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Interspecific hybridization and embryo rescue in breeding of lilies

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Abstract

This research investigates the hybridization of species *Lilium pumilum* Delile, *L. regale* Wils., *L. candidum* L., *L. monadelphum* Bieb., *L. martagon* L., *L. henryi* Baker. and cultivars of Asiatic, Trumpet and Oriental hybrids. To overcome fertilization barriers in incongruous crosses, cut-style pollination and pollination by mixed pollen were performed. The hybrid plants were obtained by *in vitro* cultivation of excised immature embryos. Cut-style pollination was efficient when Asiatic hybrids were pollinated by pollen of *L. pumilum*. Cut-style pollination and culture of excised embryo allowed receive few hybrids between Oriental hybrids and *L. henryi* or *L. regale*. Pollination of a female by a mixture of incompatible pollen was more efficient than cut-style pollination. Hybrid plants with random paternity were produced using the pollen mixture.

Key words: interspecific hybridization, pre-fertilization barriers, embryo rescue, *Lilium candidum*, *L. pumilum*, *L. monadelphum*, *L. regale*, *L. henryi*, *L. martagon*.

Introduction

The genus *Lilium* comprises more than 90 species. The involvement of wild species is promising for the production of novel cultivars (Van Tuyl et al. 1986; Van Tuyl et al. 2000).

The factors limiting interspecific crosses in *Lilium* can be separated into pre- and post-fertilization barriers (Van Tuyl et al. l991). To overcome pre-fertilization barriers, various pollination methods have been tested: applying a mixture of pollens from several species, cut-style, grafted-style, placenta pollination and *in vitro* ovule pollination (Asano, Myodo 1977; Van Tuyl et al. 1982; Van Tuyl Jet al. 1991; Chi 2000). Embryo rescue, ovary slicing and ovule culture have been used to attempt to overcome post-fertilization barriers (Chi 2002).

Asiatic hybrid lilies (AH) are important today in the flower trade. However, they lack fragrance and are sensitive to *Botrytis* blight. The Oriental hybrids (OH) are ornamental but sensitive to virus. *Lilium pumilum* Delile is a hardy early flowering species. *L. regale* Wils. and *L. henryi* Baker. are outstanding and extensively used for breeding of Trumpet hybrids (TH). *L. candidum* L. and *L. monadelphum* Bieb. are rarely employed in breeding, but they possess value due to early flowering and fragrance. *L. martagon* L. is rare used in crosses within distantly related species. The goal of this research was to involve the

gene pool of distantly related lilies in Asiatic hybrids in breeding, and to obtain hybrids between *L. martagon* and cultivars from the divisions AH, TH or OH.

Materials and methods

Ten cultivars and hybrids from division 1 (AH), eight cultivars from division 7 (HT), nine cultivars from division 8 (OH) and the species *Lilium candidum* L., *L. monadelphum* Bieb., *L. regale* Wils., *L. pumilum* Delile, *L. henryi* Baker., and *L. martagon* L. were used for crossing. Plants were grown in field and in greenhouse. To prevent self-pollination, flowers were emasculated before opening. For pollination, the pollen was applied on the stigma or on the surface of a cut by 10 % sucrose solution smear. After pollination the stigma was isolated with an aluminium foil cap. The embryos were isolated 30 days after pollination from swelled ovary and cultured *in vitro* until germination on solid Murashige and Skoog (1962) medium supplemented with 3 % sucrose at 25 °C in dark.

Results

The combinations of performed distant crosses and the respective number of different pairs are presented in the Table 1. No less than five flowers were pollinated in each cross. All crosses were performed in both reciprocal directions, except when the apomictic species *L. pumilum*, *L. monadelphum* and *L. regale* participated. These species were used only as males.

The crosses of two cultivars ('Toscana' and 'Bell Ami') from division AH with *L. pumilum* were promising when the style was cut to assist pollination. Seedpods consistently developed when pollen of *L. pumilum* was applied on the native stigma. Hybrid plants were obtained only in the case when embryos were isolated before full ripening of the seedpod. In these cases, 20 hybrids were obtained: 15 from 'Toscana' × *L. pumilum* and five from 'Bell Ami' × *L. pumilum*. Six crossing combinations between AH and *L. monadelphum* were successful. The pollination of AH with *L. monadelphum* by the cut-style technique did not improve fertilization. Three hybrids were obtained in crosses AH × *L. regale* – one from 'Tiger Babys' × *L. regale* and two from 'Lollypop' × *L. regale*. Ten hybrids were produced after crossing of *L. martagon* with AH cultivars 'Lollypop', 'Stones' and 'Toscana'. All of these successful crosses involved native uncut-style pollination. No hybrids developed in reciprocal crosses of AH with *L. candidum*, *L. henryi* and cultivars from divisions TH and OH, nor in crosses between *L. martagon* and TH or OH. The hybridization of cultivars from division OH with *L. henryi*, *L. regale* and TH allowed to obtain a few hybrids in the cases 'Sorbone' × *L. regale* and *L. henryi* × 'Acapulco' after cut-style pollination.

To achieve greater success in distant crosses, pollination was performed using mixtures of incongruous pollen. The amounts of different pollen in the mixture was equal. The results in some crosses were promising and hybrids with random paternity were obtained (Table 2).

Discussion

The failure to produce interspecific hybrids may be explained in most cases by

Table 1. Fertility of crosses between distantly related species and cultivars of lilies. *, in parentheses – number of different pairs used in each crossing combination. **, confidence intervals (95 %) are given in parentheses

Cross combination*	Frequency of swelled ovaries after pollination (%)**		Viability of embryos (%)**	Obtained hybrids
	Usual pollination	Cut-style pollination		
AH × *L. pumilum* (2)	0	90.0 (35.6 - 100.0)	56.6 (25.0 - 8.2)	20
AH × *L. monadelphum* (6)	0	0	-	-
AH × L. *regale* (6)	9.7 (0 - 33.3)	0	10.3 (0 - 16.6)	3
AH × *L. candidum* (7)	0	0	-	-
L. candidum × AH (7)	0	0	-	-
AH × *L. henryi* (5)	0	0	-	-
L henryi × AH (5)	0	0	-	-
AH × *L. martagon* (5)	0	0	-	-
L. martagon × AH (3)	80.0 (9.3 - 100.0)	0	38.5 (9.2 - 49.5)	10
AH × TH (6)	0	0	-	-
TH × AH (7)	0	0	-	-
AH × OH (9)	0	0	-	-
OH × AH (7)	0	0	-	-
OH × TH (4)	0	20.0 (0 - 30.5)	0	-
TH × OH (5)	0	12.0 (0 - 25.0)	0	-
OH × *L. regale* (3)	0	50.0 (24.5 - 100.0)	1.0 (0 - 2.0)	1
OH × *L. henryi* (3)	0	0	-	-
L. henryi × OH (3)	0	25.5 (0 - 55.5)	0.1 (0 - 10.1)	1
OH × *L. martagon* (5)	0	0	-	-
L. martagon × OH (5)	0	0	-	-
TH × *L. martagon* (5)	0	0	-	-
L. martagon × TH (5)	0	0	-	-

incompatibility of style and growing pollen. Cut-style pollination has been used to overcome incongruity in interspecific crosses of lilies (Asano, Myodo 1977; Van Tuyl et al. 1986; Van Tuyl et al. 1991; Van Creij et al. 1993; Van Tuyl et al. 2000). We found that cut-style pollination was most efficient in crosses AH × *L. pumilum*, which may

Table 2. Fertility of incongruous crosses in lilies by pollination of style with mixed pollen of incompatible partners. *, in parentheses – number of different pairs used in each crossing combination. **, confidence intervals (95 %) are given in parentheses

Cross combination*		Frequency of swelled ovaries after pollination (%)**	Viability of embryos (%)**	Obtained hybrids
Maternal plant	Pollen plants			
AH (3)	*L. regale*, *L. monadelphum*	0	-	-
AH (4)	*L. regale*, *L. candidum*	25.0 (0 - 25.5)	8.8 (0 - 10.0)	3
AH (2)	*L. regale*, *L. martagon*	12.0	-	-
AH (3)	*L. candidum*, *L. monadelphum*	12.2	- (0 - 32.0)	-
AH (3)	*L. candidum*, *L. henryi*	11.1 (0 - 33.3)	0	-
AH (4)	*L. candidum*, *L. martagon*	0	-	-
AH (2)	*L. monadelphum*, *L. henryi*	8.0 (0 - 42.3)	0	-
AH (2)	*L. monadelphum*, *L. henryi*	60.0 (9.3 - 70.5)	0 (22.0 - 68.1)	-
AH (2)	*L. regale*, *L. monadelphum* *L. candidum*	70.3 (18.0 - 100.0)	70.5 (41.3 - 99.9)	63
AH (2)	TH, OH	50.0 (0 - 98.5)	73.6 (11.1 - 85.5)	29
L. martagon (2)	TH, OH	45.5 (0 - 48.0)	26.7 (0 - 45.6)	11

be explained by a short stigma: the pollen tube of *L. pumilum* can not penetrate a long distance and fails to reach the ovules if a seed parent has longer style than the pollen parent. However, when pollen from a male with a long tube was used to pollinate by cut-style the efficiency of fertilization was low. According to Chi (2000) in cut-style pollination, the generative pollen cell divides into two sperm cell later than pollen tube penetration in to the ovule micropyle.

It is supposed that the more compatible pollen induces receptivity in the stigma, allowing the penetration of less compatible pollen. This method is a variant of the so called "mentor pollination method" when pollen of incongruous species is mixed with killed compatible pollen (Van Tuyl et al. 1982). The use of mixed incompatible pollen allowed to overcome pre-fertilization barriers in incongruous crosses (Table 2). The ancestry of hybrids received after pollination by incongruous pollen mixtures will be analysed.

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Ovule Culture and Embryo Rescue Facilitate Interspecific Hybridisation in Blueberry. About 80 blueberry (Vaccinium) accessions represent four species in New Zealand collections. They are highly heterogeneous and constitute a valuable resource as parental material for breeding.Â To increase the variation available to breeders, and to introgress characters from different species into parental breeding populations, interspecific, interploidy crosses were made. Ovules were cultured aseptically 14, 28 and 35 days after pollination. Embryos were dissected from the enlarged ovules and cultured on regeneration media. From fourteen of these crosses, more than 200 plants regenerated within 6-10 weeks. Here, we summarize major breeding goals, the role of an integrated method of cut-style pollination and fertilization followed by embryo rescue and mitotic and meiotic polyploidization involved in new assortment development. Both crops have been subjected to extensive interspecific hybridization followed by selection. Additionally, spontaneous polyploidization has played a role in their evolution. In lilies, there is a tendency to replace diploids with polyploid cultivars, whereas in tulip a majority of the cultivars that exist today are still diploid except for triploid Darwin hybrid tulips. Abstract: New FA (L. formolongi Ã— Asiatic 'A95-14'), FO (L. formolongi Ã— Oriental 'O54'), OH (Oriental 'Casa Blanca' Ã— L. henryi) and OA (Oriental 'Casa Blanca' Ã— Asiatic 'Sgl Pepper') hybrids were obtained through stigmatic, cut style pollination and embryo rescue in immature statues. The interspecific hybrids obtained were backcrossed and FAA (FA hybrid Ã— Asiatic 'A95-14') hybrid plants were obtained.Â 'OA 05-1' lily line has strong fragrance, upward-facing flower direction and shorter growing period. 'OH 02-1' has downward-facing flower direction, big flower size, no spot and vigorous growth.Â The pollen fertility of interspecific hybrid lily was recovered up to 40% in tetraploid level. Article - full text (enhanced PDF format, 845838 bytes).