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Relevance of sexual polyploidization for crop improvement – A review

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Summary

Colchicine induced polyploids have not directly contributed for crop improvement in the past. On the other hand, the so-called natural polyploids, derived from the functioning of numerically unreduced (*2n*) gametes have been shown to be more relevant for crop improvement in many cases. Different types of cytological abnormalities during meiosis can give rise to *2n* gametes and the genetic composition of these gametes is variable. Depending on the type meiotic abnormalities, various types of *2n* gametes, such as first division restitution (FDR), second division restitution (SDR), indeterminate meiotic restitution (IMR) and post meiotic restitution (PMR) gametes, among others, have been described in recent years. For the improvement of autopolyploids such as potato, alfalfa, *Vaccinium* spp., and some of the fodder grasses, FDR gametes have been proved to be highly useful. However, the use of *2n* gametes for the improvement of allopolyploid crops has received much less attention so far. Some of the investigations on allopolyploids, derived from *Festuca-Lolium*, *Alstroemeria* and *Lilium* species hybrids, have revealed that *2n* gametes can be most useful for the introgression of alien genes and chromosomes into cultivars. An important feature of using sexual polyploidization in the case of allopolyploids is that introgression can be achieved through recombination due to genetic crossing-over between alien chromosomes as well as addition of alien chromosomes, which is extremely difficult or impossible to achieve in the case of colchicine induced allopolyploids. Because of the recent developments in the field of plant molecular biology, methods have become available for the analysis of *2n* gametes and sexual polyploid progenies more accurately and to develop systematic breeding approaches. The methods include DNA in situ hybridization (GISH and FISH) and molecular mapping (AFLP, RFLP, RAPDs). In addition to providing basic information on the genetic and genome composition of the polyploid progenies, these methods can be potentially useful for a more efficient creation of desirable breeding material and cultivars.

Introduction

It was almost a century ago that the phenomenon of polyploidy was discovered in plants (Strasburger, 1910; Winkler, 1916). This was important for plant evolutionary biologists as well as plant breeders in view of the widespread occurrence of polyploids in the wild as well as in cultivated plants. Early on, two types of polyploids were recognized based on the chromosome constitution of the individuals, viz., auto- and allopolyploids (which are also often referred to as polysomic and disomic polyploids respectively in the recent literature but in this article the traditional terminology is retained). The term autopolyploid is used when individuals have three or more homologous chromosome sets (genomes) derived from a single species. On the contrary, allopolyploids consist of homoeologous chromosome sets derived from two or more distinct species. The frequencies of polyploids in angiosperms have been estimated to be anywhere between 47% to 70%, depending on the criteria used by different authors (Grant, 1981; Masterson, 1994), and regarded as an important mechanism of speciation and adaptation in plants. Among the cultivated plants,

some of the important crops such as wheat, potato, cotton, oat, sugarcane, banana, groundnut, tobacco and numerous horticultural crops are all polyploids.

With a hope that induced polyploids might be a shortcut for crop improvement, numerous attempts were made during the early part of the last century to synthesize both auto- and allopolyploids, mostly through colchicine treatment. Literature on various aspects of polyploids can be found in some of the reviews (Stebbins, 1950; 1980; Jackson, 1976: Lewis, 1980; Grant, 1981; Levin, 1983). Emphasis on the usefulness of synthetic polyploids was overly optimistic and these have contributed little, if any, to crop improvement directly. Nevertheless, unlike synthetic ones, the natural polyploids have been quite successful in view of their predominance among the flowering plants as well as their success of being important crops. After recognizing the distinction between the synthetic and natural polyploids, Skiebe (1958) made the first systematic effort to synthesize polyploids through the use of numerically unreduced (*2n*) gametes. This author assumed that natural polyploids might have originated through the functioning of *2n* gametes and demonstrated their superiority in the case of *Primula melocoides* Franchet. Although the origin of polyploids through *2n* gametes was recognized much earlier (see Darlington, 1939; Stebbins, 1950), the importance of such polyploids was underestimated. This was because of the assumption that the occurrence of 2n gametes in plants is rare and sporadic, and therefore, might have contributed little to the origin of polyploids (Stebbins, 1950). Contrary to this assumption, by a survey of literature, Harlan & De Wet (1975) showed that almost all plant species produce *2n* gametes in some frequencies and further argued that all polyploids in plants have originated through the functioning of *2n* gametes.

Polyploids that originate through the functioning of *2n* gametes are called sexual polyploids and their usefulness for crop improvement has been demonstrated in some of the crops. Examples are: potato (Mendiburu & Peloquin, 1971; Mendiburu et al., 1974), alfalfa (Bingham, 1980; Veronesi et al., 1986), red clover (Smith et al., 1985), blueberry (Lyrene et al., in this issue), among others, which are all autopolyploids. The literature on these crops has been reviewed in the past (Veilleux, 1985; Mariani & Tavoletti, 1992; Bretagnolle & Thompson, 1995). Besides autopolyploids, there are numerous instances of sexual polyploidization in the case of allopolyploid crops, which have not been reviewed so far. Nevertheless, it is apparent that sexual polyploidization is as important in the case of allopolyploids as they have been in autopolyploids. In the case of autopolyploid crops, sexual polyploidization has been useful for the maximization of heterozygosity in the sexual polyploid progenies (Bingham, 1980) and, secondly, for germplasm enhancement through the so-called analytic breeding method (Chase, 1963; Mendiburu et al., 1974). Both of these objectives can also be achieved to a certain extent in the case of allopolyploids but introgression of genes and chromosomes from alien species into the cultivars is more important in the latter.

Generally, *2n* gametes originate due to deviating meiosis in plants. Deviations can occur in plants with normal chromosome pairing as well as in those with disturbed chromosome pairing as, for example, in distant interspecific hybrids or synaptic mutants. The process that leads to *2n* gamete formation is called meiotic nuclear restitution that occurs either during micro- or megasporogenesis. Depending on the particular meiotic stages at which nuclear restitution occurs, different restitution mechanisms have been recognized, viz., first division restitution (FDR), second division restitution (SDR) (Mok & Peloquin, 1972; Ramanna, 1979). Besides these, other mechanisms such as indeterminate meiotic restitution (IMR) and post meiotic restitution (PMR), among others, have also been recognized (Lim et al., 2001; Bastiaanssen et al., 1996). Apart from the cytological distinction, different types of restitution gametes are profoundly different from a genetic point of view. This means, sexual polyploid progenies originating from the functioning of different types of *2n* gametes are also expected to be quite different. Through the traditional cytogenetic approaches, such as half-tetrad analyses, genetic differences between FDR and SDR gametes, and the progenies they give rise to, have been elucidated to some extent, especially in potato (Mok & Peloquin, 1972; Douches & Quiros, 1987; Jongedijk et al., 1990). Nevertheless, the traditional approaches have been proved to be highly inadequate to unravel the chromosome constitution and genetic composition of the sexual polyploid progenies derived from the functioning of different types of restitution gametes. Fortunately, the introduction of molecular genetic and cytogenetic methods for the analysis of polyploids in recent years has dramatically increased our knowledge regarding the natural and synthetic polyploids. These methods include different genetic mapping techniques (RFLP, AFLP, SSR, RAPDs) and DNA *in situ* hybridisation (genomic in situ hybridisa-

tion, GISH and fluorescent in situ hybridisation, FISH) for the detection of individual genomes, chromosomes and recombinant segments in the polyploid progenies. Obviously, these methods have enhanced the accuracy with which the polyploids can be analyzed and the knowledge applicable for crop improvement on a more systematic basis.

In this review, some of the aspects of the modes of origin of restitution gametes, their potential for the production of sexual polyploid progenies, techniques used for the analysis of polyploids and the future prospects of sexual polyploidisation for crop improvement are considered. In view of the availability of fairly comprehensive reviews of literature on autopolyploids, (Veilleux, 1985; Mariani & Tavoletti, 1992; Bretagnolle & Thompson, 1995), mostly the literature on allopolyploids has received emphasis here. Besides traditional cytogenetic analyses, attention is directed to the newly emerging molecular techniques that are useful for the analyses of polyploids.

Meiotic nuclear restitution mechanisms and the origin of *2n*-gametes

Production of genetically identical *2n* gametes in diplosporic apomictic plant species has been recognized for a long time (Gustafsson, 1946). Two important characteristics may be noted in diplosporic apomictic species: 1) the occurrence of *2n* gametes is not sporadic but regular, and 2) the process is, obviously, genetically determined. Extensive cytological investigations in various apomictic plant species have revealed that there are variations with regard to the cytological events that lead to the formation of restitution gametes. These events were described with various names, such as pseudohomoeotypic division, semiheterotypic division, mititized meiosis, Taraxacum type, Hieracium type, and many more (review, Battaglia, 1963; Nygren, 1971). But in most cases, the entire chromosome complement in the megaspore mother cell divides as in mitosis (i.e., centromeres divide and the two sister chromatids of each chromosome separate to two poles during the modified process of meiosis) and gives rise to two genetically identical *2n* megaspores. These investigations were confined exclusively to the study of *2n* egg formation during megasporogenesis in apomictic species.

Apart from apomictic species, meiotic nuclear restitution and *2n* gamete formation were also observed in several sexual species and their potential for the induction of polyploids was recognized a long time ago (Karpechenko, 1927; Kihara, 1946). Some notable differences were observed in the case of sexual species as compared to apomictic species. a) Meiotic nuclear restitution can occur in both micro- and megasporogenesis. b) Formation of *2n* gametes is highly sporadic instead of being regular events, and highly dependent on environment. c) Mostly heterogeneous populations of *2n* gametes are formed depending on the cytological mechanisms (Storey, 1956). Although the occurrences of distinct types of restitution gametes were recognized a long time ago, the terms such as FDR, SDR and other mechanisms of *2n* gamete formation are more recent. Different cytological mechanisms have been clearly defined in the case of potato, alfalfa, *Alstroemeria, Lilium*, and other plants (Mok & Peloquin, 1975b; Ramanna, 1979; Veilleux, 1985; Mariani & Tavoletti, 1992; Bastiaanssen et al., 1996; Lim, 2001) mostly based on the analyses of microsporogenesis. Since the cytological events that lead to different types of *2n* gametes are fairly complicated, each of these mechanisms is described individually and illustrated in the following section.

Cytological mechanisms

Undoubtedly, the occurrence of different types of restitution gametes can be established through careful cytological analyses of micro- or megasporogenesis in some cases. But cytological determination may not always be simple and straightforward. Interpretation can be difficult because nuclear restitution depends on various meiotic events such as chromosome pairing, chiasma formation and cytokinesis during the first meiotic division, and spindle abnormalities during metaphase II, cytokinesis and cell wall formation during telophase II of the second division. Moreover, in different groups of plants variations exist with regard to the sequences of meiotic events. For example, in most of the dicotyledonous plants cytoplasmic division during microsporogenesis occurs at telophase II giving rise to a tetrad. This is the so-called 'simultaneous type' of cytokinesis. But in most monocotyledonous plants, cytokinesis is of the successive type during microsporogenesis – meaning that at telophase I cytokinesis and cell wall formation occurs – and the second meiotic division proceeds separately in two different cells (within a pollen mother cell). In this case, following the second division, cytokinesis and

cell wall formation again occurs at telophase II giving rise to a tetrad.

Like in microsporogenesis, marked differences are present also in the case of megasporogenesis in different groups of plants. For example, more than 75% of the plant species possess the so-called monosporic, eight nucleate type of embryo sac formation and most of these are successive type (Maheswari, 1950). There are, however, several plants in which cytokinesis and cell wall formation do not occur during telophase I of megasporogenesis. Examples of this category are *Lilium* and *Tulipa*. From the existing information, roughly three different situations can be recognized among plants. 1. Those with simultaneous type of cytokinesis during microsporogenesis but successive type during megasporogenesis (e.g., potato, tomato and most of the dicots). 2. Successive type of cytokinesis during both micro- and megasporogenesis (e.g., cereals, grasses, *Alstroemeria* and several monocots). 3. Successive type of cytokinesis during microsporogenesis, and the absence of it during megasporogenesis (e.g., *Lilium* and *Tulipa*). It is essential to bear in mind the above three types of situations when considering meiotic nuclear restitution mechanisms described below.

First division restitution, (FDR) in a strict sense, occurs due to an equational division of the entire chromosome complement (as in mitosis) before telophase I stage. The two *2n* nuclei of a dyad will be genetically identical to each other as well as to the mother cell (Figure 1a,b). This type of division occurs in plants in which the homologous or homoeologous chromosome pairing is completely absent, as for example in synaptic mutants or distant hybrids. Essentially, this is a modification of meiosis in which the centromeres divide already during the first meiotic division rather than later. Examples of such modified division has been observed in *Aegilops squarrosa* × *Triticum durum* (Sasakuma & Kihara, 1981) emmer wheat × *A. squarrosa* (Fukuda & Sakamoto, 1992); durum wheat × *A. squarrosa*, and *rye* × *A. squarrosa* (Xu & Dong, 1992; Xu & Joppa, 1995); wheat × barley (Islam & Shepherd, 1980); haploids of durum wheat (Jauhar et al., 2000), *Alstroemeria* interspecific hybrids (Ramanna et al., this issue); *Lilium* interspecific hybrids (Lim et al., 2001). Complete absence of chromosome pairing in the meiotic mutants and distant hybrids occurs only rarely. In those cases where a limited amount of crossing-over occurs, FDR can give rise to *2n* gametes that are not identical, but differ with respect to recombinant segments (Figure 1c).

Failure of chromosome pairing is not a precondition for FDR *2n* gamete formation. Indeed, in spite of normal microsporogenesis, with normal chromosome pairing, FDR (or equivalent of FDR) occurs in a large number of cases due to a different reason. In these cases, meiosis proceeds normally during the first division, i.e., anaphase and telophase I stages will be normal, but during metaphase II stages the spindles 'fuse' and give rise to dyads instead of tetrads. Whereas the FDR in a strict sense produces identical *2n* gametes whose genotypes are the same as those of the parent, the division equivalent to FDR produces *2n* gametes that are not identical to each other nor to the parental genotype (Figure 1d). A notable feature of the equivalent of FDR is that it can occur only in plants that have the so-called 'simultaneous type' of cytokinesis during microsporogenesis, which is usually the case in most of the dicotyledonous plant species. It also implies that this type of FDR is precluded in the case of 'successive type' of micro- and megasporogenesis where cytokinesis and cell wall formation occurs at the end of the first meiotic division.

Besides spindle abnormalities, abnormal cytokinesis following anaphase II can also lead to FDR *2n* gamete formation as has been reported by Kamemoto in orchid (see, Storey, 1956). In this case a triad is formed in such a way that the *2n* microspore possesses the fusion product of two non-sister nuclei during telophase II giving rise to FDR gametes. Possibility of the occurrence of both FDR and SDR gametes due to abnormal cytokinesis has also been reported in potato (Ramanna, 1973) and holy grass, *Hieochloë odorata* (Ferris et al., 1992). One should expect two important consequences if *2n* gametes occur due to the mechanism that is equivalent to FDR. a) There will be a higher degree of heterozygosity for genetic loci that are proximal to the crossover point on the chromosome. b) The population of *2n* gametes can be much more heterogeneous as compared to those resulting from FDR in synaptic mutants or distant hybrids where crossing-over is much more restricted (compare Figures 1b-d).

Traditional cytogenetic methods can be fairly reliable for detecting FDR mechanism, especially in the case of synaptic mutants and distant hybrids, based on chromosome orientation, spindle abnormalities and chromosome division. Nevertheless, when more than one mechanism leads to *2n* gamete formation in one and the same plant, which is often the case, conclusions can be misleading. This bottleneck can be effectively overcome by analyzing the chromosomes

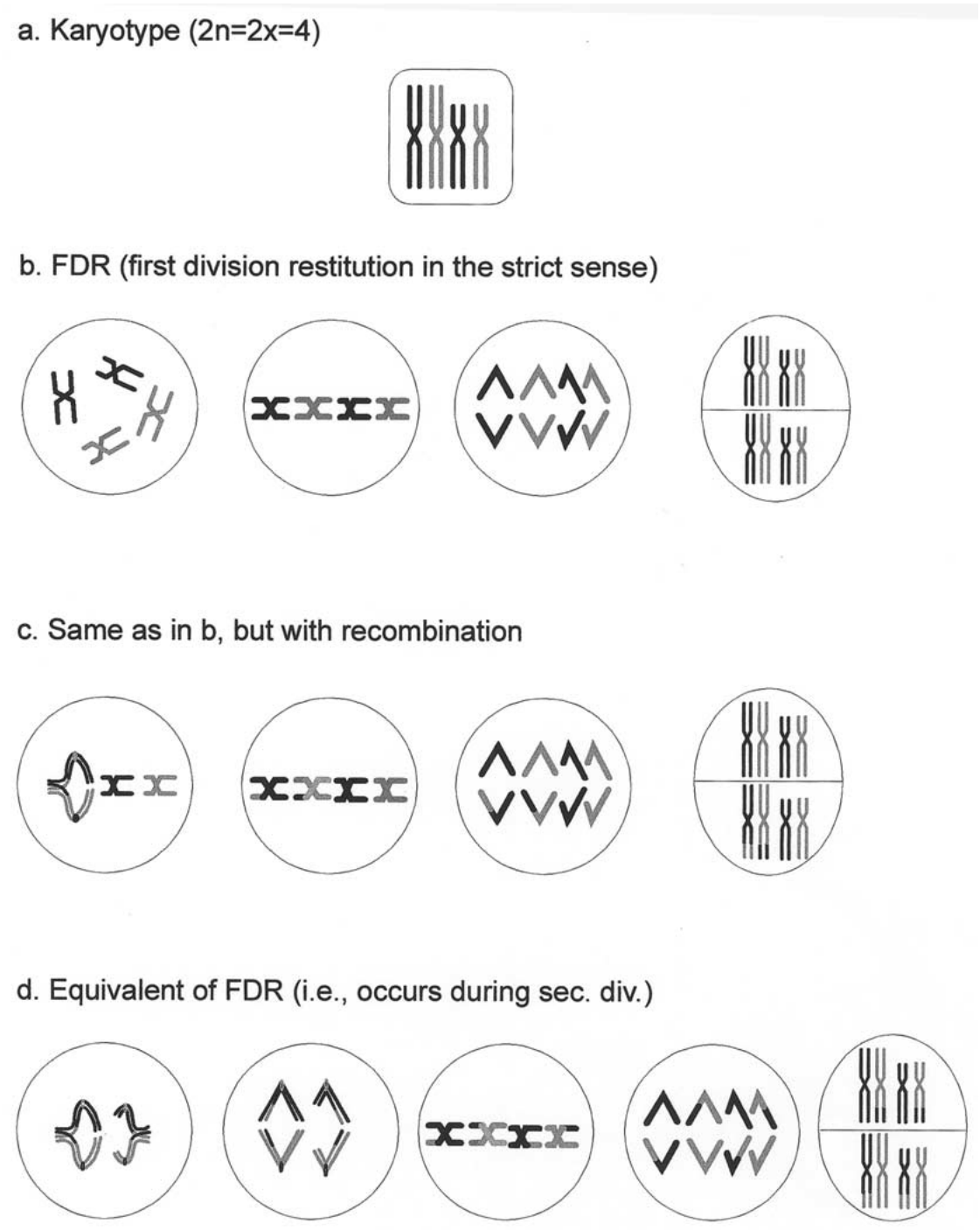

Figure 1. Consequences of FDR, without and with crossing-over in a hybrid. (In figures c-d, successive stages of metaphase I to sporads are shown from left to right) a. Somatic karyotype of hybrid. Two genomes represented by two pairs of homoeologous chromosomes (shown in black and gray). b. Meiotic stages showing FDR without crossing-over that give rise a dyad with two identical nuclei that are similar to the parent cell (Figure 1a). c. Same as in b, but with a cross-over in one pair of homoeologous chromosomes. Note: one of the nuclei in the dyad is dissimilar to the parental karyotype (Figure 1a). d. Both pairs of homoeologous chromosomes have paired, anaphase I is normal, but during the second meiotic division all chromosomes divide as single unit by forming one equatorial plate (fused spindle). Although the restitution occurs during the second division in this case, the results are similar to those shown in 1b and 1c in preserving the parental chromosomes in the restitution gametes (except for the recombinant segments in 1c and 1d).

of the parent and sexual polyploid progenies through molecular cytological techniques (GISH and FISH) or mapping methods (half-tetrad analysis) through the use of molecular genetic markers. Both of these methods have been demonstrated to be effective for detecting the mechanism of restitution and the extent of crossing-over. An important feature of the FDR mechanisms (Figure 1) is that, with the exception of crossover segments, the chromosome sets of the parental genomes remain intact in the resulting *2n* gametes.

Second division restitution and the other two mechanisms (Figure 2), on the other hand, involve the disruption of parental genomes due to independent assortment of chromosomes as well as the segregation of the recombinant segments. In the case of typical SDR, homologous or homoeologous chromosomes pair completely and the half-bivalents disjoin normally at anaphase I of meiosis. The resulting haploid products do not divide further but restitute in the sense that the centromeres divide but the chromatids do not separate to two poles (Figure 2a). As a consequence, the regions between the centromere and the first crossover point in each chromosome pair remain identical (thus homozygous) whereas the segments distal to the crossover point will be heterozygous (Figure 2a) in the resulting *2n* gametes. In those plants that have simultaneous cytokinesis during microsporogenesis, cytoplasmic division and cell wall formation occurs prematurely during either telophase I or prophase II stages already (Mok & Peloquin, 1975a). In plants with successive type of cytokinesis, which occurs predominantly during microsporogenesis in monocots and megasporogenesis in most plant species, the haploid nuclei resulting from normal disjunction restitute, i.e., centromeres divide without the separation of the chromatids. When both the nuclei restitute at two poles they give rise to a dyad, and when only one of the nuclei restitutes a triad is formed. Although the formation of a dyad or a triad can be used as a criterion for the detection of SDR, conclusive proof for SDR mechanism can be established only through genetic mapping of the sexual polyploid progenies. This is because SDR occurs in hybrids or genotypes in which the genomes are closely related so that they pair normally during metaphase I. In the absence of chromosome differentiation, DNA in situ hybridisation methods are ineffective for the elucidation of the chromosome constitution in the sexual polyploid progenies. Therefore, genetic mapping methods (half-tetrad analyses) using genetic markers such as morphological, isozyme, RFLP and RAPDs have been used for the elucidation of SDR in some of the crops (Douches & Quiros, 1988; Jongedijk et al., 1991; Tavoletti et al., 1996). In view of random assortment of chromosome pairs and a high degree of homozygosity due to chromosome doubling, SDR gametes form a highly heterogeneous population of *2n* gametes.

Generally, only two types of *2n* gametes have been recognized to occur in plants, viz., FDR and SDR. Recently, however, a mechanism that is neither FDR in a strict sense nor SDR has been reported in a *Lilium* interspecific hybrid (Lim et al., 2001). The important feature of this mechanism is that a part of the chromosome complement can divide equationally as in FDR and, simultaneously another part of the complement in the same cell can disjoin normally as in anaphase I and restitute as in SDR (Figure 2b). In other words, when both univalents and bivalents are formed during meiosis in a distant hybrid, univalents divide equationally whereas bivalents disjoin and restitute giving rise to two euploid *2n* gametes. Because this novel type of restitution cannot be characterized either FDR or SDR, the expression 'indeterminate meiotic restitution' (IMR) has been used (Lim et al., 2001). Detection of this restitution mechanism was possible because of the use of GISH that enabled monitoring of the meiosis of the F1 hybrid and the identification of the chromosomes of individual genomes in the sexual polyploid progeny. The significance of this mechanism is that the *2n* gametes originating from desynaptic genotypes need not necessarily produce FDR gametes with intact parental genomes as was previously presumed (Peloquin, 1982). On the other hand, even when synaptic mutants are used as a source of *2n* gametes, homozygosity can occur for some of the genetic loci on certain pairs of chromosomes. Further more, in allotriploids where both bivalents and univalents are most commonly formed, the occurrence of IMR might be a very common phenomenon.

There are several instances among plants where meiosis proceeds to completion and yet produce *2n,* instead of *n* or the haploid spores. Probably one of the well-known examples is that of sugarcane interspecific hybrids where the chromosome numbers of the haploid egg cells are doubled giving rise to *2n* eggs (Bremer, 1961; Price, 1961). In addition, in one of the blackberries species, *Rubus laciniatus*, apomictic progenies have been reported to arise from diploidization of the reduced egg cells (Dowrick, 1966). These events are obviously post-meiotic chromosome doubling events and involve genetic recombination

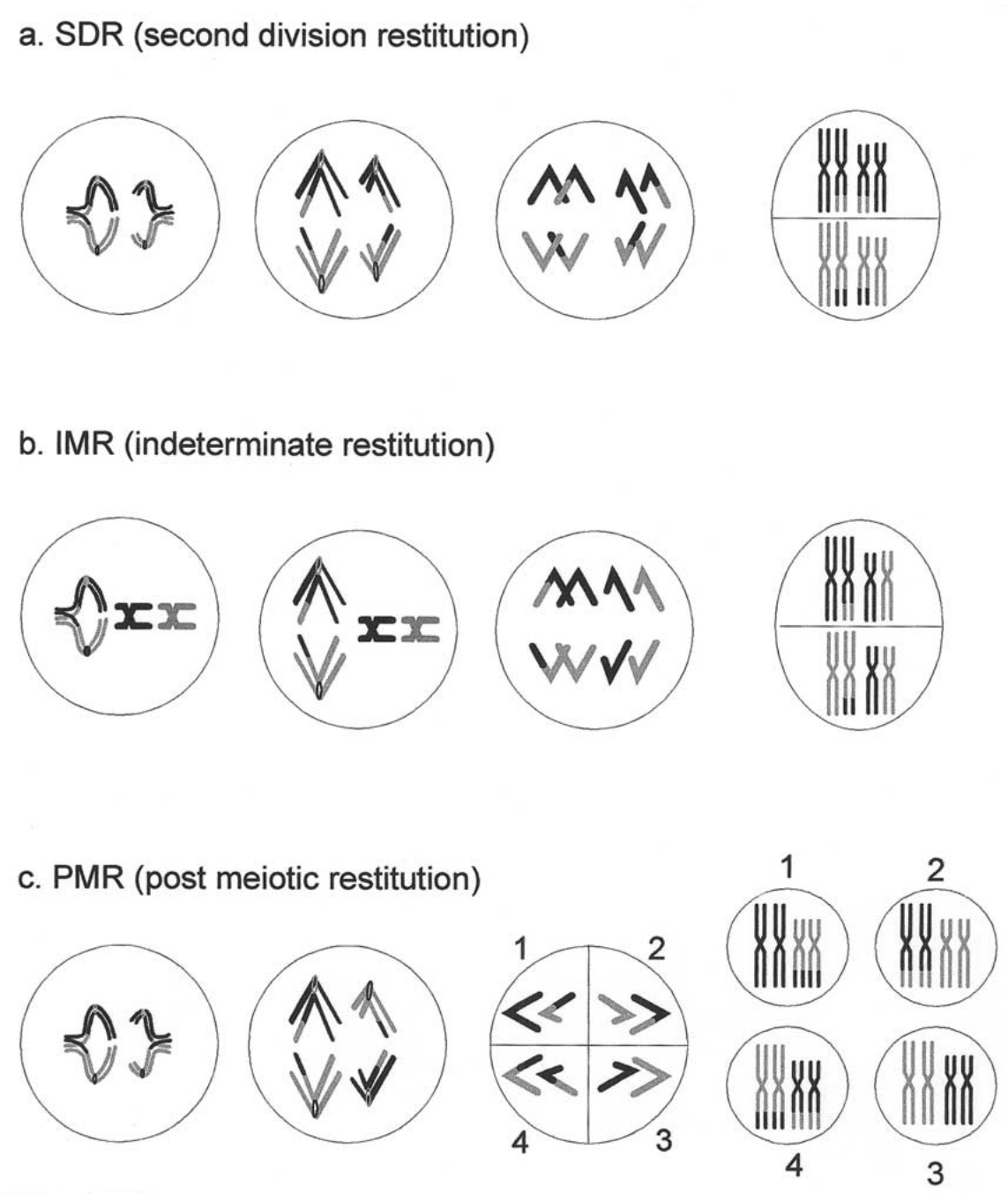

Figure 2. Restitution mechanisms in which the parental chromosome constitution (Figure 1a) is disrupted. (In all cases, metaphase I to sporad stages are shown from left to right) a. Second division restitution. Homoeologous chromosomes pair normally but after normal disjunctional separation, the nuclei at both pole restitute (centromeres divide but the chromatids do not separate). b. Indeterminate meiotic restitution. Both bivalent and univalents are formed in the meiocyte. The bivalent disjoins normally (reductional) whereas the univalents divide equationally and the nuclei restitute. c. Post meiotic restitution. Homoeologous chromosomes pair, disjoin normally giving rise to reduced haploid spores. Subsequent to normal meiosis, the chromosome numbers of the haploid spores are doubled.

and chromosome assortment that characterize normal meiosis (Figure 2c). A salient feature of post meiotic doubling is that the resulting *2n* gametes are expected to possess 100% homozygosity for the genetic loci. This has been convincingly demonstrated by using multiple chromosome specific RFLP markers in the case of diploid potato that produced *2n* eggs through post-meiotic restitution, or PMR (Bastiaanssen et al., 1998). Cytological demonstration of PMR can be proved indirectly from the fact that meiosis in such genotypes will be completely normal and produce normal *n* gametes but give rise to polyploid progeny.

Detection of *2n* gametes

Several criteria can be used for the detection of *2n* gametes in plants. In some cases these criteria can be used relatively easily whereas in others they are more indirect. The simplest way is by staining the pollen grains with traditional staining reagents, such as acetocarmine or lacto phenol acid fuchsin, the size differences can be recognized. This method is applicable in genotypes with normal meiosis and generally the larger pollen grains represent *2n* pollen and the smaller ones the *n* pollen (review, Bretagnolle & Thompson, 1995). When meiosis is abnormal as in distant hybrids, synaptic mutants or odd polyploids (such as triploids), the presence of stainable pollen is an indication for the occurrence of *2n* pollen although aneuploid pollen grains may also be stained. In both instances, the progeny will possess plants with higher ploidy levels than the parent(s). Unlike *2n* pollen, the detection of *2n* eggs in plants is much more difficult. This requires chromosome counting in the progenies, which is a laborious task. However, the formation of *2n* eggs can be more easily detected without chromosome counting in the progenies by using a curious phenomenon, called 'triploid block' that occurs in several plant species. In these cases when 2x-4x crosses are made, the expected triploid embryos resulting from the union of a haploid egg with a 2x male gamete do not survive because of the embryo-endosperm imbalance of the chromosome numbers. When such triploid block is effective in a cross, the only sporophytes that survive are tetraploids resulting from the union of *2n* eggs with 2x male gametes in a 2x-4x cross. This means, by simply making a 2x-4x cross, the frequencies of the occurrence of *2n* eggs in a diploid can be quantified on the basis of seed set. This method has been successfully used in potato, alfalfa, *Brassica*, *Dactylis* and several other plants (Stelly & Peloquin, 1986; Barcarccia et al., 2000; Heyn, 1977; van Santen et al., 1991). Besides using pollen size and 2x-4x crosses as criteria for the assessment of *2n* gamete formation, there are certain other methods that are less commonly used but have the potential for application in some of the crops. These include the production of the so-called 'metromorphic progeny' as in *Brassica* intergeneric or interspecific hybrids (Eenink, 1975; Heyn, 1977), the occurrence of diploid plants through anther culture from diploid genotypes as in tuberous *Solanum* species (Veilleux et al., 1985; Rivard et al., 1989). Instead of using pollen size as a criterion, DNA measurement of pollen grains through flow cytometry has been successful in *Lilium* interspecific hybrids (Van Tuyl et al., 1989).

Genetic basis of *2n* gamete formation

In plants, it is well-known that mutant genes can affect meiosis in various ways (Baker et al., 1976; Kaul & Murthy, 1985) and some of these can lead to the formation of *2n* gametes. Therefore, it is reasonable to assume that *2n* gamete formation in plants has a genetic basis. This assumption derives support from the following observations. a) In diplosporic apomictic species, *2n* egg formation is a regular feature and it is most likely to have a genetic basis. b) Single recessive genes have been shown to determine *2n* eggs and *2n* pollen formation in some plants (review, Bretagnolle & Thompson, 1995; Mok & Peloquin, 1975; Barcaccia et al., 2000; this issue). c) Polyhaploids (haploids derived from polyploids) mostly produce *2n* gametes, e.g., potato dihaploids (Ramanna, 1979); *Avena sativa* (Rines & Dahleen, 1990; Riera-Lizarazu et al., 1996); *Triticum durum* (Jauhar et al., 2000; Jauhar, this issue); *Rosa hybrida* (El Mokedem et al., 2002 a; 2002 b). If polyploids in the above mentioned plant species had originated through sexual polyploidization, and, if the trait of *2n* gamete production was genetically controlled, they were expected to occur also in the polyhaploids. This is indeed the case.

There are instances in which one and the same genotype produces both *2n* eggs and *2n* pollen simultaneously, but such cases are generally rare. Commonly, plants produce either only *2n* eggs or *2n* pollen indicating that these genetic traits are independent from each other. For example, in a desynaptic mutant of potato, genotypes that produced high frequencies of *2n* pollen failed to produce *2n* eggs in high frequencies

(Ramanna, 1983). On the other hand, those genotypes that produce high frequencies of only *2n* eggs have been reported in potato (Bastiaanssen et al., 1998) and alfalfa (Barcaccia et al., 2000; this issue). There are several reports which claim that the trait of *2n* gamete formation is controlled by single recessive genes (Mok & Peloquin, 1975; Peloquin, 1983), but the results are inconclusive for several reasons. First, the genes that induce meiotic nuclear restitution are highly influenced by environment and, therefore, it is difficult to establish whether it is a genetic trait or solely influenced by the environment. Second, it is extremely difficult, if not impossible, to determine whether only one or more mechanisms are involved in producing *2n* gametes in a particular genotype at a given time. Nevertheless, evidence for genetic control of meiotic nuclear restitution exists from the fact that through genetic selection the frequencies of *2n* gamete production can be significantly enhanced (Jacobsen, 1976; 1991; Barcaccia et al., this issue). Therefore, it is most likely that there might be major genes that are influenced by numerous modifier genes.

Apart from the genetically controlled traits of *2n* gamete formation, many of the interspecific and intergeneric hybrids with disturbed chromosome pairing produce high frequencies of *2n* gametes. For example, both *2n* eggs and *2n* pollen have been reported to occur in the F1 hybrids of emmer wheat × *Aegilops squarrosa* (Fukuda & Sakamoto, 1992); *Triticum turgidum* × *Secale cereale*, *T. turgidum* × *Ae. Squarrosa* (Xu & Joppa, 1995); *Alstroemeria* interspecific hybrids (Ramanna, 1992; Ramanna et al., this issue) among others. Such hybrids share two important features. 1) Both *2n* eggs and *2n* pollen are produced simultaneously by the same hybrid plant so that seeds can be obtained. 2) Neither the two parents of the F1 hybrids nor their (F2) sexual polyploid progenies possess the ability to produce *2n* gametes in any notable frequencies. Thus, it is difficult to conclude in these cases whether the *2n* gamete formation in distant F1 hybrids has the same genetic basis as in the so-called meiotic mutants. Nevertheless, single genes, or individual chromosomes controlling *2n* gamete formation have been reported in some of the distant hybrids. For example, in wheat, rye and *Aegilops* hybrids (Xu & Joppa, 1995) a single gene has been reported to determine FDR *2n* gamete formation. In oat-maize chromosome addition lines, Kynast et al., (2001) reported the highest 'fertility' in the F1 plants (haploid oat genome with an addition of a maize chromosome) with maize chromosome 2 addition, indicating the effect of a particular chromosome for FDR *2n* gamete formation. Besides these reports of single gene and chromosome controlled cases, Barcaccia et al. (2000; and this issue) have localized a recessive gene (*Tne1*) that determines the *2n* egg formation in alfalfa with respect to a DNA marker, (CA)8-GC. In conclusion, although there is evidence for the monogenic inheritance of *2n* gamete formation in some cases, the trait is highly influenced by the environment, and therefore, its basis is elusive.

Use of triploids for sexual polyploidisation

Generally, sexual polyploidisation implies that only the *2n* gametes are functional for producing polyploid progenies. However, there are situations in which ploidy level of the progeny can be increased without using *2n* gametes in a strict sense. For example, there are several instances in which triploids give rise to balanced tetraploid progenies after selfing the former. The increase of ploidy level in some of these cases occurs due to the functioning of balanced 2x gametes. There is evidence in the case of banana and plantain (Simmonds, 1962; Vuylsteke et al., 1993; Shephard, 1999), *Leucopogon juniperinus* (Smith-White, 1955), *Andropogon ternatus* (Norrmann & Quarin, 1987) among others (Ramsey & Schemske, 1998). Occurrence of 2x gametes in triploids is confirmed from the fact that balanced tetraploid progenies are produced from 3x-4x crosses. Obviously, unlike in somatic doubling, the ploidy increase in the progenies of triploids can accompany genetic recombination as in sexual polyploids. Although the autotriploids have been shown to be as fertile as allotriploids on theoretical grounds (Brandham, 1982; Kuspira et al., 1986), a survey of the literature suggests that there are several instances of fertile allotriploids as well (Table 1). From the few studies in which the progenies of allotriploids have been analyzed carefully, it is evident that genetic recombination does occur (Takahashi et al., 1997; Kamstra et al., 1999; Lim et al., 2001, 2003). Extensive GISH analyses of the progenies of allotriploid *Festuca-Lolium* hybrids have shown that intergenomic recombination does occur in allotriploids (Humphreys et al., 1996; King et al., 1998; 1999; Zwiezykowski et al., 1999; Thomas et al., this issue).

Table 1. Some examples of fertile allotriploids among plant species

Species hybrids	Purpose	Reference
Aegilops variabilis × *A. biuncialis*	Introgression	Pazy & Zohairy, 1965
Triticum turgidum × *Hordeum vulgare*	Phylogeny	Blanco et al., 1986
Narcissus hybrids*	Horticultural	Brandham, 1987
Brassica campestris × *B. oleracea*	Introgression	Inometa, 1983
Tomato × *S. lycopersicoides*	Recombination	Rick et al., 1988
Diplotaxis erucoides × *Brassica napus*	Introgression	Delourme et al., 1989
Betula nana × *B. pubescens*	Introgression	Anamthawat-Jonsson & Tomasson, 1990
Avena sativa (3x, polyhaploid)	Alien addition	Riera-Lizarazu et al., 1996
Hordeum vulgare × *H. bulbosum*	Introgression	Pickering, 1991
Tetraploid wheat × *Aegilops squarrosa*	Introgression	Fukuda & Sakamoto, 1995
Oryza australiensis × *O. sativa*	Monosomics	Multani et al., 1994
Musa acuminata × *M. balbisiana*	Introgression	Ortiz & Vuylsteke, 1995
Festuca pratensis × *Lolium perenne*	Mapping	King et al., 1998
Lolium multiflorum × *Festuca pratensis*	Recombination	Zwierzykowski et al., 1999
Lilium longiflorum × Asiatic hybrids	Introgression	Lim et al., 2003
Brassica juncea × *Diplotaxis virgata*	Introgression	Inomata (this issue)

* Numerous cases of fertile triploids have been recorded in *Narcissus* (Throckmorton, 1980).

Analyses of polyploids

In order to evaluate the advantages of sexual polyploidization, both traditional as well as molecular techniques have been used for analyses. Mostly, three aspects have received attention, viz, a) the degree of heterozygosity transferred through *2n* gametes; b) the amount of genetic crossing-over and c) transfer of alien chromosome segments. The techniques used in the case of auto- and allopolyploids obviously differ. Whereas in the case of autopolyploids the main objective is the assessment of the degree of homo- or heterozygosity that occurs in the sexual polyploid progenies, in the case of allopolyploids the detection of genomes, intergenomic recombination and introgression of alien chromosomes or their segments by molecular cytogenetics are the main objectives.

For the analysis of autopolyploid progenies, both traditional as well as molecular genetic markers have been used. For example, in potato morphological markers such as yellow tuber flesh color (*Y*), crumpled (*cr*), desynapsis (*ds*), yellow cotyledon (yc) amylose free (*amf*) have been used for the so-called half-tetrad analysis (Mendiburu & Peloquin, 1979; Jongedijk et al., 1990; Bastiaanssen, 1997). Besides these, isozyme markers have also been used similarly (Douches & Quiros, 1987; 1988; Jongedijk et al., 1991). However, because the genetic markers in these cases are highly restricted, numerous molecular genetic markers (RFLP. AFLP, RAPDs) have become available for the purpose of evaluating the autotetraploid progenies. Some examples are, potato (Van Eck et al., 1994, 1995; Bastiaanssen et al., 1998), alfalfa (Tavoletti et al., 1996; Barcaccia et al., 2000; this issue) and *Vaccinium* (Qu & Hancock, 1997). In order to determine the levels of heterozygosity transferred through the *2n* gametes, the molecular genetic markers that show codominance are the most useful ones. Theoretical basis and the advantages of using *2n* gametes for the transfer of heterozygosity has been extensively discussed and reviewed (Bingham, 1980; Bingham et al., 1994).

Unlike in autoployploids, mostly molecular cytological approaches involving GISH and FISH have been successfully used in the case of allopolyploids. An important advantage of allopolyploids for molecular cytogenetic analysis is that the constituent genomes in these can be clearly discriminated through DNA *in situ* hybridization methods. This includes the unequivocal identification of not only genomes and individual alien chromosomes but also the recombinant segments in the sexual polyploid progenies facilitating a quantitative estimate of recombination in some cases. For some unknown reasons, GISH and FISH analyses of the polyploid progenies have been confined so far to only monocotyledonous taxa. The following are only a few of the numerious examples: *Festuca-Lolium* hybrids (King et al., 1998; 1999; Zwierzykowsky et al., 1999; Canter et al., 1999), *Gasteria-Aloe* hybrids

(Takahashi et al., 1999), *Alstroemeria aurea* × *A. inodora* and other interspecific hybrids (Kamstra et al., 1999; Ramanna et al., this issue), *Lilium* interspecific hybrids (Karlov et al., 1999; Lim et al., 2000; 2002), *Musa* hybrids (D'Hont et al., 2000) and sugarcane (D'Hont et al., 1996).

It is now generally assumed that almost all polyploids in nature have originated through sexual polyploidization (Harlan & De Wet, 1985). The recent molecular cytogenetic data have revealed new information especially on allopolyploids. For example, GISH and FISH analyses of natural allopolyploid taxa have revealed the following three aspects: viz., a) genome constitution, b) intergenomic recombination or 'translocations' and c) multiple origins of allopolyploid species. Traditionally, genome constitutions of numerous allopolyploid species were determined on the basis of their taxonomic affinities to the suspected putative diploid parents as well as meiotic chromosome pairing. Through DNA in situ hybridisation, however, the genomes of allopolyploids have been more critically assigned to the diploid putative parental species. The following are some of the examples: bread wheat (Pederson & Langridge, 1997; Sánchez-Morán et al., 1999), oat (Chen & Armstrong, 1994; Jellen et al., 1994), cotton (Hanson et al., 1996), sugarcane (D'Hont et al., 2000)), tobacco (Kenton et al., 1993; Parokonny & Kenton, 1995) *Festulpia* (Bailey et al., 1993), banana (Osuji et al., 1997; D'Hont et al., 2000), *Crocus* (Ørgaard et al., 1995). In some of these cases, the so-called intergenomic translocations, some of which might well be intergenomic recombinations, have been detected (Chen & Armstrong, 1994; Parokonny & Kenton, 1995; Yang et al., 1999; Taketa et al., 1999). Differences have been observed among the related polyploid species regarding the chromosomes involved in translocations. Such differences can be explained in two ways. 1) The translocations occurred subsequent to polyploidization (Leitch & Bennett, 1997; Yang et al., 1999). 2) They occur during the process of (sexual) polyploidization. If it is the latter, then the inter- and intra specific differences that are observed in tetraploid wheats (Jiang & Gill, 1994; Badeva et al., 1994), wild *Hordeum* species (Taketa et al., 1999), *Avena* species (Leggett et al., 1994; Yang et al., 1999), among others, have originated subsequent to polyploidization events. On the assumption that sexual polyploidization in distant F1 hybrids might be a recurrent event, the occurrence of both intergenomic translocations, or recombinants, as well as multiple origins of ploypoids can be explained. This further supports the hypothesis of multiple origins of polyploid species (Soltis & Soltis, 1992) advocated on the basis of genetic evidence. Intergenomic translocations are more likely to occur in the F1 hybrids of distant species because the homoeologous chromosomes are 'forced' to pair and the *2n* gametes resulting from such meiosis are most likely to transmit recombinant chromosomes to the sexual polyploid progenies. This has been shown to occur in the progenies of hybrids of *Gasteria-Aloe* (Takahashi et al., 1997), *Alstroemeria* species (Kamstra et al., 1999; Ramanna et al., this issue) and *Lilium* species (Lim et al., 2001). Because homoeologous recombination can occur as independent cytological events in different meiocytes, the observed polymorphism for the so-called translocations is indeed expected.

Relevance of *2n* gametes for breeding

Before the discovery of colchicine, *2n* gametes were used for inducing polyploids in plants. This method of meiotic doubling was, undoubtedly, very inefficient for inducing polyploids at will. Although colchicine induced polyploids were produced in large numbers in several crops, none of the so-called synthetic crops, which were multiplied by seed, was successful, with the rare exception of *Triticale*. For seed propagated polyploids, the production of balanced gametes and high seed set are the prerequisites. In the case of *Triticale*, a broad-based breeding program was required to achieve success. This does not, however, mean that all new polyploids require such efforts for crop improvement. There are numerous instances from horticultural crops in which success has been achieved more rapidly and vindicate the superiority of polyploids as compared to diploid forms. Instances can be found in the case of roses (Darlington, 1976), *Narcissus* (Brandham, 1986), *Alstroemeria* (Ramanna, 1992), among several others. In all these cases, polyploids have originated spontaneously in the breeder's nurseries through the functioning of *2n* gametes. Ever since the demonstration of the superiority of 'natural polyploids', (Skiebe, 1958), systematic work on sexual polyploidization has progressed in some crops (review, Mariani & Tavoletti, 1992) such as potato (Peloquin, 1982; Jacobsen, 1976; Jongedijk et al., 1991), alfalfa (Bingham, 1980; Barcaccia et al., this issue) and *Vaccinium* (Lyrene et al., this issue). Initially, however, sexual polyploidisation in any crop can be difficult and laborious because of the non-availability

of desirable diploid genotypes that can be used for either in unilateral or bilateral sexual polyploidization. Once the genotypes that produce either *2n* pollen or *2n* eggs in acceptable frequencies are selected, as has been done in some crops (Clulow et al., 1995 and this issue), the task becomes manageable. The genetic basis of *2n* gamete formation is undoubtedly complex. Nevertheless, with certain amount of perceverence, *2n* gamete-producing genotypes can be selected and used successfully when necessary. There are several less well-known, but useful (auto)polyploid crops, such as sweet potato (*Ipomea*), cassava (*Manihot*), taro (*Colocassia*), yams (*Dioscoria* spp) and many more, that might be amenable for improvement through sexual polyploidization. All these crops are vegetatively propagated and may be improved by the methods similar to those used in the case of potato.

There are certain crops in which sexual polyploidization might be the only way forward. These include triploid crops such as banana, plantain and the complex polyploid crop sugarcane. In the case of banana and plantain, it has been extremely difficult to produce new cultivars because of their odd-polyploidy (2n=3x=33) in most cases and their breeding behaviour is complicated. However, some of the triploid varieties do produce haploid (x=11), diploid (2x=22) as well as *2n* gametes in low frequencies. In fact these were the main sources in most cases for producing progenies from banana and plantain (Simmonds, 1962; Ortiz & Vuylsteke, 1995). Aside from the difficulty of producing progenies, the genetics of these vegetatively propagated crops is highly complicated and the selection of varieties is, obviously, difficult. However, the development of molecular techniques (Jarret et al., 1993; Osuji et al., 1997; Guimarâes et al., 1997; D'Hont et al., 2000) for progeny analysis has added a new dimension for breeding these difficult crops.

Unlike banana and plantain, the modern sugarcane is a highly complex polyploid (2n=80) of hybrid origin. In an effort to improve this crop, the cultivated form, the so-called 'noble canes', *Saccharum officinarum,* is crossed with the wild species, *S. spontaneum.* Almost as a regular feature, the F1 plants consisted of the somatic complement of *S. officinarum* and a haploid complement *S. spontaneum* (Bremer, 1959; Price, 1963) and this was designated as *2n+n* mating. In addition, when the F1 hybrid, *S. officinarum* × *S. spontaneum* was backcrossed to *S. officinarum,* for example, it also produced *2n+n* (as well as *n+n*) progenies in many cases (Bremer, 1959; Heinz, 1980). An intreguing feature of this phenomenon is that the so-called 'nobilization' can be attained more rapidly while breeding commercial sugarcane varieties through backcrossing. Although a clear explanation for the nobilization is not yet possible, it has been speculated that the functioning of the *2n* eggs in the F1 hybrids of *S. officinarum* × *S. spontaneum*, and in the subsequent backcross generations, might increase the proportion of the chromosomes of *S. officinarum* in the progenies (see. Heinz, 1991). With the availability of molecular techniques for the identification of genomes, and recombinant chromosomes (D'Hont et al., 1996) as well as genetic mapping (Sobral, 1996; Guimarâes et al., 1997), it should be possible to elucidate the value of *2n* eggs contributed by *S. officinarum* × *S. spontanum* hybrids and their backcross progenies.

For the purpose of introgression of alien chromosomes and genes into cultivars, hybrids were usually made between distantly related species and the chromosome numbers of the F1 hybrids were doubled by colchicine treatment. Although this resulted in creating fertile allopolyploids, they did not serve the purpose of breeding for two reasons. First, the backcrossing of an allotetraploid with a diploid parent, for example, produced a triploid that was not readily suitable for further crossing. Second, due to autosyndetic pairing in a typical allotetraploid, there was no room for intergenomic recombination. This bottleneck could be avoided to a great extent if a distant (diploid) hybrid that produced *2n* gametes was selected. An important feature of meiosis in a distant hybrid is that the homoeologous chromosomes do pair to some extent resulting in intergenomic recombination. Thus, *2n* gametes from a distant hybrid can be a convenient source of intergenomic recombinant chromosomes. Through *in situ* hybridisation techniques, the occurrence of intergenomic recombination has been convincingly demonstrated in some cases, viz., hybrids of *Gasteria-Aloe*, *Alstroemeria aurea* × *A. inodora*, *Lilium* species*, Festuca-Lolium*, among others. A clear example of identification of introgressed alien chromosome segments is illustrated in the case of *Lolium perenne* into which recombinant segments are added from *Festuca pratensis* (King et al., 1998, 1999). The invaluable role of introgression for crop improvement has been highlighted in the case of wheat, the cytogenetics of which has been highly developed (review, Jiang et al., 1994).

In addition to field crops, sexual polyploidization has played a great role in producing new varieties in horticultural crops, albeit without the knowledge of

the breeders in most cases. A systematic cytogenetic knowledge of some of the cultivars or synthetic sexual polyploids has yielded and can yield even more valuable knowledge that might be potentially useful for breeding of polyploid crops in general.

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References

Anamthawat-Jónsson, K. & T. Tomasson, 1990. Cytogenetics of hybrid introgression in Icelandic birch. Hereditas 112: 65–70.

Badeva, E.D., N.S. Badev, B.S. Gill & A.A. Filatenko, 1994. Intraspecific divergence in *Triticum araraticum*. Pl Sys Evol 192: 117–145.

Baily, J.P., S.T. Bennett, M.D. Bennett & C.A. Stace, 1993. Genomic *in situ* hybridization identifies parental chromosomes in the wild grass hybrid × *Festulpia hubbardii*. Heredity 71: 413–420.

Baker, B.S., A.T.C. Carpenter, M.S. Esposito, R.E. Esposito & L. Sandler, 1976. The genetic control of meiosis. Ann Rev Genet 10: 53–134.

Barcaccia, G., E. Albertini, D. Rosellini, S. Tavoletti & F. Veronesi, 2000. Inheritance and mapping of *2n*-egg production in diploid alfalfa. Genome 43: 528–537.

Bastiaanssen, H.J.M., 1997. Marker assisted Elucidation of the Origin of 2n-gametes in Diploid Potato. PhD Thesis, Wageningen Agricultural University, The Netherlands.

Bastiaanssen, H.J.M., M.S. Ramanna, Z. Sawor, A Mincione, A. van de Steen & E. Jacobsen, 1996. Pollen markers for gene-centromere mapping in diploid potato. Theor Appl Genet 93: 1040–1047.

Bastiaanssen, H.J.M., M.S. Ramanna, D.J. Huigen & E. Jacobsen, 1998a. Selection of diploid tuberous Solanum hybrids for 2n-egg formation using 2x-4x crosses. Euphytica 101: 325–339.

Bastiaanssen, H.J.M., P.M.M.M. van den Berg, P. Lindhout, E Jacobsen & M.S. Ramanna, 1998b. Post meiotic restitution in *2n*-egg formation of diploid potato. Heredity 81: 20–27.

Battaglia, E., 1963. Apomixis. In: Recent Advances in Embryology of Angiosperms, pp. 221–264. International Society of Plant Morphology, University of Delhi.

Bingham, E.T., 1980. Maximizing heterozygosity in autopolyploids. In: W.H. Lweis (Ed.), Polyploidy, Biological Relevance, pp. 147–190. Plenum Press, NY.

Bingham, E.T., R.W. Groose, D.R. Woodfield & K.K. Kidwell, 1994. Complementary gene interactions in alfalfa are greater in autopolyploids than diploids. Crop Sci 34: 823–829.

Blanco, A., G.V. Fracchiolla & B. Greco, 1986. Intergeneric wheat × barley hybrid. J Hered 77: 98–100.

Brandham, P.E., 1982. Inter-embryo competition in the progeny of autotriploid *Aloenae* (Liliaceae). Genetica 59: 29–42.

Brandham, P.E., 1986. Evolution of polyploidy in cultivated *Narcissus* subgenus *Narcissus*. Genetica 68: 161–167.

Brandham, P.E., 1987. The chromosomes of species, hybrids and cultivars of *Narcissus* L. (Amaryllidaceae). Kew Bullitin 42: 65–102.

Bremer, G., 1959. Increase of chromosome number in interspecies-hybrids of *Saccharum* in relation to embryo-sac development. Bibliog Genet XVIII: 1–99.

Bremer, G., 1961. Problems in breeding and cytology of sugarcane IV. The origin of increase of chromosome number in species hybrids of *Saccharum*. Euphytica 10: 325–342.

Bretagnolle, F. & J.D. Thompson, 1995. Gametes with somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol 129: 1–22.

Cantor, P.H., I. Pasakinskiené, R.N. Jones & M.W. Humphreys, 1999. Chromosome constitution and recombination in the amphiploid *Lolium perenne* × *Festuca pratensis* cv. Prior (2n=4x=28). Theor Appl Genet 98: 809–814.

Chase, S.S., 1963. Analytic breeding in *Solanum tuberosum* L. – A scheme to utilizing parthenotes and other diploid stocks. Can J Genet Cytol 5: 359–363.

Chen, Q. & K. Armstrong, 1994. Genomic *in situ* hybridization in *Avena sativa*. Genome 37: 607–612.

Clulow, S.A., J. McNicoll & J.E. Bradshaw, 1995. Producing commercially attractive, uniform potato seed progenies: the influence of breeding scheme and parental genotype. Theor Appl Genet 90: 519–525.

Delourme, R., F. Eber & A.M. Chevre, 1989. Intergeneric hybridization of *Diplotaxis erucoides* with *Brassica napus* L. I. Cytogenetic analysis of F1 and BC2 progeny. Euphytica 41: 123–128.

D'Hont, A., L. Grivet, P. Feldmann, P. Rao, N. Berding & J.C. Glaszmann, 1996. Characterization of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. Mol Gen Genet 250: 405–413.

D'Hont, A., A. Paget-Goy, J. Escoute & Careel, 2000. The interspecific genome structure of cultivated banana, *Musa spp*. Revealed by genomic *in situ* hybridization. Theor Appl Genet 100: 177–183.

Darlington, C.D., 1976. Chromosome Botany and the Origins of Cultivated Plants. George Allen & Unwin Ltd., London, pp. 1–231.

Da Silva, J.A.G. & B.W.S. Sobral, 1996. Genetics of polyploids. In: B.W.S. Sobral (Ed.), The Impact of Plant Molecular Genetics, pp. 1–37. Birkhäuser, Bosten.

Douches, D.S. & C.F. Quiros, 1987. Use of 2x-4x crosses to determine gene-centromere map distances of isozyme loci in *Solanum* species. Genome 29: 519–527.

Douches, D.S. & C.F. Quiros, 1988. Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* × *S. chacoense* diploid hybrid. Heredity 60: 183–191.

Dowrick, G.J., 1966. Breeding systems in tetraploid *Rubus* species. Genet Res 7: 243–253.

Eenink, A.H., 1974. Metromorphy in *Brassica oleracea* L. III. The influence of temperature, delayed prickle pollination and growth regulators on the number of metromorphic seeds formed. Euphytica 23: 711–718.

El Mokadem, H., L. Crespel, J. Meynet & S. Gudin, 2002a. The occurrence of *2n*-pollen and the origin of sexual polyploids in dihaploid roses (*Rosa hybrida*). Euphytica 125: 169–177.

El Mokadem, H., J. Meynet & L. Crespel, 2002b. The occurrence of 2n eggs in the dihaploids derived from *Rosa hybrida* L. Euphytica 124: 327–332.

Ferris, C., R.S. Callow & A.J. Grey, 1992. Mixed first and second division restitution in male meiosis of *Hierochloë odorata* (L.) Beauv (Holy grass). Heredity 69: 21–31.

Fukuda, K. & S. Sakamoto, 1992. Cytological studies on unreduced male gamete formation in hybrids between tetraploid emmer wheats and *Aegilops squarrosa*. Japan J Breed 42: 255–266.

Grant, V., 1952. Cytogenetics of hybrid *Gilia millefoliata* × *G. achilleaefolia*. I. Variation in meiosis and polyploidy rate as affected by nutritional and genetic conditions. Chromosoma 5: 372–390.

Grant, V., 1981. Plant Speciation. New York: Columbia Univ. Press, 2nd edn.

Guimarâes, C.T., G.R. Sills & B.W.S. Sobral, 1997. Comparative mapping of Andropogonae: *Saccharum* L. and its relation to maize. Proc Nt Acad Sci US 94: 14261–14266.

Gustafsson, A., 1946. Apomixis in higher plants. I. The mechanisms of apomixis. Lunds Univ Arsskr 42: 1–66.

Hanson, R.E., M.N. Islam-Faredi, E.A. Percival, C.F. Crane, Y. Ji, T.D. McKnight, D.M. Stelly & H.J. Price, 1996. Distribution of 5S and 18S-25S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative ancestors. Chromosoma 105: 55–61.

Heinz, D.J., 1980. Thailand S. spontanium hybrid progeny as a new germplasm source in Hawaii. Proc Int Sugarcane Technol 17: 1347–1356.

Heyn, F.W., 1977. Analysis of unreduced gametes in the Brassicae by crosses between species and ploidy levels. Z Planzenzüchtg 78: 13–30.

Humphreys, M.W. & I. Pasakinskine, 1996. Chromosome painting to locate genes for drought resistance transferred from *Festuca arundenacea* into *Lolium multiflorum*. Heredity 77: 530–534.

Inomata, N., 1983. Hybrid progenies of the cross, *Brassica campestris* × *B. oleraceae* II. Crossing ability of F1 hybrids and their progenies. Jap J Genet 58: 433–449.

Islam, A.K.M.R. & K.W. Shepherd, 1980. Meiotic restitution in wheat-barley hybrids. Chromosoma 78: 363–372.

Jackson, R.C., 1976. Evolution and systematic significance 0f polyploidy. Ann Rev Ecol Syst 7: 209–234.

Jacobsen, E., M.S. Ramanna, D.J. Huigen & Z. Sawor, 1991. Introduction of an amylose free (*amf*) mutant into breeding of cultivated potato, *Solanum tuberosum* L. Euphytica 53: 247–253.

Jacobsen, E., 1976. Cytological studies on diplandroid production in a dihaploid potato clone and its correlation with seed set in 4x-2x crosses. Z Pflanzuchüchtg 77: 10–15.

Jarret, R.L., D.R. Vuylsteke, N.J. Gawel, R.B. Pementel & L.J. Dumbar, 1993. Detecting genetic diversity in diploid bananas using PCR and primers from a highly repetitive DNA sequence. Euphytica 68: 69–76.

Jauhar, P.P., M. Doğramacı-Altuntepe, T.S. Peterson & A.B. Almouslem, 2000. Seedset on synthetic haploidsof durum wheat: cytological and molecular investigations. Crop Sci 40: 1742–1749.

Jellen, E.N., B.S. Gill & T.S. Cox, 1994. Genomic in situ hybridization differentiates between A/D- and C-genome chromatin and detects intergenomic translocations in polyploid oat species (genus *Avena*). Genome 37: 613–618.

Ji, Y., D.A. Raska, T.D. Mcknight, M.N. Islam-Faredi, C.F. Crane, M.S. Zwick, R.E. Hanson, H.J. Price & D.M. Stelly, 1997. Use of meiotic FISH for identification of a newmonosome in *Gossypium hirsutum* L. Genome 40: 34–40.

Jiang, J. & B.S. Gill, 1994. Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. Chromosome Res 2: 59–64.

Jiang, J., B. Friebe & B.S. Gill, 1994. Recent advances in alien gene transfer in wheat. Euphytica 73: 199–212.

Jongedijk, E., R.C.B. Hutten, J.M.A.S.A. van der Wolk & S.I.J. Schuurmans Stekhoven, 1991a. Synaptic mutants in potato, *Solanum tuberosum* L. III. Effect of the Ds-1/ds-1 (desynapsis) locus on genetic recombination in male and female meiosis. Genome 34: 121–130.

Jongedijk, E., M.S. Ramanna, Z. Sawor & J.G.Th. Hermsen, 1991b. Formation of first division restitution (FDR) 2n-megaspores through pseudohomotypic division in ds-1 (desynapsis) mutants of diploid potato: routine production of tetraploid progeny from 2x FDR × 2x FDR crosses. Theor Appl Genet 82: 645–656.

Kamstra, S.A., A.G.J. Kuipers, M.J. De Jeu, M.S. Ramanna & E. Jacobsen, 1999. The extent and position of homoeologous recombination in a distany hybrid of *Alstroemeria*: a molecular cytogenetic assessment of first generation progenies. Chromosoma 108: 52–63.

Karlov, G.I., L.I. Khrustaleva, K.B. Lim & T.M. Van Tuyl, 1999. Homoeologous recombination in 2n-gamete producing interspecific hybrids of *Lilium* (Liliaceae) studied by genomic *in situ* hybridization (GISH). Genome 42: 681–686.

Karpechenko, G.D., 1927. The production of polyploid gametes in hybrids. Hereditas 9: 349–368.

Kaul, M.L.H. & T.G.K. Murthy, 1985. Mutant genes affecting higher plant meiosis. Theor Appl Genet 70: 449–466.

Kenton, A., A.S. Parokonny, Y.Y. Gleba & M.D. Bennett, 1993. Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Mol Gen Genet 240: 159–169.

Kihara, H., 1946. Maturation division in F1 hybrids between *Triticum dicoccoides* × *Aegilops squarrosa*. La Kromosoma 1: 6–11.

King, I.P., W.G. Morgan, I.P. Armstead, J.A. Harper, M.D. Hayward, A. Bollard, J.V. Nash, J.W. Forester & H.M. Thomas, 1998. Introgression mapping in grasses. I. Introgression of *Festuca pratensis* chromosomes and chromosome segments into *Lolium perenne*. Heredity 81: 462–467.

King, I.P., W.G. Morgan, J.A. Harper & H.M. Thomas, 1999. Introgression mapping in grasses. II. Meiotic analysis of the *Lolium perenne/ Festuca pratensis* triploid hybrid. Heredity 82: 107–112.

Kuspira, J., R.N. Bhambani, R.S. Sadasivaiah & D. Hayden, 1986. Genetic and cytogenetic analysis the A genome of *Triticum monoccum*. III. Cytology, breeding behaviour, fertility and morphology of triploids. Can J Genet Cytol 28: 867–887.

Kynast, R.G., O. Riera-Lizarazu, M.I. Vales, R.J. Okagaki, S.B. Maquieira, G. Chen, E.V. Ananiev, W.E. Oldland, C.D. Russsell, A.O. Stec, S.M. Livingston, H.A. Zaia, H.W. Rines & P.L. Phillips, 2001. A complete set of maize individual chromosome additions to the oat genome. Plant Physiol 125: 216–1227.

Leggett, J.M., H.M. Thomas, M.R. Meredith, M.W. Humphreys, W.G. Morgan, H. Thomas & I.P. King, 1994. Intergenomic translocations and genomic composition of *Avena maroccana* Gdgr. Revealed by FISH. Chromosome Res 2: 163–164.

Leitch, I.J. & M.D. Bennett, 1997. Polyploidy in angiosperms. Trends Plant Sci 2: 470–476.

Lim, K.B., M.S. Ramanna, J.H. De Jong, E. Jacobsen &J.M. Van Tuyl, 2001. Indeterminate restitution (IMR): a novel type of meiotic nuclear restitution mechanism detected in interspecific lily hybrids by GISH. Theor Appl Genet 103: 219–230.

Lim, K.-B., M.S. Ramanna, E. Jacobsen & J.M. Van Tuyl, 2003. Evaluation of BC2 progenies derived from 3x-2x and 3x-4x crosses of *Lilium*: a GISH analysis. Theor Appl Genet 106: 568–574.

Maheswari, P., 1950. An Introduction to the Embryology of Angiosperms. McGraw-Hill Book Company, Inc. New York.

Mariani, A. & S. Tavoletti, 1992. Gametes with Somatic Chromosome Number in the Evolution and Breeding of Polyploid Polysomic Species. Proc Workshop, Perugia, Tipolithographia Porziuncola-Assisi (PG) Italy, pp. 1–103.

Mendiburu, A.O. & S.J. Peloquin, 1971. High yielding tetraploids from 4x-2x and 2x-2x matings. Amer Potato J 48: 300–301.

Mendiburu, A.O., S.J. Peloquin & D.W.S. Mok, 1974. Potato breeding with haploids and 2n gametes. In: K. Kasha (Ed.), Haploids in Higher Plants, pp. 249–258. University of Guelph, Guelph, Ontario, Canada.

Mendiburu, A.O. & S.J. Peloquin, 1979. Gene-centromere mapping by 4x-2x matings in potato. Theor Appl Genet 54: 177–180.

Mok, D.W.S. & S.J. Peloquin, 1975a. Three mechanisms of 2n pollen formation in diploid potatoes. Can J Genet Cytol 17: 217–225.

Mok, D.W.S. & S.J. Peloquin, 1975b. The inheritance of three mechanisms of diplandroids (2n pollen) formation in diploid potato. Heredity 35: 295–302.

Multani, D.S., K.K. Jena, D.S. Brar, B.G. de los Reyes, E.R. Angels & G.S. Khush, 1994. Development of monosomic addition lines and introgression of genes *from Oryza australiensis* Domin. To cultivated rice *O. sativa*. Theor Appl Genet 88: 102–109.

Norrmann, G.A. & C.L. Quarín, 1987. Permanent odd polyploidy in a grass (*Andropogon ternatus*). Genome 29: 340–344.

Nygren, A., 1967. Apomixis in the angiosperms. Handb der Pflanzenphysiol 18: 551–596.

Ørgaard, M., N. Jacobsen & J.S. Heslop-Harrison, 1995. The hybrid origin of two cultivars of *Crocus* (Iridaceae) analysed by molecular cytogenetics including genomic Southern and *in situ* hybridization. Ann Bot 76: 253–262.

Ortiz, R. & D. Vuylsteke, 1995. Factors influencing seed set in triploid *Musa* spp. L. and production of euploid hybrids. Ann Bot 75: 151–155.

Osuji, J.O., G. Harrison, J. Crouch & J.S. Heslop-Harrison, 1997. Identification of the genomic constitution of *Musa* L. lines (bananas, plantains and hybrids) using molecular cytogenetics. Ann Bot 80: 787–793.

Pazy, B. & D. Zohary, 1965. The process of introgression between *Aegilops* polyploids: Natural hybridization between *A. variabilis*, *A. ovata* and I. Evolution 19: 385–394.

Pederson, C. & P. Langridge, 1997. Identification of the entire chromosome complement of bread wheat by two colour FISH. Genome 40: 589–593.

Peloquin, S.J., 1982. Meiotic Mutants in Potato Breeding. Stadler Genetic Symp 14: 11.

Pickering, R.A., 1991. The production of fertile triploid hybrids from crosses between *Hordeum vulgare* L. (2n=4x=28) and *H. bulbosum* L. (2n=2x=14). Hereditas 114: 227–236.

Price, S., 1961. Cytological studies in *Saccharum* and allied genera. VII. Maternal chromosome transmission by *S. officinarum* in intra- and interspecific crosses. Bot Gez 122: 298–305.

Price, S., 1963. Cytogenetics of modern sugarcane. Econ Bot 17: 97–106.

Qu, L. & J.F. Hancock, 1997. RAPD-based genetic linkage map of blueberry derived from an interspecific cross between *Vaccinium darrowi* and tetraploid *V. corymbosum.* J Am Hort Sci 122: 69–73.

Ramanna, M.S., 1974. The origin of unreduced microspores due to aberrant cytokinesis in the meiocytes of potato and its genetic significance. Euphytica 23: 20–30.

Ramanna, M.S., 1979. A re-examination of the mechanisms of 2n gamete formation in potato and its implications for breeding. Euphytica 28: 537–561.

Ramanna, M.S., 1983. First division restitution gametes through fertile desynaptic mutants of potato. Euphytica 32: 337–350.

Ramanna, M.S., 1992. The use of 2n-gametes in breeding polysomic polyploid species: some achievements and perspectives. In: A. Mariani & S. Tavoletti (Eds.), Gametes with Somatic Chromosome Number in the Evolution and Breeding of Polyploid Polysomic Species: Achievements and Perspectives, pp. 91–99. Perugia, Italy.

Ramsey, J. & D.W. Schemske, 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. Ann Rev Ecol Syst 29: 467–501.

Rick, C.M. R.T. Chetelat &J.W. De Verna, 1988. Recombination in sesquidiploid hybrids of *Lycopersicon esculentum* × *Solanum lycopersicoides* and derivatives. Theor Appl Genet 76: 647–655.

Riera-Lizarazu, O., H.W. Rines & R.L. Phillips, 1996. Cytological and molecular characterization of oat × maize hybrids. Theor Appl Genet 93: 123–135.

Rines, H.W. & L.S. Dahleen, 1990. Haploid oat plants produced by application of maize pollen to emasculated oat florets. Crop Sci 30: 1073–1078.

Rivard, S.R., M. Cappadocia, G. Vincent, N. Brisson & B.S. Landry, 1989. Restriction fragment length polymorphism (RFLP) analyses of plants produced by *in vitro* anther culture of *Solanum chacoense* Bitt. Theor Appl Genet 78: 49–56.

Sánchez-Morán, E., E. Benavente & J. Orellana, 1999. Simultaneous identification of A, B, D and R genomes by genomic *in situ* hybridization in wheat-rye derivatives. Heredity 83: 1–4.

Sasakuma, T. & H. Kihara, 1981. A synthesized common wheat obtained from a triploid hybrid, *Aegilops squarrosa* var. strangulata (?) × *Triticum durum* (?). Wheat Info Serv 52: 14–18.

Shephard, K., 1999. Cytogenetics of the Genus *Musa*. International Network for the Improvement of Banana and Plantain. Montpellier, France. IPGRI, Rome, pp. 1–157.

Simmonds, N.W., 1962. The Evolution of the Bananas. Longmans, London, UK, pp. 170.

Smith-White, S., 1955. The life history and genetic system of *Leucopogon juniperinum*. Heredity 9: 79–85.

Skiebe, 1958. Die Bedeutung von unreduzierten Gameten für die polyploidiezüchtung bei der Fliededprimel (*Primula melacoides* Franchet). Züchter 28: 353–359.

Soltis, D.E. & P.S. Soltis, 1992. Molecular data and the dynamic nature of polyploidy. Cri Rev Plant Sci 12: 243–273.

Stebbins, G.L., 1950. Variation and Evolution in Plants. Columbia University Press, New York, pp. 298–369.

Stebbins, G.L., 1971. Chromosome Evolution in Higher Plants. Addison Wesley, London.

Stelly, D.M. & S.J. Peloquin, 1986. Diploid female gametophyte formation in 24 chromosome potatoes: genetic evidence for the prevalence of the second meiotic division restitution mode. Can J genet Cytol 28: 101–108.

Storey, W.B., 1956. Diploid and polyploid gamete formation in orchids. Proc Amer Soc Hort Sci 68: 491–502.

Strasburger, E., 1910. Sexuelle und apogame Fortpflanzung bei Urticaceen. Jahrb Wiss Bot 47: 245–288.

Takahashi, C., I.J. Leitch, A. Ryan, M.D. Bennett & P.E. Brandham, 1997. The use of genomic *in situ* hybridization (GISH) to show transmission of recombinant chromosomes by a partially fertile bigeneric hybrid, *Gasteria lutzii* × *Aloe aristata* (Aloaceae) to its progeny. Chromosoma 105: 342–348.

Taketa, S., H. Ando, K. Takeda & R. von Bothmer, 1999. Detection of *Hordeum marinum* genome in three polyploid *Hordeum* species and cytotypes by genomic *in situ* hybridization. Hereditas 130: 185–188.

Tavoletti, S., E.T. Bingham, B.S. Yandell, F. Veronesi & T.C. Osborn, 1996. Half tetrad analysis in alfalfa using multiple restriction fragment length polymorphism markers. Pro Nat Acad Sci USA 93: 10918–10922.

Throckmorton, T.D., 1980. Daffodilsto show and grow, and abridged classified list of daffodil names. American Daffodil

Soc: Tyner, North Carolina, and Royal Horticultural Society, London.

Van Eck, H.J., J.M.F. Jacobs, P.M.M.M. van den Berg, W.J. Stikema & E. Jacobsen, 1994. The inheritance of anthocyanin pigmentation in potato (*S. tuberosum* L.) and mapping of tuber skin colour loci using RFLPs. Heredity 73: 410–421.

Van Eck, H.J., J. Van der Voort, J. Draaistra, P. Van Zandvoort, E. Van Enckevort, B. Segers, J. Peleman, E. Jacobsen, J. Helder & J. Bakker, 1995. The inheritance and chromosomal localisation of AFLP markers in a non-inbred potato offspring. Mol Breed 1: 397–410.

Van Santen, E., P.M. Hugessen & M.D. Casler, 1991. Identification and frequency of teteraploid progeny from 2x-4x crosses in *Dactylis*. Genome 34: 273–278.

Van Tuyl, J.M., J.N. De Vries, R.J. Bino & A.A.M. Kwakkenbos, 1989. Identification of 2n-pollen producing interspecific hybrids of *Lilium* using flow cytometry. Cytologia 54: 737–745.

Veilleux, R., 1985. Diploid and polyploid gametes in crop plants: Mechanisms of formation and utilization in plant breeding. Plant Breed Rev 3: 252–288.

Veilleux, R.E., J. Booze-Daniels & E. Pehu, 1985. Anther culture of 2n pollen producing clone of *Solanum phureja* Juz. & Buk. Can J Genet Cytol 27: 559–564.

Veronesi, F., A. Mariani & E.T. Bingham, 1996. Unreduced gametes in diploid *Medicago* and their importance in alfalfa breeding. Theor Appl Genet 72: 17–41.

Vuylsteke, D., R. Ortiz, C. Pasberg-Gauhl, F. Gauhl, C. Gold, S. Ferris & P. Speijer, 1993. Plantain and banana research at the International Research Institute of Tropical Agriculture. HortScience 28: 873–873, 970–971.

Winkler, H., 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeit f Bot 8: 417–531.

Xu, S. & Y. Dong, 1992. Fertility and meiotic mechanisms of hybrids between chromosome auto duplication tetraploid wheats and *Aegilops* species. Genome 35: 379–374.

Xu, S. & L.R. Joppa, 1995. Mechanisms and inheritance of first division restitution in hybrids of wheat, rye and *Aegilops squarrosa*. Genome 38: 607–615.

Xu, S. & L.R. Joppa, 2000. First division restitution in hybrids of *Langdon durum* disomic substitution lines with rye and *Aegilops squarrosa*. Plant Breed 119: 233–241.

Yang, Q., M.D. Hanson, M.D. Bennett & I.J. Leitch, 1999. Genome structure and evolution in the allohexaploid weed *Avena fatua* L. (Poaceae). Genome 42: 512–518.

Zwierzykowsky, Z., R. Tayyar, M. Brunell & A.J. Lukaszewski, 1998. Genome recombination in intergeneric hybrids between *Festuca pratensis* and *Lolium multiflorum.* J Heredity 89: 324–328.

Zwierzykowsky, Z., A.J. Lukaszewski, B. Naganowska & Lesniewska, 1999. The pattern of homoeologous recombination in triploid hybrids of *Lolium multiflorum* with *Festuca pratensis.* Genome 42: 720–726.