

# Employment of species *Lilium pumilum* DC and *L. concolor* Salisb. for breeding of small flowering lilies

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## Annotation

Cut style pollination method was used to perform interspecific crosses between cultivars of lilies from division AH (Asiatic hybrids) and two small flowering species *Lilium pumilum* DC and *L. concolor* Salisb. Hybrid progeny were received by culture *in vitro* of immature embryos. Fertility of crossings AH  $\times$  *L. pumilum* was higher than in cases AH  $\times$  *L. concolor*. Interspecific hybrids of the first generation in both combinations phenotypically resembled male species *L. pumilum* or *L. concolor*. Male fertile interspecific hybrids were backcrossed with AH cultivars to receive second generation.

**Key words:** *Lilium concolor*, *Lilium pumilum*, interspecific hybridization

## INTRODUCTION

Asiatic hybrid (AH) lilies are important in the flower trade and are the most widely grown type of hybrid lily in gardens. This group of hybrid lilies is bred largely from the earlier flowering Asian species belonging to three phylogenetically related sections of genus *Lilium* – *Sinomartagon* Comber, 1949, *Sinolirium* Vrishcz, 1968 and *Pseudolirium* Wilson, 1925 (Baranova, 1990). Despite the fact that many species were involved in breeding of AH cultivars, the genetic resources of this group are not exhausted enough. Many of the new cultivars are created only for cut-flower and forcing industries under carefully controlled conditions and are traced to the same hybrid progenitor clones. It is one of the reasons why many new cultivars do not

survive when they are grown in garden. Returning to hybridization with true species should allow to establish hybrid vigor, staying power and disease resistance in new creating cultivars of AH lilies. By the other side such species as *Lilium pumilum*, *L. concolor* possess complex of useful traits such as tolerance to virus diseases, high adaptability to soil conditions, resistance to drought and frost (Mc Rae, 1998). The other interesting trait characteristic for these two species is a small size of flowers performed in long inflorescence. However, the number of AH cultivars originated with participation of *L. pumilum* and *L. concolor* is not big. The status of *L. concolor* in lily breeding was early thought to be important because it was believed to be the parent of *L. maculatum* Tunb. thus the head of family tree of the many AH cultivars. However, recently it was shown that *L. maculatum* is not hybrid species and has no genetic relationship with *L. concolor* (Dubouzet et al., 1999). Those cultivars which really traced to *L. concolor* mainly originated after involvement to hybridization with AH the interspecific hybrids between *L. pumilum* and *L. concolor* (Mc Rae, 1998). Both species are not easy to perform interspecific crosses with other lilies since they are reputed as apomictic and in crosses can be used only as male partner (Mc Rae, 1998). The incompatibility between female pistil and germinating pollen of *L. pumilum* or *L. concolor* is another obstacle to perform crossing with AH cultivars and other species.

The goal of this research was to receive hybrids between AH lilies and species *L. pumilum* and *L. concolor* to employ them in breeding of small flowering cultivars. Since sterility of interspecific hybrids is unpredictable and depends on crossing combination evaluation of fertility in hybrids were done and backcrosses with AH were performed.

## MATERIALS AND METHODS

Two species *Lilium pumilum* DC, *L. concolor* Salisb. and 10 cultivars of lilies from group Asiatic hybrids (AH) were used for crossing. The plants were grown in field and in greenhouse. One day before anthesis flowers were emasculated and next day pollinated. For cut style pollination, the pollen was applied on the surface of a cut style smeared

by water solution of 20 % sucrose and 0.02 % boric acid. The embryos were isolated 30 days after pollination from swelled ovary and cultured *in vitro* until germination on solid Murasige and Skoog medium (Murashige and Skoog, 1962) at 25 °C in dark (Van Tuyl, 1999). After germination plantlets were grown in light and later transplanted in pot with peat under greenhouse conditions.

## RESULTS AND DISCUSSION

Since *L. pumilum* and *L. concolor* are reputed as apomictic species they were used as male partners to perform interspecific crosses with lily cultivars belonging to group Asiatic hybrids (AH). However, any seed set was received when native style of AH female was pollinated by pollen of *L. pumilum* or *L. concolor*. Incompatibility between female style and male species pollen is common prezygotic isolation barrier during interspecific hybridization. The pollination after amputation of female style has been used to overcome incongruity in interspecific crosses of lilies (Asano et al., 1977; van Tuyl et al., 1986; van Tuyl et al., 1991; van Tuyl et al., 2000). Cut style pollination method allowed us to receive hybrid progeny in crosses AH × *L. pumilum* and AH × *L. concolor*. It tallies with the fact that pollen tube can not penetrate the long distance and fails to reach the ovules if a seed parent has longer style than the pollen parent. If the short stigma and pollen tubes are characteristic for species *L. pumilum* and *L. concolor* the removing of long female style does not prevent for sperms to reach ovules (Mc Rea, 1998). However, level of fertility in crosses performed with *L. pumilum* and *L. concolor* were distinct (Table 1). Crossings with *L. pumilum* were much more successful than with *L. concolor*. 6 crossing combinations were performed where AH cultivars (*Toscana*, *Bell Ami*, *Marrakech*, *Simphony*, *Golden Chalice*, and *Landini*) were pollinated by *L. pumilum* and all of them were more or less fertile, and gave from 0.1 to 8 viable embryos per pollinated flower. However, in cases when pollination was performed with *L. concolor* only two combinations (AH seedling JP-1 × *L. concolor* and *Disco* × *L. concolor*) from 11 were fertile. Totally it was received 75 interspecific hybrids between AH and *L. pumilum*

and 5 hybrids between AH and *L. concolor*. All F<sub>1</sub> hybrids of *L. pumilum* and *L. concolor* morphologically resembled males *L. pumilum* or *L. concolor*, however with distinct heterosis and grow more vigorous than progenitor species. Both species *L. pumilum* and *L. concolor* are short living and do not increase vegetative, however the hybrids inherited from AH cultivars long vitality and possibility to propagate vegetative. Despite that *L. pumilum* was crossed with cultivars possessing upright-facing flowers all F<sub>1</sub> hybrids inherited down-facing position of turk's-cup flowers characteristic of *L. pumilum*. Hybrids AH × *L. concolor* inherited upright-facing starry flowers characteristic of male species *L. concolor*. All F<sub>1</sub> hybrids of *L. pumilum* and *L. concolor* inherited small flowers and carotene pigmentation of petals. However, intensity of petal pigmentation in hybrids depends on female partner used in crosses – the progeny of AH cultivars which possessed white and lavender flowers inherited pastel apricot colored petals.

**Table 1.** Fertility of interspecific crosses of AH cultivars with *Lilium pumilum* and *L. concolor*

Female (AH cultivars)	Male <i>L. pumilum</i>			Male <i>L. concolor</i>		
	Amount of viable embryos per 1 pollinated flower		Amount of off-spring	Amount of viable embryos per 1 pollinated flower		Amount of off-spring
	By native style pollination	By cut style pollination		By native style pollination	By cut style pollination	
Marrakech	0	8.0 ± 2.5	25	0	0	0
Symphony	0	6.7 ± 3.6	28	0	0	0
Toscana	0	4.7 ± 2.5	15	0	0	0
Bell Ami	0	2.5 ± 1.5	5	0	0	0
Golden Chalice	0	0.1 ± 0.1	1	0	0	0
Landini	0	0.1 ± 0.1	1	0	0	0
Mont Blanc	-	-	-	0	0	0
Reinesse	-	-	-	0	0	0
Vostoc 2	-	-	-	0	0	0
AH seedling JP-1	-	-	-	0	0.1 ± 0.1	2
Disco	-	-	-	0	0.2 ± 0.2	3
Totally received offspring			75			5

Some F<sub>1</sub> hybrids possessed viable pollen and were backcrossed to AH. However, larger amount of viable embryos were received in cases when not native but cut style of AH females were pollinated by F<sub>1</sub> interspecific hybrids AH × *L. pumilum* or AH × *L. concolor*.

## CONCLUSIONS

1. Cut style pollination method allows perform interspecific hybridization between female cultivars of AH and small flowered species *L. pumilum* and *L. concolor*.

2. Species *L. concolor* is more recalcitrant for crossing with AH cultivars than *L. pumilum*.

3. The complex of traits characteristic of species *L. pumilum* and *L. concolor* dominates in F<sub>1</sub> interspecific hybrids AH × *L. pumilum* and AH × *L. concolor*.

4. Inerspecific hybrids AH × *L. pumilum* and AH × *L. concolor* are male fertile and may be backcrossed to AH cultivars.

## REFERENCES

- ASANO, Y.; MYODO, H. 1977. Studies on crosses between distantly related species of lilies I. For the intrastylar pollination technique. *Journal of Japanese society for horticultural science*, vol. 46, p. 59–65.
- BARANOVA, M. V. 1990. *Lilii*. Leningrad, 384 p. ISBN 5-10-000140-2.
- DUBOUZET, J.; SHINODA, K. 1999. ITS DNA sequence relationships between *L. concolor* Salisb., *L. dauricum* Ker-Gawl. and their putative hybrid *L. maculatum* Tunb. *Theoretical and applied genetics*, vol. 98, p. 213–218.
- MCRAE, E. A. 1998. *Lilies. A guide for growers and collectors*. Portland, Oregon, 392 p. ISBN 0-88192-410-5.
- MURASIGE, T.; SKOOG, F. 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, vol. 15, p. 473–497.
- VAN TUYL, J.; M., FRANCEN, J.; JONGERIUS, R. C.; LOCK, C. A. M.; KWAKKENBOS, T. A. M. 1986. Interspecific hybridization in *Lilium*. *Acta Horticulturae*, vol. 177, p. 591–595.

VAN TUYL, J. M.; VAN DIJK, A.; CHI, H. S.; LIM, K. B.; VILLEMOES, S.; VAN KRONENBURG, B. 2000. Breakthroughs in inter-specific hybridization of lily. *Acta horticulturae*, vol. 508, p. 83–90.

VAN TUYL, J.; VAN DIEN, M. P.; VAN CRIEJ, M. G. M.; KLEINWEE, T. C. M.; FRANKEN, J.; BINO, R. J. 1991. Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in inter-specific *Lilium* crosses. *Plant science*, vol. 74, p. 115–126.

## RŪŠIŲ *LILIUM PUMILUM* DC IR *L. CONCOLOR* SALISB. PANAUDOJIMAS SMULKIAŽIEDŽIŲ LELIJŲ SELEKCIJOJE

### S a n t r a u k a

F<sub>1</sub> hibridai tarp azijinių hibridų grupės lelijų veislių (AH) ir dviejų smulkiažiedžių rūšių *Lilium pumilum* DC ir *L. concolor* Salisb. *Lilium pumilum* DC buvo gauti tik tada, kai motininių AH augalų žiedai buvo apdulkinami, pašalinus piestelės liemenėlę, o izoliuoti hibridiniai gemalai buvo auginami *in vitro* kultūroje. Kryžminimo kombinacijose AH × *L. pumilum* buvo gauta daugiau palikuonių negu AH × *L. concolor*. Pirmosios kartos tarprūšiniai hibridai panašūs į tėvines formas – *L. pumilum* arba *L. concolor*, tačiau jie vešlesni ir gerai dauginasi vegetatyviniu būdu. Kadangi hibridų žiedadulkės gyvybingos, jomis buvo apdulintos AH veislės, ir gauta antroji hibridų karta.

**Raktiniai žodžiai:** *Lilium concolor*, *Lilium pumilum*, tarprūšinė hibridizacija.