Interspecfic Hybridization in Lily: The Use of *2n* Gametes in Interspecific Lily Hybrids

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ABSTRACT

With the main goal of generating new groups of lilies, that combine agronomic characteristics of major importance, a large number of crosses were performed among the three most important groups of lilies (*Lilium*), viz., Asiatic, Longiflorum and Oriental; and to some species, to produce F_1 inter-specific hybrids. These hybrids, as well as many other F_1 hybrids, were sterile and two approaches were used to overcome sterility: i) chromosome doubling through chemicals and ii) the use of naturally occurring or induced unreduced (*2n*) gametes. The first approach produced many fertile hybrids, however, due to autosyndetic pairing in the allotetraploids, recombination was not detected through DNA *in situ* hybridization techniques (GISH), and introgression was not achieved. On the other hand, through extensive meiotic analyses and pollen germination tests, we selected hybrids that were able to produce *2n* gametes, and in some cases, hybrids in which *2n* gametes could be induced. Many plants were obtained in reciprocal backcrosses and recombination as well as introgression was detected through GISH and FISH analyses. In this chapter we present the success in breeding lilies from different taxonomical sections through the use of naturally occurring and induced *2n* gametes. The mechanisms of *2n* gamete formation are described and the genetic considerations of their use to achieve introgression and to generate variability are examined.

1. INTRODUCTION

Lily is one of the most important horticultural crops. They belong to the genus *Lilium* L. of the monocotyledonous family Liliaceae, which comprises over 80 species classified into seven taxonomic sections (Comber 1947; De Jong 1974). All species are distributed over the mountainous area in the Northern Hemisphere, mainly in Asia, North America and Europe (Lim *et al*. 2000a).

This genus contains many beautiful species and more than 7000 lily cultivars have been bred mainly by intra- and inter-specific hybridization (Leslie 1982 International Lily register). The most important hybrid groups cultivated for cut flower production are the Longiflorum, Asiatic and Oriental hybrids. These groups belong to different taxonomic sections: Leucolirion, Sinomartagon and Archelirion, respectively. The cultivars in all these groups are mostly diploid (2n=2x=24) and they are easy to hybridize within the same section. Because of their taxonomic distance, however, it is difficult to hybridize the cultivars or species that belong to different sections and the hybrids can be produced only through special techniques such as cut-style method (Asano and Myodo 1977a, 1977b), grafted style followed by *in vitro* pollination (Van Tuyl *et al*. 1991), *in vitro* pollination and rescue methods such as embryo, ovary slice and ovule culture, among others (Van Creij *et al*. 1992, 2000).

2. INTERSPECIFIC HYBRIDIZATION

In order to create completely new hybrids the most important tool is interspecific hybridization. However, in a breeding program it is important to combine and to preserve certain traits of interest in the new hybrids, but is crucial that those traits can be transferred to the progeny. There are many agronomic characteristics of major importance in lilies that are desirable to combine in order to obtain completely new hybrids, such as distinctive small or large flowers, simple or fancy shapes, up or down facing flowers, wide variety of colours, different forcing times and foliage arrangements, variation in stem length and strength, but specially, resistance to certain pathogens that are restricted only to some hybrids within

Abbreviations: FDR, first division restitution; **FISH**, fluorescent *in situ* hybridization; **GISH**, genomic *in situ* hybridization; **IMR**, indeterminate meiotic restitution; **SDR**, second division restitution

Table 1 Successful intersectional interspecific cross combinations performed to obtain some hybrids that produce *2n* gametes.

Successful combination	Group	Mechanism	# Hybrids*
L. longiflorum × Asiatic hybrid	LA	FDR, IMR (SDR)	>500
Asiatic hybrid × *L. pumilum*	APum	SDR	>100
L. longiflorum × *L. candidum*	LC	?	50
L. longiflorum × *L. henryi*	LHe	FDR	16
L. auratum × *L. henryi*	AuHe	FDR, IMR	>20
Oriental hybrid × Asiatic hybrid	OA	FDR, IMR	>700

***** the number of hybrids represent the number of hybrids obtained per each cross combination, only a few of them produces *2n* gametes and are listed in **Table 2**.

the different sections. Some lilies from the Sinomartagon section (Asiatic lilies) are resistant to *Fusarium oxysporum* and to some virus (Straathof and Van Tuyl 1994) and some lilies from the Archelirion section (Oriental lilies) are resistant to the patogenic fungus *Botrytis elliptica*. These traits would be valuable in breeding new lily hybrids (Lim *et al*. 2000a).

Interspecific hybridization of lily species and cultivars from the same taxonomic section can be done with relative ease, however, it was not possible to produce intersectional hybrids due to i) pre-fertilization barriers caused by poor pollen tube growth due to stigmatic incompatibility (Asano and Myodo 1977a; Asano 1980c) and ii) post-fertilization barriers resulting in seeds having no endosperm and very small embryos that usually abort in early developmental stages (Myodo 1975; Asano and Myodo 1977b). It was until the development of special pollination techniques such as cut-style (Myodo 1962) and intrastylar pollination (Asano and Myodo 1977a), together with embryo culture (Myodo 1975; Asano and Myodo 1977b; Myodo and Asano 1977; Asano 1978, 1980a, 1980b; Asano and Myodo 1980) which were applied to overcome pre- and post-fertilization barriers, respectively, that intersectional hybridization was possible, and a considerable number of new cultivars were produced. Even more intersectional hybrids were produced with the introduction of novel techniques that were used to overcome the fertilization barriers, some examples include: mentor pollen, *in vitro* pollination and ovary- and ovule culture (Van Tuyl *et al*. 1982, 1988, 1991; Van Creij *et al*. 1992).

With the application of these techniques to overcome fertilization barriers many intersectional hybrids have been produced. In our breeding research there is a major interest to produce intersectional hybrids in specific directions to combine disease resistances with quality traits (Van Tuyl and Van Holstein 1996). In order to achieve these combinations and to generate new groups of hybrids, our efforts have been mainly focused in the generation of intersectional hybrids from the most important hybrid groups for the cut flower market viz. Longiflorum (L) × Asiatic (A) and Oriental (O) × Asiatic (A) to develop the LA and OA hybrids respectively (**Table 1**), and in many cases, hybrids and species from these groups have been crossed to species from other taxonomic sections (*L. Longiflorum* × *L. henryi* (LHe); *L. auratum* × *L. henryi* (AuHe); Asiatic × *L. pumilium* (APum) (**Table 1**). From these crosses more than 1000 hybrids have been obtained (Van Tuyl 1988, 1990a; Van Tuyl *et al*. 1991, 2000; Barba-Gonzalez *et al*. 2004).

Although many intersectional lily hybrids were produced, the major drawback was that most of them, as in many other interspecific hybrids of other taxa, were sterile. This sterility hampers further breeding and is mainly related to low chromosome pairing during meiosis (Ohri and Khoshoo 1983; Ishizaka 1984; Yabuya 1991), besides many other abnormalities such as: chromosome aberrations, genetic incongruity, unbalanced chromosome assortment, chromosome bridges, chromosome lagging during anaphase I and II, time discrepancy between chromosome movement (Asano 1982). All these abnormalities during meiosis are lethal and lead to sterility (Asano 1982; Hermsen 1984).

3. MITOTIC POLYPLOIDIZATION

To overcome sterility in interspecific hybrids, somatic chromosome doubling is the traditional method to restore fertility. The chemicals that are utilized to double the chromosome numbers are known as “spindle poisons”, they act by inhibiting the spindle formation during mitosis (Darlington 1967; Grant 1981). The most common spindle poisons utilized for mitotic chromosome doubling are colchicine (Arisumi 1973; Asano 1982b; Yabuya 1985; Eikelboom and Van Eijk 1990; Ishizaka 1994) and oryzalin (Van Tuyl 1990b; Van Tuyl *et al*. 1992, 2000; Lim *et al*. 2000b; Takamura *et al*. 2002; Lim *et al*. 2003b; Chung *et al*. 2004). Even though fertility is restored; the allotetraploids produced by this technique are known as “permanent hybrids”, because there is no recombination between the parental genomes due to strict autosyndetic paring of the genomes (Lim *et al*. 2000b; Wendel 2000; Van Tuyl *et al*. 2002; Ramanna and Jacobsen 2003). As a consequence, all the gametes produced by these allopolyploids are similar and they offer little possibilities to create genetic variability (Figure 1B). This has been the case of inter-sectional lily hybrids, where as in an earlier attempt to combine desirable characteristics of two diploid species of *Lilium* (2n=2x=24), *L. longiflorum* Thumb. and *L. rubellum* Baker, were hybridized and the chromosome number of the F_1 (LR) hybrid was doubled through oryzalin treatment (Lim *et al*. 2000b). In the BC_1 and BC_2 progenies derived from LLRR allotetraploid, however, not even a single cross-over between the L and R genomes was ever found (Lim *et al*. 2000b; Lim and Van Tuyl 2004).

Table 2 Selection of *2n* pollen producers *Lilium* hybrids and occurrence of embryos after crossing.

Genotype	Parents		Occurrence of embryos
LA hybrids	**Longiflorum**	**Asiatic**	
88542-24	‘Gelria’	‘Whilito’	+
88542-52	‘Gelria’	‘Whilito’	+
88542-69	‘Gelria’	‘Whilito’	+
OA hybrids	**Oriental**	**Asiatic**	
951462-1	‘Romero Star’	‘Connecticut King’	+
951447-1	‘Bel Paso’	‘Gran Sasso’	-
951502-1	‘Pesaro’	‘Connecticut King’	+
951584-1	‘Acapulco’	‘Sancerre’	+
952088-1	‘Expression’	‘Au Revoir’	+
952381-5	‘Mero Star’	‘Connecticut King’	-
952400-1	‘Mero Star’	‘Gran Sasso’	+
952462-1	‘San Marco’	‘Connecticut King’	+
962119-1	‘Acapulco’	‘Connecticut King’	+
962120-1	‘Bernini’	‘Connecticut King’	+
962254-2	‘Tenerife’	‘Lanzarote’	-
APum hybrids	**Asiatic**	**Species**	
79418-2	‘Enchantment’	*L. pumilum*	+
79418-7	‘Enchantment’	*L. pumilum*	+
AuHe hybrids	**Species**	**Species**	
82111	*L. auratum*	*L. henryi*	+
LHe hybrids	**Species**	**Species**	
89356-1	*L. longiflorum*	*L. henryi*	+
89356-6	*L. longiflorum*	*L. henryi*	+

LA= *L. longiflorum* × Asiatic hybrid; OA= Oriental hybrid × Asiatic hybrid; Apum= Asiatic hybrid × *L. pumilum*; AuHe= *L. auratum* × *L. henryi*; LHe= *L. longiflorum* × *L. henryi*.

4. MEIOTIC POLYPLOIDIZATION

An alternative to the use of mitotic chromosome doubling is the use of gametes with somatic chromosome numbers, which are known as “*2n*” or “unreduced” gametes. They occur in most of the angiosperm species and many authors attribute to them the origin of polyploid plant species (reviews by Harlan and de Wet 1975; Veilleux 1985; Ramanna and Jacobsen 2003). *2n* gametes were used to produce polyploids prior to the discovery of colchicine to double the chromosome number and restore fertility. However, as the production of such gametes is highly sporadic, their use was rapidly discarded by breeders and artificial polyploids (induced by chemicals) were preferred (Ramanna and Jacobsen 2003). The main difference between the artificially induced allopolyploids and the naturally occurring sexual polyploids (originated through functioning *2n* gametes) is that the first have fixed heterozygosity (Bretagnolle and Thompson 1995; Soltis and Soltis 2000) and in sexual polyploids heterozygosity is not fixed because recombination between the alien parental genomes is present and therefore they are more promising for breeding. Another advantage of *2n* gametes is that due to recombination introgression of small chromosome segments can be achieved (Karlov *et al*. 1999; Lim *et al*. 2001a; Ramanna *et al*. 2003). Unreduced gametes have been shown to be useful in breeding; some examples include: *Alstroemeria* (Kamstra *et al*. 1999a; Ramanna *et al*. 2003), *Medicago* (Bingham 1980; Veronesi *et al*. 1986), *Primula* (Skiebe 1958), *Rhododendron* (Eeckhaut *et al*. 2006) and *Solanum* (Mendiburu and Peloquin 1971; Mendiburu *et al*. 1974) among others.

In the case of inter-sectional *Lilium* hybrids, we selected those genotypes that were able to produce *2n* pollen (**Table 2**) and they were extensively used to produce progeny (Lim *et al*. 2001a, 2001b, 2003a, 2003b; Barba-Gonzalez *et al*. 2005a, 2005b, 2005c, 2006b; Zhou 2007). The selected F_1 hybrids that produced *2n* pollen were identified either by *in vitro* pollen germination and fruit set and embryo germination after using *2n* pollen in crossing. Not only *2n* pollen has been detected in the *Lilium* hybrids but *2n* eggs (Zhou *et al*. 2007), however, the identification of *2n* eggs is more difficult because several crosses must be performed in order to detect those hybrids which produce them.

Generally, *2n* gametes originate due to deviating meiosis in plants. The process that leads to *2n* gamete formation is called meiotic nuclear restitution that occurs during micro- or megasporogenesis (Ramanna and Jacobsen 2003). A vast amount of cytological as well as genetic research has been conducted in order to elucidate the different mechanisms of *2n* gametes formation. Research in potato is a good example showing evidence for several mechanisms (Mok and Peloquin 1975; Den Nijs and Peloquin 1977; Ramanna 1979; Veilleux 1985). In the case of monocots, however, just a few genera have been the subject of research, but these have provided a good insight into the mechanisms responsible for the *2n* gamete formation. Some examples are the genus *Alstroemeria* (Kamstra *et al*. 1999a; Ramanna *et al*. 2003), *Lilium* (Lim *et al*. 2001; Ramanna and Jacobsen 2003), *Triticum* (Xu and Joppa 1995; 2000) and *Zea mays* (Roades and Dempsey 1966). There are different mechanisms responsible of the *2n* gamete formation, in a review by Veilleux (1985) several mechanisms are identified in several plant species, however, there are two main restitution mechanisms in monocot plants, where cytokinesis and the formation of a cell wall takes place after the first meiotic division (telophase I), following which, the second meiotic division

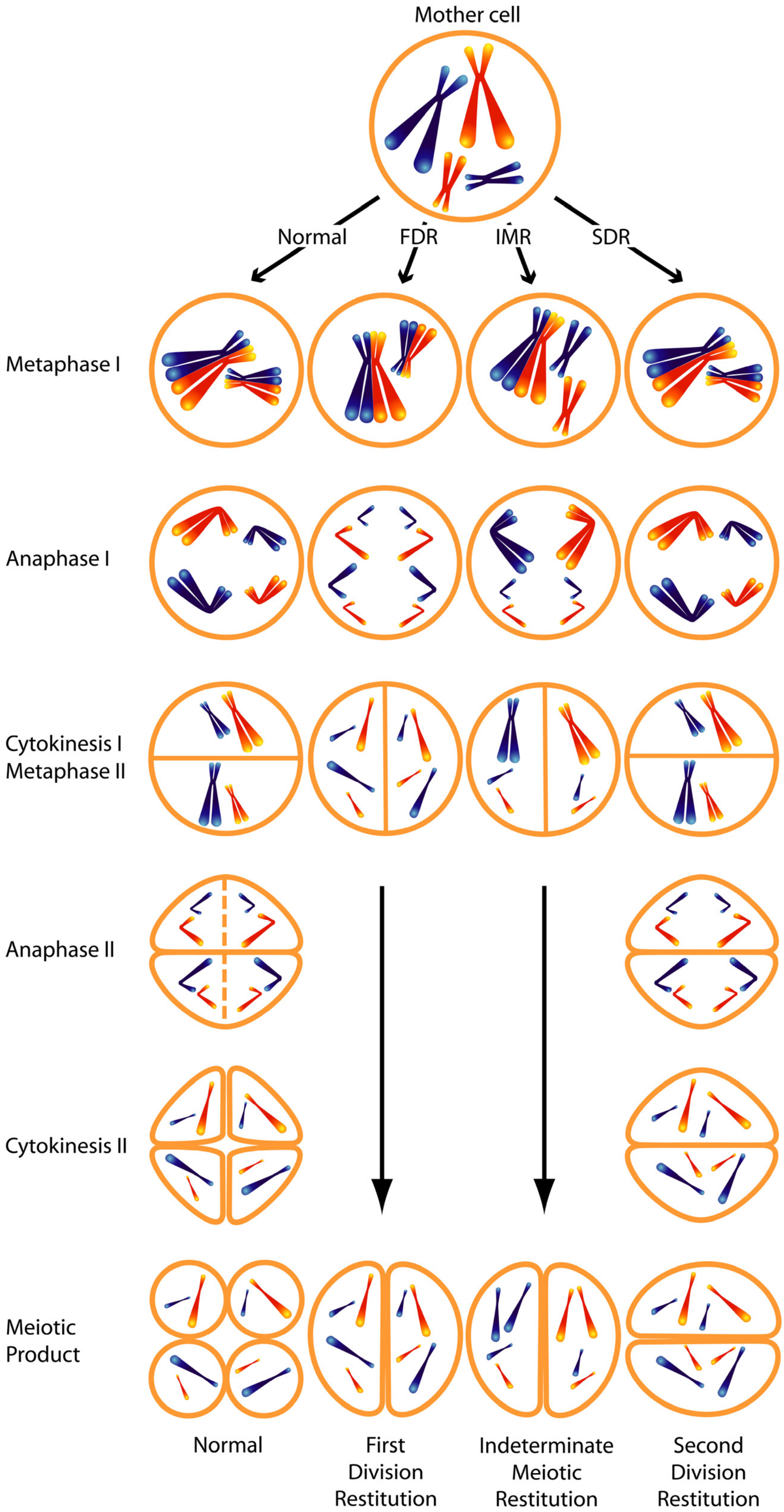

Fig. 1 Schematic representation of the meiotic process, the normal sequence and three restitution mechanisms in microsporogenesis in species having the monocotyledonous successive type of meiotic division (Lily type).

takes place in different cells within a pollen mother cell, the so-called "successive" type, In the case of dicot plants, a majority possess the so-called "simultaneous" type of microsporogenesis, where cytokinesis and cell wall formation takes place at the same time, after the second meiotic division (telophase II). Thus, when cytokinesis, chromosome disjunction and spindle abnormalities are involved in the formation of *2n* gametes, it is evident that different mechanisms are responsible for their formation (Ramanna and Jacobsen 2003). Like in dicots, there are two main restitution mechanisms in monocot plants known as First Division Restitution (FDR) and Second Division Restitution (SDR). In the case of FDR the whole chromosome complement divides "equationally" before telophase I followed by cytokinesis, leading to the formation of a dyad without further division (**Fig. 1**). In the case of SDR, the chromosomes divide "reductionally" at anaphase I and in telophase I, cytokinesis occurs, producing a dyad. However, instead of the second division, the chromatids divide but the nuclei restitute in each of the two cells of a dyad (**Fig. 1**) (Barba-Gonzalez *et al*. 2005b). Recently, a third mechanism has been identified in Longiflorum × Asiatic lily hybrids (Lim *et al*. 2001) which occurs also in other inter-sectional lily hybrids. This mechanism has been recognized as Indeterminate Meiotic Restitution (IMR) and it combines characteristics of the two mechanisms mentioned above, because during the first meiotic division some of the univalents divide equationally (as in FDR) and some bivalents disjoin reductionally (as in SDR) before telophase I (**Fig. 1**) leading to a dyad without further division.

In lily hybrids, the three mechanisms (FDR, SDR and IMR) have been identified as responsible of the *2n* gamete formation through Genomic *In Situ* Hybridization (GISH) and Fluorescent *In Situ* Hybridization (FISH) analyses (Lim *et al*. 2001, 2003a, 2004; Barba-Gonzalez *et al*. 2005a; Chung *et al*. 2006). The fact that there are different mechanisms responsible for the *2n* gamete formation has a great value in breeding. In lily hybrids, the different mechanisms have been found regarding the taxonomical distance of the hybrids. FDR and IMR has been identified in LA, OA, AuHe and LHe *Lilium* hybrids where both parents are taxonomically distant, on the contrary, SDR was identified in hybrids from *L. pumilum* × "Enchantment" (Lim *et al*. 2004), both belonging to the Sinomartagon section, and so forth, taxonomically related.

As it was mentioned above, in breeding, it is necessary to combine and preserve certain traits of agronomic value. However, to achieve considerable genetic variation and introgression of those traits it is necessary to demonstrate the presence of recombination among the parental

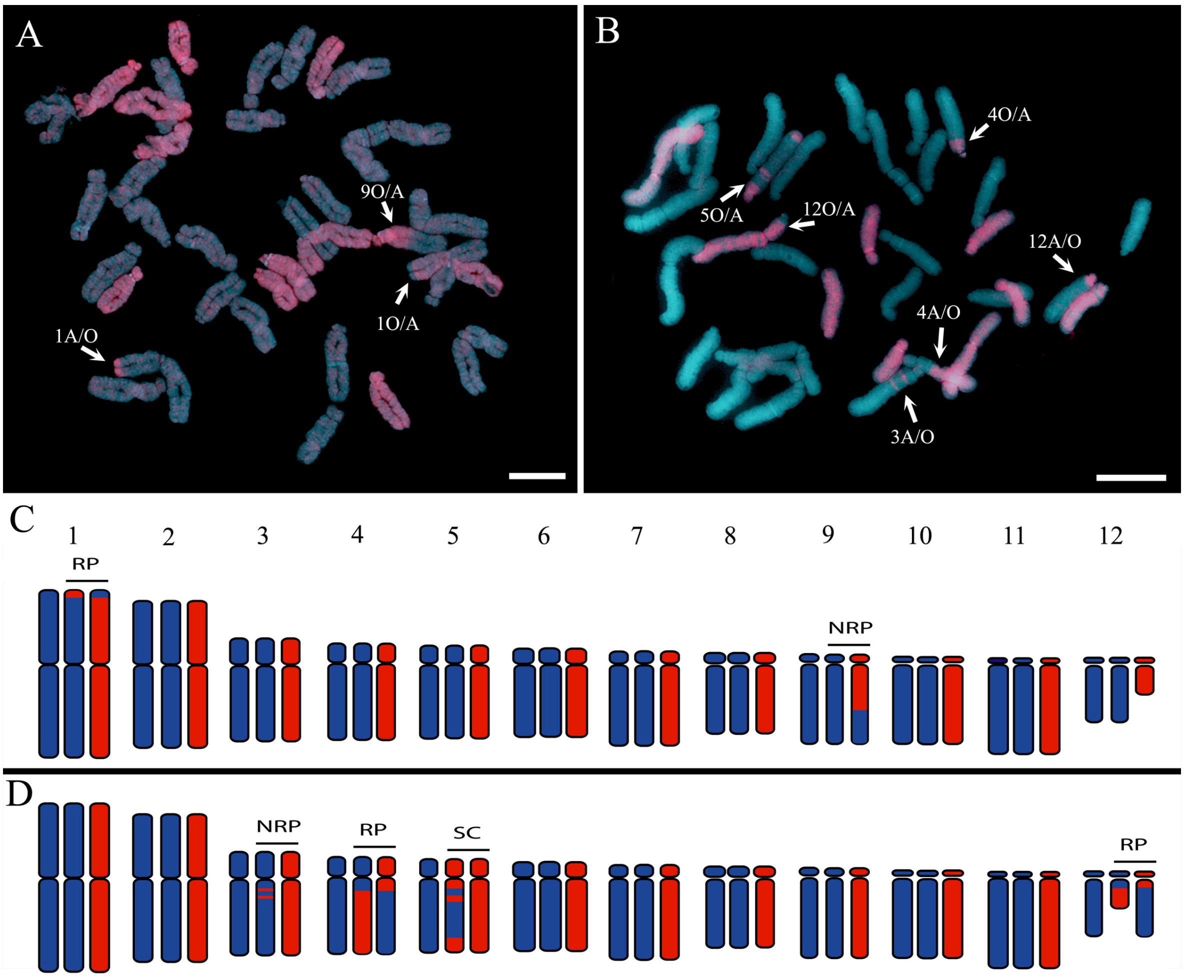

Fig. 2 Chromosome identification and detection of intergenomic recombination in two BC$_1$ progenies by Genomic *in situ* hybridization. (**A**-**B**). The recombinant chromosomes are mentioned appropriately (e.g., O/A or A/O) and the arrows indicate the recombinant segments. The ideograms (**C**-**D**) shows the recombinant chromosomes. As it can be inferred the *2n* pollen contributed with 12 Oriental (O) + 12 Asiatic (A) chromosomes and the extra chromosome set (represented in each case by the chromosome at the left) is from the haploid ovule from the Asiatic (**A**) The triploid complement of 022605-8 showing 12 (O) chromosomes + 24 (A) chromosomes with three recombinant chromosomes. The ideogram (**C**) shows that the homoeologous recombinant chromosomes 1 (1A/O and 1O/A) are the reciprocal product (RP) of a recombination event. And chromosome 9 (9O/A) is the non reciprocal product (NRP) of a recombination event, where the recombinant Oriental segment has been substituted. In this case, the mechanism that led to the pollen grain was First Division Restitution (**B**) The triploid complement of 022538-7 showing 13 (O) + 23 (A) chromosomes with six recombinant chromosomes. The ideogram (**D**) shows that the homoeologous recombinant chromosomes 4 and 12 (4 A/O, 4O/A; 12 A/O, 12O/A) are the reciprocal product (RP) of a recombination event, the recombinant chromosome 3 (3A/O) is the non reciprocal product (NRP) of a recombination event, while the recombinant Oriental chromosome 5 (5O/A) is accompanied by other non recombinant Oriental Chromosome (once its sister chromatid (SC)). This chromosome (5) has been segregated as in Second Division Restitution, however, the presence of only one chromosome for all the other cases (segregated as in First Division Restitution) demonstrates that the mechanism that took place was Indeterminate Meiotic Restitution. Bar in A and B represents 10 µm.

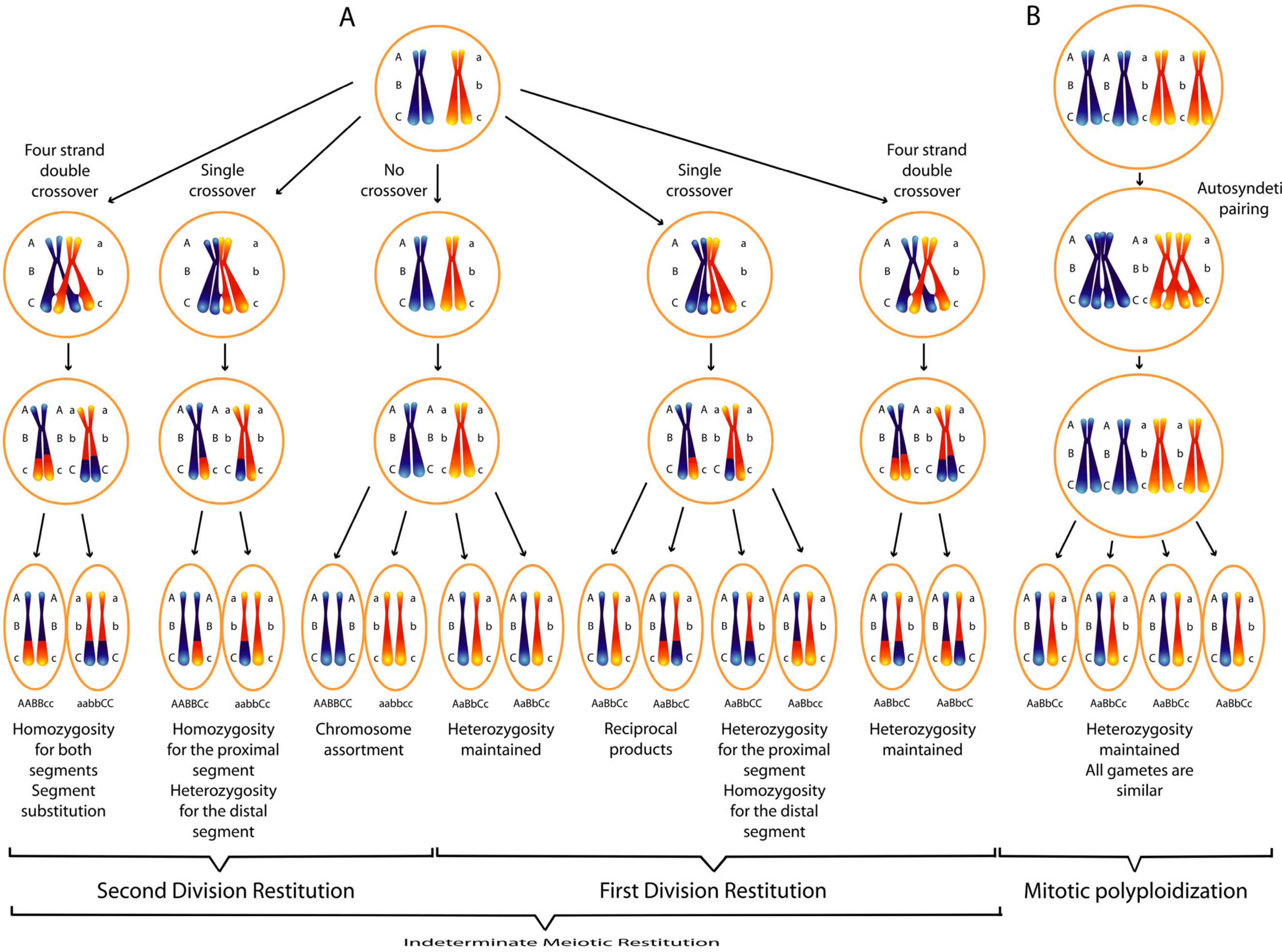

Fig. 3 Schematic representation of the genetic consequences of restitution mechanisms during meiosis. (**A**) Restitution mechanisms found in lily hybrids. In the case of Indeterminate Meiotic Restitution, some chromosomes may behave as in First Division Restitution and others as in Second Division Restitution. (**B**) Somatically doubled allotetraploid.

genomes. Considering this, the presence of these three restitution mechanisms has great advantages in creating genetic variation and achieving introgression. In the lily hybrids an enormous amount of recombination has been found through GISH analyses in the chromosomes of the progeny derived from *2n* gametes (**Fig. 2**) (Lim *et al*. 2001a, 2001b, 2003a; Barba-Gonzalez *et al*. 2004; Lim *et al*. 2004; Barba-Gonzalez *et al*. 2005a, 2005b; Zhou 2007). The implications of the presence of recombinant chromosomes are different depending on the restitution mechanism. FDR gametes will be identical to each other as well as the mother cell, when recombination is absent (**Fig. 3A**). With recombination, heterozygosity can be maintained for the part of the chromosome proximal to the crossover (in each recombinant chromosome). The "intermediate" status of IMR gametes can offer the highest degree of genetic variation, due to the random assortment of some chromosomes as in SDR and equational segregation for the others (**Fig. 3A**). Both, distal and proximal heterozygosity can be maintained if recombination is present. The genetic variation achieved by the use of *2n* gametes is clear when we observe the different phenotypes in the LA and OA progeny plants (**Fig. 4A**) obtained by reciprocal crosses to Asiatic cultivars that contrast with the similarity of each single genotype of the progeny plants obtained from reciprocal crosses to Asiatic of the mitotically doubled LA and OA hybrids (**Fig. 4B**).

5. PLOIDY LEVELS OF THE PROGENY

The ploidy level of the progeny plants has been analysed either by flow cytometric analyses (Van Tuyl *et al*. 1989) or by chromosome counting. As expected, most of the progeny obtained by the use of *2n* gametes is triploid (the unreduced gamete contributes with two chromosome sets and a third is contributed by the other gamete) (Lim *et al*. 2001b, 2003a; Barba-Gonzalez *et al*. 2004, 2005a, 2005b, 2005c; Zhou 2007), there are exceptional cases where the progeny plants are tetraploid (Barba-Gonzalez *et al*. 2005a, 2006a) in these cases, both gametes involved in the crosses were *2n* gametes. It has been generally inferred that triploid hybrids are unfertile, which makes them unattractive for breeding. However, in our research, we have found many of the triploid hybrids are able to produce viable gametes as well as progeny, but this will be discussed in the next chapter.

6. *2n* GAMETE INDUCTION

The advantages of the use of *2n* gametes over the gametes of somatic autopolyploids in breeding programs are obvious; however, there are limiting factors that hamper the use of *2n* gametes. These are: the low frequency of *2n* gamete production, the difficulty to detect them (especially *2n* eggs) and the few genotypes that produces them. There have been many attempts to increase the *2n* gamete production and

Fig. 4 Comparison of two different populations of triploid *Lilium*. (**A**) A selection of triploid hybrids obtained by crossing functioning *2n* gametes of and interspecific F_1 Oriental × Asiatic (OA) hybrid to a diploid Asiatic cultivar. Recombination among the parental genomes was present and as a result there is an enormous variation among the progeny. (**B**) A selection of triploid hybrids obtained by using fertile diploid pollen of a tetraploid interspecific Oriental × Asiatic (OA) hybrid (whose fertility was restored by somatic chromosome doubling) in crosses to a diploid Asiatic cultivar. Due to autosyndetic pairing there is no variation among the progeny. The small visible variations are the result of the variation from the ovule donor.

some attempts to induce them in interspecific hybrids, such as: genetic selection (Jacobsen 1976), high solar level (Ortiz and Vuylsteke 1995; Negri and Lemmi 1998), low temperature (Lutkov 1937; Stein 1970), heat (Lewis 1943; Lokker 2004) and caffeine treatments of immature flower buds (Levan 1939; Rasmusson and Levan 1939; Olden 1954; Lim *et al*. 2005). Nevertheless, none of the previous attempts has shown to be completely efficient. However, in recent research, flower buds containing early meiotic stages have been treated with nitrous oxide (N_2O) showing remarkable results.

N_2O is known to act as a spindle poison (Östergren 1954) and it was first utilized for somatic chromosome doubling (Östergren 1954; Nygren 1955; Östergren 1957; Tsunewaki 1962; Dvorak *et al*. 1973; Giri *et al*. 1983). However, Montezuma-de-Carvalho (1973) reported on the inhibition on the meiotic spindle at prometaphase – metaphase I stage, giving rise to nuclear restitution. With this insight, plus the characteristic of that N_2O is used as a gas under pressure, and different tissues can be treated at the same time for determined periods of time because its effect will last until the tissues are removed from the gas chamber (a process that can not be accomplished when tissues are treated with solutions), research was conducted in order to induce *2n* gamete formation in *Tulipa* (Okazaki *et al*. 2005). In the case of LA and OA *Lilium* hybrids, several different genotypes were treated with N_2O, increasing in some cases the production of *2n* gametes and in other cases inducing the formation of *2n* pollen and ovules in completely sterile OA lily hybrids (Barba-Gonzalez *et al*. 2006a, 2006c; Akutsu *et al*. 2007). The treated plants from these interspecific hybrids were utilized both, as male and female parents, in reciprocal crosses to Asiatic hybrids and many hybrids were obtained (Barba-Gonzalez *et al*. 2006a, 2006c). GISH analysis in the progeny revealed the presence of recombinant chromosomes among the parental genomes (Barba-Gonzalez *et al*. 2006a, 2006c).

7. CONCLUSION

The main goal in breeding is to combine important agronomic traits from different cultivars or species into novel varieties. However, to continue improving the vegetatively propagated varieties is crucial that those traits can be transmitted and conserved by the progeny. Interspecific hybridization has been widely used to improve cultivars, combining coveted traits from distant species that are exhibited by the new hybrids. However, the continuance of breeding programs is hampered by the F_1 hybrid sterility. The traditional method to restore fertility, doubling somatic chromosomes with chemicals, is not the best approach due to the lack of recombination among the parental genomes. If the pollen of these autopolyploids were to be used (as it has been) in further back- or reciprocal crosses in a single direction, it will not be possible to introgress and so-forth, preserve the coveted traits in the new hybrids, due to the lack of recombination. The use of *2n* gametes has shown great advantages over the use of artificially induced polyploids to achieve genetic variation. In this chapter it has been presented the success in breeding lilies from different taxonomical sections through the use of naturally occurring and induced *2n* gametes (**Table 1**). The presence of recombinant chromosomes in the progeny plants can be translated into genotypic and phenotypic variation, which contrasts with the lack of phenotypic variation in the progeny plants obtained by the use of induced allopolyploids. But more important is that the recombinant segments of chromosomes from different parental origin will be maintained in the progeny through generations, conserving the desired agronomical traits.

As was mentioned earlier, the use of *2n* gametes was discarded by breeders due to their sporadic production, however, with N_2O treatments of flower buds in early meiotic stages it has been possible to increase their production and in some cases to induce them in completely sterile hybrids, bringing new possibilities for interspecific hybridization in lilies.

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