INTERSPECIFIC LILY HYBRIDS: A PROMISE FOR THE FUTURE

J.M. van Tuyl, H.S. Chi, B.C.M. van Kronenburg and B. Meijer
DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO)
Department of Ornamental Crops
Droevendaalsesteeg 1, P.O. Box 16
6700 AA Wageningen
The Netherlands

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Abstract

In order to introduce new characters such 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 have been used for wide interspecific lily crosses with species and cultivars from the different sections of the genus *Lilium* (*L. longiflorum, L. henryi, L. canadense L. concolor, L. dauricum, L. candidum, L. rubellum, L. martagon*, Asiatic and Oriental hybrids). 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 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. At this moment a crossing programme at polyploid level with these hybrids is being carried out.

1. Introduction

In order to introduce new characters such 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; Janson *et al*, 1993; Willemse *et al.* 1995). 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.* 1992, 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 being carried out.

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).

Some of the hybrid plants produced at CPRO-DLO and used for chromosome doubling have been 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 stigmas 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) were applied as previously described.

3.3 Pollen tube penetration

Pollen tube penetration into the ovules was observed 5-10 days after pollination. One or two carpels with the ovules attached to the placenta were 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.

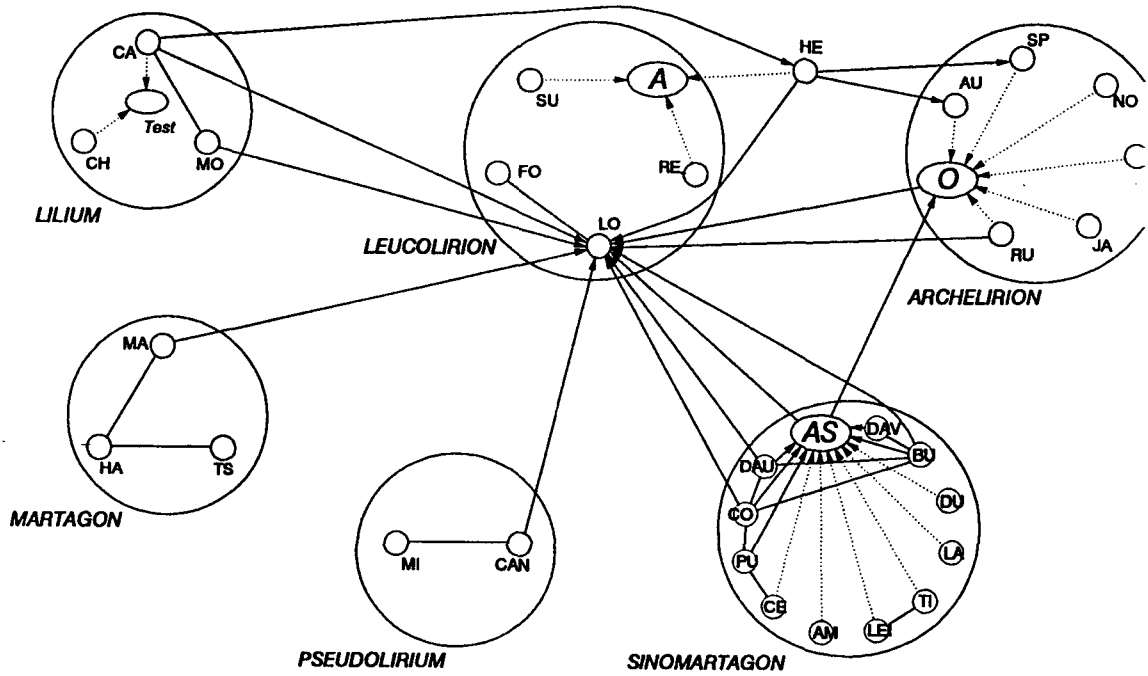

Fig. 1: A crossing polygon of the genus *Lilium* included all successful crosses of genotypes between different sections of the genus *Lilium* developed at CPRO-DLO. In this figure the connection between the Asiatic, Aurelian and Oriental hybrid groups (large ellipses) are shown by dotted lines. In successful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

In successful crosses between species (small circles) of different sections (large circles) the arrow points towards the female parent. In most cases *L. longiflorum* has been used successfully as female parent.

The hybrids, obtained after intersectional crosses, were 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 than those obtained by using the cut style method actually succeeded, but in some combinations, the number of hybrid embryos obtained per ovary substantially increased with the GSM-method. However, a problem of the grafted style method is that the pollen tubes are often 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 were demonstrated. In 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. In Table 1 the percentage ingrowth of pollen of one Asiatic father into 8 Oriental mothers are compared with the percentage hybrid plants (number of hybrids/number of pollinated flowers) obtained after cut style pollination and application of different embryo rescue methods of in total 228 ovaries. It can be concluded from this data that a high percentage of ingrowth is correlated with a higher number of hybrid plants.

From several interspecific combinations flowering plants have been obtained. The number of plantlets obtained and the number of these plants which flowered are listed in Table 2. The plants, especially the OA-hybrids, have not all flowered yet, so the number of flowering plants will increase.

The reported hybrids have partly been described by others (Asano 1980, Okazaki *et al.* 1994). The crosses between the species *L. longiflorum* x *L. martagon* var. *cattaniae*, *L. longiflorum* x *L. monadelphum*, *L. longiflorum* x *L. canadense* and *L. henryi* x *L. candidum* have not been reported before. Verification of hybrids, at an early stage when visual observation is not yet possible, was carried out by using flow cytometry (Van Tuyl & Boon, 1996).

Restoration of F1-fertility by doubling the number of chromosomes was performed successfully in *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* and L. *longiflorum x L. dauricum* using oryzalin. Using these tetraploids, backcrossings were performed on Asiatic and Oriental hybrids.

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

Table 1: The percentage pollen tube penetration of one Asiatic father into 8 Oriental mothers and the percentage of hybrids (number of OA-hybrids per 100 pollinated flowers) obtained from this cross combination.

Oriental mother	% penetration	% hybrids
No 27	23	24
No 61	21	9
No 40	16	26
No 37	7	0
No 64	6	0
No 41	5	10
No 29	5	0
No 23	4	3

Table 2: 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	>100	>100
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. bulbiferum	3	2
L. longiflorum x L. martagon	1	1
L. longiflorum x L. canadense	1	-
L. henryi x L. candidum	3	2
L. longiflorum x Oriental hybrid	25	2
Oriental x Asiatic hybrid	>100	-

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