Improvement of Breeding Efficiency for Interspecific Hybridization of Lilies in Korea

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Abstract

Interspecific hybridization of lily in Korea is focused on increasing breeding efficiency especially under Korean climate conditions. Cultivars derived from *L. formolongi* were propagated by seed and were sensitive to day length. These cultivars differ from flowering time with the Asiatic and Oriental hybrids. Therefore, to breed interspecific hybrids by using *L. formolongi*, the use of stored viable pollen is a prerequisite.

Lily pollen was stored at four different temperature conditions for one year and pollen viability was investigated using three different methods. As storage temperatures increased, pollen viability was drastically reduced. Viability of lily pollen stored was maintained effectively at temperatures lower than -20°C. FDA test was the most effective and convenient method to ascertain pollen viability.

To overcome pre-fertilization barriers in interspecific hybridization of lilies, pollen tube growth in the stigma or style, and the penetration of pollen tubes into the ovules were observed. OA (Oriental Asiatic), OL (Oriental Longiflorum), FA (Formolongi Asiatic), and FO (Formolongi Oriental) hybrids were obtained through embryo rescue and stigmatic and CSM pollination, while AO (Asiatic Oriental), AL (Asiatic Longiflorum), LA (Longiflorum Asiatic) and LO (Longiflorum Oriental) hybrids were not obtained through these methods.

Backcrosses were used in order to reduce scattered petals in interspecific hybrids. It was possible to obtain BC_{107} hybrids between FA (Fomolongi Asiatic) and Asiatic hybrids as male parent. But, it was difficult to obtain BC_1 hybrids between FA hybrid and *L. formolongi* as female parent. Normal pollination was better than CSM (Cut style pollination method) to get BC_1 hybrids.

In order to overcome the sterility of interspecific hybrids pollen, low concentration of oryzalin was more effective than high concentration of colchicine to obtain tetraploid. The fertility of pollen increased by about 40% in tetraploid level.

***Fusarium oxysporum* screening test was carried out. 'Hae-wool', 'Sinavro' and FA 96-16 were classified as highly disease resistant and 'Supia' and 'Doran' as moderately resistant to *Fusarium* bulb-rot. The FO-hybrid lilies from NHRI were susceptible.**

INTRODUCTION

Lily is one of the most economically important flower bulb crops in Korea. Korea exported lily cut flowers to Japan worth about 10 million dollars and we imported bulbs from the Netherlands worth about 4 million dollars in 2003.

There are more than 10 native lily species in Korea. Commonly cultivated lilies include the Asiatic, Oriental, and *Longiflorum* hybrids. Asiatic hybrids have no fragrance but have diverse flower color. Oriental hybrids are susceptible to virus and *Fusarium* disease but have strong fragrance and they take a very long time to produce bulb. Recently, the cultivation of *L. formolongi* hybrids, such as 'Raizan', 'F_1 Augusta' has increased. These hybrids are F_1 progeny obtained by crossing *L. longiflorum* and *L. formosanum*.

The advantages of *L. formolongi* hybrids are seed propagation, shorter generation, upward flowering type, convenience of packing and transport of cut flower, and long stem. Since the early 1990s interspecific hybridization of lily has been carried out in NHRI of Korea (Rhee, 2002).

In lily breeding, interspecific hybridization and polyploidization have greatly contributed to the breeding of commercial LA hybrids, which are becoming more important in the market (Van Tuyl et al., 2003).

Interspecific hybridization of lily in Korea has focused on increasing breeding efficiency by using *L. formolongi* as female parent especially in Korean climate conditions. To enhance breeding efficiency, several experiments were conducted in this research, the objectives of which included the following: 1) To search for adequate storage condition and to sustain the viability of the pollen stored; 2) To identify pre-fertilization barriers in incongruent crosses; 3) To improve the percentage of interspecific hybrids obtained, through in vitro culture such as embryo rescue in incongruent crosses; 4) To overcome the sterility of F_1 hybrid obtained from interspecific hybridization by in vitro chromosome doubling; and 5) To screen disease such as *Fusarium oxysporum.*

MATERIALS AND METHODS

Plant Material

Lily plants were cultivated in greenhouse and maintained at an average night and day temperature of 20 ± 5°C and 40 ± 5°C respectively. Normal culture practices for pest control, watering, ventilation, and fertilization were conducted.

Fluorescein Diacetate (FDA)

Pollen viability was evaluated using fluorochromatic reaction with FDA (0.002% with 20% sucrose) by fluorescence microscopy.

In Vitro Pollen Germination

Pollen germination test was performed in a medium containing 100 g sucrose, 5 g agar, and 20 mg boric acid per 1 L by light microscopy.

Pollen Tube Growth in Style

Pollen tube growth in stigma and style was observed using aniline blue staining and UV-fluorescence. Fixing was conducted in a solution of ethanol 3:glacial acetic acid 1 (V/V) in 4°C refrigerator for 24 hours. Softening was done in the solutions of 2N NaOH for 4 hours in Asiatic hybrids as female parents, 3N NaOH for 4 hours in Oriental hybrids, and 2N NaOH for 6 hours in Formolongi hybrids. Samples were stained with 0.1% aniline blue (W/V) for 24 hours and observed using UV-microscope.

Penetration of the Pollen Tubes to the Ovule

To determine the penetration of pollen tube in the micropyle, ovules which are 14 days after pollination were cleared in a mixture of lactic acid, glycerol and water (1:2:1) at 80°C for 1 hour, stained for 2 minutes in this solution supplemented with 1% aniline blue at the same temperature and destained again in the clearing solution at 100°C (modification of Gerlach, 1977; Janson et al., 1994). The number of ovules penetrated by a pollen tube was observed using a bright field microscope.

In Vitro Chromosome Doubling and Ploidy Analyzer

The media were composed of MS (Murashige and Skoog, 1962), supplemented with 6% (w/v) sucrose, and adjusted to pH 5.8. Colchicine and oryzalin were treated for 3 hours, with 0.1-0.001% concentration in vitro lily scales. Ploidy level was detected with the ploidy analyzer-II (Partec Co. Munster).

Screening for *Fusarium oxysporum*

Fusarium oxysporum f. sp. *lilii* (CPRO-Fol 4 and CPRO-Fol 11) were used (Straathof and Loffler 1994a.b). For soil infestation, the fungi were incubated for two weeks at 23°C in an autoclaved (120°C, 2h) 1 oatmeal: 4 soil mixture (w/w). Fully-grown cultures were ground and mixed in a 1:100 ratio with non-sterilized potting soil. The disease severity was observed visually using a disease rating according to the following ordinal scale: 1 = 'healthy', 2 = slightly rotten', 3 = 'moderately rotten', 4 = 'heavily rotten', 5 = 'very heavily rotten' and 6 = 'completely decayed'.

RESULTS AND DISCUSSIONS

Pollen Storage and Viability

Lily pollen was stored at four different temperature conditions for one year and pollen viability was investigated using three different methods. As storage temperature was high, pollen viability was drastically reduced. Viability of lily pollen stored was maintained effectively at temperatures lower than -20°C. Pollen viability by FDA was significantly ($p<0.05$) correlated with the percentage of in vitro germination and seed maturity. FDA test was the most effective convenient method to investigate pollen viability. Also, we can estimate the viability deterioration by color change in lily pollen. As pollen viability of stored Oriental hybrids was very low, fresh pollen must be used instead when Oriental hybrids are used as male parent in lily breeding program.

Pollen Tube Growth and Penetration

In intraspecific crosses, most pollen tubes reached the end of the style 2-3 days after pollination. Meanwhile, in interspecific crosses such as A x L and L x O hybrids, numerous pollen grains germinated in stigma, many pollen tubes grew along the stylar canal, and stopped growing at the upper part of the style. In other interspecific crosses such as O x L and A x O hybrids, few pollen tubes reached the end of the style. Also, in interspecific crosses such as F x A, F x O, and O x A hybrids, some pollen tubes reached the end of style. Crossing barriers in style of interspecific crosses include, the turning of many pollen tubes, the swelling of tube-tip, the abnormal growth and the coiling of tubes, and vertical direction (Fig. 1).

In intraspecific crosses (e.g. F x F, O x O, and A x A hybrids, the percentage of pollen tube penetration into ovules was more than 60% using stigmatic pollination. In contrast, it was less than 5% in the cross of O x A and O x L hybrids and less than 7% in F x A and F x O hybrids. However, pollen tube penetration into the ovule was not observed in interspecific crosses of A x L and A x O hybrids, because ovaries have shrunken already at sample collection time (Fig. 2).

Embryo Rescue for Preventing Post-fertilization Barriers

Interspecific embryos between different lily sections were rescued under light microscope. Ovaries used *Formolongi* cultivars as female parent. These were collected starting from less than 35 days after pollination. For ovaries that used Oriental hybrids as female parent, these were collected from 60 days after pollination. In interspecific crosses such as O x A, O x L, F x A, and F x O, hybrid embryos could be obtained by using embryo, ovule, and embryo-sac culture technique. According to cultivars, pollination methods, and pollen status, the number of ovules and embryos obtained from interspecific crosses is considerably different. In some interspecific crosses combinations such as A x O, A x L, L x O, and L x A, no embryo could be obtained because all ovaries died within 2 weeks after stigmatic and CSM pollination.

Backcross

To supplement characteristics of malformed F_1 hybrids, backcross was conducted using male and female parents. The number of FAA (BC_1) embryos obtained in cross combination of FA96-29 (F_1 progeny of F x A) x A95-14 (Asiatic hybrid), was 124 and 32

ovules by stigmatic and CSM pollination, respectively. In case of FA96-24 x A95-14, 10 ovules were obtained at only CSM pollination. The number of FAF (BC_1) hybrids obtained in crossing between FA96-4 (F_1) and F_1 'Augusta', Formolongi hybrid, were 1 and 13 ovules by stigmatic and CSM pollination. In the case of backcrosses with FO97-9 (F_1 progeny of F x O) x F_1 Augusta (F) and FA96-24 (F_1) x F_1 Augusta (F), no ovule was obtained by both pollination methods. Results of backcrosses can be divided into two groups according to the pollen grains used. In one group pollen grains were obtained from the female parent, and in the other group, from the male parent, in the crosses made for obtaining F_1 hybrids. Obviously, if interspecific hybrids are backcrossed with the parent's pollen, the crosses are more successful when the male parent was used again as a pollen source.

Characteristics of FA (F x A) Hybrids

FA progenies, FA97-9, FA96-10 (Fig. 4C 'Supia'), FA96-29, FA96-9 and FA99-1 were obtained from a cross between 'Raizan' (Fig. 4A) and A95-14 (Fig. 4B). As to the flower color of these progenies, the rate of white and pink was 1:4. If the flower color of female parent was white and that of male parent was red, intermediate color of parents, pink color was dominant color. Flower characteristics such as trumpet shape and unspotted petal of the FA progenies were similar to those of their parents. Plant height, number of flowers, flower width, and flowering time of FA progenies were distributed more diversely than those of royjrt parents. Among the FA progenies, 'Supia' (FA96-10) was bred as a new cultivar at NHRI in 2000. This cultivar had characteristics such as unspotted petal, light pink color, small size and trumpet-shaped flower, upward-facing, early flowering, high plant height and sweet fragrance.

Characteristics of FO (F x O) Hybrids

FO progenies, FO97-3, FO97-12, FO97-4 (Fig. 4D), FO97-1, FO-97-10, and FO97-16 were obtained from cross combination between 'Raizan' (F) and 'O54' (O). These progenies flowered in 1997. The characteristics of FO progenies were similar to those of the oriental hybrids. These include the creamy ivory color of the female flower, deep pink color of the male flower, big flower, broad leaf shape, and strong fragrance. The advantage of FO hybrids compared to Oriental hybrids is short generation. Originally, breeding problems of Oriental hybrids are long generation time from seed to commercial bulb, long bulb production and their susceptibility to virus and *Fusarium* disease. We have worked this study to reduce breeding duration and bulb production of Oriental hybrids, using *Formolongi* hybrids as female parent, which is propagated by seeds and has short generation. In breeding program and bulb production, FO hybrids are more effective than Oriental hybrids in Korea. Among the FO hybrids, 'Hanuri' (FO97-4) was bred as new a cultivar at NHRI in 2001. It had characteristics such as unspotted petal, light pink and white color, big and Oriental-shaped flower, outward facing, early flowering and sweet fragrance.

Characteristics of FAA (FA x A) Hybrids

FAA progenies, FAA00-22, FAA01-7, FAA01-3, FAA00-4, FAA00-8 and FAA00-24 were obtained from backcrosses between FA hybrids and other Asiatic hybrids. The flower color of FAA progenies became deeper and flower shape was more stable than FA hybrids. As the flower type of pollen parent was dominant, flower type of most FAA progenies was similar to the Asiatic flower type. Also, pollen fertility of FAA lines was less than 1%. Some FAA hybrids were triploid.

Pollen Fertility and Pollen Morphology after In Vitro Chromosome Doubling

Most interspecific lily hybrids have pollen sterility. To restore the pollen sterility of interspecific hybrids, we conducted in vitro chromosome doubling with colchicines and oryzalin to obtain tetraploid hybrids. Low concentration (about 0.001-0.003%) of oryzalin was more effective to obtain tetraploid hybrids than high concentration (about 0.1%) of

colchicines.

Pollen fertility of FA96-18 was 0%, while that of tetraploid FA96-18 was 42.3% in in vitro germination test.

Most sterile pollen grains were small empty ‘shell’ shape and fertile pollen of tetraploid FA96-18 was normal ‘full oval’ shape (Fig. 3).

***Fusarium oxysporum* Screening**

‘Hae-wool’, ‘Sinavro’ and FA 96-16 were classified as highly resistant cultivars and ‘Supia’ and ‘Doran’ as moderately resistant to *Fusarium* bulb-rot disease.

In interspecific crosses between cultivars of different sections, if Oriental and *Formolongi* hybrids were used as female parents, interspecific hybrids could be obtained successfully. However, Asiatic and *Longiflorum* hybrids as female parent could not produce any interspecific hybrids. Most interspecific hybrids of lilies were obtained when *Longiflorum* hybrids were used as female parent. However, we suggest that cultivars derived from *L. formolongi* are more effective than those derived from *L. longiflorum* as female parent for interspecific hybridization (Okazaki et al., 1992).

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Figures

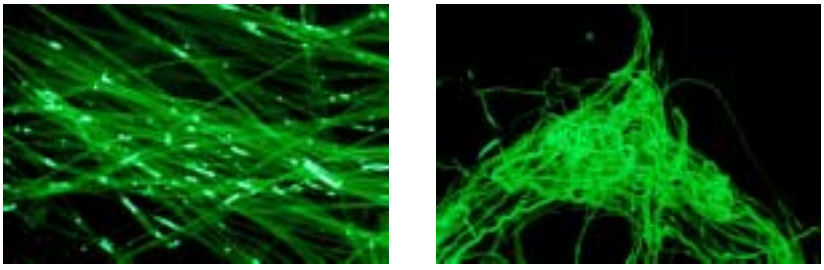


Fig. 1. Many pollen tubes grew straight along the style in compatible (A x A) cross combination (left). The bundle of pollen tubes turned back to the style and stopped in incompatible (A x O) cross combination (right).

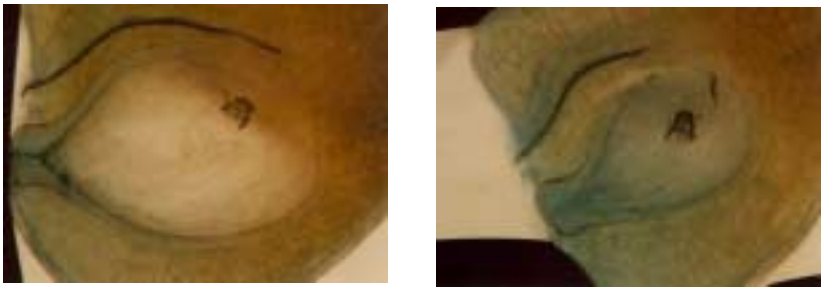


Fig. 2. Pollen tube penetration into the micropyle of lilies observed under light microscope at 14 days after pollination (left). Pollen tube penetration was failed into the micropyle (right).

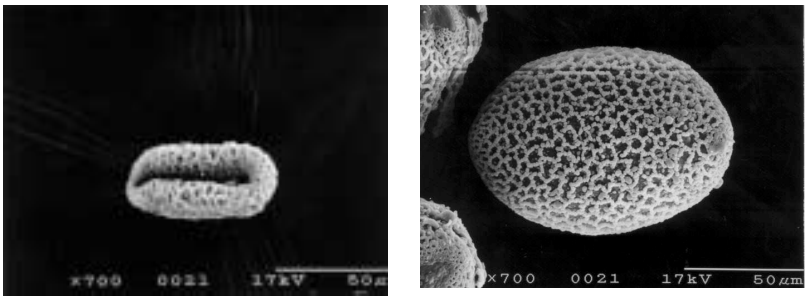
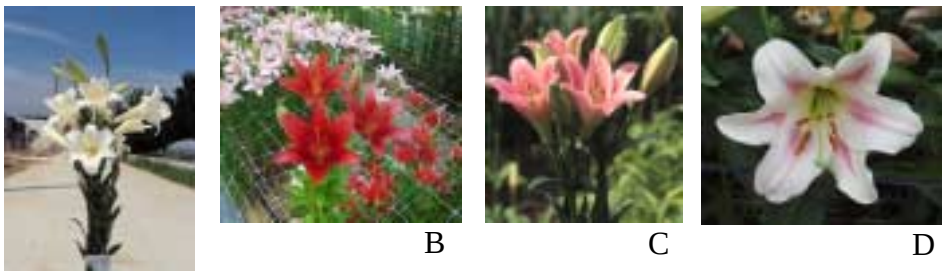


Fig. 3. The sterile pollen of FA96-18 was empty ‘shells’ shape (left) and the fertile pollen of doubled FA96-18 were normal ‘full oval’shape (right).



A B C D

Fig. 4. Flowers of parents and interspecific FA and FO hybrid lilies. A) ‘Raizan’ (Formolongi hybrid, mother parent), B) A95-14 (Asiatic hybrid, father parent), C) ‘Supia’ (interspecific FA hybrid), D) ‘Hanuri’ (interspecific FO hybrid).