Production of Polyploids and Unreduced Gametes in *Lilium auratum* × *L. henryi* Hybrid

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Abstract

Intergenomic F₁ hybrids between *L. auratum* × *L. henryi* and their BC₁ progeny were investigated through genomic in situ hybridization technique (GISH) to determine their potential value in lily breeding. We confirmed that F₁ intergenomic hybrids possessed a set of chromosomes (x=12) from both parents and that flowers of the F₁ *auratum* × *henryi* hybrid showed an intermediate morphological phenotype. Pollen size, viability and germination ability were measured through microscopic observations. F₁ intergenomic hybrids produced a relevant frequency of 2n-gametes, which were successfully used to perform crosses with Oriental hybrids, resulting in the triploid Oriental Auratum Henryi (OAuH) hybrid. Twenty BC₁ plants were generated by crossing between four different Oriental hybrid cultivars and F₁ AuH hybrids using an in vitro embryo rescue technique, after which the genome constitution and chromosome composition were analyzed by GISH. All plants were triploid, showing 12 from female parents (diploid Oriental hybrid) and 24 from male parents (diploid F₁ AuH hybrid). Overall, 16 out of 20 BC₁ progeny possessed recombinant chromosomes with 1–5 crossover sites per plant. Cytological analysis of 20 BC₁ plants by GISH verified that the occurrence of 2n pollen formation in all F₁ AuH hybrids was derived from the FDR (first division restitution) mechanism, in which the genome composition of all BC₁ plants possess 12 Oriental + 12 *L. auratum* + 12 *L. henryi* chromosomes. Allotriploids derived from the AuH hybrid were used as female for crossing with the diploid Oriental hybrid cultivar ‘Sorbonne’ and considerable numbers of plants (0–6.5 plants per ovary) were only obtained when female OAuH (BC₁) triploids were used. Taken together, the results of this study indicate that production and analysis of F₁ AuH hybrids and their progeny through sexual polyploidization can be useful for efficient creation of important horticultural traits.

Key words: Allotriploid, Polyploidization, Homoeologous recombination, Interspecific hybrid, 2n-gamete.

Introduction

There are about 80 species in the genus *Lilium*, which is taxonomically classified into seven sections [1, 2]. Currently, the most important groups for commercial breeding comprise of the Trumpet lily group, including *L. longiflorum* of the section Leucolirion, the Asiatic hybrid group of the section Sinomartagon and the Oriental hybrid group of the section Archelirion [3]. All three sections comprise species with distinct, desirable horticultural characteristics. *L. henryi* belongs to neither Archelirion nor Leucolirion, and shows intermediate phenotypic characteristics of both sections [4]. The interspecific hybridization technique has been applied to introduce some interesting traits such as virus resistance in *L.
**Materials and methods**

**Plant materials**

The F₁ AuH hybrid materials used in this experiment were developed by crossing between *L. auratum* (2n=24; hereafter Au) and *L. henryi* (2n=24; hereafter H) [13]. BC₁ progeny produced for chromosome analysis by GISH were produced by crossing male F₁ AuH hybrids with four different Oriental hybrid cultivars, ‘Stargazer’, ‘Journey’s End’, ‘Dominique’, and ‘Darlings’ [14]. Reciprocal crosses were conducted between female F₁ AuH hybrids and male *L. auratum* and *L. henryi*, and vice versa.

To produce BC₂ progeny, the BC₁ progeny were also crossed with female *L. henryi* Oriental hybrid cultivars ‘Sorbonne’ and *L. auratum* as a male. Plants were grown in pots containing a peat based soil mixture in the greenhouse at temperatures varying from 20°C-25°C during day time and 14°C-18°C at night. Present research was carried out in Wageningen University, Netherland (WUR).

**Pollen viability and germination**

To measure the pollen size and stainability, pollen were collected from fully open flowers, mounted in a drop of lactophenol acid-fuchsin and viewed under the microscope. Classification of pollen size was determined using a calibrated micrometer. Pollen were collected after flower anthesis and then cultured for 24 hours at 25°C in artificial agar medium containing 100g/L sucrose, 5g/L bacteriological agar, 20mg/L boric acid and 200mg/L calcium nitrate. The pollen was classified as large (2n) and small (n) depending on size and then counted to determine the germination range.

**Pollination and embryo rescue**

All crosses were conducted by cut style pollination method (CSM) and encapsulated with aluminum foil on top of the cut stigma for a week. Embryo rescue was then carried out before abortion. The embryo were subsequently dissected under the stereomicroscope and placed on 1/2 MS medium containing 80g/L of sucrose for germination in vitro [15]. Pre and post-fertilization were then investigated by checking ovary enlargement and embryo formation after pollination.

**Chromosome preparation**

Root tips were harvested in a saturated α-bromonaphthalene solution in the early morning and kept overnight at 4°C for accumulation of metaphase cells. Then this material was fixed in ethanol – acetic acid solution (3:1) for at least 2 hours, after which they were stored at -20°C. The root tips were then treated with a pectolytic enzyme mixture (0.3% pectolyase Y23, 0.5% cellulase RS and 0.3% cytohelicase) in 10mM citric acid buffer at 37°C for 1 hour, after which they were squashed in a drop of 60% acetic acid solution. Slides were then frozen by dipping in liquid nitrogen, after which their cover slips were removed using a razor blade. Before air-drying, the slides were dehydrated in an absolute ethanol for several
minutes, after which they were stored at 4°C for several weeks prior to in situ hybridization.

**Genomic in situ hybridization**

The GISH protocol was basically the same as that described by Lim et al. (2001) [16]. In this genomic DNA (1 – 10 kb) from *L. henryi* was used as a probe after labeling with digoxigenin by nick translation according to the manufacturer’s instructions (Boehringer Mannheim, Germany). Sheared herring sperm DNA was used to block the non-Henryi-specific DNA sequences. After detection steps, the slides were counter-stained with 5 µg/mL propidium iodide (PI). The 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. Finally, the film was scanned at 1200 dpi using an HP film scanner and the contrast and color balance were adjusted using Photoshop 5.5 (Adobe Inc. USA).

**Results**

**Analysis of 2n-gamete production of *L. auratum × L. henryi* hybrid**

To obtain progeny of the F₁ AuH hybrid, we examined the pollen viability using pollen staining (Fig. 1B; Table 1) and tested in vitro pollen germination to re-confirm Asano’s observations [10] (Table 2) and produce their BC₁ progeny of the AuH hybrid (Table 3).

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Table 1. Pollen viability of AuH (*L. auratum × L. henryi*) interspecific hybrid, 82111.

| Accession No. | Genotype | Large (2n) | Normal (n) | Total survival (%) |
|---------------|----------|------------|------------|--------------------|
|               |          | Stained (%) | Unstained (%) | Stained (%) | Unstained (%) |                  |
| 82111         | AuH      | 308 (42.0) | 19 (2.6)   | 121 (16.5) | 285 (38.9) | 58.5              |

Table 2. Pollen germination of AuH (*L. auratum × L. henryi*) interspecific hybrid, 82111, on artificial agar medium.

| Accession no. | Genome | Total | Pollen type and germination | Average germination (%) |
|---------------|--------|-------|-----------------------------|-------------------------|
|               |        |       | No. of 2n pollen (%) | Germination from 2n gametes | No. of n pollen | Germination from n-gametes |
| 82111         | AuH    | 640   | 300                         | 207 (69%)                | 340            | 6 (1.7%)                | 33.2                |

Table 3. BC₁ progeny obtained by reciprocal crosses of AuH (*L. auratum × L. henryi*) interspecific hybrid, 82111.

| Cross combination | No. of flowers pollinated | Pre-F | Post-F | No. plants derived | Av. plantlets per ovary |
|-------------------|--------------------------|-------|-------|--------------------|-------------------------|
| Female            | Male                     |       |       |                    |                         |
| 82111             | *L. auratum*             | 11    | 0     | 5                  | 3                       | 0.27                   |
|                   | 82111                    | 5     | 0     | 0                  | 4                       | 0.80                   |
| 82111             | *L. henryi*              | 10    | 0     | 8                  | 1                       | 0.10                   |
| 82111             | *L. henryi*              | 5     | 0     | 4                  | 0                       | 0.00                   |
Two types of pollen were primarily observed, aborted small pollen grains (Fig. 1A red arrow) and well-filled large pollen grains (Fig. 1A black arrow). These types of pollen were considered to be a sterile (n) gamete and fertile (2n) gamete, respectively. After pollen testing, 308 (42%) pollen grains were found to be produce viable 2n pollen (Table 1). To investigate the viability and function of pollen for crossing, these pollens were also germinated in vitro. Germination of pollen on artificial agar medium revealed that, of the 640 pollen grains tested, 300 were large pollen grains (2n), among which 207 (69%) germinated (Table 2) which is quite high percentage.

Use of 2n pollen for production of progeny

We confirmed that the F1 AuH hybrid can be utilized to obtain progeny by using the 2n gametes. To investigate the crossability and intergeneric recombination using the F1 AuH hybrid, we conducted reciprocal crosses of AuH hybrids. Generation of progeny by reciprocal crossing was more successful when female F1 AuH hybrids were used (Table 3). In addition, crossing with L. auratum led to more efficient generation of progeny. Finally, progeny were rarely obtained post-fertilization when crossing with L. henryi. These findings indicate that the F1 AuH hybrid has both female and male fertility. During ploidy levels analysis, all 20 BC1 progeny was triploid (Table 4) with equal number of chromosomes. These findings demonstrated that the F1 AuH hybrid contributed balanced diploid chromosome complements.

Chromosome constitution and intergeneric recombination

Two parental genomes of L. henryi and L. auratum in the F1 AuH hybrids (Fig. 1C) were clearly distinguishable after GISH. Probing with DIG-labeled total genomic DNA of L. henryi as a probe resulted in 12 green-labeled chromosomes, indicating that these chromosomes were derived from L. henryi and the others originated from L. auratum. Probe hybridization was uniform throughout the chromosome. These results further confirmed that L. henryi was distinct from L. auratum and belonged to the Archelirion section (Oriental hybrid group) of the genus Lilium.

To investigate chromosome number, frequency and type of recombinant chromosomes, all 20 BC1 progeny were analyzed by GISH and the results are summarized in Table 4. All 20 BC1 progeny had a triploid chromosome composed of 12 L. henryi chromosomes and 24 Oriental chromosomes. These findings were expected because a backcross of the 2n gamete from the F1 AuH hybrid contributes one set each of L. henryi and L. auratum (Oriental hybrid) genomes. These findings clearly confirmed that chromosome sets of the parental genomes of F1 AuH hybrid remain intact in resulting 2n gametes. Moreover, because the chromosome composition of the BC1 progeny comprised non-sister chromatids from a reduction division at anaphase I, all of the progeny were generated from the first division restitution (FDR) 2n pollen produced by the F1 AuH hybrid.

It was observed that among 20 BC1 progeny, four plants (20%) did not have any recombinant chromosomes (Table 4), while the other 16 (80%) contain recombinant chromosomes. The recombination (breakpoint) also occurred on 1–5 points per plant in the long or short arms, showing a variable range of recombinant chromosomes that occurred randomly (Table 4, Fig. 2).
Table 4. Genome constitution and chromosome composition of BC1 progeny in cross combinations between each Oriental hybrid and interspecific AuH hybrid, 82111, as determined by GISH.

| Accession no. | Genotype | Cross combination        | Ploidy level | Chromosome number | Chromosome constitution | No. of recombinant chromosomes |
|---------------|----------|--------------------------|--------------|-------------------|--------------------------|--------------------------------|
| 82111         | AuH      | L. auratum               | 2×           | 2n=24             | O           12        | Au 12   0       H 12   0               |
| 82396-1       | O AuH    | Journey’s End            | 3×           | 2n=36             | 12          12       12   12   0               |
| 82396-2       | O AuH    | Journey’s End            | 3×           | 2n=36             | 12          12       12   12   2               |
| 82396-3       | O AuH    | Journey’s End            | 3×           | 2n=36             | 12          12       12   12   3               |
| 82396-4       | O AuH    | Journey’s End            | 3×           | 2n=36             | 12          12       12   12   1               |
| 82396-5       | O AuH    | Journey’s End            | 3×           | 2n=36             | 12          12       12   12   2               |
| 82342-1       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   0               |
| 82342-6       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   2               |
| 83275-1       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   0               |
| 83275-3       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   1               |
| 83275-5       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   2               |
| 83275-7       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   2               |
| 83275-8       | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   1               |
| 83275-12      | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   2               |
| 83275-15      | O AuH    | Stargazer                | 3×           | 2n=36             | 12          12       12   12   1               |
| 85863-1       | O AuH    | Dorminique               | 3×           | 2n=36             | 12          12       12   12   1               |
| 85863-2       | O AuH    | Dorminique               | 3×           | 2n=36             | 12          12       12   12   1               |
| 85864-1       | O AuH    | Darling                  | 3×           | 2n=36             | 12          12       12   12   2               |
| 85864-1       | O AuH    | Darling                  | 3×           | 2n=36             | 12          12       12   12   2               |
| 85864-1       | O AuH    | Darling                  | 3×           | 2n=36             | 12          12       12   12   2               |
| 85864-1       | O AuH    | Darling                  | 3×           | 2n=36             | 12          12       12   12   2               |
| 85864-6       | O AuH    | Darling                  | 3×           | 2n=36             | 12          12       12   12   0               |

* chromosome having a L. auratum centromere with L. henryi recombination sites. † chromosome having a L. henryi centromere with L. auratum recombination sites.

Producing BC2 progeny

To assess the possibility of producing BC2 progeny from allotriploid OAuH hybrids, we investigated the pollen viability by pollen staining and germination (Table 5) prior to using them for backcross. The range of pollen staining (0–48%) and germination percentage (0–4.3%) varied depending on the genotypes and plants. However, there was no relationship between germination percentage and pollen viability. Based on these results, we primarily used the female OAUH hybrids because of the low level of germination (0–4.3%).

Extensive reciprocal or backcrossing of OAUH (BC1) triploids followed by in vitro ovule and embryo culture generated several BC2 progeny (Table 6). Backcrosses of BC1 progeny were successful when male L. auratum was used, but no progeny were obtained from backcrosses with L. henryi. These findings were consistent with those observed when reciprocal crosses of F1 AuH hybrids were conducted to produce their BC1 progeny.

Four genotypes of OAUH triploids (primarily female, with some male) were backcrossed with the diploid Oriental hybrid cultivar ‘Sorbonne’. Successful results were only obtained when the OAUH triploid was used as the female and the number of plants per ovary varied from 0–6.5 depending on the genotypes and plants. Moreover, no progeny were produced when male OAUH triploids were used. Among the four different BC1 genotypes, BC1 plants ‘83275-5’, ‘83275-8’ and ‘83242-4’ were highly successful at production of BC2 progeny, with a maximum of 13.0 plants per ovary being obtained in plant number 83275-5 (Table 6).

Discussion

The interspecific hybrid was verified to produce the 2n gametes, indicating great potential for meiotic polyploidization in a breeding program. F1 AuH hybrids are valuable to lily breeding due to the desirable horticultural traits of its parents, which include virus resistance from L. henryi and botrytis resistance from L. auratum, therefore, in this study, we re-examined features of the F1 AuH hybrid and produced their subsequent progeny.
Table 5. Pollen viability and germination of BC1 progeny derived from AuH hybrid, 82111, on artificial agar medium.

| Access no. | Geno-type | Cross combinations | Female (Oriental hybrids) | Male (AuH) | Stained pollen (%) | Germination (%) |
|------------|-----------|--------------------|---------------------------|------------|--------------------|-----------------|
| 82396-1    | O AuH     | Journey’ End       | 82111                     | 21.9       | 0                  |
| 82396-3    | O AuH     | Journey’ End       | 82111                     | 6.9        | 0                  |
| 82396-4    | O AuH     | Journey’ End       | 82111                     | 0.0        | 0                  |
| 82396-5    | O AuH     | Journey’ End       | 82111                     | 26.2       | 3.9                |
| 82342-3    | O AuH     | Stargazer          | 82111                     | 26.4       | 0                  |
| 83275-1    | O AuH     | Stargazer          | 82111                     | 4.1        | 0                  |
| 83275-3    | O AuH     | Stargazer          | 82111                     | 44.6       | 2.7                |
| 83275-5    | O AuH     | Stargazer          | 82111                     | 21.1       | 0                  |
| 83275-7    | O AuH     | Stargazer          | 82111                     | 48.0       | 0                  |
| 83275-8    | O AuH     | Stargazer          | 82111                     | 0.0        | 0                  |
| 83275-15   | O AuH     | Stargazer          | 82111                     | 42.6       | 0                  |
| 85863-1    | O AuH     | Dominique          | 82111                     | 29.6       | 2.0                |
| 85863-2    | O AuH     | Dorminique         | 82111                     | 38.1       | 2.3                |
| 85864-1    | O AuH     | Darling            | 82111                     | 34.9       | 4.3                |
| 85864-2    | O AuH     | Darling            | 82111                     | 21.2       | 0                  |
| 85864-5    | O AuH     | Darling            | 82111                     | 0.0        | 0                  |
| 85864-6    | O AuH     | Darling            | 82111                     | 22.0       | 0                  |

Table 6. Numbers of BC2 progeny obtained by backcrosses between OAuH hybrids (BC1) and Oriental hybrid, L. auratum or L. henryi.

| Cross combinations | Geno-type | No. of flowers pollinated | Pre-fertilization barrier (%) | Post-fertilization barrier (%) | No. of plants obtained | No. of plants/ovary |
|--------------------|-----------|---------------------------|-------------------------------|-------------------------------|------------------------|----------------------|
| 82396-1            | O OAuH    | 12                        | 0(0)                          | 12(100)                       | 0                      | 0                    |
| 82396-3            | O OAuH    | 12                        | 0(0)                          | 12(100)                       | 0                      | 0                    |
| 82396-4            | O OAuH    | 5                         | 0(0)                          | 2(40.0)                       | 3                      | 0.6                  |
| 82396-5            | O OAuH    | 7                         | 0(0)                          | 2(28.6)                       | 9                      | 1.3                  |
| 82396-6            | O OAuH    | 7                         | 0(0)                          | 0(0)                          | 11                     | 1.6                  |
| 82342-4            | O OAuH    | 7                         | 0(0)                          | 3(42.9)                       | 40                     | 5.7                  |
| 83275-1            | O OAuH    | 1                         | 1(100)                        | 0(0)                          | 0                      | 0                    |
| 83275-3            | O OAuH    | 3                         | 0(0)                          | 0(0)                          | 6                      | 2.0                  |
| 83275-7            | O OAuH    | 6                         | 0(0)                          | 2(33.3)                       | 3                      | 0.5                  |
| 83275-8            | O OAuH    | 4                         | 0(0)                          | 0(0)                          | 26                     | 6.5                  |
| 85863-1            | O OAuH    | 10                        | 0(0)                          | 7(70.0)                       | 4                      | 0.4                  |
| 85863-2            | O OAuH    | 8                         | 3(37.5)                       | 4(50.0)                       | 9                      | 1.1                  |
| 85864-1            | O OAuH    | 12                        | 0(0)                          | 5(41.7)                       | 0                      | 0                    |
| 85864-2            | O OAuH    | 7                         | 7(100)                        | 0(0)                          | 0                      | 0                    |
| 85864-6            | O OAuH    | 8                         | 0(0)                          | 3(37.5)                       | 8                      | 1.0                  |
| 83275-5            | L. auratum| OAuH Au                  | 5                             | 0(0)                          | 65                     | 13.0                 |
| 83275-15           | L. auratum| OAuH Au                  | 4                             | 2(50.0)                       | 2                      | 0.5                  |
| 83275-5            | L. henryi | OAuH H                   | 8                             | 8(100)                        | 0                      | 0                    |
| 83275-15           | L. henryi | OAuH H                   | 5                             | 5(100)                        | 0                      | 0                    |
While Asano (1984) reported that 15% of F₁ AuH hybrid pollen grains germinated abnormally while only 1.6% germinated normally [10], Van Tuyl et al. (1989) found that 91.2% of the pollen cells were present as dyads at the tetrad stage in the same hybrid plant [9]. Our findings also showed a high percentage of pollen staining (42%) and germination (69%). The variation between these studies could be explained by the effects of environmental factors on the formation and viability of 2n pollen. It has been reported that high temperatures caused a low frequency of 2n-gametes [9]. Additionally, Chung et al. (2009) showed that production of 2n pollen by OA hybrids differed depending on the season in which it was measured [17]. Pollen performance is also influenced by the pollen genotype: some genotypes exhibited higher pollen germination %age whereas some genotypes showed very low germination percentage under same environmental conditions [18].

GISH techniques have been applied to determine the origin of 2n gametes in Lilium and offered a new perspective for elucidation of restitution mechanisms, the extent of genetic recombination and composition of 2n-gametes. As expected, all of the BC₁ progeny originated from the F₁ AuH hybrid were triploid, without any aneuploid progeny. Production of aneuploid BC₁ progeny is not common [17, 19, 20, 21, 22], although LA interspecific hybrids showed the potential to produce a large amount aneuploid pollen [23].

Many studies of lilies have shown that F₁ interspecific hybrids produced functionally unreduced gametes and were successfully used for production of BC₁ progeny [19, 20]. However, success in introgression is related to both the level of homoeologous recombination between parental genomes during meiosis in the F₁ hybrids and their fertility [24]. The percentage of recombinant triploid BC₁ progeny was 62.5% and 65.8% in ALAs and AOA, respectively [20, 25].

It is well known that intergenomic translocations are more likely to occur in the F₁ hybrids of distantly related species because the homoeologous chromosomes are forced to pair and 2n gametes resulting from such meioses are most likely to transmit recombinant chromosomes to progeny with sexual polyploidy [26]. This was observed in the progeny of many F₁ interspecific hybrids including Gasteria-Aloe [27], Alstroemeria species [28, 29] and Lilium species [17, 30]. Our data showing a high rate of recombination again confirmed that L. henryi is distinct from L. auratum (Oriental hybrid group) [4]. Moreover, it should be noted that the percentage of recombinant chromosomes in the BC₁ progeny was variable depending on the genotypes used as the 2n gamete donor in OA hybrids from 35.7% with 952400-1 to 79.1% with 951502-1 [20]. Therefore, it might be desirable to screen diverse populations of F₁ interspecific hybrids producing 2n gametes for frequencies of chromosome pairing and chiasma formation.

Based on the above research, it was assumed that chromosome pairing and crossing over are genetically controlled and thus genotype dependent; accordingly, a high percentage of recombination might be attributed to high genome divergence between L. auratum and L. henryi when compared with OA hybrid genomes in Lilium. In conclusion, we confirmed that the 2n gametes of the F₁ AuH hybrid are highly valuable to polyploid breeding of lilies and the genetic variation of 2n gametes caused by intergeneric recombination dramatically increases the chances of selecting new cultivars from the BC₁ population.

In addition to intergeneric recombination, the mechanism of 2n gamete formation is another important aspect for sexual polyploidization. From the cytogenetic point of view, unreduced gametes are known to be formed via three different mechanisms; (i) an incomplete first meiotic division (first division restitution, FDR), (ii) an incomplete second meiotic division (second division restitution, SDR) and (iii) an indeterminate meiotic restitution (IMR) [31, 32, 33]. In the present study, all triploid BC₁ progeny of AuH hybrid resulted from FDR 2n pollens because of the presence of 12 centromeres in each of the two sets of homoeologous chromosomes of L. henryi and L. auratum in the AuH hybrids [34], together with a complete set of the genome of the Oriental hybrid as female. The value of FDR gametes is in transferring heterosis and parental gene combinations intact in sexual polyploids.

Previous studies elucidated that the mechanisms of 2n pollen derived from F₁ interspecific hybrids in lilies are FDR and IMR [17, 19], and that FDR is the predominant phenomenon involved in production of 2n gametes from F₁ OA hybrids [20, 21] and from F₁ LA hybrids [35]. Taken together, these data suggest that the chromosomal compositions of FDR gametes are more balanced than those of IMR gametes, resulting in FDR gametes being more viable and having a higher transmission rate than IMR gametes because of being suitable for selection as cultivars in nature. These findings are in accordance with Zhou et al. 2008 studies [36]. Indeed, since Lim et al. (2001) discovered the IMR mechanism in LA interspecific hybrids [17], Barba-Gonzales (2005a) found that some F₁ OA hybrids produced 2n gametes via an IMR mechanism [20].

BC₁ progeny were produced from a large number of backcrosses of F₁ AuH hybrids by crossing with Oriental hybrids through the use of 2n gametes. These BC₁ progeny have important features; namely,
moeologous recombinant chromosomes. However, allotriploids generally cannot be easily used as parents in lily breeding because of their high degree of sterility due to unbalanced meiosis. Despite this restriction, there are several instances among crop plants in which allotriploids have been successfully used as parents, such as *Arachis hypogaea* [37], *Triticum-Aegilops* hybrids [38, 39], *Triticum-Hordeum* hybrids [40], *Alstroemeria* species hybrids [41], and *Festuca-Lolium* hybrids [42].

In lily, allotriploids (ALA) of *Longiflorum* × Asiatic hybrids have been successfully used for production of BC2 progeny. These triploids can be crossed with both diploid and tetraploid parents to yield aneuploid progeny consisting of near-diploids or near-tetraploid to pentaploid offspring [35]. Furthermore, it has been reported that allotriploids AOA genotypes can be used as parents in crosses with diploid or tetraploid individuals to produce considerable numbers of BC2 progeny [43].

Interestingly, the product of BC2 progeny was only successful in cases in which the BC1 progeny (82396-1) were female (Table 6). These results well support Zhou’s hypothesis, “Five same genomes of endosperm are essential for its development in allotriploid x diploid/tetraploid crosses of *Lilium* [44]. Indeed, Lim et al. (2003) demonstrated that egg cells of triploid BC1 (ALA) produce BC2 progeny with a fairly wide range of chromosomes and this wide range of chromosomes could contribute to the viability of egg cells and chromosome balance of embryos and endosperms [35].

**Conclusion**

BC2 production derived from F1 AuH interspecific hybrids has potential value because an introduction of segments of the recombinant chromosomes can be transmitted to further generations. However, more studies of the BC2 progeny to identify the recombinant chromosomes transmitted are necessary to establish systematic and meaningful procedures for polyploidy breeding.

**Competing Interests**

The authors have declared that no competing interest exists.

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