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Homoeologous Recombination in Interspecific Hybrids of *Lilium*

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Abstract : Two different types of polyploid progenies were analyzed by genomic *in situ* hybridization (GISH) in order to estimate the extent of homoeologous recombination. One set of these had originated from a diploid F_1 hybrid between *Lilium longiflorum* × *L. rubellum* (**LR**, 2n=2x=24) followed by somatic chromosome doubling and crossing the tetraploid (**LLRR**, 2n=4x=48) to *L. longiflorum* (**LL**) as well as to the Oriental hybrid (**OO)**. These crosses gave rise to two types of triploids (2n=3x=36) of the genomic composition **LLR** and **OLR**, through the functioning of alloploid 2x-gametes, from **LLRR.** The GISH analysis revealed no homoeologous recombination in the case of **LLR**- and **OLR**-triploids. This could be explained by preferential pairing of homologous chromosomes in **LLRR** hybrids. The second sets of progenies were derived from unreduced (2n) gametes of the diploid F_1 hybrid between *L. longiflorum* × Asiatic hybrid (**LA**, 2n=2x=24). **ALA**-triploids and **AALA**-tetraploids showed considerable intergenomic recombination explainable by homoeologous pairing in **LA**-hybrid during the origin of 2n-gametes.

Key words : unreduced gametes, intergenomic recombination, genomic *in situ* hybridization (GISH), mitotic polyploidization, meiotic polyploidization

INTRODUCTION

The genus *Lilium* has been classified into seven sections with more than 80 species (Comber 1949). The species of the sections Leucolirion, Sinomartagon and Archelirion are the most important ones for lily breeding. The species of three sections have several valuable characters such as plant height, disease resistance, and flower color and size. Interspecific hybridization is inevitable in order to combine desirable characteristics into new cultivars. Interspecific hybrids of distantly related species are generally male and female sterile due to disturbed chromosome pairing during meiosis. One of the methods to overcome these barriers is by doubling the somatic chromosome number to produce fertile allotetraploids (Van Tuyl *et al*. 1992). However, it still has a major limitation although it restores fertility. In allotetraploids, homologous chromosomes pair preferentially during meiosis and no intergenomic recombination occurs between the alien chromosomes. This prevents introgression of species-specific monogenetic traits into a cultivar, during introduction of whole chromosomes in lily hybrids.

Spontaneously occurring unreduced (2n) gametes could be used alternatively to somatic chromosome doubling of F_1 hybrids. In fact, 2n-gametes occur in most plant species as well as in *Lilium* species hybrids (Harlan and de Wet 1975, Jena and Khush 1989, Van Tuyl 1990). Such gametes have been successfully used for producing sexual polyploids (Karlov *et al*. 1999, Lim *et al.* 2001). An advantage of 2n-gametes is that the F_1 hybrids can directly be used for further crossing. Additionally, intergenomic recombination is expected to occur between the alien genomes in the F_1 hybrid during meiosis. When introgression of specific traits is the aim, the use of 2n-gametes and sexual polyploidization is preferable as compared to mitotic chromosome doubling.

We studied the extent of intergenomic recombination in two different types of polyploid progenies derived from two interspecific *Lilium* hybrids. These included two diploid hybrids (2n=2x=24): *L. longiflorum* × *L. rubellum* (**LR**) and *L. longiflorum* × Asiatic hybrid (**LA**). The chromosome number of **LR** hybrid was doubled somatically to produce an allotetraploid (**LLRR**, 2n=4x=48) and backcrossed to *L. longiflorum* or crossed to the Oriental hybrid in order to obtain allotriploid (**LLR** and **OLR**, 2n=3x=36). In case of **LA** hybrids, 2n-gametes were used to produce allotriploid progeny (**ALA** and **OLA**, 2n=3x=36). These two types of triploid progenies were analyzed by genomic *in situ* hybridization (GISH) to determine the extent of intergenomic recombination.

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MATERIALS AND METHODS

Plant materials

Two species and one Asiatic hybrid (2n=2x=24) were used for producing two F_1 hybrids (genome composition is shown in parenthesis in each case): *Lilium longiflorum*, c.v. 'Gelria' (**LL**); *L. rubellum* (**RR**), and Asiatic hybrid 'Whilito' (**AA**). In one of the two F_1 hybrids, **LR** hybrids were somatically doubled (*L. longiflorum* × *L. rubellum* **LLRR**, 2n= 4x=48). The other one (**LA**) involved *L. longiflorum*, c.v. 'Gelria' × Asiatic hybrid, c.v. 'Whilito'. One of the backcrosses involved *L. longiflorum*, c.v. 'Snow Queen', resulting in triploid progeny (**LLR**, 2n=3x=36). **LLRR** was also crossed as male parent to Oriental hybrid (**OO**) 'Acapulco' in order to produce **OLR**-allotriploids. The other backcross involved the Asiatic hybrid × F_1, **LA**, which also produced triploid plants (**ALA**, 2n=3x=36) because of the functional 2n-gametes from **LA**-hybrid as pollen parent. In latter cross, three different diploid cultivars of Asiatic hybrids, viz., 'Montreux', 'Puccini' and 'Meribel', and accession number '78251' were used as female parents. In addition, the 2n-pollen producing **LA**-hybrid was also crossed as male parent to the Oriental hybrid 'Opus One' in order to obtain allotriploid (**OLA**) progeny. All plants were grown in a greenhouse at 17-20°C during day and 14-18°C during night. In all cases, embryos were rescued by *in vitro* rescue (Van Tuyl *et al*., 1991).

Chromosome preparation

Roots were kept overnight in saturated α-bromonaphtalene solution at 4°C and fixed in ethanol acetic acid (3:1) for at least 2 h. Root tips were treated with 0.3% pectolyase Y23, 0.3% cellulase RS and 0.3% cytohelicase in 10 mM citric acid buffer for about 1 h at 37°C, washed twice with water and squashed in 60% acetic acid. The slides were then frozen by dipping in liquid nitrogen before removal of cover slips by a razor blade. Then slides were dehydrated in absolute ethanol for a few minutes, dried and stored at -20°C until use.

DNA isolation and probe preparation

The methods for the isolation of genomic DNA, sonication, nick translation for labeling of probe DNA was as described by Lim *et al*. (2001).

In situ hybridization

In situ hybridization was carried out according to Lim *et al*. (2001). The hybridization mixture contained 2x SSC, 50 % formamide, 10% (W/V) sodium dextran sulfate, 0.25% SDS, 3.0 ng/μl of labelled *L. longiflorum* 'Snow Queen' DNA and 50-60 ng/μl herring sperm DNA. The hybridization mixture was heated at 70°C for 10 min and cooled on ice for about 10 min, 40 μl were dropped on each slide, denatured at 80°C for 10 min, and incubated overnight at 37°C in a humidified chamber. Digoxigenin labelled probe DNA was detected with the FITC-antidigoxigenin detection system (Boehringer Mannheim, Germany). Chromosomes were counterstained with DAPI (4,6-diaminido-2-phenylindole) and/or propidium iodide. Photographs were taken on ASA 400 color negative film.

RESULTS

Morphological observation of the F_1 and BC_1 progenies

In the F_1 interspecific hybrids (**LA** and **LR**) showed intermediate phenotypic characters of the parents. However, the BC_1 progenies (**ALA**) derived from 2n-gametes (**LA**) showed genetic segregation, for flower color, spots on flower and leaf shape, with respect to the characters of their parents. All **LLR**-hybrids (BC_1) were highly homogeneous and intermediate with respect to plant height, leaf-shape and flower color.

Meiosis and pollen fertility in LR and LA hybrids

The parental species of both **LR**- and **LA**-hybrids belong to different taxonomic sections, the chromosome pairing during microsporogenesis as well as pollen fertility were studied. In both hybrids the frequencies of bivalents and univalents varied in different pollen mother cells. The average number of bivalents per cell was 3.2 for **LR** (Fig. 1a) and 3.4 for **LA**-hybrid. Pollen of **LR**-hybrids was highly sterile. However, in the case of **LA**-hybrids viable 2n-pollen grains (5-30%) were present. Although the majority of the pollen mother cells had disturbed cell division, a considerable number of PMCs showed modified meiosis. In this case, after metaphase I, either all the 24 univalents or the univalents and half-bivalents were aligned within the equatorial plane and divided equationally (Fig. 1b). This modified meiotic cell division yielded unreduced pollen, the so-called first division restitution (FDR) gametes containing 24 chromatids without homoeologous recombination or also with recombinant chromatids due to cross-over between homoeologous chromosomes. An additional type of restitution occurred in

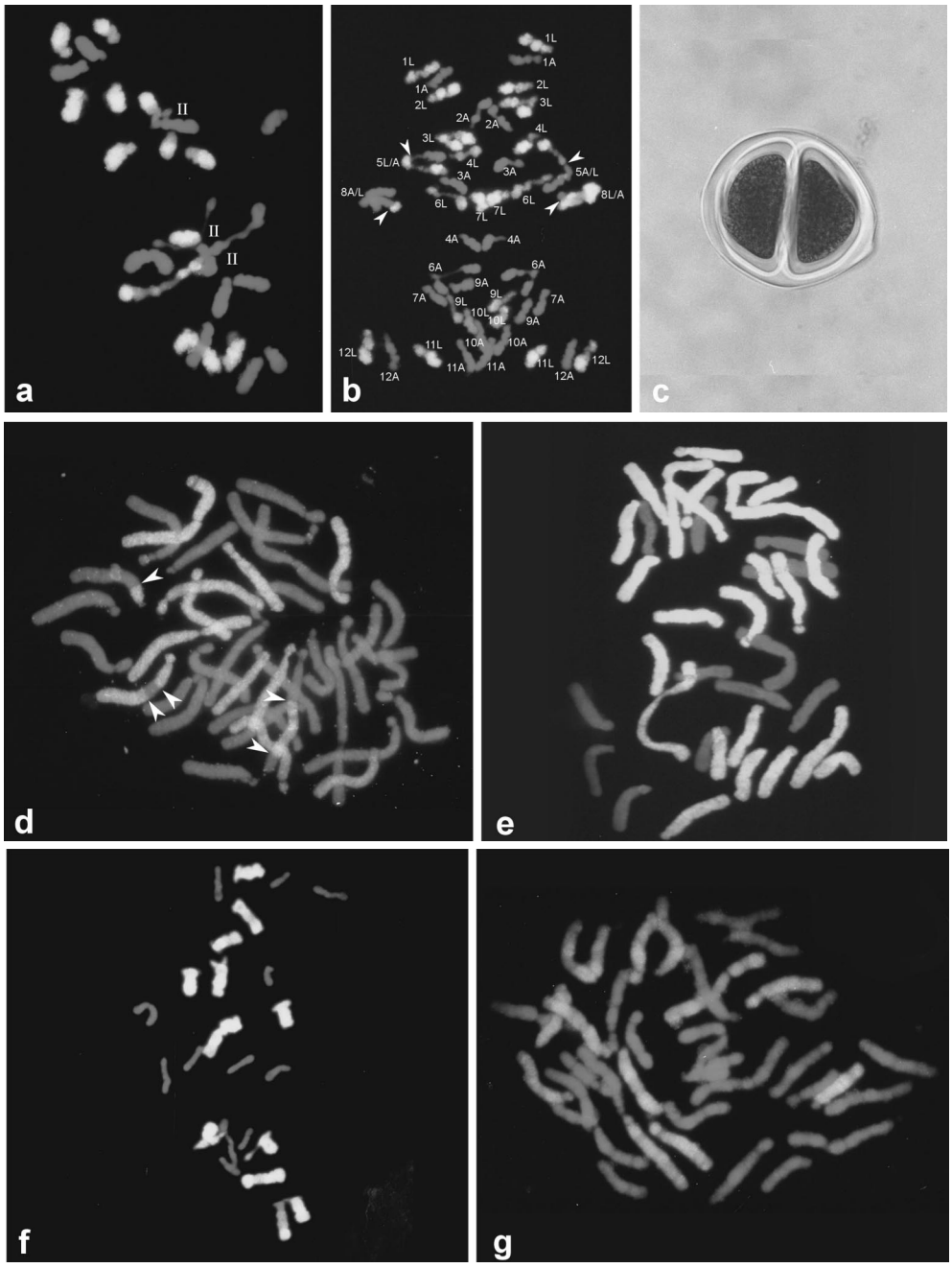

Fig. 1. Meiotic stages and mitotic chromosome complements of the F_1 **LR**- and **LA**-hybrids as well as further cross progenies, **ALA**, **LLR** and **OLR**. (**a**) Male metaphase I of a **LR**-hybrid after GISH. (**b**) A modified anaphase I stage of a **LA** hybrid showing equational division of univalents and reductional division of bivalents. Arrowheads indicate the breakpoint of homoeologous recombination between **L** and **A** genomes. (**c**) Dyads of a **LA**-hybrid that can lead to the formation of 2n-pollen. (**d**) Somatic chromosomes of an **ALA**-hybrid (BC_1) showing 24 **A** (dark) and 12 **L** chromosomes (white). Note the presence of three homoeologous recombinant segments (arrowheads). (**e**) Triploid of BC_1 (**LLR)** showing 24 **L** (white) and 12 **R** chromosomes (dark) without recombinant chromosome segments. (**f**) Metaphase I of an **LLR**-hybrid showing 12 bivalents of **L**-**L** (white) and 12 univalents of **R** (dark). (**g**) Mitotic chromosome complement of an **OLR**-hybrid with 24 chromosomes of **O** and **R** genomes (dark) and 12 chromosomes of the **L** genome (white). Because the genomic DNA of **L** genome was used as a probe, the other two, **O** and **R**, genomes (both of the section Archelirion) in the trigenomic hybrid could not be differentiated. Note the absence of homoeologous recombination between **L** and **R** chromosomes.

LA-hybrids. In this case, the centromeres of univalents divided equationally as in FDR whereas the bivalents disjoined as they usually do during anaphase I. This unique type of restitution has been called as Indeterminate Meiotic Restitution (IMR) (Lim *et al*. 2001). The genetic consequence of indeterminate meiotic restitution is different from other types of meiotic nuclear restitutions. FDR gametes possess both of parental chromatids (Mendiburu and Peloquin 1977). In contrast, SDR (second division restitution) gametes contain homologous chromatids from one of parents (Mok and Peloquin 1975, Ramanna 1979). However, the IMR gametes are considered in having heterologous and homologous chromatids depending on pairing at metaphase I.

Meiotic chromosome disjoining of the **LR**-hybrid at anaphase I was chaotic at the end of meiosis and tetrads were formed with unbalanced chromosome numbers. There was complete pollen sterility. The meiosis of **LLRR**-hybrids showed perfect pairing between homologous chromosomes and regular disjoining at anaphase I. At the end of meiosis, tetrads were formed with 2x-gametes.

Chromosome constitution of allopolyploid progenies

Twenty plants were analyzed by GISH including BC_1 from four different crosses of F_1 hybrids to check for homoeologous recombination (Table 1). The progenies were derived from crossing of 2n-pollen producing **LA**-hybrids as male parent to Asiatic hybrids and Oriental hybrids, and of 2x-pollen of the **LLRR**-hybrid crossed to *L. longiflorum* and Oriental hybrids. The progenies were mainly triploid (Table 1 and Fig. 1d-g) and in a few cases tetraploid with the expected genomic constitution. In the crosses **AA** × **LA** and **OO** × **LA**, 2n-pollen of **LA** was functional, and in the cross **AA** × **LA** occasionally also 2n-eggs were functional. Therefore, the triploid progeny **ALA** possessed 24 chromosomes of **A** genome (dark fluorescence) and 12 chromosomes of **L** genome (white fluorescence) (Fig. 1d). Two plants showed 48 chromosomes with 3 sets of the Asiatic genome and one of the *L. longiflorum* (**AA LA**). In addition, five out of 9 plants revealed homoeologous recombinations (Table 1 and Fig. 1d). Recombinant chromosomes were absent in all progenies derived from crosses with the somatically doubled **LLRR**-hybrid (Table 1, Figs. 1e-g). In this case meiotic metaphase I pairing was also analyzed to verify whether there was any pairing between **L**- and **R**-chromosomes in **LLR**-triploids. The two sets of **L**-genome chromosomes strictly paired forming 12 bivalents (Fig. 1f) while the chromosomes of the **R** genome remained as univalents (Fig. 1f). Thus, it was evident that homoeologous recombination did not occur either in **LLRR** amphidiploid or in the **LLR**-triploid BC_1.

DISCUSSION

It is evident from this investigation that sexual polyploidization is the most desirable approach if alien recombinant segments have to be added into the cultivars of lily. Previously, colchicine treatment was used to increase fertility of amphidiploid interspecific hybrids by somatic chromosome doubling. However, in most cases subsequent crosses could not be performed due to sterility. In contrast, numerous polyploid cultivars have been derived spontaneously from diploid F_1 and subsequent crosses in some horticultural crops (Bingham and Saunders 1974, Hahn *et al*. 1990, Ramanna 1992). During the last two decades, the value of 2n-gametes for sexual polyploidization has received considerable attention. However, the focus has been exclusively on autopolyploids such as potato, alfalfa and *Dactylis* (for reviews see Veilleux 1985, Mariani and Tavoletti 1992a,b, Bretagnolle and Thompson, 1995). Although sexual polyploidization is equally important to allopolyploids, relatively few reports exist in this context.

We have shown by GISH that intergenomic recombination occurs during formation of 2n-gametes in distantly related F_1 hybrids in *Lilium* species. These data are helpful to identify the species appropriate for intergenomic recombination.

We have used triploids successfully in crossing with both diploid and tetraploid genotypes and produced large numbers of aneuploid progenies (unpublished data). The proge-

Table 1. Homoeologous recombination observed in the progenies of **LA** and **LLRR** F_1-hybrids.

Type of crosses [z]	**AA** × **LA**	**OO** × **LA**	**LL** × **LLRR**	**OO** × **LLRR**
Genome constitution	**A LA** or **AA LA**	**O LA**	**L LR**	**O LR**
No. of genotypes analyzed	8	1	9	2
No. of genotypes with homoeologous recombinations	5	0	0	0

[z]**AA**=diploid Asiatic lily hybrid, **LA**=2n-gamete of the **LA**-hybrid, **OO**=Oriental lily hybrid, **LL**=*L. longiflorum*, **LLRR**=tetraploid **LR**-hybrid after mitotic chromosome doubling.

nies of somatically induced allopolyploids are phenotypically uniform for leaf shape, flower color and plant height, whereas progenies resulting from 2n-gametes of diploid hybrids showed considerable phenotypic variation for flower color. We attribute this variation to the segregation of alien recombinant segments as well as homoeologous chromosome assortment during the formation of different types of 2n FDR gametes. In view of genetic variability, sexual polyploid progenies derived from FDR gametes of dihaploids are potentially more useful for selecting desirable genotypes as compared to progenies derived from parents with mitotically doubled chromosome complements.

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