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The efficiencies of various embryo rescue methods in interspecific crosses of *Lilium*

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Abstract. The purpose of this research was to compare the efficiencies of various embryo rescue methods in interspecific hybridization of lily. This research included ten combinations of crosses with six crossing types (*Lilium longiflorum* X Asiatic hybrid, LA; Oriental hybrid X Asiatic hybrid, OA; Oriental hybrid X *Lilium longiflorum*, OL; *Lilium longiflorum* X Oriental hybrid, LO; Asiatic hybrid X Oriental hybrid, AO; and Asiatic hybrid X *Lilium longiflorum*, AL). Four culture methods—ovary slice culture (OSC), ovule with placenta culture (OPC), young single ovule culture (YOC), and embryo (sac) rescue method (ESR)—were used to culture the fertilized ovules after cut style pollination on plants. The OSC method could be used to obtain hybrid plants in each of the crossing types (0.1-2% success), except for in the OA, AO, and AL crossings. With the OPC method, ovule germinated (0.2-0.8% success) only when *Lilium longiflorum* "Gerlia" was used as the maternal parent. OSC and OPC required more complicated manipulations. When using the YOC method, ovules were harvested 10 days after the interspecific crosses, and embryo could be germinated from various genotype combinations (0.1-1.1% success). The number of days after pollination (DAP) required for ovule germination in this method (60-125 DAP) was fewer than in the OSC (73-155 DAP) and OPC (57-152 DAP) methods. More plantlets were obtained by the ESR method. The value of the ESR method is that, in the OA crossing, the embryo could only be germinated (0.2-0.3% success) after about 2-3 months of cut style pollination. Apomixes were found in the ovules in some of the cases in this crossing type. No plantlets were obtained in the AL or AO crossings with any of the culture methods tested. Based on the data obtained here, the ESR appears to be the most effective method for obtaining hybrid plants in interspecific hybridization of lily.

Keywords: Apomictic embryo; Cut style pollination; Embryo (sac) culture; *In vitro* culture; Interspecies crosses; *Lilium*; Ovary slice culture; Ovule with placenta culture; Young single ovule culture.

Abbreviations: **A**, Asiatic hybrid; **O**, Oriental hybrid; **L**, *Lilium longiflorum*; **AP**, Oriental hybrid "Acapulco"; **CB**, Oriental hybrid "Casa Blanca"; **CK**, Asiatic hybrids "Connecticut King"; **MB**, Asiatic hybrids "Mont Blanc"; **G**, *Lilium longiflorum* "Gelria"; **IS**, *Lilium longiflorum* "Indian Summer"; **SQ**, *Lilium longiflorum* "Snow Queen"; **DAP**, days after pollination; **E (sac)**, embryo and/or embryo sac; **CSM**, cut style pollination method; **OSC**, ovary slice culture; **OPC**, ovule with placenta culture; **YOC**, young single ovule culture; **ESR**, embryo (sac) rescue method.

Introduction

In the genus *Lilium*, interspecific hybridization has been conducted to produce novel hybrids which can combine the *Botrytis* resistance of Orientals, the virus resistance of Asiatics, and the *Fusarium* resistance of trumpets with attractive blooms and good growing qualities. Various techniques have been applied successfully to overcome pre- and post-fertilization of incompatibility. A comparison of various pollination methods concluded that pre-fertilization barriers could be overcome by using the cut style technique (Van Tuyl et al., 1982, 1988). This method applies the pollen on the stylar surface after removing the stigma and part of the style, which inhibit pollen tube growth. Once fertilization has occurred, the growth of the hybrid embryos may still be restricted by post-fertilization barriers. These may be partly overcome by using the embryo rescue method in lily as described by several authors (North and Wills, 1969; Asano and Myodo, 1977; Asano, 1980; Van Tuyl et al., 1986).

While post-fertilization barriers may be overcome by placenta culture (Janson, 1993; Niimi et al., 1995) and ovary slices may be cultured successfully starting 40 days (Kanoh et al., 1988) or 5-8 days after pollination (Van Tuyl et al., 1991), the integrated use of cut-style pollination followed by embryo rescue has generated some new interspecific hybrids (Van Tuyl et al., 1991). A drawback associated with the cut-style method, however, is the low seed set (Van Tuyl et al., 1982, 1986), presumably caused by the premature arrival of pollen tubes in the ovary (Janson et al., 1993) and the low rate of pollen tube penetration into the micropyle of ovules instead of sperm cells formation (Chi, 2000). Van Tuyl et al. (1991) suggested that the low number of hybrid plants recovered might be attributable to the relatively long interval between pollination and embryo rescue (Van Tuyl et al., 1991). A simple method for obtaining seedlings from very young embryos was established in *L. formosamum* by the young single ovule culture (YOC) technique (Niimi et al., 1995). Niimi et al. (1995) found that seedlings could be obtained from

embryos 10 days after self-pollination by culturing the single ovules, although the success rate was relatively low (below 5%). This method may be used for producing novel cultivars from crosses between distantly related species in which death of hybrid embryos occurs at a very early stage (Niimi et al., 1995).

In this report, young embryos (10 days after pollination) of *Lilium* interspecific crosses were used to determine the efficiency of embryo germination among four embryo rescue techniques.

Materials and Methods

Plant Materials

Seven cultivars from three groups, namely Oriental hybrids (O): "Acapulco" (AP) and "Casa Blanca" (CB); Asiatic hybrids (A): "Connecticut. King" (CK) and "Mont Blanc" (MB), and *Lilium longiflorum* (L): "Gelria" (G), "Indian Summer" (IS), and "Snow Queen" (SQ) were used to perform interspecific crosses in a phytotron (26°C). Before pollination, the plant materials were grown in a greenhouse with temperatures varying from 15°C at night to 20-25°C during the day with summer peaks of 30-35°C. There were a total of six interspecific crossing types (AO, OA, OL, LO, AL, LA) and ten combinations of crosses (Table 1).

Methods

The cut style method (CSM) was used for interspecific crossing (Van Tuyl et al., 1991). Ovaries were harvested 10-11 days after pollination and were surface-disinfected with 70% ethanol for 1 min and then with a commercial bleach solution containing 1.8% chlorine for 10 min, followed by three rinses with sterilized distilled water.

Four rescue methods were used. The ovary slice culture (OSC), young ovule culture (YOC), and ovule with placenta culture (OPC) methods were initiated 10-11 DAP, and the embryo (sac) rescue (ESR) method was started 38-71 DAP, depending on the crossing type and combination. The culture media are given in Table 2.

Ovary slice culture. The ovary was transversely sectioned 10 DAP into six to eight slices of 3-4 mm thickness and polarly placed on the ovary slice culture medium (Table 2) followed by ovule culture 40-86 DAP. The ovules were excised one by one from the ovary slice disks and cultured on ovule culture medium until germination (Table 2).

Ovule with placenta culture. Ovules with placenta were excised from ovaries and incubated on MS medium (Table 2), the ovules were excised individually 40-71 DAP from the placenta and cultured on ovule culture medium until germination (Table 2).

Young single ovule culture. Ten DAP, young ovules were excised from ovaries without placenta and ovary tissue and incubated on MS medium until germination (Table 2).

Embryo/embryo sac culture method (E/Esac). Embryo (sac) culture was applied 38-70 DAP, depending upon the crossing type. Embryos and/or embryo sacs were removed from the ovules under a dissecting microscope and cultured on the embryo culture medium until germination, depending on genotypes and combinations (Table 2).

Following the rescue procedure, all of the materials were incubated at 25°C in the dark. The number of ovules germinating were recorded daily for six months.

Table 1. The efficiency of the ovary slice culture method in various crossing types in lilies.

Crossing type	DAP of slice ovary culture[1]	Ovule culture		Ovules germination		
		DAP of ovule culture	Total ovules in culture	DAP of ovules germinated[2]	No. of ovules germinated	% of germination
LA (G X MB)	11	86	1019	73-155	19	2
LA (G X CK)	11	66	905	81-146	10	1
OA (CB X MB)	11	71	525	0	0	0
OA (CB X CK)	11	71	1077	0	0	0
OL (AP X SQ)	11	64	1110	93-101	2	0.2
OL (AP X IS)	10	41	1079	111	1	0.1
LO (G X AP)	11	62	488	0	0	0
LO (IS X AP)	11	60	1398	86	1	0.1
AO (MB X AP)	11	40	582	0	0	0
AL (MB X G)	11	40	638	0	0	0

[1]Days after pollination.

[2]DAP of first ovule germinated and last ovule germinated.

A, Asiatic hybrid; **O**, Oriental hybrid; **L**, *Lilium longiflorum*; **AP**, Oriental hybrid "Acapulco"; **CB**, Oriental hybrid "Casa Blanca"; **CK**, Asiatic hybrids "Connecticut King"; **MB**, Asiatic hybrids "Mont Blanc"; **G**, *Lilium longiflorum* "Gelria"; **IS**, *Lilium longiflorum* "Indian Summer"; **SQ**, *Lilium longiflorum* "Snow Queen"; **LA**: *Lilium longiflorum* as the mother to cross with Asiatic hybrid; **OA**: Oriental hybrid as the mother to cross with Asiatic hybrid; **OL**: Oriental hybrid as the mother to cross with *Lilium longiflorum*; **LO**: *Lilium longiflorum* as the mother to cross with Oriental hybrid; **AO**: Asiatic hybrid as the mother to cross with Oriental hybrid; **AL**: Asiatic hybrid as the mother to cross with *Lilium longiflorum.*

Table 2. Media used for ovary slice, ovule, and embryo (sac)-rescue culture in lily.

Culture	Medium	Salts	Sucrose (%)	NAA (mg/l)	pH	Agar (%)
Ovary slice	MS	Full strength	9	1.0	6.0	0.4
Ovule	MS	Full strength	5	0.1	5.8	0.4
Ovule with placenta	MS	Full strength	5	0.1	5.8	0.4
Embryo(sac)	MS	Half strength	6	0.01	5.8	0.4

Results and Discussion

Ovary Slice Culture (OSC)

The highest ovule germination was obtained with the LA crossing type. The number of ovules germinated was higher in *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Montblanc" (G X MB) (19 ovules germinated; 2%) than in *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Connecticut king" (G X CK) (10 ovules germinated; 1%). The second place was the OL crossing type. Two (0.2%) ovules germinated in the Oriental hybrid "Acapulco" X *Lilium longiflorum* "Snow Queen" (AP X SQ) whereas only one (0.1%) germinated in the Oriental hybrid "Acapulco" X *Lilium longiflorum* "Indian Summer" (AP x IS). The third was the LO crossing type; only one ovule germinated (0.1%) in *Lilium longiflorum* "Indian Summer" X Oriental hybrid "Acapulco" (IS x AP) (Table 1).

Fernandez et al. (1996) suggested that the initiation time of ovary slice culture greatly influenced the recovery of seedlings. Ovary slice culture can enhance the chance of success in developing new hybrids if initiated at least 10 DAP (Fernandez et al., 1996). Kanoh et al. (1988) suggested that the ovary wall or placenta tissue in the MS medium may supply nutrients or hormones not available in the culture medium but would stimulate hybrid embryo germination (Kanoh et al., 1988). In these experiments, almost all of the crossing types involved could be used in this method (except for the OA, AO, and AL) to obtain plantlets even though the efficiency (the range of percentage of ovule germination was about 0.1-2%) was not high in comparison with the large number of ovules (8821) cultured. However, transferring the ovules individually from the ovary slice to the ovule culture medium is time-consuming. In some cases, the ovules germinated directly on the ovary slice (Figure 1). In the OA, AO, and AL crossing types, no germination occurred.

The amount of time requiring for ovule germination in this culture method was 73-155 DAP, depending on crossing type and genotype used.

Ovary with Placenta Culture (OPC)

The highest ovule germination was obtained with the LA crossing type. The number of ovules germinated (3; 0.4%) in *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Montblanc" (G x MB), was the same (3; 0.3%) as in *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Connecticut King" (G x CK). In the LO crossing type, only one ovule germinated (0.2%) in *Lilium longiflorum* X Oriental hybrid

Table 3. The efficiency of the ovule with placenta culture method in various crossing types of lilies.

Crossing type	DAP of slice ovary culture[1]	Ovule culture		Ovules germination		
		DAP of ovule culture	Total ovules in culture	DAP of ovules germinated[2]	No. of ovules germinated	% of germination
LA (G X MB)	11	42	734	57-143	3	0.7
LA (G X CK)	11	60	870	60-136	3	0.8
OA (CB X MB)	11	71	1094	0	0	0
OA (CB X CK)	11	71	1194	0	0	0
OL (AP X SQ)	11	64	981	0	0	0
OL (AP X IS)	10	41	1347	0	0	0
LO (G X AP)	11	62	500	152	1	0.2
LO (IS X AP)	11	60	1990	0	0	0
AO (MB X AP)	11	40	582	0	0	0
AL (MB X G)	11	40	638	0	0	0

[1]Days after pollination.

[2]DAP of first ovule germinated and last ovule germinated.

A, Asiatic hybrid; **O**, Oriental hybrid; **L**, *Lilium longiflorum*; **AP**, Oriental hybrid "Acapulco"; **CB**, Oriental hybrid "Casa Blanca"; **CK**, Asiatic hybrids "Connecticut King"; **MB**, Asiatic hybrids "Mont Blanc"; **G**, *Lilium longiflorum* "Gelria"; **IS**, *Lilium longiflorum* "Indian Summer"; **SQ**, *Lilium longiflorum* "Snow Queen"; **LA**: *Lilium longiflorum* as the mother to cross with Asiatic hybrid; **OA**: Oriental hybrid as the mother to cross with Asiatic hybrid; **OL**: Oriental hybrid as the mother to cross with *Lilium longiflorum*; **LO**: *Lilium longiflorum* as the mother to cross with Oriental hybrid; **AO**: Asiatic hybrid as the mother to cross with Oriental hybrid; **AL**: Asiatic hybrid as the mother to cross with *Lilium longiflorum*.

"Acapulco" (G x AP) (Table 3). In this method, the hybrid embryo was able to germinate only when the mother plant was *Lilium longiflorum* "Gerlia."

Niimi et al. (1995) found that the ovule with placenta culture method seems to be more practical than the ovule and ovary slice culture method for rescuing young embryos 10 days after self-pollination in *L. formosanum* Wallace. They suggested that this method might be applicable for producing novel cultivars from crosses between distantly related species (Niimi et al., 1995). In this study, the range of ovule germination was 0.2-0.4% with this method. However, its efficiency (0.2-0.8%) was lower than that of the ovary slice culture method (0.1-2%), and it was also time-consuming to transfer the ovules from the placenta to the ovule culture medium. In some cases, the ovules germinated directly on the placenta (Figure 2). In the OA, AO, and AL crossing types, no ovules germinated.

The amount of time requiring for ovules germination in this culture method was 60-152 DAP, depending on the crossing type and genotype used.

Young Single Ovule Culture (YOC)

The highest ovule germination was obtained in the LA crossing type. The number of ovules germinated (8; 1.1 %) was higher in *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Connecticut King" (G x CK) than in *Lilium longiflorum* "Gerlia X Asiatic hybrid "Montblanc" (G x MB) (4; 0.7%). Only one ovule germinated in the Oriental hybrid "Acapulco" X *Lilium longiflorum* "Snow Queen" (AP X SQ; OL crossing type; 0.1%) (Figure 3) or *Lilium longiflorum* "Gelria" X Oriental hybrid "Acapulco" (G x AP; LO crossing type; 0.2%) (Table 4).

In *L. pumilum,* the endosperm nucleus divides by mitosis into two nuclei 8-14 days after pollination. At this time, the embryo is found to be a ball of 4-8 cells. The endosperm is the tissue that nourishes the young embryo from fertilization to the time when the green leaves become functional. During the next 14-21 days, the endosperm grows rapidly (Brandram and Dowrick, 1969). For crosses between distantly related species death of hybrid embryos often occurs at a very early stage. Brandram and Dowrick (1969) found that in the cross, *L. taliense* × *L. lankongense,* abnormalities were seen in endosperms 12-13 days after pollination. After 14 days, many nuclei became degenerated. Abnormalities (most often two or three nuclei joining together) were observed in some ovules at varying periods up to 18 days after pollination. A well developing embryo was associated with a degenerate endosperm in some samples (Brandram and Dowrick, 1969). Therefore, the young ovule culture could be started at 10 days after interspecific crosses and embryo could be germinated (Figure 3). The range of ovule germination in this method was 0.1-1.1%. In this method, one more crossing type (LA, LO, and OL) germinated ovules than in the ovule with the placenta culture method (LA and LO). And in the LA crossing type, more ovules germinated (0.7-1.1%) than in the ovule with the placenta culture method (0.7-0.8%). The total time required to reach ovule germination in the single ovule culture method (60-125 DAP) was less than in the ovule with the placenta culture (57-152 DAP) and the ovary slice culture (73-155 DAP) methods. The single ovule culture method (0.1-1.1%) had a higher percentage of ovule germination than the ovule with the placenta culture method (0.2-0.4%) but a lower percentage than the ovary slice culture (0.1-2%). In the OA, AO, and AL crossing types, no ovules germinated.

Table 4. The efficiency of the young single ovule culture method in different crossing types in lilies.

Crossing type	Young single ovule culture		Young single ovules germination		
	DAP of young single ovule culture[1]	Total ovules in culture	DAP of ovules germinated[2]	No. of ovules germinated	% of germination
LA (G X MB)	11	609	60-72	4	0.7
LA (G X CK)	11	716	89	8	1.1
OA (CB X MB)	11	640	0	0	0
OA (CB X CK)	11	940	0	0	0
OL (AP X SQ)	11	1001	125	1	0.1
OL (AP X IS)	10	1468	0	0	0
LO (G X AP)	11	460	112	1	0.2
LO (IS X AP)	11	690	0	0	0
AO (MB X AP)	11	320	0	0	0
AL (MB X G)	11	620	0	0	0

[1]Days after pollination.

[2]DAP of first ovule germinated and last ovule germinated.

A, Asiatic hybrid; **O**, Oriental hybrid; **L**, *Lilium longiflorum*; **AP**, Oriental hybrid "Acapulco"; **CB**, Oriental hybrid "Casa Blanca"; **CK**, Asiatic hybrids "Connecticut King"; **MB**, Asiatic hybrids "Mont Blanc"; **G**, *Lilium longiflorum* "Gelria"; **IS**, *Lilium longiflorum* "Indian Summer"; **SQ**, *Lilium longiflorum* "Snow Queen"; **LA**: *Lilium longiflorum* as the mother to cross with Asiatic hybrid; **OA**: Oriental hybrid as the mother to cross with Asiatic hybrid; **OL**: Oriental hybrid as the mother to cross with *Lilium longiflorum*; **LO**: *Lilium longiflorum* as the mother to cross with Oriental hybrid; **AO**: Asiatic hybrid as the mother to cross with Oriental hybrid; **AL**: Asiatic hybrid as the mother to cross with *Lilium longiflorum.*

Table 5. The efficiency of the embryo (sac) culture method in various crossing types of lilies.

Crossing type	Embryo (sac) culture		Embryo germination				
	DAP of embryo (sac) culture[1]	No. of E(sac) in culture / No. of capsules	DAP of embryo germinated[2]	No. of embryo germinated	% of embryo germination[3]	% of fertilization[4]	Actual % of embryo germinated[5]
LA (G X MB)	38	113/15	76-82	38	34	1	0.3
LA (G X CK)	38	77/13	67-69	20	26	1	0.3
OA (CB X MB)	71	168/13	122-128	14	8	3	0.2
OA (CB X CK)	64	41/3	92	4	10	3	0.3
OL (AP X SQ)	38	22/12	119	3	14	0.4	0.1
OL (AP X IS)	0	0/1	0	0	0	0	0
LO (G X AP)	40	1/1	0	0	0	0	0
LO (IS X AP)	40	2/1	98	1	50	0.3	0.2
AO (MB X AP)	38	0/5	0	0	0	0	0
AL (MB X G)	38	0/5	0	0	0	0	0

[1]DAP: days after pollination

[2]DAP of first ovule germinated and last ovule germinated

[3]No. of Embryo germinated / No. of Embryo (sac) in culture

[4]No. of embryos (sac) in culture/ No. of capsules multiplied by No. of ovules/capsule- about 600 (*L. longiflorum*); No. of embryos in culture/ No. of capsules multiplied by No. of ovules/capsule- about 500 (oriental hybrid); No. of embryos in culture/ No. of capsules multiplied by No. of ovules/capsule- about 300 (asiatic hybrid).

[5]Actual % of embryo germination: [3]%of embryo germinated multiplied by [4]% of fertilization.

Note: The number of ovules per ovary was obtained from the average of 20 ovaries. In *Lilium longiflorum* about 600 ovules per ovary; in Oriental hybrid, about 500 ovules; and in Asiatic hybrid about 300 ovules.

A, Asiatic hybrid; **O**, Oriental hybrid; **L**, *Lilium longiflorum*; **AP**, Oriental hybrid "Acapulco"; **CB**, Oriental hybrid "Casa Blanca"; **CK**, Asiatic hybrids "Connecticut King"; **MB**, Asiatic hybrids "Mont Blanc"; **G**, *Lilium longiflorum* "Gelria"; **IS**, *Lilium longiflorum* "Indian Summer"; **SQ**, *Lilium longiflorum* "Snow Queen"; **LA**: *Lilium longiflorum* as the mother to cross with Asiatic hybrid; **OA**: Oriental hybrid as the mother to cross with Asiatic hybrid; **OL**: Oriental hybrid as the mother to cross with *Lilium longiflorum*; **LO**: *Lilium longiflorum* as the mother to cross with Oriental hybrid; **AO**: Asiatic hybrid as the mother to cross with Oriental hybrid; **AL**: Asiatic hybrid as the mother to cross with *Lilium longiflorum.*

The Embryo (sac) Culture (ESR)

Although the actual rate of fertilization may be higher because some embryos died before being rescued, the number of rescued embryos can be used to estimate the minimum rate of fertilization. In the embryo rescue method, the product of this rate of fertilization and the percentage of germination is defined as the real percentage of embryo germination (Table 5). Crossing type OA has the highest rate of fertilization (3%) followed by the LA (1%), OL (AP x SQ, 0.4%), and LO (IS x AP, 0.3%). In almost all crossing types (except the AO and AL crossing types), embryos germinated in this embryo rescue method. In the LA crossing type, most of the embryos were cultured 38 DAP, and 26-34% embryo germination was obtained 67-82 DAP. The number of ovules germinated was higher in *Lilium longiflorum* "Gelria" X Asiatic hybrid "Montblanc" (G x MB) (38; 34%) than in *Lilium longiflorum* "Gelria" X Asiatic hybrid "Connecticut King" (G x CK) (20; 26%). In the OL crossing type, the embryo sacs were cultured 38 DAP, but only three embryos germinated (14%) in the Oriental hybrid "Acapulco" X. *Lilium longiflorum* "Snow Queen" (AP x SQ) 119 DAP. In the LO crossing type, two embryo sacs were cultured 40 DAP, and only one embryo germinated in *Lilium longiflorum* "Indian Summer" X the Oriental hybrid "Acapulco" (IS x AP) 98 DAP. In the OA crossing type, the embryo sac was rescued starting 64-71 DAP, 8-10% embryo germination was obtained 92-128 DAP. The percentage of embryos germinated in the Oriental hybrid "Casablanca" X Asiatic hybrid "Connecticut King" (CB x CK, 4, 10%) was higher than in the Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB x MB, 14, 8%) (Table 5). No embryos germinated in the AO or AL crossing types.

In the OA hybridization, the embryo, which grew fairly well in the embryo sac without the endosperm (Figures 4-6) was rescued and after that embryo germinated fairly well under in vitro conditions (Figure 7). It seemed that embryo development stopped during the 2-3 months of the embryo growing period by post fertilization barriers, probably due to the lack of endosperm when embryos of OA developed under in vivo conditions (Figures 4-6).

Among these four embryo rescue techniques, the actual percentage of ovule germination in the embryo rescue method was the lowest. The value of the embryo rescue method is that in OA crossing only the embryo rescue method resulted in embryo germination. The time needed to germinate embryos after pollination was shorter than in other culture methods in the LA crossing type.

Apomictic Embryos Formation in the Genus Lilium

In the OA hybridization ("Casablanca" x "Montblanc"), twin embryos were obtained in the same embryo sac (Figure 8), but they couldn't germinate afterwards. This is

Figure 1. In ovary slice culture, ovule germinated directly on the ovary slice disk 73 DAP in the combination of *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Montblanc" (G X MB) (LA crossing type).

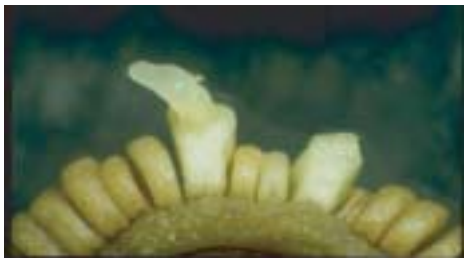

Figure 2. In ovule with placenta culture, ovule germinated directly on the placenta 60 DAP in the combination of *Lilium longiflorum* "Gerlia" X Asiatic hybrid "Connecticut King" (G X CK) (LA crossing type).

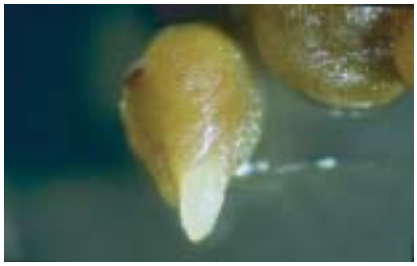

Figure 3. In young single ovule culture, ovule germinated 125 DAP in the Oriental hybrid "Acapulco" X *Lilium longiflorum* "Snow Queen" (AP X SQ) (OL crossing type) (13×).

Figure 4. Embryos and embryo sacs (without endosperm) were picked up under a dissecting microscope from the ovules 71 DAP and cultured on the embryo culture medium. Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB X MB) (OA crossing type) (13×).

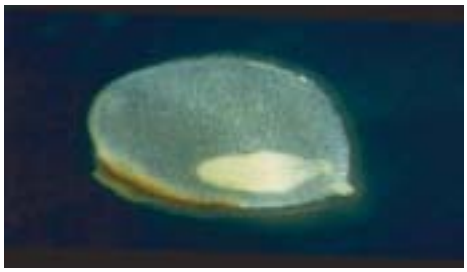

Figure 5. Embryo sac culture 71 DAP in the Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB X MB) (OA crossing type) (15×).

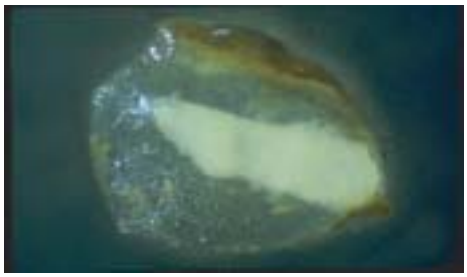

Figure 6. Embryo sac culture 71 DAP in the Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB X MB) (OA crossing type) (15×).

Figure 7. In the embryo sac culture, embryo germinated 125 DAP in the Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB X MB) (OA crossing type) (13×).

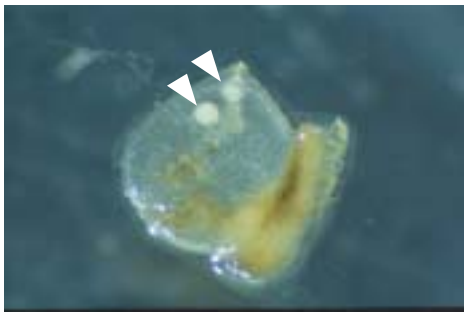

Figure 8. Two embryos in the same embryo sac (indicate by arrows). Embryo sac culture in the Oriental hybrid "Casablanca" X Asiatic hybrid "Montblanc" (CB X MB) (OA crossing type) 71 DAP. A group of cells of the nucellor tissue divided and formed an apomictic budding. The apomictic embryo was in the vicinity of the micropyle. (13×).

consistent with the finding of Myodo (1975) that, in the cross of *L. formosanum* × *L. leichtlinii* var. maximowiczii, a group of cells in the nucellor tissue began to form an apomictic budding. The location of the cells that formed an apomictic embryo was not constant, but in most cases it was in the vicinity of the micropyle. The frequency of their location in the nucellar tissue was 87% of all the observed apomictic embryos initiated in the nearest portion to the micropyle (Myodo, 1975). Nogler (1984) also observed that aposporous initials develop in a zone adjacent to the progenitor cells of a sexual megagametophyte.

In applying these four embryo rescue techniques, it is important to determine the optimal timing for each method and cross. For lily, the abortion of the embryo is often caused by the degeneration of the endosperm or absence of the endosperm (Asano and Myodo, 1977). Therefore, embryo rescue methods (ESR) have to be applied. However, between fertilization and the start of embryo culture, a lot of embryos are already lost. Employing OSC, OPC, or YOC, it is possible to rescue embryos in an earlier phase. However, in interspecific crosses using Asiatic hybrid lilies as the female parent, no embryo germination was obtained in any of the four methods. This may give certain combinations of genes that are either not viable themselves or which are incompatible with the embryo or maternal tissues.

Literature Cited

Asano, Y. 1980. Studies on crosses between distantly related species of lilies. J. Jap. Soc. Hort. Sci. **49:** 114-118.

Asano, Y. and H. Myodo. 1977. Studies on crosses between distantly related species of lilies. 1. For the intrastylar pollination technique. J. Jap. Soc. Hort. Sci. **46:** 59-65.

Brandram, S.N. and G.J. Dowrick. 1969. Hybridisation in lilies-The importance of the endosperm. North Amer. Lily Soc. Yearb. **22:** 7-17.

Chi, H.S. 2000. Interspecific crosses of lily by in vitro pollinated ovules. Bot. Bull. Acad. Sin. **41:** 143-149.

Fernandez, A. M., T. Nakazaki, and T. Tanisaka. 1996. Development of diploid and triploid interspecific hybrids between *Lilium longiflorum* and *L. concolor* by ovary slice culture. Plant Btreed. **115:** 167-171.

Janson, J. 1993. Placental pollination in *Lilium longiflorum* Thunb. Plant Sci. **90:** 105-115.

Janson, J., M.C. Reinders, J.M. van Tuyl, and C.J. Keijzer. 1993. Pollen tube growth in *Lilium longiflorum* following different pollination techniques and flower manipulations. Acta Bot. Neerl. **42:** 461-472.

Kanoh, K., M. Hayashi, Y. Serizawa, and T. Konishi. 1988. Production of interspecific hybrids between *Lilium longiflorum* and *L. x elegance* by ovary slice culture. Japan. J. Breed. **38:** 2788-282.

Myodo, H. 1975. Apomictic seed formation in the Genus *Lilium*. North Amer. Lily Soc. Yearb. **28:** 66-69.

Niimi, Y., M. Nakano, and M. Goto. 1995. Comparison of seedling production among several embryo-rescue techniques in *Lilium formosanum* Wallace. Plant Tiss. Cult. Lett. **12:** 317-319.

Nogler, G.A. 1984. Gametophytic apomixis. *In* B.M. Jori (ed.), Embryology of Angiosperms, (Berlin: Springer-Verlag), pp. 475-518.

North, C. and A. B. Wills. 1969. Inter-specific hybrids of *Lilium* lankongense Franchet produced by embryo-culture. Euphytica **18:** 430-434.

Van Tuyl, J.M., M.C. Marcucci, and T. Visser. 1982. Pollen and pollination experiments. VII. The effect of pollentreatment and application method on incompatibility and incongruity in *Lilium*. Euphytica **31:** 613-619.

Van Tuyl, J.M., R.C. Franken, C.A. Jongerius, M. Lock, and T.A.M. Kwakkenbos. 1986. Interspecific hybridization in *Lilium*. Acta. Hort. **177:** 591-595.

Van Tuyl, J.M., T.P. Straathof, R.J. Bino, and A.A.M. Kwakkenbos. 1988. Effect of three pollination methods on embryo development and seedset in intra- and interspecies crosses between seven *Lilium* species. Sex. Plant Rep. **1:** 119-123.

Van Tuyl, J.M., M.P. Van Dien, M.G.M. Vzn Creij, T.C.M. Van Kleinwee, J. Franken, and R.J. Bino. 1991. Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in interspecies *Lilium* crosses. Plant Sci. **74:** 115-126.

不同胚培養方法對百合種間雜交胚拯救效率之影響

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本研究目的是評估不同胚培養方法（子房切片培養法，胚珠結合胎座培養法，單一胚珠培養法及胚拯救法）對百合種間雜交效率之影響。在本研究中百合之種間雜交共有六種雜交型（LA, OL, LO, OA, AO, 和 AL）和十種不同的雜交組合。結果以子房切片培養法，幾乎能使所有不同雜交型之受精胚萌芽（0.1-2%）(除了 OA, AO 和 AL 雜交型之外)。胚珠結合胎座培養法只有以鐵炮百合為母本之雜交組合有受精胚的萌芽 (0.2-0.8%)。而此兩種方法（子房切片培養法及胚珠結合胎座培養法）之缺點為其操作之手續較複雜，且所耗費之時間與勞力亦較多。單一胚珠培養法亦能使幾乎所有不同雜交型之受精胚萌芽 （0.1-1.1%)(除了 OA, AO 和 AL 雜交型之外)，且胚發芽所需之日數（60-125 DAP）較子房切片培養法（73-155 DAP）和胚珠結合胎座培養法（57-152 DAP）少。在胚拯救之方法中，胚被拯救後其萌芽之成功率高約 8-50%；其中值得一提的是在四種培養方法中只有胚拯救法能成功的使 OA 雜交型之受精胚萌芽 (0.2-0.3%)；另外在 OA 雜交型之胚囊中亦發現有無性胚（apomixes）之存在。不論那一種培養方法，以亞洲型雜交百合為母本之種間雜交 (AO 和 AL)，皆未有任何雜交胚萌芽。由以上結果顯示，胚拯救法為四種胚培養法中較有效率的方法，雖然此法須要較高之技巧。

關鍵詞：無性胚；切柱受粉法；胚 (胚囊)培養；生體外培養；種間雜交；百合；子房切片培養；胚珠結合胎座培養；含未成熟胚的單一胚珠培養。