

## Meiotic polyploidization in five different interspecific *Lilium* hybrids

Jaap M. van Tuyl, Rodrigo Barba-Gonzalez, Alex A. van Silfhout,  
Ki-Byung Lim & M.S. Ramanna  
Plant Research International, Wageningen University and Research Centre,  
P.O. Box 16, 6700 AA Wageningen, The Netherlands  
Fax: +31 317 418094  
E-mail: jaap.vantuyl@wur.nl

### Abstract

With the aim of transferring important horticultural and disease resistance characters that are available in different *Lilium* species ( $2n=2x=24$ ) into the cultivars of lilies, an extensive interspecific hybridization program is in progress at The Plant Research International, Wageningen. In this context, we have made thousands of interspecific hybrids, investigated them for the occurrence of  $2n$  gametes, sexual polyploid progenies were produced and they were cytologically analyzed through genomic *in situ* hybridization (GISH). Out of the five different sets of interspecific hybrids that produce  $2n$ -gametes, the following four hybrids involved species that belonged to different taxonomic sections: *L. longiflorum* x Asiatic hybrids; *L. longiflorum* x *L. henryi*; Oriental x Asiatic hybrids and *L. auratum* x *L. henryi*. One was an intra-sectional species hybrid involving the cultivar 'Enchantment' x *L. pumilum*. In all the four inter sectional hybrids meiosis was highly irregular during meiosis and produced almost exclusively first division restitution (FDR) gametes. On the contrary, the single hybrid, 'Enchantment' x *L. pumilum* had normal chromosome pairing and produced only second division restitution (SDR) gametes. Although the frequency of  $2n$ -gamete production in all cases were greatly influenced by the environment, it was possible to produce backcross progenies in all cases. Analyses of backcross progenies through GISH proved that intergenomic recombination occurred in the case of FDR gametes that were produced in all the four interspecific hybrids. Further more, analyses of BC<sub>2</sub> and BC<sub>3</sub> progenies in some cases proved that recombinant chromosomes were transmitted normally. These investigations have shown that meiotic polyploidization is highly promising for lily breeding.

**Keywords:**  $2n$ -gametes, homoeologous recombination, introgression, FDR, SDR, IMR

### INTRODUCTION

Since 1960 about 7.000 lily cultivars has been registered (Leslie, 1982). Active lily breeding work started in Japan and the United States between the 1920's and 1940's. During the past 25 years it has been predominantly carried out in the Netherlands. The acreage raised from 100 hectares in 1970 till almost 4800 in 2000. Breeding was in the beginning mainly focused on Asiatic hybrids, the 1980's the Oriental hybrids became popular. Wide interspecific lily cultivars, with the LA-hybrids first, became commercial in the 1990's. Meanwhile it is clear that more groups including OT, LO and OA's will follow in the near future. The development of methods for overcoming fertilization barriers was essential in order to achieve the successes in interspecific lily breeding. A range of techniques were investigated and applied, besides pollination methods (cut-style, grafted style) to overcome pre-fertilization barriers and a number of techniques for post-fertilization barriers are needed. Embryo-rescue methods (ovary-slice, ovule and embryo culture) are needed to circumvent the problems with the embryo-endosperm development, mitotic and meiotic polyploidization (chromosome doubling

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using oryzalin or colchicine or by 2n-gametes) could overcome the F<sub>1</sub>-sterility which most frequently occurs. The last method to prove and speed up introgression of characters in interspecific hybrids is the so-called GISH (Genomic in situ hybridization), which can distinguish the parental genomes of interspecific hybrids. Mitotic polyploidization or somatic chromosome doubling results in tetraploid interspecific hybrids with recovered fertility, however, no homoeologous recombination can be seen in subsequent progenies (Lim *et al.* 2000). On the other hand, meiotic polyploidization has shown high frequencies of homoeologous recombination in lily hybrids (Lim *et al.* 2001; Lim *et al.* 2003; Van Tuyl *et al.* 2003), which is needed for introgression of characters. Therefore the search of 2n-gamete producing genotypes is essential for breeding with sterile interspecific hybrids. In this paper four different types of interspecific lily hybrids, for which the occurrence of 2n-gametes are described and the probable approaches for using the sexual polyploids are discussed.

## MATERIAL AND METHODS

### *Plant material*

The plant material used originated from the PRI-breeding programme (Van Tuyl *et al.* 1989, 1991, 2000) as shown in Table 1, except the *Lilium auratum* x *L. henryi* hybrid which was created by Asano (1981).

Table 1. Plant material selected for meiotic and for GISH research.

Genome type	Access no.	Cross combination
AA	Revival	Asiatic hybrid
APum	79418-2	Asiatic hybr. "Enchantment" x <i>L. pumilum</i>
APum	79418-7	Asiatic hybr. "Enchantment" x <i>L. pumilum</i>
AurHen	82111	<i>Lilium auratum</i> x <i>L. henryi</i>
LA	88542-52	<i>L. longiflorum</i> "Gelria" x Asiatic hybr. "Whilito"
LA	88542-80	<i>L. longiflorum</i> "Gelria" x Asiatic hybr. "Whilito"
LHen	89356-1	<i>L. longiflorum</i> "Gelria" x <i>L. henryi</i>
LHen	89356-6	<i>L. longiflorum</i> "Gelria" x <i>L. henryi</i>
OA	951502-1	Oriental hybr. "Pesaro" x Asiatic hybr. "Connecticut King"
OA	952400-1	Oriental hybr. "Merostar" x Asiatic hybr. "Gran Sasso"

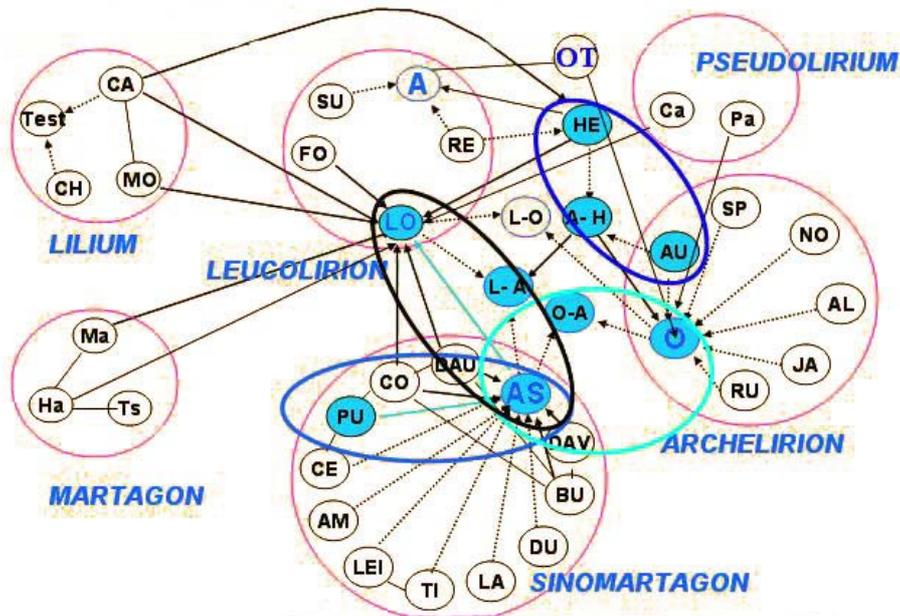


Figure 1. Crossing polygon genus *Lilium*

#### ***Chromosome preparation and in situ hybridization***

Chromosomes were prepared by squashing fixed cells onto clean microscope slides. The method was modified from that of Karlov *et al.* (1998). Well squashed preparations were dehydrated in a graded ethanol series after taking off cover slip and air dried. Genomic in situ hybridization was performed according to Lim *et al.* 2001.

#### **RESULTS AND DISCUSSION**

From Figure 1, the crossing polygon of the genus *Lilium*, it is clear that many interspecific cross combinations were successful. In all these cases through mitotic polyploidization fertile tetraploids were produced. It was proved however that these tetraploids were not suitable for inducing any intergenomic recombination, which is essential for introgression characters (Lim *et al.* 2000). Therefore 2n-gametes producing genotypes are needed. This phenomenon is, however, rare among *Lilium* interspecific hybrids. During a period of 25 years in lily in 5 different type of hybrids 2n-gametes producing genotypes were found.

Hybrids between “Enchantment” x *L. pumilum*, an intra sectional hybrid, appeared not to be fertile like almost all inter sectional hybrids, but they produce both n and 2n pollen (Van Tuyl *et al.* 1989).

Using the *Lilium auratum* x *L. henryi* hybrid obtained by Yoshito Asano (1977), we produced a range of triploid Oriental-henryi hybrids in the early eighties. Now, 20 years later, using the GISH-technique, we could demonstrate that in many of these hybrids genetic recombination between the *L. henryi* and *L. auratum* chromosomes took place during meiosis of F<sub>1</sub> hybrid. In Table 3, GISH-results are presented for a number of hybrids we obtained in

1982, 1983 and 1985 using Journey's End, Stargazer, Dominique and Darling as female in crosses with F<sub>1</sub> hybrid of *L. auratum* × *L. henryi* (Van Tuyl *et al.* 2002).

Through a similar approach, we produced a large number of F<sub>1</sub> hybrids between *L. longiflorum* × Asiatic hybrids and selected for 2n gamete producing genotypes (Lim *et al.* 2000; 2001). These were successfully back crossed to the parental species and obtained the BC<sub>1</sub>, triploid ALA progenies. GISH analyses of the ALA genotypes showed considerable frequencies of intergenomic recombination between the chromosomes of Longiflorum and Asiatic hybrids. Despite having an odd polyploid number (3x), some of the ALA genotypes were successfully used as parents in crosses with both 2x and 4x parents and produced a large number of near diploid as well as near pentaploid progenies. From GISH analyses, of triploid and aneuploid progenies it was proved that whole genomes, individual chromosomes as well as recombinant chromosomes were transferred to the BC<sub>1</sub> progenies.

More recently, we have produced F<sub>1</sub> hybrids between Oriental and Asiatic hybrids (OA) and selected 2n gamete forming genotypes. Using these, we have produced a large number of BC<sub>1</sub>, triploid, OOA and OAA progenies. GISH analyses of these triploids have demonstrated that there is considerable frequency of intergenomic recombination between the chromosomes of Oriental and Asiatic species.

In all the four inter sectional hybrids meiosis was highly irregular during meiosis and produced almost exclusively first division restitution (FDR) gametes. On the contrary, the intra sectional hybrid, 'Enchantment' × *L. pumilum* had quite normal chromosome pairing and produced only second division restitution (SDR) gametes.

From the three instances described above the following conclusions can be made:

- a) Through proper screening, it is possible to select genotypes of F<sub>1</sub> hybrids of lilies that can produce fairly high frequencies of 2n gametes and these can be successfully used for generating BC progenies.
- b) Unlike somatic chromosome doubling, use of 2n gametes can successfully produce intergenomic recombination. This facilitates the expression of recessive phenotypes in the BC<sub>1</sub> progenies already and this could be a reason why cultivars have been selected by using the BC<sub>1</sub> progenies produced in the case of both AH and LA hybrids.
- c) GISH analysis of BC<sub>1</sub> and subsequent progenies can provide valuable information for developing rational approaches for –breeding lilies more efficiently.

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Table 2. Frequency of chromosome association at metaphase I in ten hybrids of *Lilium*.

Pairing con- figuration		Cells ob- served	12II 0I	11II 2I	10II 4I	9II 6I	8II 8I	7II 10I	6II 12I	5II 14I	4II 16I	3II 18I	2II 20I	1II 22I	0II 24I	Mean frequency
'Revival'	AA	125	115	8	2	-	-	-	-	-	-	-	-	-	-	11.8II+0.2I
79418-2	APum	99	95	3	1	-	-	-	-	-	-	-	-	-	-	11.9II+0.1I
79418-7	APum	118	108	6	4	-	-	-	-	-	-	-	-	-	-	11.8II+0.2I
88542-80	LA	32	-	-	-	-	-	-	5	1	12	8	3	4	0	3.7II+16.6I
88542-52	LA	87	-	-	-	-	-	1	-	2	13	20	13	28	10	2.1II+19.8I
82111	AurHen	117	-	-	-	-	-	-	-	-	2	19	34	39	23	1.5II+21.0I
89356-6	Lhen	148	-	-	-	3	3	6	5	2	17	35	26	31	20	2.6II+18.8I
89356-1	Lhen	191	-	-	-	-	-	-	-	-	7	29	64	61	30	1.6II+20.8I
951502-1	OA	296	-	-	-	-	-	-	1	3	13	26	63	91	99	1.25II+21.49I
952400-1	OA	50	-	-	-	-	-	-	-	-	1	3	13	14	19	1.06II+21.88I

Table 3. Chromosome composition of progeny derived from Oriental × (*Auratum-henryi*) hybrids

Cross no	Female	Male	Ploidy level	Chromosome no	Originated from	
					Female	Male <sup>z</sup>
82111	<i>L. auratum</i> (Au)	<i>L. henryi</i> (H)	2x	24	12Au	12H
82396-1	Journey's End	82111	3x	36	12O	12(1Au/H)+12(2H/Au)
82396-2	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82396-3	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82396-4	J E	82111	3x	36	12O	12(1Au/H)+12H
82396-5	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82342-3	Star Gazer	82111	3x	36	12O	12Au+12H
82342-6	SG	82111	3x	36	12O	12Au+12(2H/Au)
83275-1	SG	82111	3x	36	12O	12Au+12H
83275-3	SG	82111	3x	36	12O	12Au+12(1H/Au)
83275-5	SG	82111	3x	36	12O	12(2Au/H)+12(3H/Au)
83275-7	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
83275-8	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
83275-12	SG	82111	3x	36	12O	12(1Au/H)+12(2H/Au)
83275-15	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
85863-1	Dominique	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
85863-2	Dominique	82111	3x	36	12O	12Au+12(1H/Au)
85864-1	Darling	82111	3x	36	12O	12(2Au/H)+12(2H/Au)
85864-2	Darling	82111	3x	36	12O	12Au+12H
85864-5	Darling	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
85864-6	Darling	82111	3x	36	12O	12Au+12H

<sup>z</sup>Numbers in parenthesis indicate the breakpoint of *L. auratum* (Au) chromosome with *L. henryi* (H) chromosome segment(s) and *L. henryi* chromosomes with *L. auratum* chromosome segment(s).