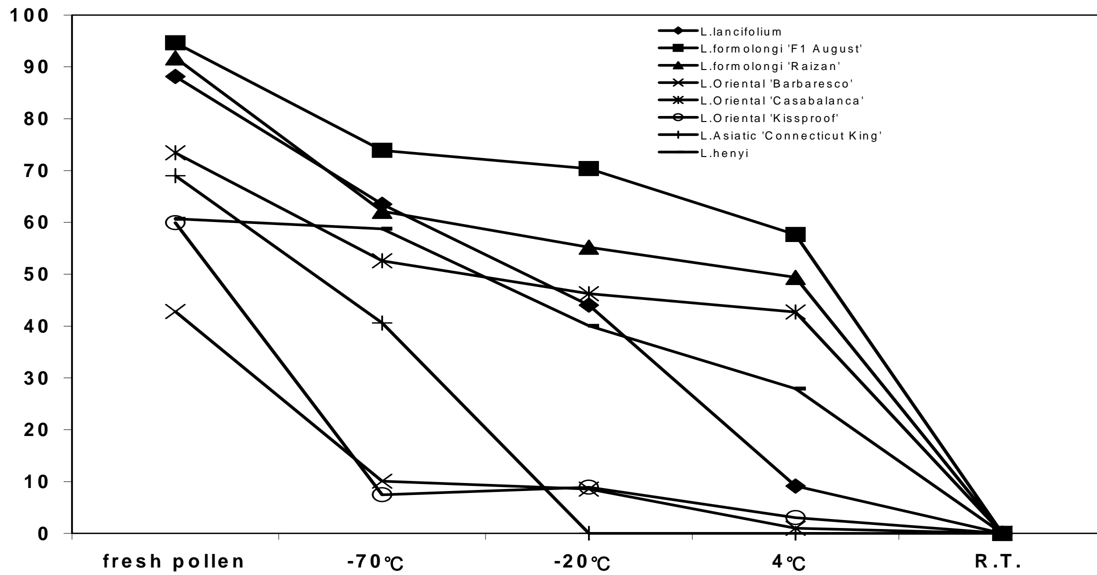

Fig. 2. Pollen viability in different lily species and cultivars affected by storage temperatures for 1 year. (A) *L.* Asiatic hybrid and *L. lancifolium*.(B*) L. henryi* and *L.* Oriental hybrids (C) *L. formolongi* hybrids.

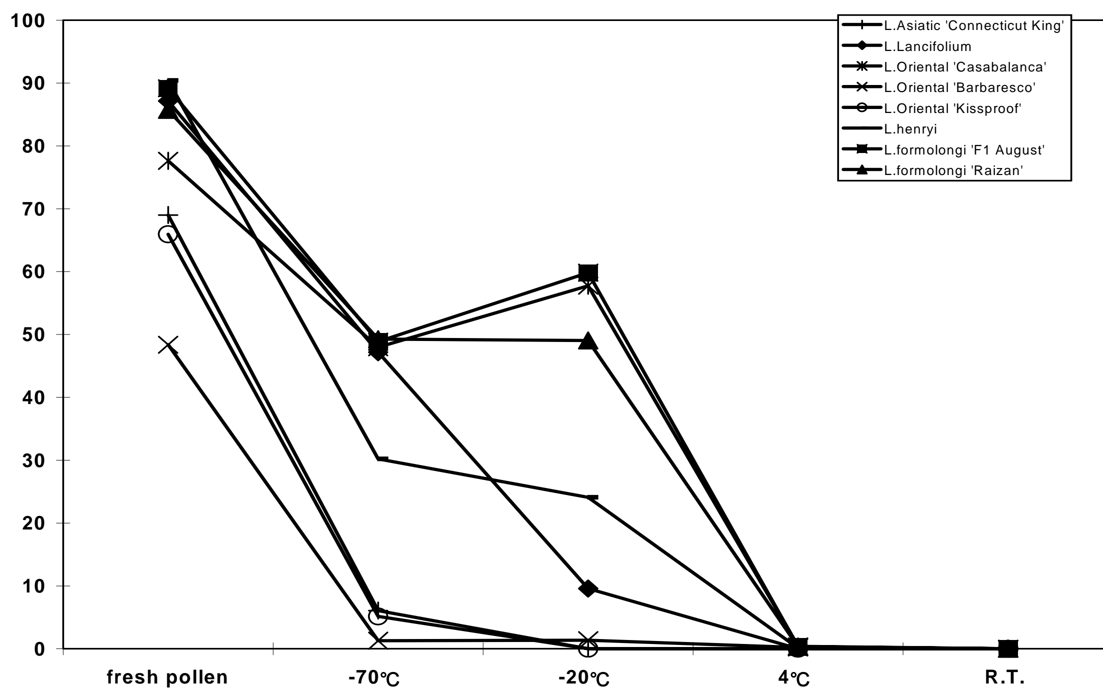

Fig. 3. Pollen germination rate in different lily species and cultivars affected by storage temperatures for 1 year. (A) *L.* Asiatic hybrid and *L. lancifolium*. (B) *L. henryi* and *L.* Oriental hybrids (C) *L. formolongi* hybrids.