Occurrence of SDR 2N-gametes in Lilium Hybrids

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The mechanism of SDR 2n-pollen formation was analyzed in two intra-sectional diploid (2n = 2x = 24) Lilium hybrids (Enchantment × L. pumilum). Variable frequencies of 2n-pollen were found. Meiotic analysis indicated that the intra-sectional hybrids showed perfect chromosome pairing in most cases at metaphase I and normal anaphase I movement of pollen mother cells (PMCs), but produced 2n-pollen by second division restitution (SDR). A high bivalent formation (11.9II and 11.8II, respectively) at metaphase I, irregular meiotic division such as unbalanced chromosome separation and chromatic fragmentation resulted yet in acceptable pollen fertility for cross-pollination. The hybrids were fertile, and when used as male parents, offspring could be generated. The significance of the occurrence of 2n-pollen for the breeding of lilies was analyzed.

Key Words: SDR, sexual polyploid, meiosis, pollen viability, intra-sectional hybrid.

Introduction

The cultivated lilies consist of mainly three distinct groups, e.g. the Longiflorum hybrids, Asiatic hybrids and Oriental hybrids. The hybrid cultivars in each of these groups are derived from inter-crossing of the diploid species (2n = 2x = 24) within each of the taxonomic sections, viz., Leucolirion (Longiflorum hybrids), Sinomartagon (Asiatic hybrids) and Archelirion (Oriental hybrids) (Van Tuyl *et al.* 2000). Species within each section are crossable although not easily; the meiosis of these hybrids is normal and results in the production of fertile pollen. On the other hand, the species belonging to the six different taxonomic sections of the genus *Lilium* are more difficult to hybridize. Meiosis in intersectional hybrids is highly irregular and consequently, the hybrids are sterile (Asano 1982, 1984, Lim *et al.* 2001).

Although most of the lily cultivars are diploid, polyploid cultivars have become more important in recent years. Polyploids have been successfully induced through chemical treatment (somatic doubling) and have been used in

Communicated by K. Okazaki Received February 10, 2003. Accepted October 1, 2003. breeding for more than 20 years (Van Tuyl *et al.* 1992). However, in order to exploit the advantages of sexual polyploidization, it is necessary to identify genotypes that produce 2n-gametes in order to use them for the generation of both polysomic and disomic polyploids in lilies (Lim *et al.* 2003).

In plant species and hybrids, normal diploid (2n = 2x) plants produce genetically four different male gametes (n, n, n, n) through complex pathways of microsporogenesis. Numerically unreduced (2n) gametes occur occasionally instead of the expected haploid (n) gametes. Chromosome pairing, centromere division, spindle formation or cytokinesis may be the main factors causing 2n-gamete formation during micro- and megasporogenesis (Veilleux 1985). First division restitution (FDR) is the most common mechanism of 2n-gametes' formation in interspecific hybrids (Mendiburu and Peloquin 1977). However, depending on the genetic distance between parental genomes, the mechanism underlying 2n-gamete production could be either FDR or SDR (second division restitution).

Interspecific and intergeneric hybrids are outstanding materials for the study of 2n-gametes. These hybrids are mainly sterile and viable pollen is derived from abnormal meiotic division mainly resulting in 2n-gametes. We have shown that some interspecific lily hybrids produce 2ngametes. Although, FDR was the most common mechanism among them (Lim et al. 2001), IMR (indeterminate meiotic restitution) and SDR were occasionally observed. In potato, genetic control of these mechanisms has been described (Watanabe and Peloquin 1989, Peloquin et al. 1999). Some sporocytes show parallel spindles (ps) in the second division, and after cytokinesis, 2n-microspores were formed. The ps, pseudohomoeotypic division, fused spindle (fs) are equivalent to the FDR mechanism, while premature cytokinesis (pc), omission of the second meiotic division (os), failure of cytokinesis (fc) are equivalent to the SDR mechanism (Jongedijk and Ramanna 1988, Peloquin et al. 1999).

As a genetic consequence, three types of 2n-gametes can be obtained, corresponding to FDR, SDR and IMR. Due to the equational division of sister chromatids, FDR gametes contain an equal number of parental chromosomes. On the other hand, in the case of SDR, sister chromosomes move to the same daughter cell. Therefore, FDR gametes maintain maximum heterozygosity and SDR gametes display maximum homozygosity in the absence of crossing-over (for review, Hermsen 1984, Veilleux 1985, Peloquin *et al.* 1999).

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Since the IMR gametes display a mixture of FDR and SDR depending on the degree of bivalent formation, the genetic consequence of IMR is more complex than that of FDR or SDR (Lim *et al.* 2001).

The existence of 2n-gametes allows potato breeders to broaden the genetic basis of cultivated Solanum tuberosum, by introducing both new genes for the improvement of traits of interest and allelic diversity to maximize heterozygosity (Peloquin et al. 1999). When cultivars or their hybrids produce 2n-gametes in reasonable frequencies, they can be used for producing, so-called, sexual polyploids. The breeding value of sexual polyploids has been convincingly demonstrated in the case of potato (Peloquin 1982, Hermsen 1984, Jongedijk et al. 1991, Carputo et al. 2000), alfalfa (Bingham and McCoy 1979) and clover (Parrot et al. 1985) among other crops. In these cases, emphasis has been placed in maximizing the hybrid vigor in sexual polyploid progenies by 2n-gametes. Unlike polysomic polyploids, hardly any attention has been paid so far to disomic polyploids (allopolyploids) in which 2n-gametes can be used for the introgression of genes from alien species. There are only a few examples among plants so far, in which the 2n-gametes have been utilized for inducing both polysomic and disomic polyploids in one and the same crop in order to broaden the genetic basis for crop improvement (Carputo et al. 2000, Lim et al. 2001). In the case of Lilium, however, it might be possible to utilize 2n-gametes for inducing both polysomic and disomic polyploids.

There are two types of polyploidization, namely mitotic and meiotic polyploidization. Mitotic polyploidization or somatic chromosome doubling takes place in mitotic cells and a new cell contains two times the original set of chromosomes. At the tetraploid level, although sterile interspecific hybrids can recover their fertility, no homoeologous recombination can be seen in subsequent progenies (Lim *et al.* 2000). On the other hand, meiotic polyploidization by FDR and IMR has resulted in high frequencies of homoeologous recombination in lily hybrids (Lim *et al.* 2001, 2003). Meiotic analysis of SDR gamete formation in lily has not been reported. The present paper presents the mechanism of formation of SDR 2n-gametes in intra-sectional lily hybrids from the cross 'Enchantment' $\times L.$ *pumilum*.

Materials and Methods

The lily genotypes and their pedigree are listed in Table 1.

 Table 1. Pedigree of the Asiatic Lilium hybrids used in this experiment

Genotype	Pedigree
Revival	Eurovision×Rosefire
79418-2	Enchantment×L. pumilum
79418-7	Enchantment×L. pumilum

The hybrids were grown after normal cross-pollination and sowing of the seeds (Van Tuyl et al. 1986). The ratio of nand 2n-pollen was analyzed using flow cytometry (Van Tuyl et al. 1989). The plant materials were grown in a greenhouse and used for meiotic analysis as well as pollen germination studies. Anthers were fixed for two hours in acetic-alcohol (1:3) at room temperature and transferred to 70% ethanol for storage until use. The materials were digested with a pectolytic enzyme mixture (0.3% pectolyase Y23, 0.3% cellulase RS and 0.3% cytohelicase in 10 mM citric acid buffer, pH 4.5) at 37°C for 1 hour and squashed in a drop of 60% acetic acid solution. Slides were stained with 2% acetoorceine for the meiotic chromosomes. Fresh sporads were also stained with a lacto-phenol fuchsin solution for the observation of viability and nucleus restitution. Pollen germination was estimated on an artificial medium consisting of 10% sucrose, 20 mg/L boric acid and 8 g/L of agar.

Results

Meiotic behavior and pollen viability

The meiotic analysis of one Asiatic hybrid and two intra-sectional hybrids is presented in Table 2. In all three cases, there was a high frequency of bivalent formation at metaphase I (Table 2 and Fig. 1a) with 92% for the control cultivar 'Revival', and 96% and 92% for the 2n-gametes producing intra-sectional hybrids, respectively. Table 2 shows that meiotic division from prophase I to metaphase I was normal in the three genotypes. Although meiosis I appeared to be normal, many cells skipped cytokinesis II, resulting in SDR unreduced gametes. Therefore, a high frequency of dyads was formed after microsporogenesis (Fig. 1b). The pollen germination of the intra-sectional hybrids '79418-2' was high (44%) and among them, the formation of 2n-gametes reached a value of 25%. Along with meiotic pairing, pollen viability and in vitro pollen germination were presented in Table 3 and Table 4.

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

Hybrids No. of cells observed		Configurations / cells													
		1211	11II	11II 10II 2I 4I	911 61		7II	7II 6II 10I 12I	511 141	4II 16I	3II 18I	211 201	1II 22I	0II 24I	Mean frequency
	observed		2I				10I								
Revival	125	115	8	2	-	_	-	_	_	-	_	-	-	-	11.8II+0.2I
79418-2	99	95	3	1	_	-	_	-	-	-	_	_	-	_	11.9II+0.1I
79418-7	118	108	6	4	-	_	-	_	-	-	-	-	-	-	11.8II+0.2I

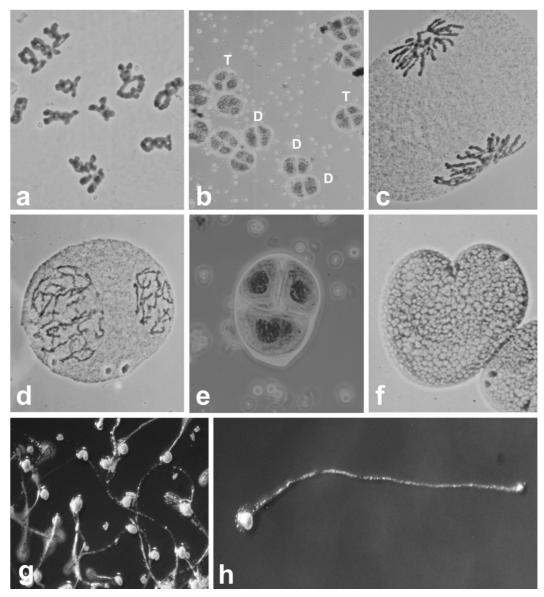


Fig. 1. Microsporogenesis and pollen germination of the interspecific hybrid 79418-2. a. 12 bivalents at MI.
b. Sporads after tetrads' stage show dyads (D), triads (T) and tetrads. c. Balanced movement of homoeologous chromosomes at late anaphase I. d. Unbalanced chromosome distribution. This type of disjunction occurs the most commonly during microsporogenesis in distantly related interspecific hybrids. e. Triad shows a clear division of three nuclear formations by successive cytokinesis. f. Heart-shaped SDR 2n-pollen shows that the second cytokinesis had been skipped. g–h. Germination of heart-shaped 2n-pollen.

Table 3. Frequency of types of sporocytes at tetrad stage in Lilium hybrids

Hybrids	Monad	Dyad	Triad	Tetrad	Others	Total
Revival	-	$1(1)^{1)}$	3 (2)	124 (97)	_	128 (100)
79418-2	8 (5)	113 (64)	18 (10)	29 (16)	8 (5)	176 (100)
79418-7	11 (6)	97 (53)	23 (13)	45 (25)	5 (3)	181 (100)

¹⁾ The percentage is given in brackets.

Formation of 2n-pollen

In order to determine the types of sporads and microspores that were formed, the late meiotic stages (i.e., subsequent stages of telophase II) were analyzed in the anthers. The frequencies of different types of sporads, which were classified into five categories, varied among the hybrids (Table 3). In the case of 'Revival', nearly all of them were tetrads whereas in the two intra-sectional hybrids ('79418-2'

and '79418-7'), dyads and triads were present in addition to tetrads (Fig. 1b). Due to normal chromosome pairing at metaphase I in these hybrids, the anaphase I disjunction was normal (Fig. 1c), except for a few abnormalities (Fig. 1d), implying that the formation of dyads and triads in the intrasectional hybrids was due to the lack of second division (Fig. le and Fig. 1f). The 2n-pollen that was observed in both '79418-2' and '79418-7' was therefore of the SDR type. Heart-shapes sporads that were often produced by the SDR mechanism due to the omission of second cytokinesis (Fig. 1f) and heart-shaped mature 2n-pollen could germinate (Fig. 1g and Fig. 1h).

Ganatura	Pollen	No. of	Germinated	Percentage		
Genotype	type	pollen	pollen	In group	In total	
Revival	n	251	173	68.9	68.1	
	2n	3	0	0	0	
	All	254	173		68.1	
79418-2	n	130	41	31.5	19.1	
	2n	85	54	63.5	25.1	
	All	215	95		44.2	

Table 4. Pollen germination of n and 2n-gametes

 Table 5. Crossing results with different Asiatic hybrids and SDR 2n-gametes

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Female	Male	No. of flowers crossed	No. of plants derived ¹⁾
Revival	79418-2	10	29
Orlito	79418-2	9	31
Connecticut King	79418-2	9	69

¹⁾ Number of plants indicated is the total number of plants that germinated from normal harvested seed. Therefore, the number of seeds harvested is higher than the number of plants that germinated.

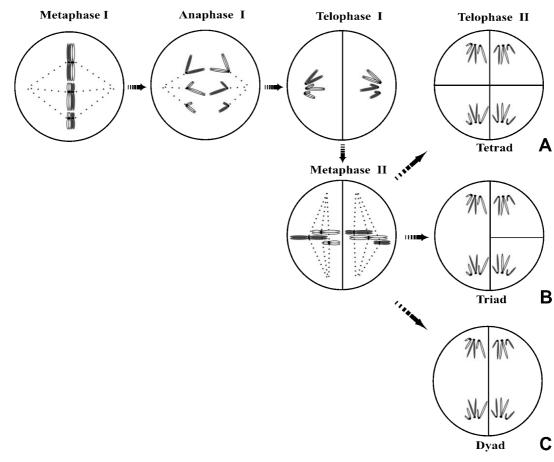


Fig. 2. Schematic diagram of three possible pathways for sporad formation during microsporogenesis in a diploid intra-sectional hybrid in the case of 2n = 2x = 6. There might be many crossing-overs between parental chromosomes, but the breakpoints of crossing-over are not marked in this figure. The disjunction of homoeologous chromosomes at anaphase I was random in this diagram. A. Normal meiosis pathways resulting in tetrad formation with four n-gametes. Because of the genetic similarity between the parental species, the omission and addition of certain chromosomes may not affect the pollen survival. **B.** Triad consisting of one 2n-gamete and two n-gametes due to the omission of cytokinesis II in one part. **C.** Cytokinesis II had been skipped in this case, resulting in the formation of two SDR 2n-gametes.

Production of subsequent progenies

Several crosses listed in Table 5 were made using the pollen of '79418-2'. Two types of progenies were produced either through in vitro embryo rescue or normal seed set. When plants were produced by in vitro embryo rescue, the two types of progenies were produced, namely either triploid from 2n-gametes or diploid from n-gamete of the male parent. However, most of the plants produced by normal seed harvest, were diploid or tetraploid, because of the triploid block. Table 5 shows the number of plants that germinated from normal harvested seeds. In addition to the results shown in Table 5, from a backcross of 'Enchantment' and '79418-1', four tetraploid cultivars 'Puchanta', 'Pumivetta', 'Pumenta' and 'Pumena' were produced and registered in the RHS lily register (Leslie 1982). Apparently 2n gametes from both male and female were responsible for this progeny.

Discussion

Meiotic behavior and pollen viability

This investigation demonstrates that SDR pollen grains in 2 hybrids of 'Enchantment' $\times L$. *pumilum* occurred mainly because of a high chromosome association and omission of the second division in the pollen mother cells. It is well known as a consequence of SDR, homologous or homoeologous chromosomes assort independently during anaphase I (Mendiburu and Peloquin 1977, Hermsen 1984).

The chromosome behavior until the anaphase I of perfect-pairing cells was mainly normal without any unbalanced chromosome separation. Fig. 2 shows a diagram of the formation of SDR 2n-gametes by three types of cytokinesis. Abnormal cytokinesis is one of the main reasons for the formation of SDR 2n-gametes, which differs from FDR by the presence of equational division.

Although SDR pollen may not contain some of the parental chromosomes, the germination rate of this pollen was high in the intra-sectional hybrids '79418-2' and '79418-7'. These results are in agreement with previous observations (Van Tuyl *et al.* 1989), where '79418-2' showed the highest pollen viability. It is possible that the high genetic similarity between parental genomes is close enough to compensate for pollen lethality.

Formation of SDR 2n-pollen and genetic consequence

The consequence of SDR is that the parental gene combination is not completely retained in the resulting 2ngametes. A large genetic variation maybe expected in the case of sexual polyploid progenies of the intra-sectional hybrids, '79418-2' and '79418-7'. On the average, 40% of the parental heterozygosity and epistatic relationship is retained in the case of one crossing-over on a chromosome arm (Hermsen 1984). However, if there is no crossing-over in certain chromosomes, the SDR gamete displays 100% homozygosity. Since the two intra-sectional hybrids '79418-2' and '-7' showed a perfect pairing between parental chromosomes, crossing-over may have occurred all over the chromosomes. It appears that a high chromosome association is a prerequisite step in meiosis I followed by the absence of cytokinesis II for the production of SDR 2n-gametes.

In the case of disomic polyploids, 2n-gametes are highly attractive for the introgression of specific horticultural characters as well as for the introduction of heterosis. In this context, the occurrence of 2n-gametes in *Lilium* (Van *Tuyl et al.* 1989, Karlov *et al.* 1999, Lim *et al.* 2001) is highly significant for breeding of this crop. Since most of the cultivars are intra-sectional hybrids and since, in recent years, intersectional hybrids have become increasingly popular in the horticultural industry, 2n-gametes could be used for the improvement of both polysomic and disomic polyploids of lilies.

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