Interspecific Lily Hybrids with the Ability to Flower Precociously and to Produce Multiple Flower Stalks from *Lilium formosanum*

Hiroki Saruwatari¹, Yuka Shuto-Nakano¹, Kanehiro Nakano¹, Michikazu Hiramatsu^{2*}, Yukio Ozaki² and Hiroshi Okubo²

¹Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

²Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Lilium formosanum Wallace has remarkable traits such as ‘precocious flowering’ ability, i.e., it reaches anthesis within 12 months from seed germination, with multiple shooting of flower stalks. To verify the possibility of the usefulness of these traits in lily breeding, nine combinations of interspecific crosses (*L. formosanum* as the seed parent; *L. auratum*, *L. speciosum*, *L. regale*, ‘Lollypop’, ‘Pink Tiger’, ‘Zaza’, ‘Le Reve’, ‘Marco Polo’, and ‘African Queen’ as pollen parents) were carried out with cut-style pollination and ovary-slice culture. Germination was observed in all nine interspecific crosses and 53 hybrids were obtained. Thirty (56.6%) of the 53 hybrids and two self-pollinated progenies of *L. formosanum* reached anthesis within 24 months from germination through ovary-slice culture. Multiple shooting of flower stalks was recognized in 11 (36.7%) of those flowered hybrids. Four hybrids, the pollen parents of which were Asiatic hybrid lilies with colored flowers, expressed ‘precocious flowering’ ability, multiple shooting of flower stalks, and entirely colored flowers simultaneously. These results suggest the possibility of breeding new types of cultivars with triple favorable traits from the cross between *L. formosanum* and Asiatic hybrid lilies with colored flowers.

Key Words: Breeding, *Lilium formosanum*, Multiple shooting of flower stalks, ‘Precocious flowering’.

Introduction

Most *Lilium* cultivars are commercially propagated vegetatively by ‘scaling’ (McRae, 1998), which reduces the effort and time required for flowering and rapid propagation of many clones. It usually takes two years for Oriental hybrid lilies propagated by scaling to achieve the size of a flowering plant (Enami, 2005). *Lilium* species take several years to start flowering from the time of seed sowing; for example, *L. auratum* Lindley, *L. speciosum* Thunb., and *L. rubellum* Baker take 4–5 years (Shimizu, 1971). *Lilium formosanum* Wallace, native to mainland Taiwan, exceptionally reaches anthesis within 12 months from the time of seed germination without temperature control during the entire cultivation (Hiramatsu et al., 2002). The strong ‘precocious flowering’ ability of this species is a highly attractive trait in lily cut flower production for obtaining flowers in a short cultivation period and for rapid rotation of the breeding cycle.

The ‘precocious flowering’ ability has been utilized in interspecific hybrid between *L. formosanum* × *L. longiflorum* Thunb. from 1951 (Shimizu, 1971). The hybrid cultivar is now taxonomically named *L. × formolongi* and it is mainly propagated by seeds to produce its cut flowers (Shimizu, 1971). Recently, many intersectional hybrids have been produced by crossing *L. × formolongi* with other species and their cultivars (Matsumoto et al., 1998; Mitsunaga et al., 1996, 2002; Okada et al., 1997; Okazaki et al., 1992). Their ‘precocious flowering’ ability, however, has not been definitely evaluated.

Another attractive trait of *L. formosanum* is the multiple sprouting of flower stalks (Inagaki, 2002; Shii, 1983). Although the trait is considered useful for developing novel lily cultivars, it has also never been used for extensive breeding. Thus, we attempted to obtain intersectional hybrids between *L. formosanum* and other *Lilium* species or cultivars to evaluate the heritability of the two above-mentioned traits of *L. formosanum* in hybrid progenies.

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* Corresponding author (E-mail: m-hirama@agr.kyushu-u.ac.jp).

Materials and Methods

Plant materials

Lilium formosanum (section *Leucolirion* 6b) grown in our experimental field was used as the female parent. *L. formosanum* (control), *L. auratum* (*Archelirion*), *L. speciosum* (*Archelirion*), *L. regale* Wilson (*Leucolirion* 6a), three Asiatic hybrids ('Lollypop', 'Pink Tiger', and 'Zaza'), two Oriental hybrids ('Le Reve' and 'Marco Polo'), and one trumpet hybrid ('African Queen') grown in plastic pots in a greenhouse were used as male parents. Their pollen was collected at anthesis and kept at -20°C .

Cut-style pollination and ovary slice culture

Cut-style pollination (Asano and Myodo, 1977; Van Tuyl et al., 1991) was performed in August 2002 and 2003. Ovaries were collected 15 days after cut-style pollination and surface sterilized as described by Obata et al. (2000). Ovary-slice culture (Hayashi et al., 1986; Kanoh et al., 1988) was then carried out. Ovaries were sliced into approximately 2-mm thick disks. Each disk contained an average of approximately 30 ovules (Hayashi et al., 1986). The sliced disks were placed horizontally on MS medium (Murashige and Skoog, 1962) in a test tube ($\phi = 18 \times 180$ mm). The MS medium was supplemented with $40 \text{ g}\cdot\text{L}^{-1}$ sucrose, $40 \text{ g}\cdot\text{L}^{-1}$ D-mannitol, and $2.5 \text{ g}\cdot\text{L}^{-1}$ gellan gum, and the pH was adjusted to 5.8 prior to autoclaving at 121°C for 20 min.

The explants were incubated under dark conditions at 25°C for four weeks and then placed at 25°C under illumination (16 h daylength, $45 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Ovule germination was recorded until eight months after the culture. The germinated ovules were detached from the placental tissue and subcultured in a test tube ($\phi = 18 \times 180$ mm) containing 15 mL of subculture medium comprising half-strength MS medium supplemented with $20 \text{ g}\cdot\text{L}^{-1}$ sucrose and $2.5 \text{ g}\cdot\text{L}^{-1}$ gellan gum at 25°C under illumination.

Hybrid confirmation

The produced plants were subjected to isozyme analysis to confirm their hybridity. Three enzymes, isocitrate dehydrogenase (IDH; E.C. 1.1.1.41), phosphoglucomutase (PGM; E.C. 2.7.5.1), and 6-phosphogluconate dehydrogenase (6-PGD; E.C. 1.1.1.43), were analyzed according to the method of Hiramatsu et al. (2001).

Growth performance and flower characteristics of acclimated plants

After subculturing, the plantlets were transplanted to plastic pots ($\phi = 9$ cm: one plantlet per pot) containing vermiculite and kept in a humid environment for acclimatization at 20°C . After 2–6 months of acclimatization culture, they were transplanted to plastic pots ($\phi = 15$ cm: one plantlet per pot) containing potting

medium (Redi-earth, Sun Gro Horticulture Canada Ltd., British Columbia, Canada) and grown in a greenhouse without controlling temperature, i.e., the minimum was 5°C in winter and the maximum was 43°C in summer. A liquid fertilizer (Hyponex, Hyponex Japan Co. Ltd., Osaka, Japan) was applied approximately once a month. The number of flowering plants, flower stalks, and days to flowering and flower color were recorded.

Pollen germination of flowered plants

Pollen was harvested at anthesis and stored at -20°C . Pollen fertility was estimated by a germination test performed on solid medium modified by Niimi and Yoshikawa (1992) as follows: 1.0% (w/v) agar, 10% (w/v) sucrose, and 0.1% (w/v) boric acid on Petri dishes. Pollen grains were scattered over the surface of the medium, and the Petri dishes were placed at 25°C for six hours in dark conditions. Germination was observed under an optical microscope (E800, Nikon Co., Tokyo, Japan). Pollen grains were considered to have germinated when their pollen tube length was longer than the diameter of the pollen grain. For each plant, 200 pollen grains were observed.

Results

Germination of cultured ovules was observed in all nine interspecific cross combinations, and the germination rate was in the range of 0.2–7.7% (Table 1). All 53 acclimated plants from interspecific crosses possessed both maternal and paternal allozyme marker bands of IDH, 6-PGD, and PGM.

Two of three self-pollinated *L. formosanum* progenies and 30 of 53 acclimated progenies bolted and reached anthesis during the second year of cultivation (Table 2). It took 651 (in *L. formosanum* $\times$ 'Pink Tiger') to 707 (in *L. formosanum* $\times$ *L. speciosum*) days from the time of pollination to anthesis. Eleven of the flowered progenies produced two or more flower stalks (Table 3). A maximum of four flower stalks was observed in *L. formosanum* $\times$ 'Pink Tiger' (Table 3).

Obtained plants expressed various flower shapes and perianth colors (Table 4). Bowl-shaped flowers were observed in interspecific crosses with *L. auratum*, 'Lollypop' and 'Pink Tiger'; on the other hand, trumpet-shaped flowers were observed with *L. speciosum*, *L. regale*, and 'African Queen'. Entirely colored flowers were induced in crosses with 'Lollypop' and 'Pink Tiger'. *L. formosanum* $\times$ 'Lollypop' plants produced pale reddish purple flowers with a yellowish cream brush mark toward the inner base of perianths (Fig. 1A). The cross combination of *L. formosanum* $\times$ 'Pink Tiger' produced two types of progenies; three had pink perianths with carmine spots (Fig. 1B); while seven had a white perianth with carmine spots (Fig. 1C). *L. formosanum* $\times$ *L. auratum* had white perianths with pink stripes along nectary furrows (Fig. 1D) and *L. formosanum* $\times$ *L. speciosum* showed white flowers

Table 1. Ovule germination in self-pollinated *Lilium formosanum* and nine interspecific cross combinations using *Lilium formosanum* as the female parent.

Pollen parent	Taxonomic group ^z	Number of ovules cultured ^y	Number of genimations	% of germination ^x
Control				
<i>L. formosanum</i>	<i>Leucolirion 6b</i>	900	65	7.2
Interspecific cross				
<i>L. auratum</i>	<i>Archelirion</i>	2190	74	3.4
<i>L. speciosum</i>	<i>Archelirion</i>	630	11	1.7
<i>L. regale</i>	<i>Leucolirion 6a</i>	300	6	2.0
'Lollypop'	Asiatic hybrid	1260	39	3.1
'Pink Tiger'	Asiatic hybrid	1560	120	7.7
'Zaza'	Asiatic hybrid	990	37	3.7
'Le Reve'	Oriental hybrid	720	17	2.4
'Marco Polo'	Oriental hybrid	300	4	1.3
'African Queen'	Trumpet hybrid	1050	2	0.2

^z According to Comber (1949).^y Number of ovules cultured = number of disks × 30 (Hayashi et al., 1986).^x % of germination = Number of germinated/cultured ovules × 100.**Table 2.** Growth performance of self-pollinated progenies of *Lilium formosanum* and hybrids from nine interspecific combination crosses using *L. formosanum* as the female parent.

Pollen parent	Number of plants acclimatized	Number of plants (%) flowering in the second year	Days from pollination to first anthesis (Ave. (max–min))	Number of plants		
				Flowered in the third year	Died in the third year	Died before flowering
Control						
<i>L. formosanum</i>	3	2 (66.7)	716.0 (712–720)	0	3	1
Interspecific cross						
<i>L. auratum</i>	15	9 (60.0)	659.3 (655–665)	0	15	6
<i>L. speciosum</i>	5	2 (40.0)	706.5 (706–707)	1	4	3
<i>L. regale</i>	3	3 (100)	656.7 (654–658)	0	3	0
'Lollypop'	3	2 (66.7)	668.5 (667–670)	0	2	1
'Pink Tiger'	15	10 (66.7)	663.3 (651–681)	2	11	3
'Zaza'	4	1 (25.0)	656	0	4	3
'Le Reve'	3	1 (33.3)	663	0	3	2
'Marco Polo'	3	1 (33.3)	666	0	2	2
'African Queen'	2	1 (50.0)	655	0	1	1
Total ^z	53	30 (55.6)	666.0 (651–707)	3	45	20

^z Self-pollinated progenies of *L. formosanum* were excluded.

with brushed pink toward the inner base (Fig. 1E). A pure white flower was observed in *L. formosanum* × *L. regale* (Fig. 1F) and white perianths with narrow pale purple stripes outside were observed in *L. formosanum* × 'African Queen' (Fig. 1G). All flowers obtained from *L. formosanum* × 'Zaza', *L. formosanum* × 'Le Reve', and *L. formosanum* × 'Marco Polo' were white, but their flowers developed abnormally and perianths were separated (data not shown).

Except for progenies from crosses with 'Le Reve', 'Marco Polo', and 'Zaza', most flowered progenies developed anthers normally and produced many pollen grains. No pollen germination on artificial medium was observed (data not shown). In the third year of cultivation, 48 of the acclimated plants (45 progenies

from the interspecific crosses and three of self-pollinated progenies of *L. formosanum*) produced shoots with malformed leaves and flowers, and they ultimately died (Table 2).

Discussion

Isozyme analysis suggests that plants obtained from the nine intersectional crosses were hybrids. To our knowledge, there are only three studies in which interspecific hybrids were produced using *L. formosanum*, i.e., *L. formosanum* × *L. longiflorum* (Shimizu, 1971), *L. formosanum* × *L. speciosum* (Asano, 1978), and *L. formosanum* × *L. japonicum* Thunb. (Inagaki and Otsuka, 2001). Hybrids from eight cross combinations are produced for the first time in the present study. This

result indicates that *L. formosanum* has a wide range of cross-compatibility as a female parent with regard to producing intersectional hybrids.

About 22–24 months after pollination, 30 (56.6%) of the 53 hybrids obtained in this study reached anthesis (Table 2), indicating that they reached anthesis within

Table 3. Multiple shooting of flower stalks in self-pollinated progenies of *Lilium formosanum* and hybrids from nine interspecific combination crosses using *L. formosanum* as the female parent.

Pollen parent	Number of plants tested ^z	Number of plants with indicated number of flower stalks			
		1	2	3	4
Control					
<i>L. formosanum</i>	2		2		
Interspecific cross					
<i>L. auratum</i>	9	6	3		
<i>L. speciosum</i>	2	1	1		
<i>L. regale</i>	3	2	1		
‘Lollypop’	2	1	1		
‘Pink Tiger’	10	6	2	1	1
‘Zaza’	1	1			
‘Le Reve’	1	1			
‘Marco Polo’	1	1			
‘African Queen’	1			1	
Total ^y	30	19	8	2	1

^z Reached anthesis in the second year after pollination.

^y Self-pollinated progenies of *L. formosanum* were excluded.

24 months after germination. Although *L. formosanum* progenies established from natural mature seeds normally reach anthesis within about 12 months after germination (Hiramatsu et al., 2002; McRae, 1998; Wilson, 1925), self-pollinated *L. formosanum* progenies derived from ovary-slice culture yielded similar results to the interspecific hybrids obtained in this study (Table 2). Anthesis in the second year could be attributed to difficulty in achieving maturation and the subsequent germination of extremely immature embryos aged only 15 days in the present experiment. It seems that the ‘precocious flowering’ ability of *L. formosanum* appeared dominantly, at least in hybrids that reached anthesis within 24 months after germination. Our method could be applicable to intersectional hybrids obtained using *L. × formolongi* as a female plant through embryo-rescue techniques that flowered in the second year of acclimatization (Fukai et al., 2002; Fukai and Tsuji, 2004; Suzuki and Shoji, 1992).

Lilium formosanum showed a maximum differentiation of 6.2 flower stalks within a year when grown outdoors (Inagaki, 2002). In most of the other species and commercial cultivars of *Lilium*, only one flower stalk emerges within a year (McRae, 1998). Thus, the expression of multiple shooting with more than two flower stalks in 11 (36.7%) of the flowered hybrids (Table 3) also indicates the inheritance of this trait from *L. formosanum*. Multiple shooting was also observed in ‘Li-9’, which is a hybrid between ‘Mona’ × *L. concolor* Salisb. var. *pulchellum* (Fisch.) Baker (Oomiya et al.,

Table 4. Flower shapes and perianth colors of pollen parents, self-pollinated progenies of *Lilium formosanum* and hybrids from nine interspecific combination crosses using *L. formosanum* as the female parent.

Pollen parent			Produced hybrid		
Species or cultivar	Flower shape	Perianth color	Flower shape	Perianth color	Number of hybrids obtained ^z
Control					
<i>L. formosanum</i>	Trumpet	White	Trumpet	White	2
Interspecific cross					
<i>L. auratum</i>	Bowl	White with golden-yellow central stripes and carmine spots and papillae	Bowl	White with pink stripes along nectary furrows	9
<i>L. speciosum</i>	Turk’s-cup	Pink to crimson with very undulate margins, sprinkled with carmine spots and papillae	Trumpet	White with brushed pink toward inner base	2
<i>L. regale</i>	Trumpet	White with a chrome-yellow throat inside, rose-purple outside	Trumpet	Pure white	3
‘Lollypop’	Bowl	White with bright cherry pink tips	Bowl	Pale reddish purple with white stripes	2
‘Pink Tiger’	Bowl	Pink with carmine spots	Bowl	Pink with carmine spots	3
‘Zaza’	Bowl	White with carmine spots	Bowl	White with carmine spots	7
‘Le Reve’	Bowl	Light-pink with carmine spots	Abnormal	White with carmine spots	1
‘Marco Polo’	Bowl	White infused with light pink	Abnormal	White	1
‘African Queen’	Trumpet	Brownish purple outside, yellow to apricot inside	Abnormal	White	1
			Trumpet	White with narrow pale purple stripes outside	1

^z Reached anthesis in the second year after pollination.

Fig. 1. Flowers of interspecific hybrids. (A) *L. formosanum* × 'Lollypop', (B, C) *L. formosanum* × 'Pink Tiger', (D) *L. formosanum* × *L. auratum*, (E) *L. formosanum* × *L. speciosum*, (F) *L. formosanum* × *L. regale*, (G) *L. formosanum* × 'African Queen'. Scale bars = 5 cm. Arrows indicate flower stalks.

2005); nevertheless, the factors controlling the trait have not been revealed. Convenient artificial methods for controlling the multiple shooting trait will allow its commercial promotion.

The five hybrids obtained in this study had colored flowers, four of which showed both 'precocious flowering' and multiple shooting. Hybrids with all three traits were observed in crosses of *L. formosanum* × Asiatic hybrids with colored flowers. Breeding new cultivars with these three favorable traits is possible by cross combinations of *L. formosanum* × Asiatic hybrids.

Intersectional hybrids appear to inherit not only favorable traits but also unfavorable traits from *L. formosanum*. In the third year of cultivation, 45 hybrids produced shoots with malformed leaves and flowers and some bulbs finally rotted (Table 2). This is quite similar to the growth features of *L. formosanum* after virus infection (McRae, 1998). Viral disease is a serious concern in the cultivation of *L. formosanum* (Hiramatsu et al., 2006; McRae, 1998) and species of section *Archelirion* (Shimizu, 1971). This is consistent with our result that growth abnormality was quite severe in intersectional hybrids between *L. formosanum* and *L. auratum* of section *Archelirion*.

To utilize the traits of 'precocious flowering', multiple shooting, and flower colors and to avoid virus-disease infection, the intersectional hybrids produced in this experiment should recover their sexual reproductive fertility and should be propagated by seeds. Many interspecific lily hybrids are sterile at the diploid level (Asano, 1978, 1980; Van Tuyl et al., 1986). F₁ sterility

is, however, restored at the tetraploid level (Asano, 1982; Van Tuyl et al., 1997). Induction of amphidiploids by chromosome doubling and crosses within or among induced amphidiploids may provide better recombinant types.

Therefore, in conclusion, breeding new hybrids with 'precocious flowering' ability, multiple shooting of flower stalks in *L. formosanum*, and with colored and bowl-shaped flowers like the paternal plant is possible; however, it is necessary to overcome fertility problems to supply the hybrids commercially by seeds.

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