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Evaluation of BC_2 progenies derived from 3*x*-2*x* and 3*x*-4*x* crosses of *Lilium* hybrids: a GISH analysis

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Abstract An allotriploid (**ALA**, 2n=3*x*=36) BC_1 plant was obtained by backcrossing a diploid F_1 interspecific hybrid (**LA**, 2n=2*x*=24), derived from a *Lilium longiflorum* (**L** genome) and an Asiatic hybrid (**A** genome), to the latter parent. This allotriploid was backcrossed to a diploid Asiatic hybrid (2n=2*x*=24) and to an allotetraploid (**LLAA**, 2n=4*x*=48) **LA** hybrid. A total of 25 plants of these crosses were examined for ploidy level, and 12 individuals were analyzed for their genome constitution through genomic in situ hybridization (GISH). In most cases the progenies from the triploid-diploid (3*x*-2*x*) crosses consisted of aneuploids. Further more, there was evidence for the formation of near-haploid (*x*=12+2) to triploid (3*x*=36) gametes in the allotriploid BC_1 plant. The progenies of triploid-tetraploid (3*x*-4*x*) cross also consisted of mostly aneuploids but in this case the triploid female parent had contributed predominantly near-triploid (2n) gametes for the origin of BC_2 progenies. The different ploidy levels observed between 3*x*-2*x* and 3*x*-4*x* crosses are possibly caused by preferential fertilization or survival resulting in a different ratio of chromosome numbers between the embryo and endosperm. Though *Lilium* has a tetrasporic, eight-nucleate type of embryo sac formation (*Fritillaria* type), the observed difference between the progeny types in 3*x*-2*x* and 3*x*-4*x* crosses is comparable to that of observed in monosporic eight nucleate types (*Polygonum* type) that predominate in most genera of Angiosperms. An important feature of the genome constitution of the progenies was that the homoeologous recombinant chromosomes were transmitted intact from BC_1 to BC_2 progenies in variable numbers. In addition, there was evidence for the occurrence of new homoeologous recombinations in the triploid BC_1. Of the two euploid BC_2 plants one had originated through the parthenogenetic development of a 2n egg and the other had originated through indeterminate meiotic restitution (IMR).

Keywords Indeterminate meiotic restitution (IMR) · Embryo-endosperm ratio · Introgression breeding · Interploidy cross · *L. longiflorum* · Asiatic hybrids

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Introduction

Triploid plants are generally not useful in breeding because of their high degree of sterility. However, there are several instances among crop plants in which triploids have been successfully used as parents (reviewed by Brandham 1982; Kuspira et al. 1986). All these crops, however, are the so-called autotriploids. The reason why the autotriploids might be fertile has been explained as due to a higher degree of chromosome pairing during meiosis (Brandham 1982). There is evidence that autotriploids can produce balanced haploid (*x*), diploid (2*x*), triploid (3*x*) and aneuploid gametes that can be functional. The recovery of these gametes in the progenies of triploids appears to depend upon the types of crosses that are made. For example, in 3*x*-2*x*, or reciprocal, crosses the progeny will be predominantly diploid or near-diploid. On the other hand, in the case of 3*x*-4*x*, or reciprocal, crosses the progeny will be predominantly tetraploid or near-tetraploid.

Unlike in autotriploids, chromosome pairing can be more restricted during meiosis in the case of allotriploids because of the differentiation of chromosomes of alien genomes. This can lead to the formation of a higher frequency of univalents leading to more unbalanced meiosis and, consequently, a higher degree of sterility. De-

Table 1 Numbers and their parentage of the plant material (BC_2) used in this study

Accession	Parentage
997139-1~11	921238-1 (**ALA**) × 'Connecticut King' (**AA**) = [Asiatic hybrid 'Montreux' × (*L. longiflorum* 'Gelria' × 2*x* Asiatic hybrid 'Whilito')] × Asiatic hybrid 'Connecticut King'
997118-1~13	921238-1 (**ALA**) × 952047-1 (**LLAA**) = [Asiatic hybrid 'Montreux' × (*L. longiflorum* 'Gelria' × Asiatic hybrid 'Whilito')] × (4*x L. longiflorum* × 4*x* Asiatic hybrid 'Orlito')

spite this theoretical expectation (Brandham 1982), there are several instances among crop plants in which allotriploids have been successfully used as parents and the resulting progenies have been used in backcrossing programs. For example, allotriploid banana and plantain (*Musa accuminata* and *Musca balbisiana*) (Shepherd 1999), *Arachis hypogea* (Simpson and Davis 1983), *Festuca-Lolium* hybrids (King et al. 1998, 1999; Morgan et al. 2001), *Triticum-Aegilops* hybrids (Vardi and Zohary 1967; Xu and Dong 1992), *Triticum-Hordeum* hybrids (Blanco et al. 1986), *Zea-Tripsacum* hybrids (De Wet and Harlan 1970), *Brassica* species hybrids (Inomata 1983), *Diplotaxix-Brassica* hybrids (Batra et al. 1990), *Alstroemeria* species hybrids (Kamstra et al. 1999) and *Lycopersicon esculentum-Solanum lycopersicoides* (Rick et al. 1988) among others. But these data, to our knowledge, has not been systematically reviewed and very little information is available on the consequences of 3*x*-2*x* and 3*x*-4*x* crosses in the case of allotriploids.

In an attempt to transfer chromosomes and genes between distantly related diploid species (2n=2*x*=24) of *Lilium*, viz., *Lilium longiflorum* (**L** genome) and Asiatic hybrids (**A** genome), we produced allotriploid hybrids involving **A** and **L** genomes (indicated hereafter as **ALA** hybrids) (Lim et al. 2001). These BC_1 triploids were the sexual polyploid progenies derived from backcrossing the F_1 interspecific hybrid (**LA**) with the female parent, the Asiatic hybrid (A). An important feature of these sexual polyploids was that they possessed homoeologous recombinant chromosomes in their complements. In spite of being allotriploids, these could be successfully used as parents in 3*x*-2*x* as well as 3*x*-4*x* crosses. In this article, the analyses of BC_2 progenies, using genomic in situ hybridisation (GISH), are described. In addition, embryo-endosperm relationship with regard to the type of progenies obtained in the so-called tetrasporic, eight-nucleate female gametophytes (*Fritillaria* type) is discussed.

Materials and methods

Plant material

Interploidy crosses were made between triploid BC_1 (**ALA**) as a female, and a diploid (2n=2*x*=24) Asiatic hybrid and an allotetraploid **LLAA** (2n=4*x*=48) as male parents (Table 1). Detailed cross combinations and their BC_2 plants are listed in Table 1. The origin of the **ALA** hybrid has been reported earlier (Lim et al. 2001).

Crossing and embryo rescue

Lily bulbs were planted in pots and grown in the greenhouse with the temperature ranging from 14–16 °C during the night, to 20–22 °C during the day. All crosses were made using the cut-style pollination method (CSM) and encapsulated with aluminum foil on top of the cut-stigma for 7 days. Embryo rescue was carried out at 45–60 days after pollination depending on the maturation of the ovary. Embryos were dissected under the stereomicroscope and placed on 1/2 MS medium with supplement of 80 g/l of sucrose. After the embryos were germinated in vitro, the leaf tissue of the plantlet was used for DNA measurement by flowcytometry.

Flowcytometric measurement

The flowcytometric measurement was followed as described by Van Tuyl and Boon (1997). DNA values in the present paper are expressed as 'units' rather than 'picograms' because the DNA values were estimated by using only DAPI staining which is known to be base-biased (Peterson et al. 1999).

Chromosome preparation

Root tips were harvested in a saturated α-bromonaphthalin solution during early morning and kept overnight at 4 °C for accumulation of the metaphase cells. The next morning, the material was fixed in ethanol – acetic acid solution (3:1) for at least 2 h following washing with mQ water three times and stored at –20 °C until use. Root tips were treated with a pectolytic enzyme mixture (0.3% pectolyase Y23, 0.3% cellulase RS and 0.3% cytohelicase) in 10-mM citric acid buffer at 37 °C for about 1 h and squashed in a drop of 60% acetic acid solution. Slides were dipped in liquid nitrogen for few seconds and their cover slips were removed by a razor blade. Before air-drying the slides were dehydrated in absolute ethanol for a few minutes.

Genomic in situ hybridisation (GISH)

GISH protocol is basically the same as Lim et al. (2001). Briefly, sonicated genomic DNA (1–10 kb) from *L. longiflorum* was used as a probe after labeling with digoxigenin by nick translation according to manufacturer's instruction (Boehringer Mannheim, Germany). Sheared herring sperm DNA was used for blocking the non-hybridised DNA sequences. After detection steps, slides were counter-stained with 5 μg/ml of propidium iodide (PI). Images were photographed with a Zeiss Axiophot microscope equipped with epi-fluorescence illumination and single band filters for FITC and Cy3/PI using 400 ISO color negative film. The film was then scanned at 1,200 dpi using a HP film scanner, and the contrast and color balance was adjusted in the digital processing software program 'Photoshop 5.5' (Adobe Inc. USA).

Results

Ploidy level and chromosome composition was determined in the BC_2 progenies through the flowcytometric

Table 2 Chromosome number through flow cytometric and microscopic analysis of two different crosses

Genotypes	Flowcytometric results		Chromosome number by microscopic observation
	DNA content[a]	Chromosome number by calculation[b]	
921238-1	149.20	37.2	36
Connecticut King	100.80	24.0	24
952047-1	201.30	48.0	48
3*x* × 2*x* (921238-1 × 'Conn. King'			
997139-1	242.30	61.2	61
997139-2	123.60	31.2	30
997139-4	146.50	37.2	36
997139-5	127.90	32.4	31
997139-7	105.70	26.4	26
997139-8	124.60	31.2	28
997139-10	124.40	31.2	31
997139-11	114.60	28.8	27
3*x* × 4*x* (921238-1 × 952047-1)			
997118-1	242.45	58.8	NA[c]
997118-2	223.10	54.0	54
997118-3	243.50	58.8	NA[c]
997118-5	242.84	58.8	NA[c]
997118-6	241.65	58.8	60
997118-7	246.03	60.0	62
997118-8	243.56	58.8	NA[c]
997118-9	184.15	44.4	NA[c]
997118-10	168.93	40.8	NA[c]
997118-11	245.66	60.0	NA[c]
997118-12	243.81	58.8	63
997118-13	239.25	57.6	NA[c]

[a] Expressed in units, because estimation is based on DAPI staining only
[b] Predicted by average single chromosome DNA value from internal standard cultivars, 'Snow Queen', 'Star Gazer' and 'Connecticut King'
[c] NA indicates data not available

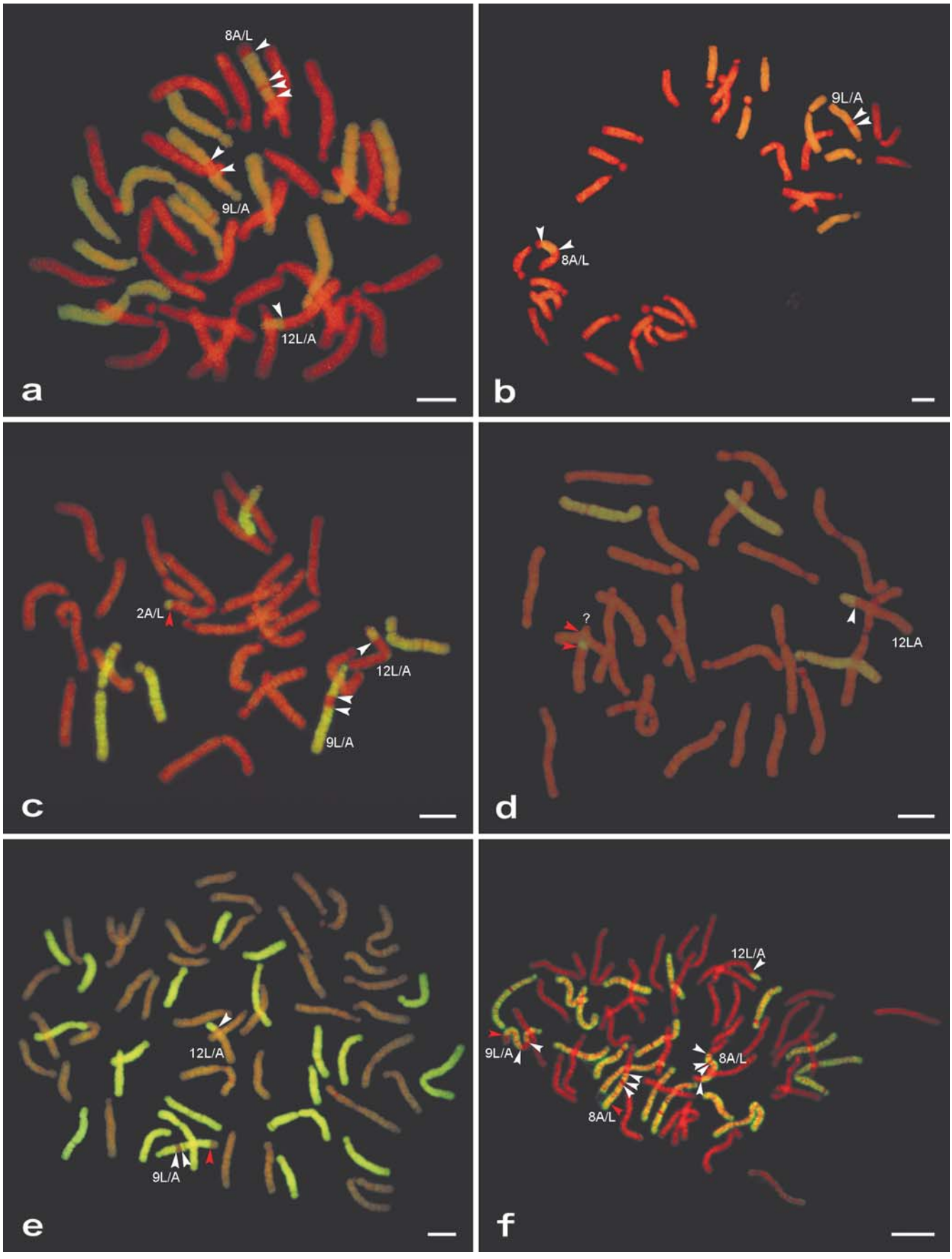
estimation of 2C DNA values and GISH. By dividing the DNA value of the progeny by that of the diploid parent it was possible to determine the approximate chromosome number of the former. In most of the cases the actual chromosome number counted was nearly concurrent with the predicted number (Table 2). Out of the 13 progenies of the 3*x*-2*x* and 3*x*-4*x* crosses in which the chromosome numbers were counted, only two (997139-4 and 997118-6) had a euploid number, the rest being aneuploids (Table 2).

Assuming that the diploid male parent had contributed normal n (*x*=12) gametes in a 3*x*-2*x* cross, the chromosome numbers of the egg cells ranged from 14 to 24 (Table 3). Thus, in the case of the 3*x*-2*x* cross the aneuploid female gametes with a near-haploid chromosome number were functional. The one exceptional plant, 997139-1, was the result of the functioning of 2n gametes (2n = 36) from both parents.

On the other hand, in the case of the 3*x*-4*x* cross where the male parent had contributed 2*x* gametes (i.e., 2*x* = 24) and the chromosome number of the egg cells ranged from 30 to 39 which was a higher number than in the 3*x*-2*x* cross (Table 3). Thus, a tendency of the survival of different types of progenies in different crosses was observed (compare also DNA values in Table 2). With two exceptions, 997118-9 and -10, the progenies were near-pentaploid or pentaploid in the 3*x*-4*x* cross. This could be an indication that although the triploid parent produced gametes with a range of chromosome numbers, there was a difference in the functioning of gametes in different crosses.

The genome compositions determined through GISH in the triploid **ALA** parent and 12 of the BC_2 progenies

Fig. 1a–f Somatic metaphase chromosome complements of the allotriploid BC_1 parent (**ALA**) and its BC_2 progenies. In all cases, DNA of *L. longiflorum* (**L**) was used as probe labeled with FITC (*yellow-green fluorescence*) and counterstained with propidium iodide (*orange-red fluorescence*) to make visible of the chromosomes of Asiatic hybrid. Homoeologous recombinant chromosomes are marked (*L/A* or *A/L*) and *arrow-heads* indicate recombinant break points. The new homoeologous recombination breakpoints are indicated by a *red-colored arrow-head*. In all cases the bar indicates 10 μm. **a** Allotriploid complement of **ALA**, 921238-1, (2n=3*x*=36) showing three recombinant chromosomes. **b** A near triploid chromosome complement (2n=3*x*–5=31) of the BC_2 plant, 997139-5, showing 7**L**+24**A** chromosomes. Note: of the two recombinant chromosomes, 8**A/L** is further modified by a second recombination. **c** A near diploid (2n=2*x*+4=28) complement of 997139-8 showing 6**L**+22**A** chromosomes. Note: of the three recombinant chromosomes, 2**A/L** has a new recombination. **d** A near diploid (2n=2*x*+3=27) of 997139-11 showing 4**L**+23**A** chromosomes. 10**A/L** is a new recombinant chromosome originated from recombination in the triploid **ALA**. **e** The pentaploid (2n=5*x*=60) complement of 997118-6 showing 11**L**+49**A** chromosomes. The unreduced egg cell in this case has contributed 36 chromosomes. The peculiar feature is that only 11**L** (instead of 12**L**)+25**A** (instead of 24**A**) chromosomes have been included in the restitution nucleus. Such anomalous balanced chromosome constitution of a gamete occurs due to indeterminate meiotic restitution (IMR). **f** Aneuploid constitution (2n=4*x*+6=54) of 997118-7 showing 8**L**+46**A** chromosomes. Of the four recombinant chromosomes, two (9**L/A** and 8**A/L**) have new recombinant segments


8A/L
9L/A
12L/A
a
9L/A
8A/L
b
2A/L
12L/A
9L/A
c
?
12LA
d
12L/A
9L/A
e
12L/A
9L/A
8A/L
8A/L
f

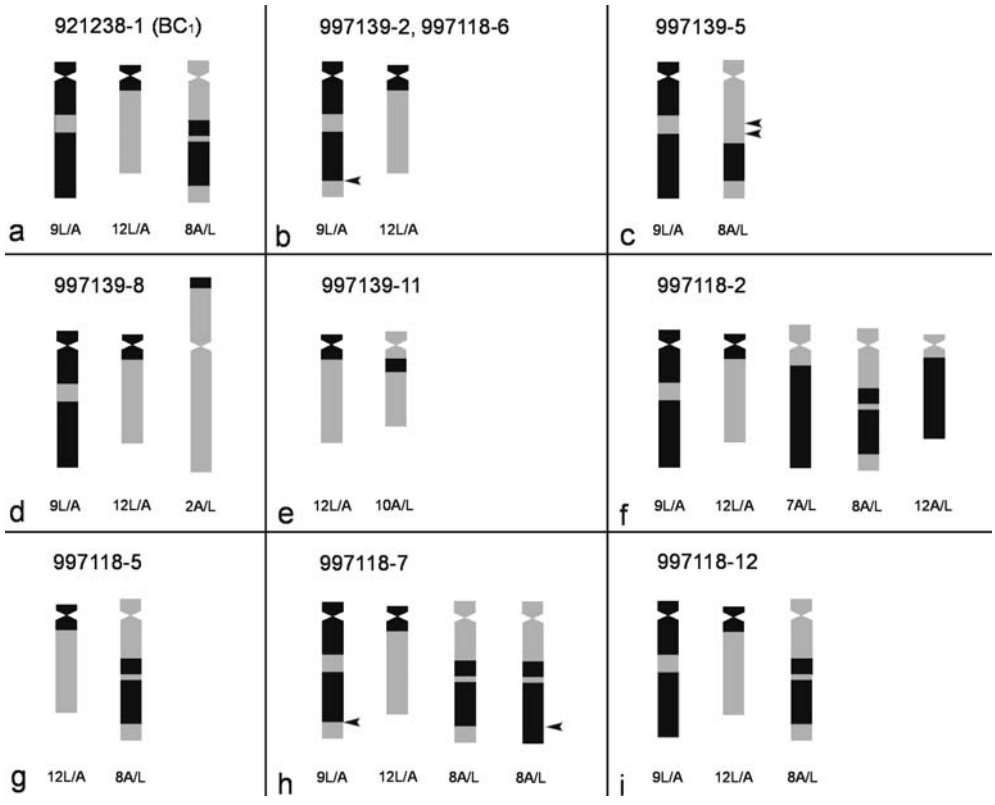


Fig. 2a–i Diagrammatic representation of the recombinant chromosomes in some of the BC_1 (**ALA**) and nine of the BC_2 progenies. It was possible to distinguish different types of homoeologous recombinations in BC_2 as follows: (1) the same type as in BC_1 (Fig. 2i; 997118-12), (2) missing one recombinant chromosome (Fig. 2g; 997118-5), (3) addition of new recombinant chromosomes (Fig. 2f; 997118-2), (4) addition of new recombinant chromosomes with a second cycle of recombination (Fig. 2h), (5) absence of one of the recombinant chromosomes in the second cycle of homoeologous recombination (Fig. 2b and c; 997139-2, 997139-5 and 997118-6), and (6) not only absence of recombinant chromosomes but also addition of new homoeologously recombinant chromosomes (Fig. 2d and e; 997139-8 and 997139-11)

Table 3 Genome composition and embryo/endosperm ratio of BC_2 progenies derived from $3x \times 2x$ and $3x \times 4x$ crosses determined through GISH

Genotypes	Chromosome number	No of chromosomes contributed by		No (types) of recombinant chromosomes[b]	Embryo:endosperm ratio[c]
		A (female)[a]	**B** (male)		
$3x \times 2x$ cross					
997139-1	61 ($5x + 1$)	37 (13**L** + 24**A**)	24	3 (2**L/A**; 1**A/L**)	1:1.6
997139-2	30 ($2x + 6$)	18 (5**L** + 13**A**)	12	2 (2**L/A**)	1:2.8
997139-4	36 ($3x$)	24 (12**L** + 12**A**)	12	3 (2**L/A**; 1**A/L**)	1:2.3
997139-5	31 ($3x - 5$)	19 (7**L** + 12**A**)	12	2 (1**L/A**; 1**A/L**)	1:2.7
997139-7	26 ($2x + 2$)	14 (2**L** + 12**A**)	12	2 (2**A/L**)	1:3.2
997139-8	28 ($2x + 4$)	16 (6**L** + 10**A**)	12	3 (2**L/A**; 1**A/L**)	1:3.0
997139-10	31 ($3x$-5)	19 (6**L** + 13**A**)	12	6 (3**L/A**; 3**A/L**)	1:2.7
997139-11	27 ($2x + 3$)	15 (4**L** + 11**A**)	12	2 (2**L/A**)	1:3.1
$3x \times 4x$ cross					
997118-2	54 ($4x + 6$)	30 (10**L** + 20**A**)	24	5 (2**L/A**; 3**A/L**)	1:1.7
997118-6	60 ($5x$)	36 (11**L** + 25**A**)	24	3 (2**L/A**; 1**A/L**)	1:1.6
997118-7	54 ($4x + 6$)	30 (8**L** + 22**A**)	24	4 (2**L/A**; 2**A/L**)	1:1.7
997118-12	63 ($5x + 3$)	39 (12**L** + 27**A**)	24	3 (2**L/A**; 1**A/L**)	1:1.5

[a] The number of chromosomes (centromeres) of *L. longiflorum* (**L**) and Asiatic hybrid (**A**) transmitted from the female parent (**ALA**) is shown in parenthesis

[b] Type of recombination defined as **L/A** and **A/L**, indicating a *L. longiflorum* centromere with Asiatic chromosome segment(s) and an Asiatic centromere with *L. longiflorum* chromosome segment(s), respectively

[c] Embryo:endosperm ratio is calculated by (72+B)/A+B

are given in Table 3, and some are illustrated in Figs. 1 and 2. From this analysis it was possible to: (1) determine the contribution of the number of **L** and **A** genome chromosomes from the female and male parents, and (2) the number and types of homoeologous recombinant chromosomes in the BC_1 as well as each of the BC_2 progenies (Table 3 and Fig. 2b–i). Of the three homoeologous recombinant chromosomes observed in the BC_1, namely 9**L/A**, 12**L/A** and 8**A/L** (Fig. 2a), at least two of these were present in the BC_2 progenies derived from both $3x$-$2x$ and $3x$-$4x$ crosses. Surprisingly, there were also new recombinant chromosomes in the BC_2 proge-

nies that were not originally present in the BC_1 parent. These new homoeologous recombinant chromosomes (Figs. 2d, e, f,) had originated during meiosis in the triploid BC_1 parent. This was surprising because in the allotetraploid (**LLAA**) parent there was no homoeologous recombination owing to the preferential pairing of chromosomes of alien genomes (data not shown).

A maximum of six recombinant chromosomes were, for example, found in one BC_2 plant, 997139-10, of which three were the same as in the BC_1 parent whereas three were new recombinant chromosomes (Table 3). There were also instances in which the original recombinant chromosome was involved in the second cycle of homoeologous recombination as in the case of BC_2 plant, 997118-7, which had two such cases, i.e., 9**L/A** and 8**A/L** (Figs. 1f and 2h).

With the exception of two BC_2 plants, 997139-4 and 997118-6 that were euploids, all others were aneuploids (Table 3). The balanced triploid constitution observed in 997139-4 could be explained as due to the functioning of a balanced first-division restitution (FDR) egg cell, which had developed parthenogenetically. However, in the case of the other euploid, 997118-6, that had a balanced pentaploid chromosome constitution, the explanation was not that straight forward. At first sight it appeared as if the 2n egg from the **ALA** parent had originated through FDR, accounting for 36 (12**L**+24**A**) chromosomes, and the tetraploid **LLAA** parent had contributed a 2*x* gamete (12**L**+12**A**) that accounted for the pentaploid constitution of 997118-6. If this explanation was correct, then this pentaploid was expected to possess 24**L**+36**A** chromosomes. Unexpectedly, this pentaploid possessed 23**L**+25**A** chromosomes in its complement (Fig. 1e). This means that the 36 chromosomes contributed by the 2n egg did not possess the expected 12**L**+24**A** chromosomes but had 11**L**+25**A** chromosomes. An obvious explanation was that in the case of 997118-6 the 2n egg was not of FDR origin but had occurred due to the indeterminate meiotic restitution (IMR) that we have described earlier (Lim et al. 2001). In this mechanism, euploid gametes with a different number of chromosomes of the alien genomes can occur.

A notable feature of the triploid BC_1 parent was that it produced egg cells with a fairly wide range of chromosome numbers (range: 14 to 39, Table 3) and these egg cells were indeed functional in producing BC_2 progenies. At least in two cases, 997139-1 and 997118-12, there was evidence that egg cells possessed more chromosomes (37 and 39 respectively) than the triploid parent (36). Gametes with such excessive chromosome numbers could be expected to occur in allotriploids because of the abnormal meiosis. In view of the viability of egg cells with such a wide range of chromosome numbers, as well as the production of BC_2 progenies, it was interesting to examine the embryo-endosperm relationship in these progenies. The embryo sacs of *Lilium* species being tetrasporic, a eight-nucleate type (*Fritillaria*-type, Maheshwari 1950), the secondary nucleus was expected to possess 72 chromosomes regardless of the chromosome numbers of the egg cells. On this basis, the expected chromosome numbers of embryo and endosperm were estimated and the ratios calculated (Table 3). In the case of 2*x*-3*x* crosses the embryo-endosperm ratio was close to 1:3 whereas in the case of 3*x*-4*x* cross it was either 1:2 or lower.

Discussion

The results of this investigation show that allotriploids of *Lilium* species hybrids can be successfully used as parents with the aid of embryo-rescue techniques. As in autotriploids, there appears to be a marked difference in the ploidy levels of the progenies depending on the types of crosses. Thus, in 3*x*-2*x* crosses the progeny are predominantly near-diploid whereas in the case of 3*x*-4*x* crosses they are mostly near-pentaploid. There was evidence for the production of 2*x* gametes in the triploid parent but, for an unknown reason, there seems to be a preferential occurrence of pentaploid progenies. Two explanations may be proposed as follows: (1) there might be preferential fertilization of the near-3*x* egg cells with 2*x* pollen, giving rise to pentaploids, and (2) there might be preferential survival of pentaploid sporophytes due to embryo-endosperm balance. Since the data are few, no definite conclusions can be made at this stage. Besides that, there are no analogous studies on progenies derived from tetrasporic eight-nucleate gametophytes in other taxa for comparison. However, the differences in the ploidy levels observed in the present study are comparable to those described in autotriploid crops (Brandham 1982).

An important feature of interploidy crosses, such as the ones investigated in this study, is that the type of progeny obtained depends on the ratio of chromosome numbers of the embryo and endosperm that result after fertilization. In most of the flowering plants in which embryo-endosperm relationships have been investigated so far they possess the so-called monosporic eight-nucleate type of embryo sac (*Polygonum* type). In these cases, the chromosome number of the secondary nucleus will be invariably twice the number of chromosomes that is found in the egg cell (i.e., a 1:2 ratio). By contrast, in the case of tetrasporic eight-nucleate embryo sacs (*Fritillaria* type), the ratio will be quite different. Especially in triploids, due to irregular meiosis during megasporogenesis, the ratio of chromosomes between the egg cell and the secondary nucleus in the embryo sacs can be highly variable. Despite such variation, it is noteworthy that in 3*x*-2*x* crosses the progeny is diploid or nearly so and in 3*x*-4*x* crosses the progeny is either penta- or tetraploid. This result is very similar to that found in the monosporic eight-nucleate type of embryo sac. More studies are necessary in order to establish whether or not there are any similarities or differences between the two types of embryo sac with respect to the production of euploid and aneuploid progenies.

One of the important features of the triploid BC_1 plant is that it is a product of sexual polyploidisation possessing three homoeologous recombinant chromosomes. For

a meaningful utilization of this valuable genotype in further introgression breeding, it is essential to monitor the transmission of the recombinant chromosomes to the BC_2 and subsequent generations. Accurate identification of recombinant chromosomes through GISH in the BC_2 progenies of this study is a step forward for in-breeding lily on a rational basis. Besides the original homoeologous recombinant chromosomes in the BC_1, new recombinants were also produced in the triploid parent. This means it should be possible to map some of the horticultural traits in lilies. This requires, however, the production of substitution lines for recombinant segments. Similar studies have been conducted in *Festuca-Lolium* hybrids where some of the agronomic traits have been assigned to specific chromosome segments (King et al. 1998).

Allotriploids have been shown to produce balanced haploid (x), diploid ($2x$) and triploid (2n) gametes in some crops such as banana, plantain and peanuts. In some cases, especially in banana and plantain, they occur in high frequencies (Shepherd 1999). There is no convincing explanation for the occurrence of balanced gametes in triploids. Such events might be assumed to be random events but only the balanced gametes are viable. In the case of 2n gametes in triploids, however, balanced gametes can originate through the FDR mechanism. In this case, the entire chromosome complement divides equationally giving rise to two identical, or nearly identical, 2n gametes. Besides FDR, there is also a prospect of the occurrence of balanced 2n gametes, i.e. with 36 chromosomes in the present case, with another mechanism that we have described as indeterminate meiotic restitution (IMR) in *Lilium* species hybrids (Lim et al. 2001). In this case euploids can originate with a different number of alien chromosomes in a 2n gamete. The chromosome complement of 997118-6 proves our earlier hypothesis discovered in a diploid hybrid.

Allotriploids involving species of different genera such as *Festuca* and *Lolium* have been shown to be useful for the introgression of desirable characters into cultivars (Humphreys and Thomas 1993; Humphreys et al. 1995; Morgan et al. 2001). Besides this practical application, such triploids have also been used for the so-called introgression mapping (King et al. 1998, 1999). The important feature is that the homoeologous recombination that is observed in the allotriploid *Lilium* species hybrid (Fig. 2) is similar to that observed in *Lolium-Festuca* hybrids. Such recombination events could be detected only because of the availability at present of the GISH technique. Thus, the present investigation demonstrates that allotriploids can be used successfully for introgression. Furthermore, the progenies can be critically monitored for the presence of introgressed recombinant alien segments in the BC_2 progenies. This can be a step forward for developing more systematic and meaningful procedures for breeding polyploid cultivars.

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