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# The Significance of Polyploidy for Bulbous Ornamentals: A Molecular Cytogenetic Assessment

**M. S. Ramanna[1] • Agnieszka Marasek-Ciolakowska[2] • Songlin Xie[1*] • Nadeem Khan[3] • Paul Arens[1] • Jaap M. Van Tuyl[1]**

[1] Plant Breeding, Wageningen University and Research Centre , P.O. Box 16 6700 AA Wageningen, The Netherlands
[2] Institute of Horticulture, Department of Physiology and Biochemistry, Konstytucji 3 Maja Str. 1/3 , 96-100 Skierniewice, Poland
[3] Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, S-90187 Umeå, Sweden

*Corresponding author*: * **songlin.xie@seadc.com**

## ABSTRACT

Most of the bulbous crops, viz., *Crocus*, *Narcissus*, *Tulipa*, *Alstroemeria* and *Lilium* that are commercially important, share certain common characteristics. The present day cultivars are all derived from hybrids between distantly related species, and in almost all cases spontaneous polyploidization has played a prominent role and there is a tendency to replace diploids by polyploid cultivars. Molecular cytogenetic techniques such as genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH), along with other techniques, have greatly facilitated our understanding of the modes of origins of polyploids. Because the bulbous crops generally have large chromosomes, the parental genomes, individual chromosomes, as well as intergenomic recombinant chromosomes, can be accurately identified in the interspecific hybrids and their backcross progenies. This enables an assessment of the potential genetic variation that might occur in the progenies as well as the extent of introgression. Although the superiority of polyploids as compared to their diploid parents is beyond doubt, the actual explanation for their superiority is still elusive. Of the several explanations, chromosome dosage, optimal amounts of 4C DNA values of the complements, heterozygosity and favourable gene interactions transmitted by the *2n* gametes to polyploid progenies are some of the factors that might be considered at present. Undoubtedly, more studies on the bulbous ornamental crops using molecular techniques might be rewarding.

**Keywords:** *Alstroemeria*, *Crocus*, FISH, GISH, lily, *Narcissus*, polyploids, tulip
**Abbreviations: FDR**, first division restitution; **FISH**, fluorescence *in situ* hybridization; **GISH**, genomic *in situ* hybridization; **IMR**, indeterminate meiotic restitution; **NBS**, nucleotide-binding site; **PMR**, post meiotic restitution; **rDNA**, ribosomal DNA; **SDR**, second division restitution

## CONTENTS



## INTRODUCTION

Hundreds of plant species that are grown as bulbous ornamental crops, are known as ornamental geophytes in the global flower industry. Among all the different genera, eight commercially important genera include *Tulipa*, *Lilium*, *Narcissus*, *Gladiolus*, *Hyacinthus*, *Crocus*, *Alstroemeria* and *Iris* (Benschop *et al*. 2010). A common feature of most bulbous ornamentals is that the existence of polyploid cultivars (Van Tuyl *et al.* 2002a), which in several cases prove to be superior to their diploid parents. In fact, during the course of a century, polyploid cultivars have almost fully replaced diploid forms, such as *Narcissus* (Brandham 1986). Remarkably, in several cases, polyploids have originated spontaneously through the functioning of numerically unreduced (*2n*) gametes in the breeder's nurseries. Unconsciously, the breeders have selected such polyploid forms because of their superiority of vigour, growth, larger flowers or sturdier stems, among other qualities, as compared to their diploid parents. It should be noted further that in many cases, as in *Narcissus* (Brandham and Kirton 1987), Darwin hybrids tulips (Marasek *et al*. 2006) and *Crocus* (Ørgard *et al.* 1995), interspecific hybridization has played a pivotal role in the origins of polyploid cultivars. Apart from these polyploid forms of spontaneous origins, polyploid cultivars have also been synthesized intentionally by using *2n* gametes from interspecific hybrids in some crops, such as *Lilium* (Van Tuyl *et al*. 2002b), *Alstroemeria* (Buitendijk and Ramanna 1996; Kamstra *et al*. 1999a, 2004) and *Tulipa* (Marasek *et al.* 2006; Marasek and Okazaki 2008), as well as through somatic chromosome doubling (Van Tuyl *et al*. 2002b).

In view of the superior performance, as well as pervasive presence of polyploid cultivars in bulbous ornamen-

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tals, it is important to gain insight into their genome compositions, modes of their origins, and their genetic and cytological behaviour in order to effectively use them as parents in breeding new cultivars and for introgression. To this end, molecular cytogenetic methods involving DNA in situ hybridization (GISH and FISH) have been extremely useful to unravel the above mentioned aspects. Although molecular cytogenetic approaches have been used only in a limited number of bulbous crops so far, viz., *Lilium*, *Tulipa*, *Alstroemeria*, *Crocus* and *Narcissus*, a considerable amount of useful information has been reported in recent years. An attempt is made in this chapter to review the literature and highlight some of the significant aspects of polyploids in these crops.

## DETERMINATION OF GENOME COMPOSITION

To a large extent, the knowledge on the genome composition of some of the above mentioned crops has emerged from historical accounts as well as karyotype studies based on classical cytology, e.g., *Crocus* (Brighton *et al.* 1980), *Narcissus* (Brandham and Kirton 1987). In other crops like tulips, lily and *Alstroemeria* some information on the genome constitution was available; nevertheless, more accurate information became available only after the advent of molecular cytogenetic techniques. In the following section, the molecular cytogenetic information available on the genome composition of some of the crops is described individually.

### *Crocus*

Saffron Crocus (*Crocus sativus* L.) has been cultivated as an economic plant for several centuries. The species that are grown as horticultural cultivars are only considered in this section. Although considerable amount of knowledge was available on the origin of *Crocus* cultivars (Brighton *et al*. 1980), the first instance in which the potential of molecular methods for unravelling the genome composition of any ornamental crop was proved in *Crocus* (Ørgaard *et al*. 1995). By using DNA in situ hybridization (GISH and FISH) as well as genomic Southern hybridization methods, Ørgaard *et al.* (1995) demonstrated the hybrid origins of two common cultivars of *Crocus*, 'Stellaris' (2n=2x=10) and 'Golden Yellow' (2n=3x=14). The diploid cultivar 'Stellaris' is a hybrid possessing two distinct genomes with two different basic chromosome numbers (viz., x=4; x=6). Thus, one of the parents, *C. flavus* (2n=2x=8), has contributed four chromosomes and *C. angustifolius* (2n=2x=12) has contributed six chromosomes. The triploid cultivar 'Golden Yellow' (2n=3x=14) comprise of two genomes of *C. flavus* (i.e., 8 chromosomes) and one genome of *C. angustifolius* (i.e., 6 chromosomes). As additional proof, the number and location of 18S-5.8S-26 rDNA sites in the parents and the two cultivars were in agreement. These results were further confirmed by the Southern Hybridization proving the origin of cv. 'Golden Yellow'.

Besides the cultivar 'Golden Yellow', some of the accessions of the wild relatives of *Crocus vernus* Hill. (2n=2x=8) that has given rise to the so-called large Dutch Crocus, have also been investigated through FISH (Frello and Heslop-Harrison 2000). By using 18S-25S rDNA, 5S rDNA and a cloned DNA sequence, pKB8 from *C. vernus* as probes for FISH, they discovered extensive polymorphisms of hybridization sites as well as size differences among chromosomes of 10 accessions of *C. vernus*. A notable feature was that in a tetraploid accession (2n=4x=16) the karyotype was so different that it did not resemble any of the diploid parents studied. This highlights the possibility of extensive chromosomal alterations that might be prevalent in these taxa.

The presence of more than one basic chromosome numbers in different groups of the genus *Crocus* has given rise to what appears to be aneuploids in many cultivars. One example is that of *C. vernus* group of cultivars having 2n=20, 21, 23, 24, 25, 28, 29 and 32, all derived from a polyploid series based on x=8 and x=10 basic chromosome numbers (Karasawa 1935, 1943; Brighton *et al.* 1980). A clarification of the origins of such series obviously requires the application of molecular cytogenetic techniques.

### *Narcissus*

Within a span of about 150 years, a few species of the genus *Narcissus* L. have given rise to some thousands of cultivars (Brandham and Kirton 1987; Brandham 1992) through hybridization, both spontaneous and deliberate, followed by selection. Impressive amounts of information on the origins and genome compositions of the cultivars have been obtained through traditional karyotype analyses (Moore 1982; Brandham and Kirton 1987). An important fact that emerges from these studies is that polyploidy has played a prominent role in the origin of numerous cultivars. So much so, the original diploid cultivars have been replaced by polyploid cultivars (Brandham and Kirton 1987; Brandham 1992). These polyploids include the species from two subgenera, *Narcissus* L. and *Hermione* (Haw) Spach. as parents. The species of these two subgenera have different basic chromosome number. The subgenus *Narcissus* species possess a basic chromosome number of x=7, forming a natural polyploid series ranging from diploid (2n=2x=14) up to octoploid (2n=8x=56). On the other hand, subgenus *Hermione* species are comprised of different basic chromosome numbers of x=5, 10 and 11 and have given rise to a group of polyploids that include diploids (2n=2x=10), tetraploids (2n=4x=20) and hexaploids (2n=6x=30) as in *N. serotinus* (Moore 1982). Although there are tens of thousands of cultivars of *Narcissus*, one outstanding cultivar called 'Tête-á-Tête', has received considerable attention because of its attractive horticultural characteristics as well as performance. 'Tête-á-Tête' is known to have derived from a cross of the 'diploid cultivar 'Cyclataz' (2n=2x=17), which is a hybrid between 'Soleil d'Or' from *N. tazetta* (2n=2x=20) of the subgenus *Hermione* and *N. cyclamineus* (2n=2x=14) of the subgenus *Narcissus* (thus 10+7=17 of 'Cyclataz'), with an unknown diploid parent with 2n=14 (Brandham and Kirton 1987). Accordingly, it was assumed that a *2n* gamete from 'Cyclataz' fused with an *n* gamete of (x=7) from a parent like *N. cyclamineus* and gave rise to the allotriploid Tête-á-Tête' (2n=3x=24+1B chromosome). This, however, needed further confirmation in view of its complex history of origin. By using GISH and molecular markers (NBS profiling), it was possible to prove unequivocally that, the allotriploid 'Tête-á-Tête' has indeed two genomes of *N. cyclamineus* and one genome of *N. tazetta* together with a B chromosome (Wu *et al*. 2011).

Besides the cultivar 'Tête-á-Tête', GISH has also been successfully used for the elucidating the genome composition of allopolyploids from natural populations of the subgenus *Hermione* (Diaz Lefonte *et al*. 2009). The genome constitution of a tetraploid (as the authors call it), *N. obsoletus* (2n=4x=30) was determined by using the genomic DNA of the two putative parental species, *N. serotinus* L (2n=2x=10) and *N. elegans* (Haw) Spach (2n=2x=20) as probes. In the allotetraploid they have detected clearly differentiated 10 chromosomes of *N. serotinus* and 20 chromosomes of *N. elegans* in the painted complement.

### *Alstroemeria*

Compared to other bulbous ornamental crops, *Alstroemeria* is a relatively recent crop in terms of cultivation and breeding. Registered cultivars appeared in the early 1960s (Goemans 1962). All the species of this genus are endemic to South America, mainly distributed in Chile and Brazil, with most of the species are diploid (2n=2x=16) with only one basic chromosome number (x=8). Similar to the other bulbous crops, interspecific hybridization followed by polyploidization is most common in the origins of several hundreds of cultivars. Based on the species used as parents, two

main types of cultivars may broadly be distinguished: A) those derived from inter-Chilean species hybrids (so-called "Orchid type") and B) those originated from crosses between the Chilean and Brazilian species ("Butterfly type"). The Orchid type cultivars were likely derived from crosses between several Chilean species but the information has not been disclosed or made available. From information obtained from some of the breeders (Ramanna 1992), it appears that the following Chilean species are involved: *A. aurea* Graham; *A. magenta* Herb; *A. pulchra* Sims; *A. pelegrina* L; among others. The genomes of all the Chilean species are highly differentiated and the F1 interspecific hybrids are highly sterile (Ramanna 1992). The only viable gametes that are produced are the *2n* gametes, which can give rise to polyploid progenies. Among the Brazilian species, *A. inodora* Herb; *A. psittacina* Lehm and *A. braziliensis* Sprengel have been mainly used for hybridization with the Chilean species. The Brazilian species genomes are highly differentiated from those of Chilean species and like the Chilean hybrids, the F1 Brazilian hybrids are highly sterile and produce viable *2n* gametes giving rise to polyploid progenies (Buitendijk *et al*. 1995; Kamstra *et al*. 1999a).

Although none of the cultivars have been analysed for their genome constitution through GISH to date, sexual polyploid progenies (which are almost like cultivars) have been analysed through *in situ* hybridization methods. In an attempt to analyse homoeologous chromosome pairing, intergenomic recombination and the behaviour of individual chromosomes during meiosis, the F1 hybrids between *A. aurea* × *A. inodora* and their back progenies (BC1), in which *A. inodora* was the recurrent parent, were critically analysed using GISH and sequential FISH (Kamstra *et al.* 1999a, 1999b). The BC1 progenies consisted of a diploid (2n=2x=16) aneuploids (2n=2x=16+1) as well as triploids (2n=3x=24). It was evident that the F1 hybrids produced normal haploid, aneuploidy, as well as balanced *2n* gametes. A special feature of the cytological analyses was that in addition to genomic DNA, two clones of species specific repetitive DNA and rDNA sequences were used as probes to identify the chromosomes and genomes in the progenies. In the triploid BC1 progenies recombinant chromosome numbers varied between 4 to 10 per complement of the triploid progenies. The number of crossover points also varied from 1 to 4 per chromosome. From the limited data based on the analysis of six plants, it was concluded that as compared to aneuploids (or diploid), the triploid progenies possessed fewer crossover per chromosome, indicating a reduced pairing and recombination prior to *2n* gamete formation in the meiocyte. Besides intergenomic recombination, remarkably, chromosome structural alterations (translocation and inversion) were also discovered. At least one inversion and an ancient translocation involved two chromosomes of *A. inodora*: the chromosome 1 and 2 in one genotype.

Besides BC1 progenies of *A. aurea* × *A. inodora*, another F1 hybrid between *A. inodora* × *A. pelegrina* and its F2 and F3 progenies were also analysed through GISH (Ramanna *et al*. 2003). A notable feature of this analysis was that the F2 population was produced through bilateral sexual polyploidization of the F1 hybrid. This means the progeny were all tetraploids (2n=2x=32). By using one genotype (P6C49-15) that possessed the reciprocal products of a homoeologous recombination for chromosome 1 and formed a ring quadrivalent, an F3 population was created and analysed cytologically. A notable conclusion was that the recombinant chromosomes that formed quadrivalents showed a segregation pattern in the F3 progenies, comparable to that which occurs in polysomic polyploids but not as in translocation heterozygotes. In other words, ring quadrivalent formation in P6C49 did not give rise to duplication-deficiency gametes. This was a clear demonstration that an allotetraploid, or the so-called "permanent hybrid" will not be a permanent hybrid if recombinant chromosomes are present in the complement.

## *Tulipa*

Tulips, *Tulipa* L. (Liliaceae), have been in cultivation in Western Europe for more than four centuries. Of the thousands of presently available cultivars, there are two prominent groups. The most prevalent type is the so-called *T. gesneriana*, the exact origin of which is not clearly known. Probably it has originated through hybridization of closely related species of section *Tulipa* in genus *Tulipa.* The cultivars of *T. gesneriana* are mostly diploid (2n=2x=24) but some polyploids also exist (Van Scheepen 1996). The other group is the Darwin hybrids, derived from crossing *T. gesneriana* × *T. fosteriana*. Both of these species are diploid (2n=2x=24), but the original Darwin hybrids were triploid (2n=3x=36). The triploid Darwin hybrids were of spontaneous origin (thus not produced intentionally) and proved to be a phenomenal success in tulip cultivation. Paradoxically, despite the success of triploid tulips, little systematic efforts appear to have been made to induce polyploids in this crop because a majority of the cultivars that exist today are still diploid (Zeilinga and Schouten 1968a, 1968b; Van Scheepen 1996). Some attempts to induce ploypoids have been made by using interploidy crosses (Van Scheepen 1996; Straathof and Eikelboom 1997; Okazaki and Nishimura 2000). Another approach of polyploidization in tulip was by using for pollination of *2n* pollen induced via laughing gas treatment (Okazaki 2005; Okazaki *et al*. 2005; Barba-Gonzalez *et al*. 2006; see also Marasek-Ciolakowska *et al.* 2012).

Similar to other bulbous crops, interspecific hybridization followed by polyploidization is evident in the case of Darwin hybrids. Among Darwin hybrids, diploid (2n=2x=24), triploid (2n=3x=36) and tetraploid (2n=4x=48) cultivars have been recorded (Marasek *et al.* 2006; Marasek and Okazaki 2007, 2008). Because this group of cultivars possess the two differentiated genomes, viz., *T. gesneriana* (G) and *T. fosteriana* (F), they are amenable for GISH and FISH analyses (**Fig. 1A, 1B**). Thus by using GISH and Southern hybridization, the presence of two genomes of *T. gesneriana* and one genome of *T. fosteriana* was demonstrated for the first time in triploid Darwin hybrid cultivar 'Yellow Dover' (Marasek *et al.* 2006). Using simultaneous GISH and FISH, the genome constitutions were analysed in diploid Darwin hybrid 'Purissima' and its six BC1 progenies. These progenies consisted of five diploids and one triploid. All of these progenies, except the triploid, possessed variable numbers (1-6) of recombinant chromosomes that were clearly detected by GISH (Marasek and Okazaki 2008). Similarly, GISH was successfully used for the elucidation of genome composition of tetraploid cultivars 'Ollioules' (Marasek and Okazaki 2007) and 'Judith Leyster' (Marasek and Okazaki 2008). In cv. 'Ollioules' GISH clearly distinguished 36 chromosomes representing genome

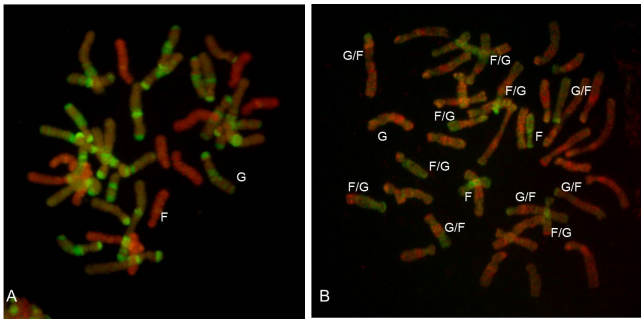


**Fig. 1 GISH chromosome images.** (**A**) Chromosome complement of triploid (2*n* = 36) Darwin hybrid tulip comprising of 24 chromosomes of *T. gesneriana* (green) and 12 chromosomes of *T. fosteriana* (red). (**B**) Chromosome complement of aneuploid Darwin hybrid tulip (2*n* = 42) resulted from 3x × 2x (♂2n gamete producer) cross comprising of 33 chromosomes of *T. gesneriana* (5 G/F recombinant chromosomes) (red) and 8 chromosomes of *T. fosteriana* (6 F/G recombinant chromosomes) (green).

of *T. gesneriana* and 12 chromosomes of *T. fosteriana*. Chromosome recombination was not detected in this cultivar (Marasek and Okazaki 2007). In contrast, 4 recombinant chromosomes were observed in tetraploid cultivar 'Judith Layster' ($2n = 4x = 48$) which complement comprised of 36 chromosomes of *T. gesneriana*, and 12 chromosomes of *T. fosteriana.* There were two types of recombinant products: *T. gesneriana* chromosome (G centromere) with a recombinant segment of *T. fosteriana* (F segment), indicated as G/F. Conversely, an F chromosome (centromere) with G segment indicated as F/G. Generally, each recombinant chromosome was the result of a single crossover event during meiosis (Marasek and Okazaki 2008).

### *Lilium*

Lilies (*Lilium* L.) have been in cultivation from ancient times (probably 5,000 years) and certain distinct types, such as Easter lily, Oriental and Asiatic lilies are recognisable. These are, in fact, the main hybrid groups of lilies that are the most commercially important at present. The cultivars of all these groups are derived from hybridization of diploid forms (2n=2x=24) within each of the three groups. The Asiatic lily cultivars are derived from the hybridization of about 12 closely related *Lilium* species of the botanical section of Sinomartagon of the genus *Lilium* in the Liliaceae. The Oriental lily cultviars are hybrids derived from crossings of five closely related species of the Archelirion section. The well-known Easter lily has been derived from intraspecific crosses within *L. longiflorum* in the section *Leucolirion*, and are referred to as Longiflorum group. The genomes of the species or cultivars of these three groups, viz., Asiatic (genome L), Oriental (O) and Longiflorum (L) are highly differentiated. Interspecific hybridization between cultivars from different sections is difficult (in vitro rescue methods required), as the F1 hybrids are highly sterile as a result of disturbed chromosome pairing during meiosis. In spite of this, there is a desire to combine desirable horticultural characters using intersectional species hybrids. To this end, distant species hybrids have been made on a large scale in the last two or three decades and the backcross progenies have been analysed through GISH and FISH in recent years (Lim *et al*. 2003; Van Tuyl *et al*. 2002b; Barba-Gonzalez *et al*. 2005a; Zhou *et al*. 2008; Khan *et al*. 2009a). These molecular cytogenetic investigations have yielded very valuable insights into a) the mechanisms of meiotic crossing-over and homoeologous recombination; b) mechanisms of meiotic nuclear restitutions and sexual polyploidyzation; and c) the genome composition of the sexual polyploid progenies, among others, in some *Lilium* species hybrids and their progenies. One example is the F1 hybrids of Longiflorum × Asiatic (LA) which are generally highly sterile due to the failure of chromosome pairing. However, these hybrids do produce *2n* gametes in some cases and from these, triploid backcross progenies can be produced (Lim *et al*. 2001a; Zhou *et al*. 2008a, 2008b; see also Khan *et al*. 2012). Interestingly, there are certain LA hybrids genotypes in which, despite being distant species hybrids, the homoeologs of chromosomes pair and form chiasmata during meiosis, as normally as in the diploid parental cultivars, giving rise to both haploid (*n*) and *2n* gametes (Zhou *et al*. 2008; Khan *et al*. 2009a). GISH analysis of meiosis in such LA hybrids and somatic chromosome complements in their backcross progenies, has provided evidence to the extent and distribution of crossovers in the genomes of the parental species. A striking feature is that the crossovers are distributed highly unevenly among the chromosomes in all three genomes analysed. In other words, whereas there are high frequencies of crossovers on some individual chromosomes (e.g. 7, 8, 9, 10 and 11), there are hardly any in the case of some chromosomes (e.g., 1, 2, 4 and 6). Analyses of genome compositions have also been conducted in other cases, such as in AOA backcross progenies and has facilitated the construction of cytological maps in all of the three different genomes, viz., L, A and O (Khan *et al*. 2009b). One feature that has emerged from determining the genome compositions of the triploid BC1 progenies is that there is a potential to generate genetic variation in the first backcross generation, and homozygosity can be attained for some of the recombinant segments at this stage (see later). Besides LA, OA and their BC1 progenies, similar intersectional hybrids and their backcross progenies have been produced and the genome compositions of BC1 progenies have been analysed. Some other examples are: *L. auratum* × *L. henryi* back crossed to *L. henryi* (AuHH) (Van Tuyl *et al*. 2002b); *L. martagon* × Asiatic (MA) backcrossed to Asiatic (MAA) (unpublished results); Oriental × Trumpt (OT) backcrossed to Oriental hybrids (OTO) (unpublished results).

## MODES OF ORIGIN OF POLYPLOID CULTIVARS

It is well recognized that polyploids in plants can arise either through somatic chromosome doubling (somatic doubling) or through the functioning *2n* gametes (meiotic doubling). In bulbous ornamental crops there might be two main reasons why meiotic doubling has played a predominant role in the origins of polyploid cultivars. Firstly, interspecific hybridization involving distantly related species is very common. Such hybrids are generally highly sterile (with a few rare exceptions), due to the lack of normal haploid (n) gamete production, whereas only *2n* gametes of spontaneous origin are expected to be functional. Unintentionally, plant breeders have utilized such *2n* gametes and produced polyploid progenies. Secondly, because the polyploids are generally superior to the diploid progenies in terms of vigour, sturdiness of stems or larger flowers, (among other positive characheristics), such individuals are preferentially selected so that they might have become cultivars in some cases. It has been generally recognized that polyploid plants originating from the process of meiotic polyploidization are superior to those produced from somatic doubling. This has been explained from the fact that somatic doubling merely increases the chromosome number of an individual without adding anything new attributes to the individual. On the contrary, if polyploids are formed through the functioning of *2n* gametes, a high degree of heterozygosity and favourable gene interactions (e.g., epistatis, over dominance etc) are transmitted from the hybrid parent to the progenies through *2n* gametes (Bingham 1980; Bingham *et al*. 1994). Such transmission of heterozygosity and gene interactions, however, depend on the types of *2n* gametes that are produced by the parent. There are four different types of restitution mechanisms that produce 2n gametes that have been reported to occur in plants. All of them originate as a result of abnormal behaviour of meiosis called meiotic nuclear restitution. Depending on the meiotic stage at which the nucleus restitutes, variable types of genetically different *2n* gametes are produced. These are: the first division restitution (FDR), the second division restitution (SDR), indeterminate meiotic restitution (IMR) and post meiotic restitution (PMR). Although the distinction between different types of restitution have been detected through mostly genetic methods, very limited amount of cytological evidence was previously available. This was because most of the earlier studies were confined to plants with small chromosome such as potato, clover, alfalfa and grasses (Ramanna and Jacobsen 2003). And most importantly, GISH and FISH techniques were not used for the elucidation of restitution mechanisms in the aforementioned cases. However, there was a radical change when plants with large chromosomes, such as bulbous ornamentals, were used for investigating meiotic restitution mechanisms using GISH and FISH which yielded direct cytological evidence regarding meiotic nuclear restitution mechanisms. Comprehensive studies have been conducted so far in the case of *Alstroemeria* (Kamstra *et al.* 1999a, 1999b; Ramanna *et al*. 2003; Kamstra *et al.* 2004) and *Lilium* (Lim *et al.* 2001a, 2003; Barba-Gonzalez *et al*. 2004, 2005a, 2005b, 2006; Khan *et al.* 2009a, 2009b). These

investigations have proved that there are two types of FDR gametes: those without intergenomic crossover chromosomes and those with intergenomic crossover chromosomes. The occurrence of these types of gametes in the case of both *Alstroemeria* and *Lilium* could be proved directly from GISH analysis of meiosis as well as from the analysis of the somatic chromosome karyotypes of the BC progenies derived from the functioning of the FDR gametes. In the case of non-crossover FDR, segregation of characters is not expected to occur whereas in the case of FDR with crossover chromosomes, the progeny has the potential to segregate. In the case of *Lilium*, the unique occurrence of the IMR *2n* gametes was demonstrated for the first time (Lim *et al.* 2001a). In this case, whereas some pairs of homoeologous pairs of chromosomes divide equationally as in FDR, other chromosomes in the complement segregate reductionally, leading to the inclusion of sister chromatids in one and the same *2n* gamete. Taking into account of the occurrence of FDR with crossovers and IMR during the origin of *2n* gametes, one should expect considerable amount of genetic variation in the polyploidy progenies. This situation is in striking contrast to the somatic doubling of species hybrids that give rise to allopolyploids which behave as 'permanent hybrids' due to the autosyndetic pairing of homologous chromosomes in such cases.

One of the drawbacks of polyploids that result from the functioning of *2n* gametes is that it leads to the production of triploids which can be highly sterile. Moreover, even if they produce functional gametes, the progenies will be aneuploid. However, GISH analyses of the progenies of interploidy crosses such as 3x-2x (or reciprocals) in *Lilium* has proved that allotriploids can produce balanced haploid as well as aneuploid gametes and give rise to diploid or circa diploid progenies (Khan *et al.* 2009a). Furthermore, the drawback of producing triploids can be circumvented by crossing genotypes that produce *2n* eggs and *2n* pollen (bilateral sexual polyploidization), as has been done in *Alstroemeria* interspecific hybrid, *A. inodora* × *A. pelegrina* (Ramanna *et al*. 2003) and in *Lilium* (LA) hybrids (Khan *et al.* 2010). A notable feature of the allotetraploids resulting from such bilateral sexual polyploidization is that they possess intergenomic recombinant segments in their complements. This obviously leads to segregations of those pairs of chromosomes that possess recombinant segments and segregate during meiosis as they behave in a polysomic polyploid. This means allotetraploids derived from bilateral sexual polyploidization will not be permanent hybrids but do segregate for characters that are present on recombinant chromosomes.

An interesting fact that is common to all the five bulbous crops considered above is that the triploid cultivars have been important to begin with. In the case of *Crocus,* the cultivar 'Golden Yellow' (2n=3x=14) was a success story. In *Narcissus*, the cultivar 'Tête-á-Tête' (2n=3x=24+1B) is still prominent; as was mentioned earlier, the commercial cultivation of *Alstroemeria* began with the so-called "Parigo Hybrids" which were triploids (Goemans 1962). Darwin tulip hybrids are a well-known case of the most successful triploid cultivars. In *Lilium*, although the cultivars were predominantly diploid till the late 20$^{th}$ century, triploid cultivars are becoming prominent. This may be incidental because at least in *Narcissus* and *Alstroemeria*, tetraploid cultivars have almost replaced diploid forms. Brandham and West (1993) and Brandham *et al.* (1995) have proposed a hypothesis based on the comparison of the amount of 4C DNA values in *Narcissus*, *Hyacinthus* and *Tulipa*. According to these authors, the optimal 4C DNA values ranging from 100 to 120 pg, plants with lower or higher than these values may suffer growth or vigour disadvantage and are not selected. As an argument, they consider that a majority of tulip cultivars are diploid because the 4C DNA value of these is already 118.32 for diploid and is ideal. In the case of triploid tulip cultivars, according to their estimate, the 4C DNA value of 166 pg which is too high a value to be ideal. Nevertheless, these authors have not taken into consideration the triploid Darwin hybrids which have been so successful. Moreover, the DNA values of diploid *Lilium* species are as high as in those of *Tulipa*, but triploid cultivars appear to be quite superior to diploid cultivars. However, it might be important to examine and evaluate the hypothesis of Brandham and West (1993).

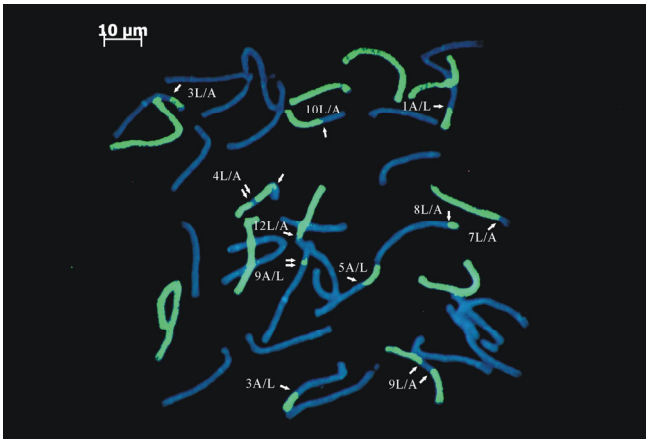


**Fig. 2 Genome composition of a triploid (2n=2x=36) derived from backcrossing of the diploid F1 hybrid, Longiflorum × Asiatic lily (2n=2x=24) to the Asiatic parent.** In this case, the F1 hybrid had contributed unreduced (*2n)* gamete to give rise to triploid progeny. There are 11 recombinant chromosomes, 7L/A+4A/L, marked with arrows. Green fluorescence represents chromosomes from Longiflorum and blue represents chromosome from Asiatic genome. The recombinant chromosomes have not resulted from translocations in the newly synthesized polyploids.

In recent years, there have been reports on the extensive chromosomal rearrangements such as translocations, duplications and deletions, in the newly synthesized polyploids (Neo-polyploids) in some plant species (see review Gaeta and Pires 2010). Because the chromosomes of *Lilium* hybrids are some of the largest among plants, they are amenable for GISH analysis, the newly synthesized polyploids of lily have been analysed recently (Xie *et al.* 2010). These analyses have proved that the so called translocations, duplications and deletions are not the result of chromosomal mutations but the result of intergenomic recombinations (**Fig. 2**).

## FUTURE PERSPECTIVES

The gap between chromosomes and molecular markers still need to be filled in the future. Marker assisted selection (MAS) has become a main tool to speed up the breeding process, not only in main crops, but also in bulbous ornamentals. However, it is still difficult to locate the markers on chromosomes due to the limited size of selected markers and the big chromosomes of the bulbous flowers. Furthermore, it is still difficult to distinguish individual chromosomes in many bulbous ornamentals. A few efforts have been made to distinguish lily chromosomes with different banding techniques, but only afew pairs of chromosome could be identified. In view of this, it is necessary to develop techniques, such as bacterial artificial chromosomes (BACs) to solve these problems. BACs with euchromatin region could be used as bridges to link molecular markers and chromosomes, and BACs with repetitive sequences could be used to identify individual chromosomes.

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