BREAKTHROUGHS IN INTERSPECIFIC HYBRIDIZATION OF LILY

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Abstract

In order to introduce new characters as resistances, flower shape and colour, from wild species into the cultivar assortment of lily it is necessary to overcome interspecific crossing barriers. Several techniques, as the cut-style method, the grafted-style method and the in vitro isolated ovule pollination technique, have been developed to overcome pre-fertilization barriers. Post-fertilization barriers can be circumvented by in vitro pollination and/or rescue methods as embryo, ovary-slice and ovule culture.

Using these techniques wide interspecific lily crosses with species and cultivars from the different sections of the genus *Lilium* (*L. longiflorum, L. henryi, L. canadense, L. pardalinum, L. concolor, L. dauricum, L. candidum, L. rubellum, L. martagon*, Asiatic and Oriental hybrids) could be made. Hybrids originating from intersectional crosses (e.g. *L. longiflorum x L. concolor, L. longiflorum x L. dauricum, L. longiflorum x L. henryi, L. longiflorum x L. martagon, L. longiflorum x L. candidum, L. longiflorum* x Asiatic hybrids (LA), *L. longiflorum* x Oriental hybrids (LO), *L. longiflorum x L. rubellum, L. longiflorum x L. canadense*, Oriental x *L. pardalinum*, Oriental x Asiatic hybrids (OA) and *L. henryi x L. candidum*) have been produced. Especially the Oriental x Asiatic hybrids are a break-through in lily breeding and a promise for the future.

In general wide interspecific lily hybrids show F1-sterility. Using chromosome doubling techniques tetraploids with restored fertility are produced from these diploid hybrids. Introgression of characters of in interspecific hybrids into the next generations is studied using *in situ* hybridization techniques (GISH).

1. Introduction

In order to introduce new characters as resistances, flower shape and colour, from wild species into the cultivar assortment of lily it is necessary to overcome interspecific crossing barriers. Several techniques, as the cut style method, the grafted-style method and the in vitro isolated ovule pollination technique, have been developed to overcome pre-fertilization barriers (Asano & Myodo, 1977ab; Van Tuyl *et al.*, 1991). Post-fertilization barriers can be circumvented by in vitro pollination and/or rescue methods as embryo, ovary-slice and ovule culture (Asano, 1980; Van Tuyl *et al.*, 1991; Okazaki *et al.*, 1994).

In cooperation with eleven Dutch breeding companies crossing barriers especially between the Oriental and Asiatic hybrids are being investigated. In general wide interspecific lily hybrids show F1-sterility. Using chromosome doubling techniques tetraploids with restored fertility are produced from these diploid hybrids (Van Tuyl *et al.*, 1992; Van Tuyl, 1993). At this moment a crossing programme at polyploid level with these hybrids is been carried out. Some recent results are described.

2. Plant material

Lilium hybrids and species which were used, originated from the CPRO-DLO collection and are derived from different sections (between brackets) of the genus *Lilium*: *L. longiflorum* (Leucolirion), *L. henryi* (Leucolirion or Archelirion), *L. canadense* (Pseudolirium), *L. bulbiferum, L. dauricum* (Sinomartagon), *L. candidum* (Lilium), *L. rubellum* (Archelirion), *L. martagon* (Martagon), Asiatic hybrids (Sinomartagon) and Oriental hybrids (Archelirion).

A part of the hybrid plants produced at CPRO-DLO and used for chromosome doubling are described in earlier papers (Van Creij *et al.*, 1993; Van Tuyl *et al.*, 1991).

3. Methods

3.1. Pollination methods

The cut-style method (CSM) and the grafted style method (GSM) have been used most frequently. CSM: the style was cut with a razor blade 0-2 mm above the ovary, stigmatic fluid was applied followed by pollen. GSM: pollen was deposited on compatible stigma and after one day the style with germinating pollen was cut 1-2 mm above the ovary and attached to an ovary of the incongruent mother plant (Van Tuyl *et al.*, 1991).

3.2. *In vitro* methods

Ovary culture, ovule and embryo culture (Van Tuyl *et al.*, 1991) and chromosome doubling (Van Tuyl *et al.*, 1992) are applied as described before.

3.3. Pollen tube penetration

Pollen tube penetration in the ovules: 5-10 days after pollination ovaries are used. One or two carpels with the ovules attached to the placenta are destained in a mixture of water, glycerol and lactic acid (1:2:1) and subsequently stained in a solution of 1% aniline blue in the same mixture followed by destaining. Penetration was observed using a light microscope (Janson *et al.*, 1994).

4. Results

In Figure 1 a crossing polygon is presented of the genus *Lilium* All successful crosses between different sections of the genus *Lilium* obtained at CPRO-DLO are included. In this figure the connection between the Asiatic, Aurelian and Oriental hybrid groups (ellipses) are shown by dotted lines. In successful crosses between species (small circles) of different sections (large circles) the arrow point towards the female parent. In most cases *L. longiflorum* has been used successfully as female parent.

The hybrids, obtained after intersectional crosses, are produced by using the cut-style method in combination with embryo culture or with ovary-slice and ovule culture. It has not been proved that by using the grafted style method other combinations succeeded than after using the cut style method, but the number of hybrid embryos obtained per ovary increased, in some combinations, substantially with the GSM-method. However, a problem of the grafted style method is that the pollen tubes often are not able to enter the style of the mother ovary because of the inadequate attachment of the styles.

In this study reciprocal differences in crossing barriers are demonstrated. In the crosses with *L. longiflorum* this species was only successful when used as mother. Especially the Oriental x Asiatic hybrids, a combination of the two commercially important lily groups, are a break-through in lily breeding and a promise for the future. This combination appeared to be more difficult than other crosses. The percentage of hybrids per pollinated flower appeared to be very low and genotype dependent.

From several interspecific combinations flowering plants have been obtained. The number of obtained plantlets and the number of these plants which flowered are listed in

Table 1. The plants, especially the OA-hybrids, have not all flowered yet, so the number of flowering plants will increase.

The reported hybrids are partly described by others before (Asano, 1980, Okazaki *et al.*, 1994; Van Tuyl *et al.*, 1997). The crosses between Oriental hybrids x *L. pardalinum* and Oriental x *L. hansonii* have not been reported before. Verification of hybrids, in an early stage when visual observation is not yet possible, was carried out using flow cytometry (Van Tuyl & Boon, 1997).

Restoration of F1-sterility by doubling the number of chromosomes was performed successfully in the following crosses *L. henryi x L. candidum*, *L. longiflorum x* Asiatic hybrids, *L. longiflorum x L. candidum*, *L. longiflorum* x *L. concolor*, *L. longiflorum x L. henryi*, *L. longiflorum x L. rubellum* and *L. longiflorum x L. dauricum*. Using these tetraploids backcrossings were performed on Asiatic and Oriental hybrids and *L. longiflorum*. An interesting backcross appeared to be *L. longiflorum x* (*L. longiflorum x L. rubellum*), resulting in a triploid population, showing all characters of a pink longiflorum. The characters were intermediate between *L. longiflorum* and *L. rubellum* (Table 2, Fig. 2). All seedlings were at least 2 weeks earlier in forcing than *L. longiflorum* had a pink flower colour and had a broad range in plant length (Fig. 2).

For the near future tetraploid *Lilium*-hybrids originated from a range of different genotypes (*L. longiflorum*, *L. henryi*, *L. rubellum*, Asiatic hybrids, Oriental hybrids), which could not be combined up till now, open complete new and promising possibilities for innovating the lily assortment.

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Table 1 - Total number of *in vitro* plantlets and the number of plants which produced flowering plants obtained from several interspecific cross combinations, using ovary-slice culture and other embryo rescue techniques.

SUCCESSFUL COMBINATIONS	NUMBER	
	plantlets	flowering
L. longiflorum x Asiatic hybrid	>200	>150
L. longiflorum x L. candidum	>100	28
L. longiflorum x L. concolor	21	8
L. longiflorum x L. henryi	16	15
L. longiflorum x L. dauricum	>100	56
L. longiflorum x L. rubellum	5	4
L. longiflorum x L. bulbiferum	3	2
L. longiflorum x L. martagon	1	1
L. longiflorum x L. canadense	1	1
L. henryi x L. candidum	3	2
L. longiflorum x Oriental hybrid	25	15
Oriental hybrid x *L. pardalinum*	15	1
Oriental hybrid x *L. hansonii*	10	1
Oriental x Asiatic hybrid	> 500	

Table 2 - Plant height, flower length and forcing time of *Lilium longiflorum* 'Gelria' and 'Snow Queen' compared with the average of the triploid *L. longiflorum x (L. longiflorum x L. rubellum)* (LLR) population and the tetraploid *L. longiflorum x L. rubellum)* (LR) population in a forcing experiment with bulbs of size 12/14 cm.

	Plant height cm	Flower length Cm	Forcing time days
'Gelria'	94.0	15.9	96.7
'Snow Queen'	116.3	18.2	96.7
LLR	79.4	15.9	75.2
LR	47.9	11.2	51.7

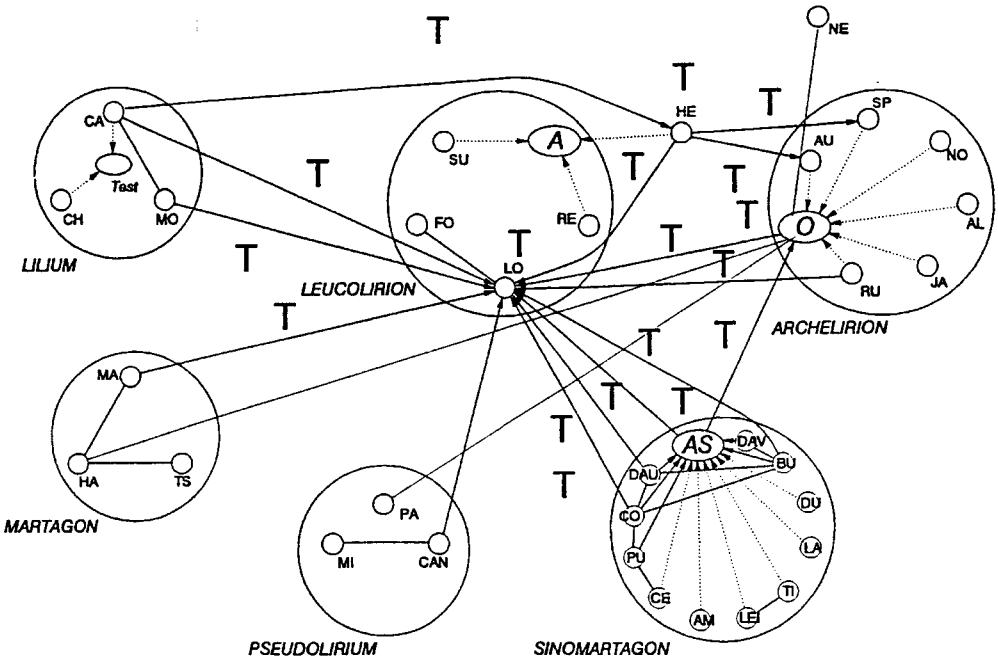

Figure 1 - A crossing polygon of the genus *Lilium* included all successful crosses of genotypes between different sections of the genus *Lilium* developed at CPRO-DLO. In this figure the connection between the Asiatic, Aurelian and Oriental hybrid groups (large ellipses) are shown by dotted lines. In successful crosses between species (small circles) of different sections (large circles) the arrow point towards the female parent.

Abbreviations: A: Aurelian hybrids; AL: *L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; BU: *L. bulbiferum*; CA: *L. candidum*; CAN: *L. canadense*; CE: *L. cernuum*; CH: *L. chalcedonicum*; CO: *L. concolor*; DAU: *L. dauricum*; DAV: *L. davidii*; DU: *L. duchartrei*; FO: *L. formosanum*; HA: *L. hansonii*; HE: *L. henryi*; JA: *L. japonicum*; LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; MA: *L. martagon*; MI: *L. michiganense*; MO: *L. monadelphum*; NO: *L. nobilissimum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE: *L. regale*; RU: *L. rubellum*; SP: *L. speciosum*; SU: *L. sulphureum*; TI: *L. tigrinum*; TS: *L. tsingtauense*; Test: *L. x testaceum*.

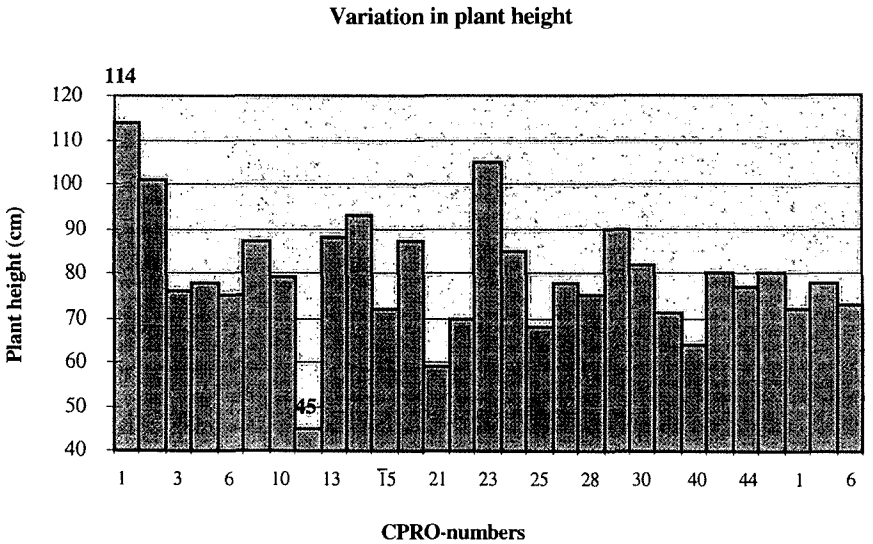

Figure 2 - Variation in plant height in the triploid *L. longiflorum x* (*L. longiflorum x L. rubellum)* population.