Overcoming Interspecific Hybrid Sterility in *Lilium*¹

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Summary

For the purpose of overcoming interspecific hybrid sterility in *Lilium*, the following methods were attempted: 1) Further use of embryo culture in the cross of F₁ and 2) Use of amphidiploids induced from F₁. When F₁ hybrids were used as female parents, embryos were developed in 0.3.4% of their ovules. A total of 134 seedlings from nine backcross (or the similar cross) combinations were obtained through embryo culture. A seedling from the cross of F₁ as male parent flowered and proved to be a triploid. Amphidiploids could be used as pollen donors and eight B₁ plants were produced from the cross of the amphidiploid of *L. longiflorum* × *L. henryi*.

Three new interspecific hybrids in *Lilium* produced by embryo culture were introduced.

Introduction

The combined techniques of cut-style pollination with embryo culture *in vitro* allowed us to obtain a number of interspecific hybrids in *Lilium* which had not been produced by the usual crossing methods(1, 2, 3, 5). Some of the hybrids had horticulturally desirable characters such as disease resistance, flower color, fragrance, etc.. However, most of them were highly sterile and only a few B₁ progenies were obtained from those F₁. Next generations must be produced by overcoming these hybrid sterilities to attain such gene recombinations as to produce ideal horticultural hybrid lilies. For this purpose, the following methods were attempted.

- 1) Further use of embryo culture in the cross of F₁
 - 2) Use of amphidiploids induced from F₁
- The present paper reports these results and in addition, three new interspecific hybrids in *Lilium* produced by embryo culture are introduced.

Materials and Methods

Interspecific hybrids from 19 cross combinations were used in backcrosses or crosses

with the relatives. All of the hybrids were raised at our laboratory in recent years. Crosses were mostly made at the experimental field using the pollen either collected fresh or stored under refrigeration. Cut-style pollination technique was applied again in those crosses. As soon as capsules started turning a little tan colored, they were harvested, sterilized in 70% alcohol and examined aseptically for embryos. The removed embryos were grown on Murashige and Skoog medium (11) under the same conditions as reported previously(2). Vermiculite was used as the transplant medium when seedlings were successfully obtained after 2–4 months culture. Two amphidiploid plants were induced from the hybrid *L. longiflorum* × *L. henryi* by 0.1% colchicine solution treatment of the scales one week after scaling(10). Amphidiploid 'Black Beauty' (*L. speciosum* × *L. henryi*) was donated by Mr. Woodriff, Oregon, U. S. A. Observation of meiotic chromosome pairing in PMCs of the amphidiploid was made by fixing in Newcomer's fluid(12) and iron aceto-carmines smearing. Somatic chromosome number, pollen fertility and pollen germination rate were examined in the same way as in the previous report(3).

¹ Received for publication January 4, 1982.

Results and Discussion

Crosses of F_1 as female parents

Many of the F_1 hybrids are completely pollen sterile and can not be used as male parents. However, when they were pollinated with the pollen from their parents or the relatives, especially with their paternal pollen, fertilization took place and a few seeds were produced in several hybrids(3,5). From this observation, male parents (or the relatives) of the hybrids were mainly used as pollen donors in the present crosses. The results of the crosses are shown in Table 1. Embryos were produced in several hybrids including a triploid at an average low of 0.1 to an average high of 13.0 per capsule. These hybrid ovaries contained 234-426 ovules so that the frequency of ovules forming embryos varied from 0.0-3.4%. Almost all of the embryos were immature and were

associated with none or liquid-semisolid abnormal endosperms. They were, however, often larger in size than those of F_1 hybrids and reached the stage of elongated boat-shape. A total of 134 seedlings from nine backcross (or the similar cross) combinations were obtained through embryo culture.

A similar result of overcoming hybrid sterility by using the hybrid as the female parent was reported by Ronald and Ascher(13) in completely pollen sterile *L. 'Black Beauty'*. They gave two explanations for the possible reasons of this slight female functionality: 1) the difference in nutritive surroundings between female and male gametophytes and 2) mutual complement among deficient female genomes resulting from a specific megasporogenesis in *Lilium*, in which all four meiotic products participate in the formation of the female gametophyte.

In the interspecific cross, the failure of

Table 1. The results of the crosses of F_1 hybrids as female parents : cut-style pollination.

♀ (Hybrid)	♂	No. of crosses	No. of capsules obtained	No. of embryos /capsule	Freq. of ovules forming embryos (%)	Length of embryos (mm)	No. of seedlings obtained ^y
(<i>L. longiflorum</i> × <i>L. dauricum</i>) × <i>L. dauricum</i>		8	8	4.4	1.3	0.4~3.6	13
^z (" × <i>L. 'Utagoe'</i>) × <i>L. dauricum</i>		43	23	13.0	3.4	0.3~7.8	78
(" × <i>L. 'Shuho'</i>) × <i>L. dauricum</i>		20	10	4.8	1.2	0.2~2.6	10
^z (" × <i>L. 'Karakoma'</i>) × <i>L. dauricum</i>		8	0				
(" × <i>L. 'Hagoromo'</i>) × <i>L. maximowiczii</i>		4	3	2.0	0.5	0.5~1.8	1
(" × <i>L. amabile</i>) × <i>L. amabile</i>		3	0				
(" × <i>L. maximowiczii</i>) × <i>L. maximowiczii</i>		11	0				
(" × <i>L. pumilum</i>) × <i>L. longiflorum</i>		4	2	6.0	1.9	0.6~2.6	2
^z (" × <i>L. 'Sugehime'</i>) × <i>L. 'Sugehime'</i>		15	1	0			
(" × <i>L. 'Beninoizumi'</i>) × <i>L. dauricum</i>		10	9	1.1	0.3	0.6~3.2	6
(" × <i>L. henryi</i>) × { <i>L. longiflorum</i>		20	0				
		20	0				
(<i>L. speciosum</i> × <i>L. henryi</i>) × { <i>L. henryi</i>		10	10	2.1	0.7	0.2~0.7	1
		10	3	0.7	0.2	0.2	
(<i>L. auratum platyphyllum</i> × ") × <i>L. henryi</i>		48	39	2.7	1.1	0.2~1.7	
(<i>L. 'Sakuakaka'</i> × ") × <i>L. henryi</i>		10	3	0			
		10	3	3.0	1.3	0.2~0.7	
		10	2	0			
		5	0				
(<i>L. 'Shikayama'</i> × ") × <i>L. auratum</i>		10	0				
		10	0				
		10	3	0			
		10	3	0			
(<i>L. 'Shikayama'</i> × <i>L. nobilissimum</i>) × <i>L. nobilissimum</i>		9	0				
(<i>L. 'Royal Gold'</i> × <i>L. speciosum</i>) × <i>L. speciosum</i>		9	8	0.1	0.0	0.4	
(<i>L. regale</i> × <i>L. maximowiczii</i>) × <i>L. maximowiczii</i>		11	11	5.1	1.7	0.2~3.8	21
(<i>L. 'Okani'</i> × <i>L. cernuum</i>) × <i>L. cernuum</i>		9	1	2.0	0.9	0.9~1.5	2

^z Triploid. ^y After embryo culture.

Table 2. The results of the crosses of F_1 hybrids as male parents : cut-style pollination.

♀	♀ (Hybrid)	No. of crosses	No. of capsules obtained	No. of embryos /capsule	Length of embryos (mm)	No. of seedlings obtained
<i>L. longiflorum</i>	$\times (L. longiflorum \times L. maximowiczii)$	30	0			
<i>L. longiflorum</i>	$\times (L. longiflorum \times L. 'Hagoromo')$	5	0			
<i>L. 'Hagoromo'</i>		5	0			
<i>L. henryi</i>		10	0			
<i>L. 'Aurelian'</i>	$\times (L. auratum platyphyllum \times L. henryi)$	15	0			
<i>L. auratum</i>		5	2	0		
<i>L. speciosum</i>		10	1	0		
<i>L. henryi</i>	$\times (L. 'Shikayama' \times L. henryi)$	28	2	0		
<i>L. 'Aurelian'</i>		64	8	1.8	1.4-mature	3
<i>L. 'Royal Gold'</i>		10	5	0		
<i>L. 'Shikayama'</i>		5	0			
<i>L. speciosum</i>		10	0			
<i>L. 'Sasaotome'</i>		18	0			
<i>L. 'Okani'</i>	$\times (L. 'Okani' \times L. cernuum)$	25	3	0.7	0.5~2.3	

endosperm development is caused by the disturbance of genetic balance in the genomic constitution MMMM+P (polar nucleus + sperm nucleus) in the nuclei(7). (M: maternal genome, P: paternal genome) Endosperm failure in the backcross indicates that genetic imbalance exists in a similar fashion in the genomic constitution MMPP+P.

Crosses of F_1 as male parents

Among the F_1 hybrids we obtained, some were found to be partially pollen fertile. Such five hybrids were used as pollen donors and crossed with their parents or the relatives. The results of the crosses are shown in Table 2. The pollen tube growth of those hybrids on artificial medium, was often abnormally short and was followed by tip-bursting. This is suspected to be the primary cause of the very poor results. However, from one cross combination, *L. 'Aurelian' 7111' $\times (L. 'Shikayama' \times L. henryi)$* , a few matured seeds were obtained and they germinated without the aid of embryo culture. One of the seedlings flowered in 1980 and proved to be a triploid (Fig. 4, Table 7). The karyotype showed that two of the three genomes were derived from the male parent. Meiotic observation of the PMCs of the hybrid *L. auratum platyphyllum $\times L. henryi$* showed the formation of unreduced gametes due to only equational division (unpublished). Probably, the hybrid *L. 'Shikayama' $\times L. henryi$* might have produced unreduced male gametes by a similar meiotic process. The

Fig. 1. Flowers of diploid (left) and amphidiploid (right) of *L. longiflorum $\times L. henryi$* .Table 3. Comparison of some characters between diploid and amphidiploid of *L. longiflorum $\times L. henryi$* : The average of two plants, respectively.

	Diploid ($2n=24$)	Amphidiploid ($2n=47, 48$)
Plant height	155	158 cm
Diameter of stem*	13.5	16.5 mm
No. of leaves ^y	90	63
No. of flowers	8	5.5
Diameter of flower	15.5	19.5 cm
Flowering time ('81)	Aug. 2	Aug. 4
Pollen : Fertility	0	85.4%
Germination rate*	0	11.6%

* At the base.

^y Below the lowest pedicel.

* On the artificial medium.

pollen of this hybrid also successfully produced a number of progenies in the crosses with 'Oriental', the relatives of *L. 'Shikayama'*, with the aid of embryo culture (van Tuyl, personal communication).

Crosses of amphidiploids

Two colchicine-induced amphidiploids of

Table 4. The results of the crosses of amphidiploids : cut-style pollination.

♀	♂	No. of crosses	No. of capsules obtained	No. of embryos /capsule	Length of embryos (mm)	No. of seedlings obtained ^z
Amphidiploid	$\left\{ \begin{array}{l} L. longiflorum \\ L. henryi \end{array} \right\} \times L. henryi$	6	0			
$(L. longiflorum \times L. henryi) \times$	$\left\{ \begin{array}{l} L. longiflorum \\ L. henryi \end{array} \right\}$	6	0			
$L. 'Black\ Beauty' \times$	$\left\{ \begin{array}{l} L. speciosum \\ L. henryi \end{array} \right\}$	5	0			
$L. longiflorum \left. \begin{array}{l} L. henryi \\ L. 'Aurelian' \\ L. speciosum \end{array} \right\} \times$	Amphidiploid $(L. longiflorum \times L. henryi)$	79	13	0.8	0.2~0.3	8
$L. 'Sakuakaka' \times$	$L. 'Black\ Beauty'$	20	1	6.2	0.2~4.1	
$L. henryi \left. \begin{array}{l} L. 'Aurelian' \\ L. 'Sakuakaka' \end{array} \right\} \times$	$L. 'Black\ Beauty'$	10	0	2.0	0.3	
$L. 'Aurelian' \times$	$L. 'Black\ Beauty'$	58	13	0.6	0.3~1.1	
Between amphidiploids of $L. longiflorum \times L. henryi$		16	13	0.4	0.3~0.6	
		10	0			
		10	0			
		7	0			

^z After embryo culture.

Table 5. Chromosome pairing frequency at metaphase I in PMCs of the amphidiploid (2n=48) of *L. longiflorum* × *L. henryi*.

Configurations/cell													Total
Quadrivalent	: 1	1	1	1									
Trivalent	: 1				1	1	1	1					
Bivalent	: 20	22	21	20	22	21	20	19	24	23	22	21	20
Univalent	: 1		2	4	1	3	5	7		2	4	6	8
Cells observed	1	4	1	1	1	3	3	1	40	56	28	10	151

Fig. 2. Meiotic metaphase I in the PMC of the amphidiploid of *L. longiflorum* × *L. henryi* (2n=48) : 23 bivalents+2 univalents.

Fig. 3. Pollen germination of the amphidiploid of *L. longiflorum* × *L. henryi* on artificial medium. Note the short pollen tube growth and tip-bursting.

L. longiflorum × *L. henryi* have chromosome numbers of 47 and 48. As compared with the original diploids, they show larger and more fleshy flowers and thicker stems, but fewer flowers and leaves(Fig.1, Table3). Pollen fertility and some pollen germinating ability are restored. The results of the crosses using amphidiploids are shown in Table 4. It shows that amphidiploids from completely

pollen sterile hybrids can be used as pollen donors especially when they are backcrossed with their female parents or the relatives, although embryo culture is still required to obtain seedlings. Eight B₁ plants were produced from the cross *L. longiflorum* × amphidiploid (*L. longiflorum* × *L. henryi*).

Fig. 4. Flowers of new interspecific hybrids in *Lilium*.

A : *L.* 'Aurelian 7111' $\times$ (*L.* 'Shikayama' $\times$ *L. henryi*)

B : *L. longiflorum* $\times$ *L. leichtlinii maximowiczii*

C : *L.* 'Sakuakaka' $\times$ *L. henryi*

D : *L. nobilissimum* $\times$ *L. henryi*

Table 6. Characters of new interspecific hybrids in *Lilium*.

	<i>L.</i> 'Aurelian 7111' × (<i>L.</i> 'Shikayama' × <i>L. henryi</i>)	<i>L. longiflorum</i> × <i>L. leichtlinii</i> <i>maximowiczii</i>	<i>L.</i> 'Sakuakaka' ^z × <i>L. henryi</i>	<i>L. nobilissimum</i> × <i>L. henryi</i>
Stem : color	Pale purplish brown	Light green	Pale purplish brown	Pale purplish brown
Leaf : shape	Narrow lanceolate	Linear-lanceolate	Lanceolate	Lanceolate
color	Light green	Green	Light green	Green
Flower : facing	Sub-pendulous	Sub-pendulous	Sub-pendulous	Sub-pendulous
type	Funnel	Recurved	Strongly recurved	Funnel
diameter (cm)	17.3	13	10.5	18
color ^y	Deep purplish red	Light orange	Pale yellow orange	Yellowish gray
spots	Dark red	Purple	Brown	Pale purple
pollen	Brown	Brown	Brown	Brown
scent	Weakly sweet	None	Weakly sweet	Weakly sweet
Flowering time	Late Jul.	Early Aug.	Mid Aug.	Mid Aug.

^z *L. auratum platyphyllum* × *L. speciosum*^y Refer to 'Guide to color standard' (9).

Table 7. Chromosome numbers, pollen fertilities and pollen germination rates of new interspecific hybrids.

Parents		No. of observation	Chromosome No. (2n)	Pollen	
♀	♂			Fertility (%)	Germination rate ^z (%)
<i>L.</i> 'Aurelian 7111'	(<i>L.</i> 'Shikayama' × <i>L. henryi</i>)	1	37	36.2	5.0
<i>L. longiflorum</i>	<i>L. leichtlinii maximowiczii</i>	1	24	52.0	0.7
<i>L.</i> 'Sakuakaka'	<i>L. henryi</i>	1	24	0	0
<i>L. nobilissimum</i>	<i>L. henryi</i>	1	24	0	0

^z On the artificial medium.

However, when crosses were made with amphidiploids as female parents, no better results were obtained than in the crosses with the original diploids.

The result of the observation of meiotic chromosome pairing in PMCs of the amphidiploid of *L. longiflorum* × *L. henryi* is shown in Table 5. Twenty six and a half percent of the PMCs(40 cells) had regular pairing configurations of 24 bivalents, and in 62.2% of the PMCs(94 cells) 1-3 pairs failed to show an association(Fig.2). The formation of multivalents may indicate some chromosomal homology between the parental genomes(6). Almost regular nuclear divisions followed the metaphase I. Similar observations of restoration of chromosome pairing and the resultant fertile pollen were reported in the amphidiploid of *L.* 'Black Beauty'(8, 14). However, in spite of this observation, the pollen tube growth of the amphidiploid on artificial medium is found to be often abnormal (Fig.3). This may indicate that

both parental genomes are genetically ill-balanced in pollens. This genomic imbalance and some incompleteness in chromosomal complements in pollens would be responsible for the tube growth abnormality and consequently, for the unexpected and very poor results of the crosses. A similar situation in female gametes might cause very poor results when amphidiploids were used as the female parents.

Three new interspecific hybrids

Three interspecific hybrids in *Lilium* were newly obtained by embryo culture(Fig. 4: B-D). Among them, the hybrid of *L. longiflorum* × *L. leichtlinii maximowiczii* was raised by nurse culture(4) using normally developing *L. longiflorum* endosperm from the intraspecific cross as a nurse tissue. Their characters are generally intermediate between their parents in all of the three hybrids except for the red color on the flower in *L.* 'Sakuakaka' which is lost from the hybrid(Table 6). As *L.* 'Sakuakaka' has

a gene responsible for anthocyanine pigment heterozygously, a genome devoid of the gene would participate in the cross. The hybrid *L. longiflorum* × *L. leichtlinii maximowiczii* has a pollen fertility of 52.0% and a slight pollen germination ability (Table 7). This will be the result of its relatively high extent of chromosome pairing at meiosis with an average of 7.5 bivalents/PMC(6). This may show some close genomic relationship between the parental species.

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ユリの種間雑種の雑種不稔性の克服

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摘 要

ユリの種間雑種の雑種不稔性を打破するために、次の方法が採られた。1) F_1 の交配における、再度の胚培養の利用、2) 複二倍体の作成。 F_1 を母親とした交配において、幾つかの組合せで、0.0—3.4% の胚珠で胚が形成された。9 戻し交雑（或はそれに近い）組合せから、胚培養を経て、合計 134 個体が得られた。 F_1 を父親とし

た交配からの 1 個体が開花し、これは三倍体であった。複二倍体は花粉親として用い得ることが認められ、テッポウユリ × キカノコユリの複二倍体との交雑から 8 個体の B_1 が得られた。

胚培養により作られた、3 組合せの新しいユリの種間雑種が紹介された。