Introgression in Interspecific Hybrids of Lily

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Abstract

In order to introduce new desirable characters into the cultivar assortment of lily a range of interspecific crossing barriers has to be overcome. By using various pollination and embryo rescue techniques pre- and postfertilization barriers were overcome and a range of wide interspecific lily hybrids between species and cultivars from the different sections of the genus *Lilium* could be made. Important breakthroughs include the development of the LA- (*L. longiflorum* ´ Asiatic hybrids), the LO- (*L. longiflorum* ´ Oriental hybrids) and the OA- (Oriental ´ Asiatic hybrids) hybrids. In general wide interspecific lily hybrids show F_1-sterility. Using somatic chromosome doubling techniques (mitotic polyploidization) tetraploids with restored fertility can be produced from these diploid hybrids. An alternative method is the use of unreduced (or 2n) gametes (meiotic polyploidization), which are rarely found in some hybrids. Introgression of alien chromosome segments from donor species into recipient cultivar through backcrossing of F_1 hybrid was studied using in situ hybridization techniques (GISH). Mitotic polyploidization showed no homoeologous recombinations between the parental genomes whereas meiotic polyploidization detected many. The use of 2n-gametes seems to be the most promising way for the introgression of desirable characters.

INTRODUCTION

Interspecific crossing barriers need to be overcome in order to introduce new characters such as resistances, flower shape and colour, from wild species into the cultivar assortment of lily. Lily appeared to be a suitable model plant for studying interspecific crossing barriers. For overcoming pre- and post-fertilization barriers a range of techniques have been developed. Barriers that occur before fertilization, such as inhibition of pollentube growth in the style can be overcome by using pollination techniques, as the cut style method, the grafted-style method and the in vitro isolated ovule pollination technique (Asano & Myodo, 1977ab; Van Tuyl et al., 1991).

Post-fertilization barriers can occur during stages of hybridization (e.g. firstly during the development of the hybrid embryo) and 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). A second type of post-fertilization barrier can be found after raising the hybrid, manifesting itself in interspecific hybrids that show F_1-sterility. Using mitotic (chromosome doubling) and meiotic polyploidization techniques amphidiploids with restored fertility can be produced from these diploid hybrids (Van Tuyl et al., 1992; Van Tuyl, 1993). The third post-fertilization barrier is a lack of introgression, caused by absence of recombination in cases when amphidiploid interspecific hybrids are used. Genomic in situ hybridization techniques offer an insight into the parental genome composition and homologous recombination breakpoints (Karlov et al., 1999, Lim et al., 2000). This paper describes some recent results of our interspecific hybridization research and the possibilities of introgression.

Plant Material

Lilium hybrids and species which were used from the collection of Plant Research International (former CPRO-DLO) 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).

Three diploid (2n=2x=24) 2n-gametes producing interspecific hybrids (**LA**) of *L. longiflorum* (**L**) 'Gelria' × Asiatic hybrid (**A**) 'Whilito' as well as their backcross progenies (**ALA**) were used. All F_1-hybrids were known to produce fertile 2n-pollen ranging from 0.1% up to 15%. The Asiatic hybrid 'Montreux' was used to produce backcross progenies.

Because F_1-hybrids of *L. longiflorum* 'Gelria' × *L. rubellum* (**LR**) showed absolute sterility, mitotic polyploidization was performed by in vitro treatment of oryzalin. The selected amphidiploid (**LLRR**) plants resulting from artificial chromosome doubling were used as male parent in the back-cross with *L. longiflorum* 'Snow Queen' (2n=2x=24) and over a hundred BC_1 plants were obtained (Lim et al. 2000). BC_1 plants were selected and pollinated with tetraploid (**LLLL**) *L. longiflorum* to produce BC_2 individuals.

METHODS

Pollination Methods:

The cut-style method (CSM) and the grafted style method (GSM) have been most frequently used. 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).

In vitro methods: Ovary culture, ovule and embryo culture and chromosome doubling are applied as previously described (Van Tuyl et al., 1991, 1992).

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 de-stained 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 before being de-stained again. Penetration was observed using a light microscope (Janson et al., 1994).

Chromosome doubling: Mitotic chromosome doubling was carried out by treating in vitro bulb scales with 0.001% oryzalin and detecting by flowcytometric DNA-measurements (Van Tuyl et al. 1992; van Tuyl and Boon, 1997).

Chromosome preparation: Chromosomes were prepared by squashing fixed cells onto clean microscope slides. The method was modified from that of Karlov et al. (1999). Well-squashed preparations were dipped for a few seconds in liquid nitrogen and, after taking off the cover slip, dehydrated in a graded ethanol series (70, 95, and 100%) and air dried. The best slides were used for GISH-work.

DNA probes: Total genomic DNA was isolated from *in vitro* young leaves of *L. longiflorum* and labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation according to the manufacturers instructions. Sheared herring sperm DNA was used for block DNA instead of *L. rubellum* DNA as a counterpart of *L. longiflorum*.

In situ hybridization: Genomic *in situ* hybridization was performed according to Khrustaleva and Kik (1998) with some modifications. Chromosome preparations were pretreated with RNase A (100 µg/mL) and pepsin (5 µg/mL), fixed with 4% ρ-formaldehyde for 10 minutes, dehydrated in an ethanol series and air dried. The hybridization mixture contained 50% deionized formamide, 10% (w/v) sodium dextran sulfate, 2× SSC , 0.25% (w/v) SDS, 1.25-2.5 ng/µl genomic probe DNA, and 25 ng/µl herring sperm DNA. The mixtures were denatured for 10 min. at 70 ºC and then directly placed on ice for over 10 minutes. 44 µL of hybridization mixture was applied on each slide. Slides were denatured at 80 ºC for 10 minutes and hybridised for overnight at 37 ºC in a humid chamber. Slides were washed for 15 minutes in 2× SSC, for 30 minutes in 0.1× SSC at 42 ºC, followed by 5 minutes in buffer 1 (0.1

M Tris-Hcl with 0.15 M NaCl, pH 7.5). Digoxigenin-11-dUTP was detected with 20 µg/mL anti-Dig-FITC (fluorescein isothiocyanate; Boehringer Mannheim) and 20 µg/mL rabbit-anti-sheep-FITC. Biotin-16-dUTP was detected with 4 µg/mL streptavidin-Cy3, 10 µg/mL biotinylated-antistreptavidin, and 4 µg/mL streptavidin-Cy3. The preparations were counterstained with 1 µg/mL DAPI and 4 µg/mL PI (propidium iodide).

RESULTS AND DISCUSSION

In Figure 1 a simplified crossing polygon is presented of the genus *Lilium*, while a complete crossing polygon is published elsewhere (Van Tuyl et al. 2000). The most important crosses between *L. longiflorum* and the Asiatic hybrids and the Oriental hybrids are presented. The cross is unidirectional in case of the LA and the OA hybrids, but appears bi-directional in case of the LO-hybrids. More than 10.000 flowers were pollinated to produce these 3 groups of hybids. The LA-combination resulted in approximate of one hybrid per 2 pollinated flowers, the LO-combination one in four and the OA-combination one in ten. However, the percentage of hybrids per pollinated flower appeared to be genotype dependent in all cases.

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 proven that by using the grafted style method other combinations can succeed inproducing other combinations than after using the cut style method, but the number of hybrid embryos obtained per ovary substantially increased in some combinations with the GSM-method. The grafted style method's problem, however, is that the pollen tubes are often not able to enter the style of the mother ovary because of inadequate attachment of the styles.

Restoration of F_1-sterility by doubling the number of chromosomes was successfully performed 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*, *L. longiflorum x* Oriental hybrids, *L. longiflorum x L. dauricum* and Oriental x Asiatic hybrids. In all these F_1-hybrids (except the OA-hybrids) the restoration was found in most autodiploids. 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 that showed all characters of a pink longiflorum. The characteristics were intermediate between *L. longiflorum* and *L. rubellum*. These triploids were backcrossed to *L. longiflorum* for introgression of the pink colour. An investigation into the possible introgression of characteristics from one species to the cultivated one was performed in the **LR**-hybrids by in situ hybridization techniques. A total absence of homoeologous recombinations was demonstrated in all 9 hybrids (Fig. 2b) (Lim et al. 2000). There was, however, a segregation of chromosomes in the BC2 (**LLLR** 2n=42, 43, 44), in which 6, 7 or 8 chromosomes of *L. rubellum* were retained with three sets of *L. longiflorum* (Fig. 2c). In general it can be concluded that due to preferential pairing of the parental chromosomes (Fig. 2c) crossovers between parental chromosomes will not occur.

In case of the **LA**-hybrids, some fertility in a number of cases was found in the diploid F_1's, caused by 2n-gametes occurring in the pollen or pollen mother cells. When investigating this material using GISH-techniques, there was clear evidence for homoeologus chromosome association and recombination at metaphase I and anaphase I stages of microsporogenesis (Fig. 2d). The occurrence of such genetic recombination in **LA**-hybrids was also convincingly proven by the analysis of chromosome composition of BC_1 (**ALA**) and BC_2 progenies. (Fig. 2ef.) (Karlov et al. 1999; Lim et al. 2000). This recombination is highly important for introgression of specific characters from donor to recipient cultivars. It has been shown that by using 2n-gametes, as in **LA**-hybrids, sexual polyploid progenies can be produced with the addition of complete alien chromosomes as well as recombinant chromosome segments of variable sizes.

In the near future polyploid *Lilium*-hybrids originated from a range of different genotypes (*L. longiflorum*, *L. henryi*, *L. rubellum*, Asiatic hybrids, Oriental hybrids), which previously could not be combined or recombined, will open up a whole range of new and promising possibilities for innovating the lily assortment.

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Figures

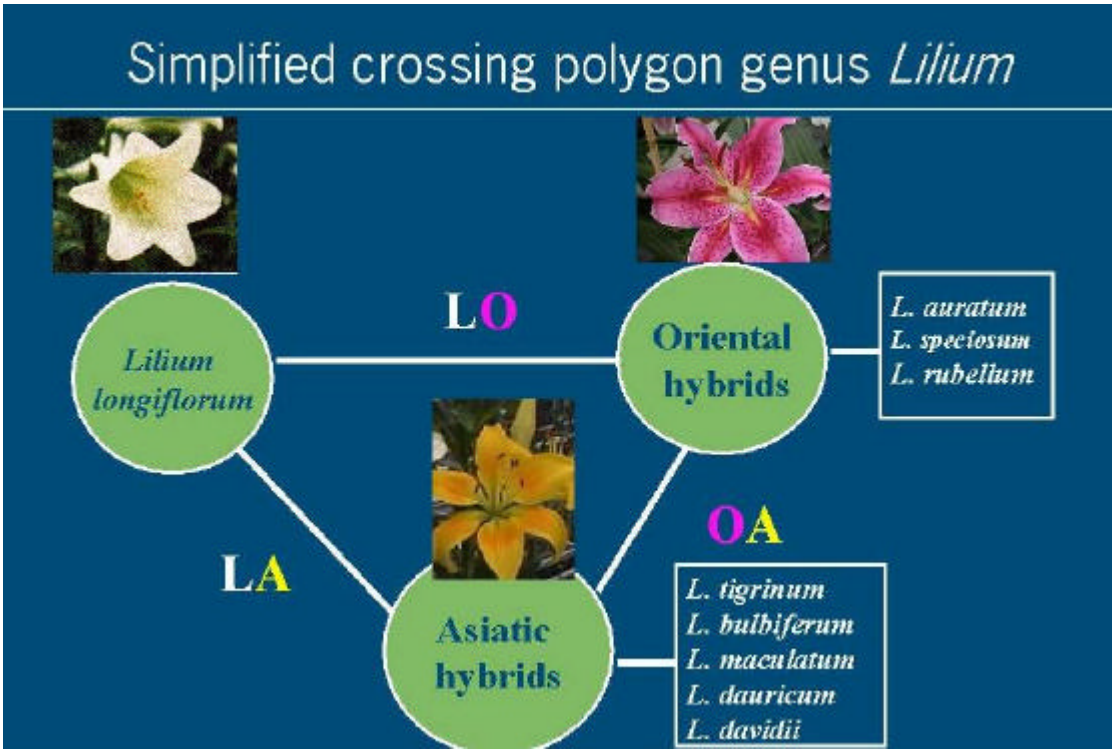

Fig. 1. A simplified crossing polygon of the most important lily hybrid groups (L=*L. longiflorum*, A=Asiatic, O=Oriental) in the genus *Lilium*, resulting in wide interspecific hybrids (**LA**, **LO** and **OA**-hybrids).

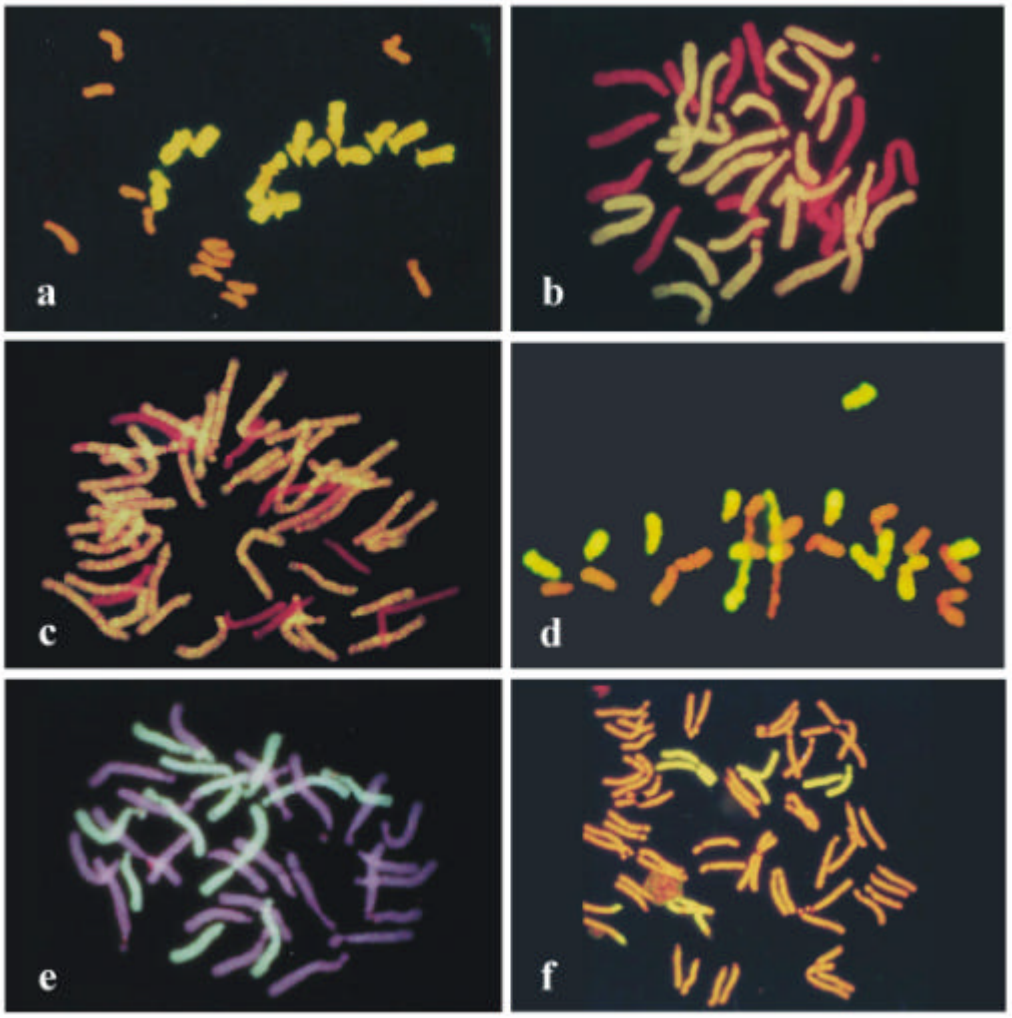

Fig. 2. Genomic in situ hybridization of mitotic chromosomes of the F_1, BC_1 and BC_2 plants of the **LR**- and the **LA**-hybrids. (a) The meiotic chromosomes of BC_1 (**LLR**) with 12 bivalents (yellow fluorescence) indicating *L. longiflorum* and 12 univalents (red fluorescence) representing *L. rubellum*. (b). 36 Chromosomes of the BC_1 plant 961003-27 without any recombinations. *L. longiflorum* (yellow fluorescence) and *L rubellum* (red fluorescence). (c) Aneuploid BC_2 plant 982211-27 from backcrossing of the BC_1 (**LLR**) to 4x *L. longiflorum* (**LLLR**). Thirty six (three sets) of *L. longiflorum* (yellow fluorescence) with 8 *L. rubellum* chromosomes (red fluorescence). (d) The meiotic chromosomes of LA 88542-52 in metaphase I with 2 bivalents indicating chromosome association of *L. longiflorum* (yellow) and Asiatic (red) (e). 36 Chromosomes of the **ALA** plant 921238-1 with three recombinations. *L. longiflorum* (yellow fluorescence) and *L rubellum* (blue fluorescence). (c) Aneuploid BC_2 plant 997139-2 (2n=30) from backcrossing of the BC_1 (**ALA**)-hybrids to diploid Asiatic. 5 chromosomes of *L. longiflorum* (green fluorescence) and 25 Asiatic chromosomes including 2 recombinant chromosomes.