

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Use of 2n Gametes in Plant Breeding

A. Dewitte¹, K. Van Laere² and J. Van Huylenbroeck²

¹KATHO Catholic University College of Southwest Flanders,
Department of Health Care and Biotechnology,

²Institute for Agricultural and Fisheries Research (ILVO),
Plant Sciences Unit,
Belgium

1. Introduction

Genome doubling (polyploidization) has played a major role in the evolution and diversification of the plant kingdom and is regarded as an important mechanism of speciation and adaptation in plants (Otto & Whitton, 2000). The term ploidy refers to the number of basic chromosome sets (represented by 'x') present in a somatic plant cell (2n) or gamete (n). Scaling whole sets of chromosomes up or down is a powerful and commonly applied strategy to produce altered genotypes for breeding purposes.

Polyploids differ from their diploid progenitors in morphological, ecological, physiological and cytological characteristics. Their wider geographical distribution reflects the variety of their ecological tolerances (Carputo et al., 2003; Chen, 2007; Knight et al., 2005; Otto & Whitton, 2000; Soltis & Soltis, 2000; Thompson et al., 2004). Polyploids have breeding value as they can have broader and thicker leaves, larger flowers, longer internodes, fewer stems per plant, fewer inflorescences, higher vegetative yields, more compact plants, greater tolerance to environmental stress, higher (additive) resistance to several diseases, increased seed weight but fewer seeds or higher chlorophyll production. Doubling the chromosome number can rescue and stabilise interspecific hybrids that would otherwise show a high degree of sterility due to failure in meiosis. Furthermore, polyploidy might result in the development of sterile cultivars (e.g. triploids), loss of self-incompatibility, flowering time variation, changes in growth vigour or shifts in reproductive system (e.g. to asexual reproduction). Apomixis, an extreme form of reproductive modification, is commonly associated with polyploidy. Negative side effects of polyploidization might be infertility (which can also be a desired trait), brittle wood, watery fruit, stunting and malformation (Andruezza & Siddiqi, 2008; Baert et al., 1992; Barringer, 2007; Bretagnolle & Thompson, 1995; Briggs & Walters, 1997; Chen, 2007; Chahal & Gosal, 2002; Comai, 2005; Contreras et al., 2007; De Nettancourt, 1997; Eeckhaut et al., 2004; Grant, 1981; Gu et al., 2005; Kermani et al., 2003; Lamote et al., 2002; Otto & Whitton, 2000; Stebbins, 1971; Thomas, 1993; Van Huylenbroeck et al., 2000; Van Laere et al., 2011).

Several phenotypic characteristics have been used as an indirect measure of ploidy level. Polyploids frequently have larger pollen sizes, stomata sizes, and seeds than diploids but have slower developmental rates. Further, the number of chloroplasts in guard cells, leaf

area index, stomatal density, or pollen fertility might be related to the ploidy level (Aryavand et al., 2003; Kessel & Rowe, 1975; Mishra, 1997; Otto & Whitton, 2000; Vandenhout et al., 1995; Zlesak, 2009).

In general, polyploidy is accompanied by genome-wide changes in gene expression and epigenetic modifications. The genotypic and phenotypic differences are caused mainly by the increased cell size, gene dosage effect, allelic diversity (level of heterozygosity), gene silencing and genetic or epigenetic interactions (Andruezza & Siddiqi, 2008; Jovtchev et al., 2006; Kondorosi et al., 2000; Leitch & Bennett, 1997; Levin, 1983; Lewis, 1980; Mittelsten Scheid et al., 1996; Osborn et al., 2003; Pikaard, 1999).

Polyploids can be induced by two mechanisms. First, mitosis spindle inhibitors such as colchicine, oryzalin and trifluralin can be applied to create artificial (mitotic) polyploids (for a review see Dhooghe et al., 2011). Second, gametes with somatic chromosome numbers, also referred to as $2n$ gametes or (numerically) unreduced gametes are considered to be the driving force behind the formation of polyploids in nature (Bretagnolle & Thompson, 1995; Otto & Whitton, 2000).

Harlan & De Wet (1975) showed that almost all plant species produce $2n$ gametes in some frequencies and argued the importance of $2n$ gametes in the origin of polyploids. Although efforts to synthesize polyploids through the use of $2n$ gametes were performed much earlier (e.g. Skiebe 1958), it was assumed that $2n$ gametes occur only rarely and with little contribution to the origin of polyploids (Ramanna & Jacobsen, 2003). The use of $2n$ gametes in plant breeding, resulting in the establishment of sexual or meiotic polyploids, has been very useful for crop improvement (Ramanna & Jacobsen, 2003). These gametes combine the genetic effects of raised ploidy level with meiotic recombination, which makes them an attractive alternative for mitotic chromosome doubling. $2N$ gametes are an effective and efficient way to transmit genetic diversity (allelic variation) to cultivated forms, including both valuable qualitative and quantitative traits (Peloquin et al., 1999). More recently plant breeders have become interested in the practical use of $2n$ gametes in breeding programmes due to the new tools available for $2n$ gamete manipulation and insights into the genetic background of their formation.

This chapter presents a review of the recent advances in the practical breeding applications of $2n$ gametes. It addresses tools for detection, induction, and enrichment of $2n$ pollen, as well as the potential to engineer $2n$ gamete production in agricultural crops.

2. $2n$ gametes: mechanisms and genetic background

$2n$ gametes originate from meiotic aberrations. In a normal meiosis, one mother cell ($2n$) divides in 4 (n) daughter cells (tetrad formation). Aberrations during chromosome pairing, spindle formation (parallel spindle, tripolar spindle, fused spindle, sequential spindle or lack of spindle) or cytokinesis might result in the formation of triads, dyads or monads (Bretagnolle & Thompson, 1995; Dewitte et al., 2010c; Taschetto & Pagliarini, 2003). This finally results in pollen grains with the same DNA content ($2n$ in the case of dyads or triads) or a doubled DNA content ($4n$ in the case of monads) compared to the somatic $2n$ plant cell. Premeiotic and postmeiotic chromosome doubling and cytomixis have also been proposed as possible mechanisms for the production of $2n$ gametes (Bastiaanssen et al., 1998; Falistocco et al., 1995; Ghaffari, 2006; Lelley et al., 1987; Singhal & Kumar, 2008).

Two main categories of 2n gamete formation have been described: first division restitution (FDR), and second division restitution (SDR) (Bretagnolle & Thompson, 1995; Ramanna & Jacobsen, 2003; Zhou et al., 2008). In FDR, the pairing and/or the separation of the homologous chromosomes at meiosis I does not occur (univalent formation) or occurs only at low frequencies, while the second division occurs normally with the two sister chromatids of each chromosome moving to opposite poles. With the exception of cross-over segments, the resulting FDR gametes retain all parental chromosomes. In SDR, the pairing and the separation of the homologous chromosomes during meiosis I occurs normally (bivalent formation). In meiosis II, the centromeres of the half-bivalents divide, but the chromatids do not migrate to the poles. Finally, SDR gametes contain only half of the parental chromosomes as in normal gametes (random combinations), but each of these chromosomes is present twice. Besides these two main categories, several other meiotic aberrations exist where the final chromosome constitution in the microspores is equivalent to the FDR or SDR pathways as described above (Bretagnolle & Thompson, 1995). Less frequently reported are indeterminate meiotic restitution (IMR) and post meiotic restitution (PMR). IMR has been detected in lily (Lim et al., 2001), and shows characteristics similar to both SDR and FDR. PMR, where chromosomes duplicate after meiosis, was observed by Bastiaanssen et al. (1998) in potato. Genomic *in situ* hybridisation (GISH) has made detection of the restitution mechanism possible by monitoring the meiosis of hybrids and identifying the chromosomes of individual genomes in the sexual polyploid progeny.

Examples in maize (*Zea mays*; Rhoades & Dempsey, 1966), potato (*Solanum tuberosum*; Mok & Peloquin, 1975b; Veilleux, 1985; Watanabe & Peloquin, 1989), red clover (*Trifolium pratense*; Parrot & Smith, 1986), rye (*Secale cereale*; Lelley et al., 1987), alfalfa (*Medicago sativa*; Barcaccia et al., 2000; Ortiz & Peloquin, 1991) and banana (*Musa*; Ortiz, 1997) have shown the complexity of the genetic base of 2n gamete formation. Often, one major locus is responsible for 2n gamete formation while several other genes controls its frequency. Some authors tried to map 2n gamete production or developed molecular markers associated with 2n gamete production (e.g. Barcaccia et al., 2000, 2003; Zhang et al., 2007). However, research on the model plant thale cress (*Arabidopsis thaliana*) has led to many recent advances in elucidating the molecular mechanisms as well as the first genes in which mutations result in the production of viable 2n gametes (for a review see Brownfield & Köhler, 2011). d'Erfurth et al. (2008) were the first to successfully isolate and characterize a gene involved in 2n gamete production. The *Arabidopsis thaliana* Parallel Spindle1 (*AtPS1*) gene is involved in abnormal orientation of spindles at meiosis II, which controls diploid 2n gamete formation in *Arabidopsis thaliana*. Parallel, fused or tripolar spindles are different phenotypic expressions of this gene. A similar male-specific dyad-triad phenotype was observed and characterised in another mutant called *jason* (De Storme & Geelen, 2011; Erilova et al., 2009). Mutants of two other genes, OSD and TAM (CYCA1;2), were shown to omit the second meiotic division in both male and female sporogenesis at high frequency resulting in the formation of both 2n pollen and egg cells (d'Erfurth et al., 2009, 2010).

3. Sources of 2n gametes

Three important sources of 2n gametes are commonly reported (Bretagnolle & Thompson, 1995), beginning with interspecific hybrids. In many genera interspecific or intergeneric hybrids have produced 2n gametes e.g. lily (*Lilium*; Barba-Gonzalez et al., 2005a; Lim et al.,

2001; Lim et al., 2004), Peruvian lily (*Alstroemeria*; Ramanna et al., 2003), wheat (*Triticum*; Xu & Joppa, 1995; Xu & Joppa, 2000; Zhang et al., 2010), *Impatiens* (Stephens, 1998), *Citrus* (Chen et al., 2008), rose of sharon (*Hibiscus*; Van Laere et al., 2009), clover (*Trifolium*; Meredith et al., 1995), and the *Festuca/Lolium* complex (Gymer & Whittington, 1973; Morgan et al., 1995). Frequently, these interspecific hybrids show abnormal or absent chromosome pairing and the presence of univalents, lagging chromosomes and chromosome bridges (Islam & Shepherd, 1980; Del Bosco et al., 1999; Trojak-goluch & Berbeć, 2003). Interspecific hybrids usually share two important features. First, both $2n$ eggs and $2n$ pollen are produced simultaneously by the same hybrid. Second, neither the two parents of the F_1 hybrids nor their (F_2) sexual polyploid progenies can produce $2n$ gametes in any notable frequencies (Ramanna & Jacobsen, 2003).

Meiotic mutants are a second source of $2n$ gametes. A mutation in a gene active in meiosis might disturb during spindle formation or cytokinesis, resulting in $2n$ gametes. As different genes are active within the micro- and macrosporogenesis, $2n$ pollen can be formed independent from $2n$ egg cells and vice versa. Meiotic mutants have been described in potato (Jongedijk et al., 1991; Peloquin et al., 1999; Ramanna, 1983), red clover (Parrot & Smith, 1984), alfalfa (Barcaccia et al., 2003), wheat (Jauhar, 2003; Roberts et al., 1999) and *Arabidopsis* (d' Erfurth et al., 2008; Yang et al., 1999), among others.

A third source are odd polyploids. For instance, crosses with triploids revealed that euploid gametes of triploids can be $1x$, $2x$ or $3x$ (Husband, 2004; Van Huylenbroeck et al., 2005). Although these $2x$ gametes are not exactly $2n$ gametes (they are $3x$ gametes), they result in higher ploidy levels of the progeny and mostly act as a bridge between diploids and tetraploids (Köhler et al., 2010).

4. Practical use of $2n$ gametes in plant breeding

4.1 Detection of $2n$ gametes

$2n$ gametes must be correctly identified when used in ploidy breeding programmes. Most detection techniques focus on pollen, since it is more convenient to isolate than egg cells. $2N$ pollen can be detected in four ways (Bretagnolle & Thompson, 1995): pollen size measurements, flow cytometric detection of pollen DNA content, analysis of the microsporogenesis and ploidy analysis of the progeny. In Table 1, an updated overview is given of the different methods used in several crops since Bretagnolle & Thompson's 1995 review. Only the last two of the above-mentioned techniques (ploidy and macrosporogenesis analysis) can be used to detect $2n$ egg formation. The frequency of $2n$ egg formation has often been estimated after crosses between $2x \times 4x$ plants. A cross between a diploid seed parent and tetraploid pollen parent will only result in good tetraploid seed when $2n$ egg cells are present (Conicella et al., 1991; De Haan et al., 1992; Erazzu & Camadro, 2007; Estrada-Luna et al., 2004; Jongedijk, 1987; Lamote et al., 2002; Ortiz & Peloquin, 1991; Veronesi et al., 1986; Van Laere et al., 2009; Werner & Peloquin, 1987). In *Triticum-Aegilops* hybrids, Zhang et al. (2007a, 2010) used the selfed seedset as a good indication for the formation of $2n$ gametes.

An easy and commonly used method to screen for $2n$ pollen is searching for large pollen within a population. Large pollen has frequently been attributed to $2n$ pollen in many genera. This association is caused by the positive correlation between DNA content and cell

Detection method				Crop	Reference
PS	FC	AP	MS		
x			x	<i>Achillea borealis</i>	Ramsey, 2007
		x	x	<i>Actidinia spp.</i>	Yan et al., 1997
x		x	x	<i>Alstroemeria spp.</i>	Ramanna et al., 2003
x				<i>Anthoxanthum alpinum</i>	Bretagnolle, 2001
x	x	x	x	<i>Begonia spp.</i>	Dewitte et al., 2009
			x	<i>Brachiaria spp.</i>	Gallo et al., 2007
	x			<i>Brassica napus</i>	Pan et al., 2004
x		x		Cacti hybrids	Tel-Zur et al., 2003
		x	x	<i>Capsicum annuum</i>	Yan et al., 2000
x	x			<i>Cupressus spp.</i>	Pichot & El Maataoui, 2000
x	x	x		<i>Diospyros kaki</i>	Sugiura et al., 2000
x			x	<i>Diospyros spp.</i>	Xu et al., 2008
	x	x	x	<i>Durum wheat</i>	Jauhar, 2003
x	x	x	x	<i>Hibiscus spp.</i>	Van Laere et al., 2009
x			x	<i>Ipomoea batatas</i>	Becerra Lopez-Lavalle & Orjeda, 2002
	x	x	x	<i>Lilium spp.</i>	Lim et al., 2004
x	x	x		<i>Lilium spp.</i>	Akutsu et al., 2007
x		x		<i>Lilium spp.</i>	Barba-Gonzalez et al., 2004
x		x	x	<i>Lotus tenuis</i>	Negri & Lemmi, 1998
x				<i>Musa spp.</i>	Ortiz, 1997; Ssebulita et al., 2008
			x	<i>Paspalum spp.</i>	Pagliarini et al., 1999
			x	<i>Pfaffia spp.</i>	Taschetto & Pagliarini, 2003
			x	<i>Populus tomentosa</i>	Zhang & Kang, 2010
x			x	<i>Populus hybrid</i>	Wang et al., 2010
x			x	<i>Rhododendron spp.</i>	Jones & Ranney, 2009
x			x	<i>Rosa hybrida</i>	Crespel et al., 2006
x		x	x	<i>Rosa hybrida</i>	El Mokadem et al., 2002a
	x			<i>Rosa rugosa</i>	Roberts, 2007
x				<i>Rosa spp.</i>	Zlesak, 2009
x		x		<i>Trifolium pratense</i>	Simioni et al., 2006
x	x	x		<i>Tulipa spp.</i>	Okazaki et al., 2005
x			x	<i>Ziziphus jujube</i>	Xue et al., 2011

Table 1. Overview of studies on the detection of 2n pollen since Bretagnolle & Thompson’s 1995 review. FC = Flow cytometry on pollen; PS = Pollen size measurements; AP = Ploidy analysis of progeny; MS = Microsporogenesis analysis

volume which in turn influences pollen diameter. In crops as Japanese persimmons (*Diospyros kaki*), banana, rose (*Rosa*) and sweet potato (*Ipomoea batatas*), the diameter of the 2n pollen was approximately 30% larger than that of the n pollen (Becerra Lopez-Lavalle & Orjeda, 2002; Crespel et al., 2006; Ortiz, 1997; Sugiura et al., 2000). In Chinese jujube (*Ziziphus jujube*) 2n pollen was more than 1.5 times larger compared to n pollen (Xue et al., 2011). The presence of large 2n pollengrains results in a bimodal pollengrain size

distribution instead of a normal distribution. Although the size distribution of normal and giant pollen grains show some overlap, a threshold value of the pollen grain size is often used to select individuals that produce $2n$ gametes (e.g. Crespel et al., 2006; Ortiz, 1997; Sugiura et al., 2000). The presence of large pollen only indicates the presence of $2n$ pollen but does not present proof of doubled DNA content. Another disadvantage of this screening technique is the broad overlap in size distribution between small and large pollen in some genera such as grasses. In these cases, the frequency of $2n$ pollen based on pollen size only is difficult to determine (Jansen & Den Nijs, 1993). A bimodal size distribution, on the other hand, can also be related to a population of small unviable and normal pollengrains as was observed in *Hibiscus* (Van Laere et al., 2009). Other methods are necessary to confirm the association between large and $2n$ pollen, and supplementary evaluation of pollen viability is necessary for breeding purposes. Besides pollen size also pollen shape (spherical instead of ellipsoidal) has been associated with the ploidy level, which simplifies the determination of $2n$ pollen (Akutsu et al., 2007; Dewitte et al., 2009; Ramanna et al., 2003).

A more stringent method is the direct quantification of nuclear pollen DNA using flow cytometry. To determine the DNA content of pollen, nuclei have to be released from pollen and purified from the pollen wall. Several enzymatical, chemical, mechanical and osmotical methods for nuclear isolation of pollen have been used in different plants, e.g. maize, *Plumbago zeylanica*, lily hybrids, tobacco (*Nicotiana tabacum*) and rape (*Brassica napus*) (Dupuis et al., 1987; Pan et al., 2004; Russel, 1991; Van Tuyl et al., 1989; Xu et al., 2002; Zhang et al., 1992). The presence of a complex outer exine layer on the pollen surface is the main obstacle in releasing the nuclei from pollen. This outer layer is a biopolymer that is highly resistant to enzymatic breakdown and hydrolytic decomposition in strong acid or alkaline media (Bohne et al., 2003). The isolation of nuclei from pollen is often difficult and the efficiency of nuclear isolation techniques must be investigated each time when a new genus is explored.

Flow cytometric analysis compares the DNA content of pollen nuclei to the DNA content of somatic leaf tissue. Pollen nuclei are expected to have only half of the DNA content (1C) compared to nuclei from somatic cells (2C) of the same plant. Consequently, $2n$ pollen have a nuclear DNA content equal to somatic cells. This is true for species as *Brassica napus* or *Triticum aestivum* (Pan et al., 2004). However, several reports on crops from the genus *Begonia* (Dewitte et al., 2009), *Lilium* (Van Tuyl et al., 1989), *Cupressus* (Pichot & Maâtaoui, 2000), *Hibiscus* (Van Laere et al., 2009) and *Rosa* (Roberts et al., 2007) have shown that pollen nuclei can be at the G2 phase of cell division, and have a temporary doubled DNA content (2C) which is equal to the DNA content of somatic cells. In *Begonia* for instance, analysis of normal (binucleate) pollen results in 2 peaks in a flow cytometric histogram at the 1C (vegetative nuclei) and 2C level (generative nuclei). If $2n$ pollen are present, a peak at the 4C level of the $2n$ generative nuclei can be observed (Dewitte et al., 2009).

Although flow cytometric screening of $2n$ pollen can be used routinely in breeding programmes, sample preparation for flow cytometric screening generally requires more time than for microscopic evaluation. Therefore, the use of flow cytometry can be limited to genotypes which produce pollen grains with highly variable sizes to confirm the occurrence of $2n$ pollen grains. Some quick physical techniques to isolate pollen nuclei, such as bead beating (Roberts, 2007) or chopping of pollen grains (Pichot & Maâtaoui, 2000; Sugiura et al., 2000; Van Laere et al., 2009; Van Tuyl et al., 1989), may speed up flow cytometric screening of $2n$ pollen.

Moreover, Dewitte et al. (2006, 2009) developed a nuclear isolation protocol which only releases nuclei from germination tubes. As a result, flow cytometric analysis is based only on viable pollen grains and no supplementary evaluation of pollen viability is necessary.

The presence of 2n pollen is associated with the occurrence of monads, dyads or triads during microsporogenesis, except when 2n gamete formation is the result of pre- or postmeiotic restitution. Analysis of microsporogenesis may therefore provide an alternative method to confirm the presence of 2n pollen but this method does not provide any information about pollen viability. Moreover, what is observed in the meiocytes is not necessarily reflected in the mature pollen (Dewitte et al., 2010a), since the production of n and 2n pollen also depends on balanced chromosome segregation during meiosis and further maturation steps after meiosis. Scoring the meiotic products is thus not the best method to determine the frequency of the final 2n pollen. Although these observations give no information about the viability of 2n pollen, they provide insight in the mechanisms (FDR, SDR) behind 2n gamete formation. The identification of the mechanisms behind the formation of 2n gametes is however complex, because different cytological mechanisms may operate within one individual. The use of molecular cytological techniques (genomic in situ hybridization, GISH or fluorescent in situ hybridization, FISH) or marker analysis (such as amplified fragment length polymorphism, AFLP) on meiocytes or polyploid progeny may provide more accurate or additional information on the mechanisms behind 2n gamete formation (Barba-Gonzalez et al., 2005b; Crespel et al., 2002; Lim et al., 2001). Molecular cytological approaches have been successfully used in the case of allopolyploids, where the constituent genomes can be clearly discriminated. This includes the unequivocal identification of not only genomes and individual alien chromosomes but also recombinant segments in the sexual polyploid progenies. Through DNA in situ hybridisation, genomes of allopolyploids can be more critically assigned and intergenomic translocations and recombinations can be detected such as in *Gasteria-Aloe* hybrids (Takahashi et al., 1997), *Alstroemeria* species (Ramanna et al. 2003) and *Lilium* species (Barba-Gonzalez et al., 2005; Karlov et al., 1999; Lim et al., 2001). As such, GISH can also be used to discover the mechanism of 2n gamete formation (Karlov et al., 1999).

Finally, ploidy analysis of the progeny (usually using flow cytometry) can reveal the presence of 2n gametes in parent plants. Progeny analysis has the advantage of indicating the existence of viable 2n pollen in parent plants, but 2n pollen in parent plants may remain unnoticed because of preferential pairing between normal gametes. This technique has the disadvantage of being very time consuming with no guarantee of information about the production frequency in the parent plant (Bretagnolle & Thompson, 1995) due to possible differences in pollen viability, germination speed or pollen tube growth between n and 2n pollen.

Although different techniques can be used to detect 2n pollen, frequencies should be considered carefully because they depend on the method used (Dewitte et al., 2009). Moreover, frequencies may vary in different populations, within a certain genotype and during time (season) due to environmental fluctuations (Bani-Aameur et al., 1992; Crespel et al., 2006; McCoy, 1982; Ortiz et al., 1998; Ortiz & Vulsteke, 1995; Parrott & Smith, 1984; Pécrix et al., 2011; Veilleux et al., 1982). In Table 2, some recently published data on 2n gamete frequency in different (hybrid and non hybrid) populations are given. In general, the proportion between normal and 2n gametes over an at random sampled population (the mean population frequency, F_m) is low, close to 1%. However, the proportion of plants

producing 2n gametes in a population (population frequency, Fp) as well as 2n gametes formation within an individual genotype (individual frequency, Fi) can be very variable. In several reports, Fp is higher than 10% and in some genera as *Ipomoea*, poplar (*Populus*) or *Pfaffia*, Fp values are noticed higher than 80%. A similar conclusion can be drawn from Fi, where values are reported from less than 1% up to 100% in *Begonia* (Dewitte et al., 2009).

As a general rule, it appears that approximately 0.1 to 2.0% of the gametes in a nonhybrid population are expected to be unreduced (Ramsey, 2007). However, the mean frequency of 2n gametes found in studies of hybrids (27.52%) was nearly a 50-fold greater than in nonhybrids (0.56%) (Ramsey and Schemske, 1998). Hence, the chance to find individuals that produce 2n gametes increases when hybrids are investigated.

Crop	No of genotypes	Fp ¹ (%)	Fi ² (%)	Fm ¹ (%)	Reference
<i>Achillea borealis</i>	50-75	34.7-50.0	15.8	0.03-0.54	Ramsey, 2007
<i>Anthoxanthum alpinum</i>	31-78	9-12.8	39.5	0.8-1.3	Bretagnolle, 2001
<i>Begonia spp.</i>	70	14.3	100	-	Dewitte et al., 2009
<i>Hibiscus spp.</i>	15	13.3	10	-	Van Laere et al., 2009
<i>Ipomoea batatas</i>	64	86	84.2	-	Becerra Lopez-Lavalle & Orjeda, 2002
<i>Lolium perenne</i>	154	9.7	-	-	Lamote et al., 2002
<i>Musa spp.</i>	156	14-56	-	-	Ortiz, 1997
<i>Paspalum spp.</i>	112	46.4	-	-	Pagliarini et al., 1999
<i>Pfaffia spp.</i>	14	100	18.9	-	Taschetto & Pagliarini, 2003
<i>Populus tomentosa</i>	224	97.3	21.9	1.8-7.5	Zhang et al., 2007b
<i>Rosa spp.</i>	53	26.4-50.9	9.6	1-2	Crespel et al., 2006
<i>Solanum okadai</i>	118	20.3	5		Camadro et al., 2008

Table 2. Examples of 2n gamete frequencies in different crops (since Bretagnolle & Thompson’s 1995 review). Fp: population frequency; Fi: individual frequency; Fm: mean population frequency. ¹Two values represent a frequency interval in which the value is dependent on the population or season. ²The highest reported value is represented

4.2 Induction of 2n gametes

To date, the major drawback to use 2n gametes in plant breeding is that only a minority of genotypes regularly produce 2n gametes. Identifying such genotypes in the breeding stock

thus requires a great deal of screening. Additionally, not many superior genotypes produce these gametes. Recently different attempts, with variable success, were made to induce these gametes in any genotype of interest. An overview of the methods used to induce 2n gametes is given in Table 3.

Induction method	Crop	Reference
N ₂ O	<i>Begonia spp.</i>	Dewitte et al., 2010
	<i>Lilium spp.</i>	Akutsu et al. 2007; Barba-Gonzales et al. 2006; Kitamura et al., 2008; Sato et al., 2010
	<i>Tulipa spp.</i>	Okazaki et al. 2005
Trifluralin	<i>Begonia spp.</i>	Dewitte et al., 2010
	<i>Zea Mays</i>	Kato, 1999
Colchicine	Chinese cabbage	Zhong et al., 2010
	<i>Lilium spp.</i>	Wu et al., 2007
	<i>Populus hybrid</i>	Li et al., 2008
	<i>Strelitzia reginae</i>	Xiao et al., 2007
Temperature	<i>Lilium spp.</i>	Lokker et al., 2004
	<i>Rosa spp.</i>	Pécrix et al., 2011
EMS	<i>Arabidopsis thaliana</i>	De Storme et al., 2007; De Storme & Geelen, 2011
GA ₃	<i>Carthamus tinctorious</i>	Kumar & Srivastava, 2009

Table 3. Methods used to induce 2n gamete formation

N₂O treatments have been reported to be an effective way to induce 2n gametes (both 2n pollen and egg cells) in tulip (*Tulipa*), *Lilium* and *Begonia* (Akutsu et al., 2007; Barba-Gonzales et al., 2006; Dewitte et al., 2010b; Okazaki et al., 2005). N₂O is suitable for treating organs inside tissues as the gas simply permeates the tissue, thereby protecting the tissues from harmful after-effects as soon as the gas is released (Kato & Geiger, 2002; Östergren, 1954). N₂O treatments are performed in a pressure tolerant vessel at 6 bar for 24 or 48h on bulbs (when microsporogenesis occurs inside the bulb) or flower buds at the stage of meiosis. In the latter case, the stage of meiosis can be estimated by the size of the flower bud (Akutsu et al., 2007; Barba-Gonzalez et al., 2006; Dewitte et al., 2010b). Consequently, bud size has been used as a criterium to start N₂O treatments. Akutsu et al. (2007) showed that effects were optimal when treatments started during pollen mother cell progression to metaphase I. By applying this technique, male sterile hybrids may produce fertile 2n gametes after treatments, but the efficiency of the treatment seems to be genotype specific (Barba-Gonzalez et al., 2006; Dewitte et al., 2010b). In *Begonia* for example, viable 2n pollen could be induced in the male sterile hybrid *B. schmidtiana* x *B.cucullata* in 14 of the 49 treated flowers, while no 2n pollen were induced in *B. fischeri* with a similar number of flowers treated.

The small number of studies on N_2O induction of $2n$ gametes calls for additional testing in other genera. Detailed studies on the exact mechanism of N_2O mediated chromosome doubling during meiosis are also limited, but it has been suggested that N_2O disrupts the spindle mechanism in both mitosis (Dvorak et al., 1973; Kato & Birchler, 2006; Kihari & Tsunewaki, 1960; Östergren, 1954) and meiosis (Akutsu et al., 2007; Barba-Gonzalez et al., 2006; Okazaki et al., 2005;). Consequently, aneuploidy is frequently reported after treatments. In *Lilium*, Barba-Gonzalez et al. (2006) showed that N_2O fumigation produced mainly first division restitution (FDR) gametes, indicating a disruption in meiosis I. Furthermore, Kitamura et al. (2009) showed in *Lilium* that microtubules were depolymerised during metaphase I, which prevented chromosomes from moving to the poles.

Dewitte et al. (2010b) tried to induce $2n$ pollen by submerging flower buds of *Begonia* in a trifluralin solution. Their treatments resulted in a disturbed meiosis and finally in $4n$ gametes but no progeny with raised ploidy level could be obtained after crosses with these gametes. Another way to increase the ploidy level in pollen was achieved in maize by spraying tassels with a trifluralin solution before flowering. As such, the generative nucleus was mitotically arrested and viable bicellular pollen was obtained (Kato, 1999). In some genera, flower buds were treated with colchicine (Table 2). These treatments resulted in the induction of both $2n$ pollen and $2n$ egg cells, and polyploid progeny was established after crosses with the treated plants.

Some authors tried to induce $2n$ gametes by manipulation of the temperature. Lokker et al. (2004) exposed four complete sterile lily genotypes to heat shock treatments and observed that three of the four genotypes became fertile by the production of viable $2n$ gametes. Also Pécrix et al. (2011) observed a production of $2n$ gametes up to 24.5% in *Rosa spp.* through an exposition of a high temperature gradient, but the range of efficient temperatures is narrow and should be applied during early meiosis. The disturbed meiosis resulted in the production of dyads and triads which mainly resulted from spindle misorientations in meiosis II. This supports the hypothesis that polyploidization events could have occurred in adverse conditions and may be of importance during species evolution.

Interspecific hybridisation is another way to induce $2n$ gametes. The occurrence of $2n$ gametes has been reported frequently in interspecific hybrids (see above). For example, in a breeding program between the tetraploids *Hibiscus syriacus* and *Hibiscus paramutabilis*, 2 of the 5 F_2 hybrids produced between 6 and 10% $2n$ pollen. These hexaploid F_2 hybrids were all raised in ploidy level compared to the tetraploid F_1 hybrids as a result of $2n$ egg cells in the F_1 population (Van Laere et al., 2009). However, even in interspecific hybrids, $2n$ gametes are rather exception than rule and many hybrids may be screened to detect $2n$ gamete producing genotypes. In *Lilium*, only 12 of the 708 (1.2%) OA F_1 hybrids produce $2n$ gametes on a regular basis, while the other seedlings were sterile (Barba-Gonzales et al., 2004).

Different attempts were made to increase the frequency of $2n$ pollen in genotypes which produce a low number of $2n$ pollen. Specific efforts have been performed using temperature variation, genetic selection, velocity sedimentation or pollen sieving (Table 4). For example, by using genetic selection, Negri & Lemmi (1998) were able to increase the frequency $2n$ pollen (Fi) in *Lotus tenuis* from 0-13% in the natural populations to 47.5-77.6% in the selected individuals. A further increase in frequency with a factor 1.5 to 3 could be obtained in a warm chamber.

Increase method	Crop	Reference
Temperature	<i>Begonia spp.</i>	Dewitte, 2010a
	Loquat tree	Wang et al., 2003
	<i>Lotus tenuis</i>	Negri & Lemmi, 1998
	<i>Prunus persica</i>	Ye et al., 2010
	<i>Prunus spp.</i>	Zhang & Liu, 1998
	<i>Rosa spp.</i>	Crespel et al., 2006
	<i>Solanum phureja</i>	McHale, 1983; Veilleux & Lauer, 1981; Werner & Peloquin, 1987
Velocity sedimentation	<i>Solanum spp.</i>	Simon & Sanford, 1990
Pollen sieving	<i>Cyclamen persicon</i>	Takamura & Miyajima, 2002
	<i>Diospyros kaki</i>	Sugiura et al., 2000
	<i>Lilium spp.</i>	Okazaki et al., 2005
	<i>Solanum spp.</i>	Eijlander, 1988
Genetic selection	<i>Lotus tenuis</i>	Negri & Lemmi, 1998
	<i>Medicago sativa</i>	Tavoletti et al., 1991b; Calderini & Mariani, 1997; Mariani et al., 2000
	<i>Solanum</i>	Ortiz & Peloquin, 1992
	<i>Trifolium pratense</i>	Parrot & Smith, 1986

Table 4. Overview of methods used to increase the frequency of 2n gametes

4.3 Use of molecular tools to engineer 2n gamete formation

The isolation of genes involved in 2n gamete production opens the way for new strategies in plant breeding programmes. More specifically, techniques that involve knockdown of RNA levels, such as RNA interference (RNAi), virus induced gene silencing (VIGS) or mutagenesis of the encoding gene using techniques such as site-directed mutagenesis could be used to knock down the level of specific proteins which play a role in the unreduced gamete formation (Brownfield & Köhler, 2010). The translation of this knowledge from the model plant *Arabidopsis* into plant breeders work still has to start. However, the vision of crop generation via designed gametes is becoming realistic.

Potential strategies to mutate genes active in meiosis and induce 2n gamete producing genotypes also include general mutagenesis strategies using chemicals such as ethyl methane sulphonate (EMS) (used in *Arabidopsis* to create 2n gametes; De Storme et al., 2007), random insertional mutagenesis or irradiation of seeds or buds (Shu-Ye.& Srinivasan, 2010).

5. Role of 2n gametes in plant breeding

2N gametes have already been used to create new cultivars at higher ploidy levels as well as creating a bridge to transfer desirable genes from wild diploid species into the cultivated polyploid gene pool (Carputo et al., 2000 Peloquin & Ortiz, 1992). Although 2n gametes have been documented in several genera, they have been extensively investigated in potato (Den Nijs & Peloquin, 1977; Mok & Peloquin, 1975a; Peloquin et al., 1999), rose (Crespel et

al., 2002; Crespel et al., 2006; El Mokadem et al., 2002a; El Mokadem et al., 2002b;), lily (Barba-Gonzalez et al., 2005a, 2005b; Lim et al., 2001a; Lim et al., 2004), and alfalfa (Tavoletti et al., 1991a; Tavoletti et al., 1991b; Barcaccia et al., 2003). The $2n$ gametes have in turn contributed to these crops' breeding programmes. For example, ploidy manipulations have been used in potato (*Solanum tuberosum*) breeding for many decades. Cultivated potatoes are tetraploid but most wild species are diploid. Haploidisation techniques can create dihaploids from cultivated potatoes. Via a series of hybridisations between selected dihaploids and $2x$ species, desirable agronomic traits from wild and closely related species can be captured. These dihaploids (producing $2n$ gametes) can then be introduced to tetraploids via interploidy crosses between $2x$ and $4x$ plants. The most successful breeding scheme for potatoes involves obtaining $4x$ progeny from $4x-2x$ crosses, where the $2x$ parent forms $2n$ pollen via the meiotic mutant *ps* (Peloquin et al., 1999). Several ploidy series have been developed in potato by using haploidisation and sexual polyploidization techniques, ranging from the monoploid to the hexaploid level (Carputo & Barone, 2005).

Other examples of the usefulness of $2n$ gametes for crop improvement in plant breeding have been demonstrated in *Alstroemeria*, carnation (*Dianthus*), primrose (*Primula*), *Triticum*, cassava (*Manihot*), blueberry (*Vaccinium*), cocksfoot grasses (*Dactylis*), *Lotus* and *Trifolium* (Carroll & Borrill, 1965; Hahn et al., 1990; Hayashi et al., 2009; Lyrene et al., 2003; Negri & Veronesi, 1989; Nimura et al., 2008; Parrot & Smith, 1984; Ramanna et al., 2003; Zhang et al., 2010), among others. $2N$ gametes also occur after haploidisation, which is useful for scaling the ploidy level upward again without artificial ploidy doubling (e.g. El Mokadem et al., 2002b; Nelson et al., 2009).

One advantage of $2n$ gametes is one-step triploid generation. Breeding for triploids may become an aim in itself. In *Citrus* for example, seedlessness is one of the most important characteristics in mandarin oranges. *Citrus* triploid plants can be recovered by sexual hybridisation of diploid plants as a consequence of the formation of $2n$ gametes at low frequency (Aleza et al., 2010). If breeding for triploids is not the aim of the breeding programme, further crosses are required to achieve the tetraploid or higher ploidy levels. For this purpose, the production of $2n$ gametes should be inherited from the diploid to the triploid plant. Several examples have been described where triploid plants produce $2n$ gametes, and often these are the only viable source of gametes. Crosses with these triploids resulted in tetraploid progeny, and even in this tetraploid progeny, $2n$ gamete production was observed (Brown, 1988; Dewitte et al., 2010d; Dweikat & Lyrene, 1988; Mok et al., 1975).

Plant breeding seeks to maximize the heterozygosity in the generations used for commercial production, and also polyploid induction will only contribute to plant improvement if substantial heterozygosity can be incorporated (Sanford, 1983). Higher heterozygosity can be achieved at the polyploid rather than at the diploid level. This is because polyploids have a greater probability of possessing three or more different alleles at the same locus whereas diploids only have two (Bingham, 1980; Lewis, 1980; Sanford, 1983). The heterozygosity present in the polyploid depends on the heterozygosity present in the parent plants and the mechanism behind $2n$ gamete formation. In general, FDR pollen are more important in producing heterozygous hybrids because of the highly heterozygous $2n$ gametes formed (Bretagnolle & Thompson, 1995). In FDR, each gamete is, except for recombinant fragments, identical to the somatic cell and thus contains a high level of heterozygosity. In SDR, chromosome assortment is random (as in normal gametes) which results in a very

heterogeneous population of gametes, but with a lower heterozygosity within one cell. For breeding purposes, 2n gametes of the FDR type are more advantageous than those obtained by SDR for transferring parental heterozygosity (Barcaccia et al., 2000; Barcaccia et al., 2003), although Hutten et al. (1994) could not confirm this FDR superiority for all agronomic characteristics investigated in potato. By using molecular techniques as AFLP, random amplification of polymorphic DNA (RAPD) or isozyme analysis, the heterozygosity transmitted through 2n gametes can be calculated. Several reports on *Solanum spp.* indicate that FDR gametes transmit roughly 70-80% of the parental heterozygosity, whereas this is only about 30-40% for SDR (Barone et al., 1995; Douches & Quiros, 1988; Werner & Peloquin, 1991a; Werner & Peloquin, 1991b). These values were also reported in other crops: roses, ryegrass, *Begonia* and *Vaccinium darrowi* (Chen et al., 1997; Crespel et al., 2002; Dewitte et al., 2010a; Vorsa and Rowland, 1997; Qu & Hancock, 1995). In general FDR is more than twice as effective in transmitting heterozygosity as SDR (Peloquin et al., 2008).

Ramanna & Jacobsen (2003) report that sexual polyploids have been much more useful for crop improvement than mitotic polyploids. Beuselinck et al. (2003) has shown that 2n gametes from *Lotus glaber* may aid intercrossing with *Lotus corniculatus* to produce progeny with a greater heterotic advantage than progeny obtained from the colchicine induced tetraploid (4x) *Lotus glaber*. In alfalfa, McCoy & Rowe (1986) showed better breeding value of 2n gametes from the diploids than n gametes from chromosome doubled tetraploids. Lim et al. (2001) has shown that recombinant chromosomes were present in 2n gametes in contrast to 2x gametes from mitotically doubled plants. Hence, 2n gametes have great potential to selectively introgress certain characteristics. The genetic consequences of 2n gametes indicate that sexual polyploidization results in greater variability, fitness and heterozygosity than does somatic doubling (Carputo et al., 2003).

One potential strategy to maximize heterozygosity in polyploids is analytic breeding: breeding for superior genotypes at the diploid level followed through sexual hybridisation using 2n gametes. The potential of analytical breeding to maximize heterozygosity and intergenomic recombination has been shown in *Lilium* using GISH. In traditional methods, mitotically doubled tetraploids are crossed with a diploid to produce triploids. However, in the tetraploid no recombination is expected to occur due to autosyndetic pairing. This results in a narrow selection of cultivars from mitotic polyploidization. In contrast, analytic breeding strategies allow intergenomic recombination to produce triploid *Lilium* varieties (Khan et al., 2009; Khan et al., 2010). Other examples of analytical breeding for crop improvement are vegetatively propagated species such as potato, sweet potato, cassava, among roots and tubers, and plantain/banana (Ortiz, 2002).

6. Conclusions

The exploitation of 2n gametes creates a plethora of opportunities for practical breeding. In general, several reports show that a) 2n gametes are mostly easy to detect, although this depends on the method used, b) 2n pollen may transmit a high level of heterozygosity (genetic variation) to the progeny, c) the ability to produce 2n gametes may be passed on to the progeny and d) 2n gamete production can be induced by artificial techniques. Besides, recent progress in identifying the genes and molecular mechanisms involved in 2n gamete production offers breeders new opportunities to design new tools and techniques to engineer 2n gamete production in specific crops and plants. The increasing reports and

knowledge about the practical use of 2n gametes in several crops and their genetic consequences show that 2n gametes are recognized as a very important tool in plant breeding. With ongoing research at the molecular level and research towards efficient methods to induce 2n gametes, the importance of 2n gametes for plant breeding is sure to increase.

7. References

- Akutsu, M.; Kitamura, S.; Toda, R.; Miyajima, I. & Okazaki, K. (2007). Production of 2n pollen of Asiatic hybrid lilies by nitrous oxide treatments. *Euphytica*, Vol.155, No.1-2, (May 2007), pp.143-152, ISSN 0014-2336
- Aleza, P.; Juárez, J. ; Cuenca, J.; Ollitrault, P. & Navarro, L. (2010). Recovery of citrus triploid hybrids by embryo rescue and flow cytometry from 2x x 2x sexual hybridisation and its application to extensive breeding programs. *Plant Cell Reports*, Vol.29, No.9, (September 2010), pp.1023-1034, ISSN 0721-7714
- Andruezza, S. & Siddiqi, I. (2008). Spindle positioning, meiotic nonreduction, and polyploidy in plants. *PLOS Genetics*, Vol. 4, No.11, (November 2008), pp. 1-2, ISSN 1553-7404
- Aryavand, A.; Ehdaie, B.; Tran, B. & Waines, JG. (2003). Stomatal frequencies and size differentiate ploidy levels in *Aegilops neglecta*. *Genetic Resources and Crop Evolution*, Vol.50, No.2, (March 2003), pp.175-182, ISSN 0925-9864
- Baert, J.; Reheul, D.; Van Bockstaele, E. & De Loof, A. (1992). A rapid method by flow cytometry for estimating persistence of tetraploid perennial ryegrass in pasture mixtures with diploid perennial ryegrass. *Biologia Plantarum*, Vol. 34, No.5-6, (September 1992), pp. 381-385, ISSN 0006-3134
- Bani-Aameur, F.; Lauer, FI. & Veilleux, RE. (1992). Frequency of 2n pollen in diploid hybrids between *Solanum phureja* Juz. & Buk. and *Solanum chacoense* Bitt. *Potato Research*, Vol. 35, No.2, (June 1992), pp. 161-172, ISSN 0014-3065
- Barba-Gonzalez, R.; Lim, K-B.; Ramanna, MS.; Visser, RGF. & Van Tuyl, JM. (2005a). Occurrence of 2n gametes in the F1 hybrids of Oriental x Asiatic lilies (*Lilium*): Relevance to intergenomic recombination and backcrossing. *Euphytica*, Vol. 143, No.1-2, (January 2005), pp.67-73, ISSN 0014-2336
- Barba-Gonzalez, R.; Ramanna, MS.; Visser, RGF. & Van Tuyl, JM. (2005b). Intergenomic recombination in F1 lily hybrids (*Lilium*) and its significance for genetic variation in the BC1 progenies as revealed by GISH and FISH. *Genome*, Vol.48, No.5, (October 2005), pp.884-894, ISSN 0831-2796
- Barba-Gonzalez, R.; Lokker, AC.; Lim, K-B.; Ramanna, MS. & Van Tuyl, JM. (2004). Use of 2n gametes for the production of sexual polyploids from sterile Oriental x Asiatic hybrids of lilies (*Lilium*). *Theoretical and Applied Genetics*, Vol.109, No.6, (October 2004), pp.1125-1132, ISSN 0040-5752
- Barba-Gonzalez, R.; Miller, CT.; Ramanna, MS. & Van Tuyl, JM. (2006). Nitrous oxide N₂O induces 2n gametes in sterile F1 hybrids of Oriental x Asiatic lilies (*Lilium*) and leads to intergenomic recombination. *Euphytica*, Vol.148, No.3, (April 2006), pp.303-309, ISSN 0014-2336
- Barcaccia, G.; Albertini, E.; Rosellini, D.; Tavoletti, S. & Veronesi, F. (2000). Inheritance and mapping of 2n egg production in diploid alfalfa. *Genome*, Vol.43, No.3, (June 2000), pp.528-537, ISSN 0831-2796

- Barcaccia, G.; Tavoletti, S.; Mariani, A. & Veronesi, F. (2003). Occurrence, inheritance and use of reproductive mutants in alfalfa improvement. *Euphytica*, Vol.133, No.1, (July 2003), pp.37-56, ISSN 0014-2336
- Barone, A.; Gebhardt, C. & Frusciante, L. (1995). Heterozygosity in 2n gametes of potato evaluated by RFLP markers. *Theoretical and Applied Genetics*, Vol.91, No.1, (July 1995), pp.98-104, ISSN 0040-5752
- Barringer, BC. (2007). Polyploidy and self-fertilisation in flowering plants. *American Journal of Botany*, Vol.94, No.9, (September 2007), pp.1527-1533, ISSN 0002-9122
- Bastiaanssen, HJM.; Van Den Berg, PMM.; Lindhout, P.; Jacobsen, E. & Ramanna, MS. (1998). Postmeiotic restitution in 2n-egg formation of diploid potato. *Heredity*, Vol.81, No.1, (June 1998), pp.20-27, ISSN 0018-067X
- Becerra Lopez-Lavalle, LA. & Orjeda, G. (2002). Occurrence and cytological mechanism of 2n pollen formation in a tetraploid accession of *Ipomoea batatas* (sweet potato). *Journal of Heredity*, Vol.93, No.3, (May 2002), pp.185-192, ISSN 0022-1503
- Beuselinck, PR.; Steiner, JJ. & Rim, YW. (2003). Morphological comparison of progeny derived from 4x-2x and 4x-4x hybridizations of *Lotus glaber* Mill. and *L. corniculatus* L. *Crop Science*, Vol.43, No.5, (September 2003), pp.1741-1746, ISSN 0011-183X
- Bingham, ET. (1980). Maximizing heterozygosity in autopoloids. In: *Polyploidy, biological relevance* (Vol 13), Lewis, WH., (ed.), 471-489, Plenum Press, ISBN 9780306403583, New York
- Bohne, G.; Richter, E.; Woehlecke, H.; Ehwald, R. (2003). Diffusion barriers of tripartite sporopollenin microcapsules prepared from pine pollen. *Annals of Botany*, Vol.92, No.2, (August 2003), pp.289-297, ISSN 0305-7364
- Bretagnolle, F. & Thompson, JD. (1995). Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopoloid plants. *New Phytologist*, Vol.129, No.1, (January 2005), pp.1-22, ISSN 0028-646X
- Bretagnolle, F. (2001). Pollen production and spontaneous polyploidization in diploid populations of *Anthoxanthum alpinum*. *Biological Journal of the Linnean Society*, Vol.72, No.2, (February 2001), pp.241-247, ISSN 0024-4066
- Briggs, D. & Walters, SM. (1997). *Plant Variation and Evolution*. Cambridge University Press, ISBN 9780521452953, Cambridge, UK
- Brown, CR. (1988). Characteristics of 2n pollen producing triploid hybrids between *Solanum stoloniferum* and cultivated diploid potatoes. *American Journal of Potato Research*, Vol.65, No.2, (February 1988), pp.75-84, ISSN 1099-209X.
- Brownfield, L. & Köhler, C. (2011). Unreduced gamete formation in plants: mechanisms and prospects. *Journal of Experimental Botany*, Vol.62, No.2, (March 2011), pp. 1659-1968, ISSN 0022-0957
- Calderini, O. & Mariani, A. (1997). Increasing 2n gamete production in diploid alfalfa by cycles of phenotypic recurrent selection. *Euphytica*, Vol.93, No.1, (January 1997), pp. 113-118, ISSN 0014-2336
- Camadro, EL.; Saffarano, SK.; Espinillo, JC.; Castro, M. & Simon, PW. (2008). Cytological mechanisms of 2n pollen formation in the wild potato *Solanum okadae* and pollen-pistil relations with the common potato, *Solanum tuberosum* L. *Genetic Resources and Crop Evolution*, Vol.55, No.3, (May 2008), pp.471-477, ISSN 0925-9864

- Carputo, D. & Barone, A. (2005). Ploidy level manipulations in potato through sexual hybridisation. *Annals of Applied Biology*, Vol.146, No.1, (January 2005), pp.71-79, ISSN 0003-4746
- Carputo, D.; Barone, A. & Frusciante, L. (2000). 2N gametes in the potato: essential ingredients for breeding and germplasm transfer. *Theoretical and Applied Genetics*, Vol.101, No.5-6, (October 2000), pp.805-813, ISSN 0040-5752
- Carputo, D.; Frusciante, L. & Peloquin, SJ. (2003). The role of 2n gametes and endosperm balance number in the origin and evolution of polyploids in the tuber-bearing *Solanums*. *Genetics*, Vol.163, No.1, (January 2003), pp.287-294, ISSN 0016-6731
- Carroll, CP. & Borrill, M. (1965). Tetraploid hybrids from crosses between diploid and tetraploid *Dactylis* and their significance. *Genetica*, Vol.36, No.1, (December 1965), pp.65-82, ISSN 0016-6707
- Chahal, GS. & Gosal, SS. (2002). *Principles and procedures of plant breeding: biotechnological and conventional approaches*. Alpha Science International Ltd, ISBN 9788173193743, Pangbourne, UK
- Chen, C.; Lyon, MT.; O'Malley, D.; Federici, CT.; Gmitter, J.; Grosser, JW.; Chaparro, JX.; Roose, ML. & Gmitter Jr., FG. (2008). Origin and frequency of 2n gametes in *Citrus sinensis* x *Poncirus trifoliata* and their reciprocal crosses. *Plant Science*, Vol.174, No.1, (January 2008), pp.1-8, ISSN 0168-9452
- Chen, C.; Sleper, DA.; Chao, S.; Johal, GS. & West, CP. (1997). RFLP detection of 2n pollen formation by first and second division restitution in perennial ryegrass. *Crop Science*, Vol.37, No.1, (January 1997), pp.76-80, ISSN 0011-183X
- Chen, ZJ. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annual Review of Plant Biology*, Vol.58, (June 2007), pp. 377-406, ISSN 1543-5008
- Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, Vol.6, No.11, (November 2005), pp.836-846, ISSN 1471-0056
- Conicella, C.; Barone, A.; Del Giudice, A.; Frusciante, L. & Monti, LM. (1991). Cytological evidences of SDR-FDR mixture in the formation of 2n eggs in a potato diploid clone. *Theoretical and Applied Genetics*, Vol.81, No.1, (January 1991), pp.59-63, ISSN 0040-5752
- Contreras, RN.; Ranney, TG. & Tallury, SP. (2007). Reproductive behavior of diploid and allotetraploid *Rhododendron* L. 'fragrant affinity'. *HortScience*, Vol.42, No.1, (February 2007), pp.31-34, ISSN 0018-5345
- Crespel, L.; Gudín, S.; Meynet, J.; Zhang, D. (2002). AFLP-based estimation of 2n gametophytic heterozygosity in two parthenogenetically derived dihaploids of *Rosa hybrida* L. *Theoretical and Applied Genetics*, Vol. 104, No.2-3, (February 2002), pp. 451-456, ISSN 0040-5752
- Crespel, L.; Ricci, SC. & Gudín, S. (2006). The production of 2n pollen in rose. *Euphytica*, Vol.151, No.2, (September 2006), pp.155-164, ISSN 0014-2336.
- D'Erfurth, I.; Jolivet, S.; Froger, N.; Catrice, O.; Novatchkova, M. & Mercier, R. (2009). Turning meiosis in mitosis. *PLOS Biology*, Vol.7, No.6, (June 2009), e1000124, ISSN 1544-9173
- D'Erfurth, I.; Jolivet, S.; Froger, N.; Catrice, O.; Novatchkova, M.; Simon, M.; Jenczewski, E. & Mercier, R. (2008). Mutations in *AtPS1* (*Arabidopsis thaliana* Parallel Spindle 1)

- lead to the production of diploid pollen grains. *PLOS Genetics*, Vol.4, No.11, (November 2008), e1000274, ISSN 1553-7404
- D'Erfurth, I.; Cromer, L.; Jolivet, S.; Girard, C.; Horlow, C.; Sun, YJ.; To, JPC.; Berchowitz, LE., Copenhaver, GP. & Mercier, R. (2010). The cyclin-A CYCA1;2/TAM is required for the meiosis I to meiosis II transition and cooperates with OSD1 for the prophase to first meiotic division transition. *PLoS Genetics*, Vol.6, No.6, (June 2010), e100989, ISSN 1553-7404
- De Haan, A.; Maceira, NO.; Lumaret, R. & Delay, J. (1992). Production of 2n gametes in diploid subspecies of *Dactylis glomerata* L. 2. Occurrence and frequency of 2n eggs. *Annals of Botany*, Vol. 69, No.4, (April 1992), pp.345-350, ISSN 0305-7364
- De Nettancourt, D. (1997). Incompatibility in angiosperms. *Sexual Plant Reproduction*, Vol.10, No.4, (August 1997), pp. 185-199, ISSN 0934-0882
- De Storme, N. & Geelen, D. (2011). The Arabidopsis mutant jason produces unreduced FDR male gametes through a parallel/fused spindle mechanism in meiosis II. *Plant Physiology*, Vol.155, No.3, (March 2011), pp.1403-1415, ISSN 0032-0889
- De Storme, N.; Van Labbeke, M-C. & Geelen, D. (2007). Formation of unreduced pollen in *Arabidopsis thaliana*. *Communications in Agricultural and Applied Biological Sciences*, Vol.72, No.1, pp.159-162, ISSN 1379-1176
- Del Bosco, SF.; Tusa, N. & Conicella, C. (1999). Microsporogenesis in a *Citrus* interspecific tetraploid somatic hybrid and its fusion parents. *Heredity*, Vol.83, No.4, (October 1999), pp.373-377, ISSN 0018-067X
- Den Nijs, TPM. & Peloquin, SJ. (1977). 2N gametes in potato species and their function in sexual polyploidization. *Euphytica*, Vol.26, No.3, (December 1977), pp.585-600, ISSN 0014-2336.
- Dewitte, A.; Eeckhaut, T.; Van Huylenbroeck, J.; Van Bockstaele, E. (2006). Flow cytometric detection of unreduced pollen in *Begonia*. *Acta Horticulturae*, Vol.714, (September 2006), pp.107-112, ISSN 0567-7572
- Dewitte, A. (2010a). Exploitation of 2n pollen to create genetic variation in the genus *Begonia*, PhD thesis. Faculty of Bioscience engineering, Ghent University, ISBN 9789059893993
- Dewitte, A.; Eeckhaut, T.; Van Huylenbroeck, J. & Van Bockstaele, E. (2010b). Induction of unreduced pollen by trifluralin and N₂O treatments. *Euphytica*, Vol.171, No.2, (October 2010), pp.283-293, ISSN 0014-2336
- Dewitte, A.; Eeckhaut, T.; Van Huylenbroeck, J. & Van Bockstaele, E. (2010c). Meiotic aberrations during 2n pollen formation in *Begonia*. *Heredity*, Vol.104, No.2, (February 2010), pp. 215-223, ISSN 0018-067X
- Dewitte, A.; Eeckhaut, T.; Van Huylenbroeck, J. & Van Bockstaele, E. (2009). Occurrence of viable unreduced pollen in a *Begonia* collection. *Euphytica*, Vol.168, No.1, (February 2009), pp.81-94, ISSN 0014-2336
- Dewitte, A.; Van Laere, K.; Van Huylenbroeck, J. & Van Bockstaele, E. (2010d). Inheritance of 2n pollen formation in an F1 and F2 population of *Begonia* hybrids. *Acta Horticulturae*, Vol.855, (February 2010), pp.95-100, ISSN 0567-7572
- Dhooghe, E.; Van Laere, K.; Eeckhaut, T.; Leus, L. & Van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell Tissue and Organ Culture*, Vol.104, No.3, (March 2011), pp.359-373, ISSN 0176-6857

- Douches, DS. & Quiros, CF. (1988). Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* x *S. chacoense* diploid hybrid. *Heredity*, Vol.60; No.2, (April 1988), pp.183-191, ISSN 0018-067X
- Dupuis, I.; Roeckel, P.; Matthys-Rochon, E. & Dumas, C. (1987). Procedure to isolate viable sperm cells from corn (*Zea mays* L.) pollen grains. *Plant Physiology*, Vol.85, No.4, (december 1987), pp.876-878, ISSN 0032-0889.
- Dvorak, J.; Harvey, BL.; Coulman, BE. (1973). The use of nitrous oxide for producing eupolyploids and aneuploids in wheat and barley. *Canadian Journal of Genetics and Cytology*, Vol.15, No.1, (March 1973), pp.205-214, ISSN 0008-4093
- Dweikat, IM. & Lyrene, PM. (1988). Production and viability of unreduced gametes in triploid interspecific blueberry hybrids. *Theoretical and Applied Genetics*, Vol.76, No.4, (October 1988), pp.555-559, ISSN 0040-5752
- Eeckhaut, TGR.; Werbrouck, SPO.; Leus, LWH.; Van Bockstaele, EJ. & Debergh PC (2004). Chemically induced polyploidization in *Spathiphyllum wallisii* Regel through somatic embryogenesis. *Plant Cell Tissue and Organ Culture*, Vol.78, No.3, (September 2004), pp.241-246, ISSN 0176-6857
- Eijlander, R. (1988). Manipulation of the 2n gametes frequencies in *Solanum* pollen. *Euphytica*, Vol.39, No.3, (December 1988), pp.45-50, ISSN 0014-2336
- El Mokadem, H.; Crespel, L.; Meynet, J. & Gudin, S. (2002a). The occurrence of 2n pollen and the origin of sexual polyploids in dihaploid roses (*Rosa hybrid* L.). *Euphytica*, Vol.125, No.2, (May 2002), pp.169-177, ISSN 0014-2336
- El Mokadem, H.; Meynet, J.; Crespel, L. (2002b). The occurrence of 2n eggs in the dihaploids derived from *Rosa hybrid* L. *Euphytica*, Vol.124, No.3, (April 2002), pp.327-332, ISSN 0014-2336
- Erazzu, LE. & Camadro, EL. (2007). Direct and indirect detection of 2n eggs in hybrid diploid families derived from haploid tbr x wild species crosses. *Euphytica*, Vol.155, No.1-2, (May 2007), pp. 57-62, ISSN 0014-2336
- Erilova, A.; Brownfield, L.; Exner, V.; Rosa, M.; Twell, D., Scheid, OM., Hennig, L. & Kohler, C. (2009). Imprinting of the Polycomb Group gene MEDEA serves as a ploidy sensor in *Arabidopsis*. *PLOS Genetics*, Vol.5, No.9, (September 2009), e1000663, ISSN 1553-7404
- Estrada-luna, AA. ; Garcia-Aguilar, M. & Vielle-Caldaza, JP. (2004). Female reproductive development and pollen tube growth in diploid genotypes of *Solanum cardiophyllum* Lindl. *Sexual Plant Reproduction*, Vol.17, No.3, (September 2004), pp.117-124, ISSN 0934-0882
- Falisticco, E.; Tosti, N. & Falcinelli, M. (1995). Cytomixis in pollen mother cells of diploid *Dactylis*, one of the origins of 2n gametes. *Journal of Heredity*, Vol.86, No.6, pp.448-453, ISSN 0022-1503
- Gallo, P.; Micheletti, P.; Boldrini, K.; Risso-Pascotto, C.; Pagliarini, M. & Valle, C. (2007). 2N gamete formation in the genus *Brachiaria* (Poaceae: Paniceae). *Euphytica*, Vol.154, No. 1-2, (March 2007), pp.255-260, ISSN 0014-2336
- Ghaffari, S.M. (2006). Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. *African Journal of Biotechnology*, Vol.5, No.16, (August 2006), pp. 1450-1453, ISSN 1684-5315
- Grant, V. (1981). *Plant speciation*. Columbia University press, ISBN 9780231051125, New York

- Gu, XF.; Yang, AF.; Meng, H.; Zhang, JR. (2005). In vitro induction of tetraploid plants from diploid *Zizyphus jujuba* Mill. cv. Zhanhua. *Plant Cell Reports*, Vol.24, No.11, (December 2005), pp. 671-676, ISSN 0721-7714
- Gymer, PT. & Whittington, WJ. (1973). Hybrids between *Lolium perenne* L and *Festuca pratensis* Huds. *New Phytologist*, Vol.72, No.2, (March 1973), pp.411- 424, ISSN 0028-646X.
- Hahn, SK.; Bai, KV.; Asiedu, R. (1990). Tetraploids, triploids and 2n pollen from diploid interspecific crosses with cassava. *Theoretical and Applied Genetics*, Vol.79, No.4, (April 1990), pp. 433-439, ISSN 0040-5752
- Harlan, J. & De Wet, J. (1975). On Ö. Winge and a prayer: the origins of polyploidy. *The Botanical Review*, Vol.41, No.4, (October 2004), pp. 361-390, ISSN 0006-8101
- Hayashi, M.; Kato, J.; Ohashi, H. & Masahiro, M. (2009). Unreduced 3x gamete formation of allotriploid hybrid derived from the cross of *Primula denticulata* (4x) × *P. rosea* (2x) as a causal factor for producing pentaploid hybrids in the backcross with pollen of tetraploid *P. Denticulata*. *Euphytica*, Vol. 169, No.1, (September 2009), pp.123-131, ISSN 0014-2336
- Husband, BC. (2004). The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society*, Vol.82, No.4, (August 2004), pp.537-546, ISSN 0024-4066
- Hutten, RCB.; Schippers, MGM.; Hermesen, JGT. & Ramanna, MS. (1994). Comparative performance of FDR and SDR progenies from reciprocal 4x-2x crosses in potato. *Theoretical and Applied Genetics*, Vol.89, No.5, (November 1994), pp.545-550, ISSN 0040-5752
- Islam, AKMR. & Sheperd, KW. (1980). Meiotic restitution in wheat-barley hybrid. *Chromosoma*, Vol.79, No.3, (July 1980), pp.363-372, ISSN 0009-5915
- Jansen, RC. & Den Nijs, APM (1993). A statistical mixture model for estimating the proportion of unreduced pollen grains in perennial ryegrass (*Lolium perenne* L.) via the size of pollen grains. *Euphytica*, Vol.70, No.3, (January 1993), pp.205-215, ISSN 0014-2336
- Jauhar, PP. (2003). Formation of 2n gametes in durum wheat haploids: sexual polyploidization. *Euphytica*, Vol.133, No.1, (July 2003), pp.81-94, ISSN 0014-2336
- Jones, JR. & Ranney, TG. (2009). Fertility of neopolyploid *Rhododendron* and occurrence of unreduced gametes in triploid cultivars. *Journal of the American Rhododendron Society*, Vol.63, No.3, pp.131-135, ISSN 0003-0821
- Jongedijk, E. (1987). A rapid methyl salicylate clearing technique for routine phase contrast observations on female meiosis in *solanum*. *Journal of Microscopy*, Vol.146, pp.157-162, ISSN 1365-2818
- Jongedijk, E.; Ramanna, MS.; Sawor, Z. & Hermesen, JGT. (1991). 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 2xFDR × 2xFDR crosses. *Theoretical and Applied Genetics*, Vol.82, No.5, (October 1991), pp.645-656, ISSN 0040-5752
- Jovtchev, G.; Schubert, V.; Meister, A.; Barow, M. & Schubert, I. (2006). Nuclear DNA content and nuclear and cell volume are positively correlated in angiosperms. *Cytogenetic and Genome Research*, Vol.114, No.1, (May 2006), pp.77-82, ISSN 1424-8581

- Karlov, GI.; Khrustaleva, LI., Lim, KB. & Van Tuyl, JM. (1999). Homoeologous recombination in 2n-gamete producing interspecific hybrids of *Lilium* (Liliaceae) studied by genomic *in situ* hybridisation (GISH). *Genome*, Vol.42, No.4, (August 1999), pp.681-686, ISSN 0831-2796
- Kato, A. (1999). Induction of bicellular pollen by trifluralin treatment and occurrence of triploids and aneuploids after fertilization in maize. *Genome*, Vol.42, No.1, (February 1999), pp.154-157, ISSN 0831-2796
- Kato, A. & Geiger, HH. (2002). Chromosome doubling of haploid maize seedling using nitrous oxide gas at the flower primordial stage. *Plant Breeding*, Vol.121, No.5, (October 2002), pp.370-377, ISSN 0179-9541
- Kato, A. & Birchler, JA. (2006). Induction of tetraploid derivatives of maize inbred lines by nitrous oxide gas treatment. *Journal of heredity*, Vol.97, No.1, (January 2006), pp.39-44, ISSN 0022-1503
- Kermani, MJ.; Sarasan, V.; Roberts, AV.; Yokoya, K.; Wentworth, J. & Sieber, VK. (2003). Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. *Theoretical and Applied Genetics*, Vol.107, No.7, (November 2003), pp.1195-1200, ISSN 0040-5752
- Kessel, R. & Rowe, PR. (1975). Production of intraspecific aneuploids in the genus *Solanum*. Triploids produced from tetraploid-diploid crosses in potato. *Euphytica*, Vol.24, No.2, (June 1975), pp.65-75, ISSN 0014-2336
- Khan, N.; Barba-Gonzalez, R.; Ramanna, MS.; Arens, P.; Visser, RGF. & Van Tuyl, JM. (2010). Relevance for unilateral and bilateral sexual polyploidization in relation to intergenomic recombination and introgression in *Lilium* species hybrids. *Euphytica*, Vol.171, No.2, (January 2010), pp.157-173, ISSN 0014-2336
- Khan, N.; Zhou, S.; Ramanna, MS.; Arens, P.; Herrera, J.; Visser, RGF. & Van Tuyl, JM. (2009). Potential for analytic breeding in allopolyploids: an illustration from Longiflorum x Asiatic hybrid lilies (*Lilium*). *Euphytica*, Vol.166, No.3, (April 2009), pp.399-409, ISSN 0014-2336
- Kihari, H. & Tsunewaki, K. (1960). Production of polyploidy wheat by nitrous oxide. *Proceedings of the Japan Academy*, Vol.36, No.10, pp.658-663, ISSN 0021-4280
- Kitamura, S.; Akutsu, M. & Okazaki, K. (2009). Mechanisms of action of nitrous oxide gas applied as a polyploidizing agent during meiosis in lilies. *Sexual Plant Reproduction*, Vol.22, No.1, (March 2009), pp.9-14, ISSN 0934-0882
- Knight, CA.; Molinari, NA. & Petrov, DA. (2005). The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany*, Vol.95, No.1, (January 2005), pp.177-190, ISSN 0305-7364
- Köhler, C.; Mittelsten Scheid, O. & Erilova, A. (2010). The impact of the triploid block on the origin and evolution of polyploid plants. *Trends in Genetics*, Vol.26, No.3, pp.142-148, (March 2010), ISSN 0168-9525.
- Kondoresi, E.; Roudier, F. & Gendreau, E. (2000). Plant cell-size control: growing by ploidy? *Current Opinion in Plant Biology*, Vol.3, No.6, (December 2000), pp. 488-492, ISSN 1359-5266
- Kumar, G. & Srivastava, P. (2009). Gibberellic acid-induced pollen mortality and abnormal microsporogenesis in safflower. *Cytologia*, Vol.74, No.2, (February 2009), pp.171-176, ISSN 0011-4545

- Lamote, V.; Baert, J.; Roldan-Ruiz, I.; De Loose, M. & Van Bockstaele, E. (2002). Tracing of 2n egg occurrence in perennial ryegrass (*Lolium perenne* L.) using interploidy crosses. *Euphytica*, Vol.123, No.2, (January 2002), pp.159-164, ISSN 0014-2336.
- Leitch, I. & Benett, M. (1997). Polyploidy in angiosperms. *Trends in Plant Science*, Vol.2, No.12, (December 1997), pp.470-476, ISSN 1360-1385
- Lelley, T.; Mahmoud, AA. & Lein, V. (1987). Genetics and cytology of unreduced gametes in cultivated rye (*Secale cereale* L.). *Genome*, Vol.29, No.4, (August 1987), pp.635-638, ISSN 0831-2796
- Levin, D. (1983). Polyploidy and novelty in flowering plants. *The American Naturalist*, Vol.122, No.1, (July 1983), pp.1-25, ISSN 0003-0147
- Lewis, W. (1980). Polyploidy in species populations. In: *Polyploidy, biological relevance* (Vol 13), Lewis, WH., (ed.), 103-144, Plenum Press, ISBN 9780306403583, New York
- Li, YH.; Kang, XY.; Wang, SD.; Zhang, ZH. & Chen, HW. (2008). Triploid induction in *Populus alba* x *P. glandulosa* by chromosome doubling of female gametes. *Silvae Genetica*, Vol.57, No.1, pp.37-40, ISSN: 00375349
- Lim, K.; Ramanna, S.; De Jong, J.; Jacobsen, E. & Van Tuyl, J. (2001). Indeterminate meiotic restitution (IMR): a novel type of meiotic restitution mechanism detected in interspecific lily hybrids by GISH. *Theoretical and Applied Genetics*, Vol.103, No.2-3, (August 2001), pp.219-230, ISSN 0040-5752
- Lim, K.; Shen, T.; Barba-Gonzalez, R.; Ramanna, M. & Van Tuyl, J. (2004). Occurrence of SDR 2n-gametes in *Lilium* hybrids. *Breeding Science*, Vol.54, No.1, pp.13-18, ISSN 1344-7610
- Lokker, AC.; Barba-Gonzalez, R.; Lim, K-B.; Ramanna, MS. & Van Tuyl, JM. (2004). Genotypic and environmental variation in production of 2n gametes of oriental x Asiatic lily hybrids. *Acta Horticulturae*, Vol.673, (May 2005), pp. 453-456, ISSN 0567-7572
- Lyrene, P.; Vorsa, N. & Ballington, J. (2003). Polyploidy and sexual polyploidization in the genus *Vaccinium*. *Euphytica*, Vol.133, No.1, (July 2003), pp.27-36, ISSN 0014-2336
- Mariani, A.; Campanoni, P.; Gianì, S. & Breviario, D. (2000). Meiotic mutants of *Medicago sativa* show altered levels of alpha- and beta-tubulin. *Genome*, Vol.43, No.1, (February 2000), pp.166-71, ISSN 0831-2796
- McCoy, TJ. (1982). Inheritance of 2n pollen formation in diploid alfalfa (*Medicago sativa* L.). *Canadian Journal of Genetics and Cytology*, Vol.24, No.3, (June 1982), pp.315-323, ISSN 0008-4093
- McCoy, TJ. & Rowe, DE. (1986). Single cross alfalfa (*Medicago sativa* L.) hybrids produced via 2n gametes and somatic chromosome doubling: experimental and theoretical comparisons. *Theoretical and Applied Genetics*, Vol.72, No.1, (April 1986), pp.80-83, ISSN 0040-5752
- McHale, NA. (1983). Environmental induction of high frequency 2n pollen formation in diploid *Solanum*. *Canadian Journal of Genetics and Cytology*, Vol.25, No.6, (December 1983), pp.609-615, ISSN 0008-4093
- Meredith, MR.; Michaelson-Yeates, TPT.; Ougham, H. & Thomas, H. (1995). *Trifolium ambiguum* as a source of variation in the breeding of white clover. *Euphytica*, Vol.82, No.2, (January 1995), pp.185-191, ISSN 0014-2336.
- Mishra, MK. (1997). Stomatal characteristics at different ploidy levels in *Coffea* L. *Annals of Botany*, Vol.80, No.5, (November 1997), pp.689-692, ISSN 0305-7364

- Mittelsten Scheid, O.; Jakovleva, L.; Afsar, K.; Maluszynska, J. & Paszkowski, J. (1996). A change in ploidy can modify epigenetic silencing. *PNAS*, Vol.93, No.9, (July 1996), pp.7114-7119, ISSN 0027-8424
- Mok, DW. & Peloquin, SJ. (1975a). Breeding value of 2n pollen (diploandroids) in tetraploid x diploid crosses in potatoes. *Theoretical and Applied Genetics*, Vol.46, No.6, (January 1975), pp.307-314, ISSN 0040-5752
- Mok, DW. & Peloquin, SJ. (1975b). The inheritance of three mechanisms of diplandroid (2n pollen) formation in diploid potatoes. *Heredity*, Vol.35, No.3, (December 1975), pp.157-164, ISSN 0018-067X
- Mok, DW.; Peloquin, SJ. & Tarn, TR. (1975). Cytology of potato triploids producing 2n pollen. *American Journal of Potato research*, Vol.52, No.6, (June 1975), pp.171-174, ISSN 1099-209X
- Morgan, WG., Thomas, H. & Humphreys, MW. (1995). Unreduced gametes in interspecific hybrids in the *Festuca/Lolium* complex. *Plant Breeding*, Vol.114, No.3, (June 1995), pp.267-268, ISSN 0179-9541
- Negri, V. & Lemmi, G. (1998). Effect on selection and temperature stress on the production of 2n gametes in *Lotus tenuis*. *Plant Breeding*, Vol.117, No.4, (September 1998), pp.345-349, ISSN 0179-9541
- Negri, V. & Veronesi, F. (1989). Evidence for the existence of 2n gametes in *Lotus tenuis* Wald. Et Kit (2n = 2x = 12); their relevance in evolution of breeding of *Lotus corniculatus* L. (2n = 4x = 24). *Theoretical and Applied Genetics*, Vol.78, No.3, (September 1989), pp.400-404, ISSN 0040-5752
- Nelson, MN.; Mason, A.; Castello, M-C.; Thomson, L.; Yan, G. & Cowling, WA. (2009). Microspore culture preferentially selects unreduced (2n) gametes from an interspecific hybrid of *Brassica napus* L. x *Brassica carinata* Braun. *Theoretical and Applied Genetics*, Vol.119, No.3, (August 2009), pp.497-505, ISSN 0040-5752
- Nimura, M.; Kato, J.; Mii, M. & Ohishi, K. (2008). Cross-compatibility and the polyploidy of progenies reciprocal backcrosses between diploid carnation (*Dianthus caryophyllus* L.) and its amphidiploid with *Dianthus japonicus* Thunb. *Scientia Horticulturae*, vol. 115, No.2 (January 2008), pp. 183-189, ISSN 0304-4238
- Okazaki, K.; Kurimoto, K.; Miyajima, I.; Enami, A.; Mizuochi, H.; Matsumoto, Y. & Ohya, H. (2005). Induction of 2n pollen by arresting the meiotic process with nitrous oxide gas. *Euphytica*, Vol. 143, No.1-2, (January 2005), pp.101-114, ISSN 0014-2336
- Ortiz, R. (2002). Analytical breeding. *Acta Horticulturae*, Vol.622, (August 2003), pp.235-247 ISSN 0567-7572
- Ortiz, R. & Peloquin, SJ. (1991). Breeding for 2n egg production in haploid x species 2x potato hybrids. *American Journal of Potato research*, Vol.68, No.10, (October 1991), pp.691-703, ISSN 1099-209X
- Ortiz, R. & Peloquin, SJ. (1992). Recurrent selection for 2n gamete production in 2x potatoes. *Journal of Genetics and Breeding*, Vol.46, pp.383-390, ISSN 0394-9257
- Ortiz, R. & Vuylsteke, D. (1995). Factors influencing seed set in triploid *Musa spp.* L. and production of euploid hybrids. *Annals of Botany*, Vol.75, No.2, (February 1995), pp.151-155, ISSN 0305-7364
- Ortiz, R. (1997). Occurrence and inheritance of 2n pollen in *Musa*. *Annals of Botany*, Vol.79, No.4, (April 1997), pp.449-453, ISSN 0305-7364

- Ortiz, R., Ulburghs, F. & Okoro, JU. (1998). Seasonal variation of apparent male fertility and 2n pollen production in plantain and banana. *Hortscience*, Vol.33, No.1, pp.146-148, ISSN 0018-5345
- Osborn, T.; Pires, J.; Birchler, J.; Auger, D.; Chen, Z.; Lee, H.; Comai, L.; Madlung, A.; Doerge, R.; Colot, V. & Martienssen, R (2003). Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics*, Vol.19, No.3, (March 2003), pp.141-147, ISSN 0168-9525
- Östergren, G. (1954). Polyploids and aneuploids of *Crepis capilaris* by treatment with nitrous oxide. *Genetica*, Vol.27, No.1, (December 1955), pp.54-64, ISSN 0016-6707
- Otto, SP. & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, Vol.34, (December 2000), pp.401-437, ISSN 0066-4197
- Pagliarini, MS.; Takayama, SY.; de Freitas, PM.; Carraro, LR.; Adamowski, EV.; Silva, N. & Batista, LAR. (1999). Failure of cytokinesis and 2n gamete formation in Brazilian accessions *Paspalum*. *Euphytica*, Vol.108, No.2, (August 1999), pp.129-135, ISSN 0014-2336
- Pan, G.; Zhou, Y.; Fowke, LC. & Wang, H. (2004). An efficient method for flow cytometric analysis of pollen and detection of 2n nuclei in *Brassica napus* pollen. *Plant Cell Reports*, Vol.23, No.4, (October 2004), pp.196-202, ISSN 0721-7714
- Parrot, WA. & Smith, RR. (1984). Production of 2n pollen in red clover. *Crop Science*, Vol.24, No.3, (May 1984), pp.469-472, ISSN 0011-183X
- Parrot, WA. & Smith RR. (1986). Recurrent selection for 2n pollen formation in red clover. *Crop Science*, Vol.26, No.6, (November 1986), pp.1132-1135, ISSN 0011-183X.
- Pécricx, Y.; Rallo, G.; Folzer, H.; Cigna, M.; Gudín, S. & Le Bris, M. (2011). Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *Journal of Experimental Botany*, Vol.62, No.10, (June 2011), pp.3587-3597, ISSN 0022-0957
- Peloquin, SJ.; Boiteux, LS.; Simon, PW. & Jansky, SH. (2008). A chromosome-specific estimate of transmission of heterozygosity by 2n gametes in potato. *Journal of Heredity*, Vol.99, No.2, (March-April 2008), pp.177-181, ISSN 0022-1503
- Peloquin, SJ. & Ortiz, R. (1992). Techniques for introgressing unadapted germplasm to breeding populations. In: *Plant breeding in the 1990s*, Stalker, TP. & Murphy, JP. (eds.), 485-507, CAB International, ISBN 0851987176, Wallingford, UK
- Peloquin, SJ.; Boiteux, LS. & Carputo, D. (1999). Meiotic mutants in potato: valuable variants. *Genetics*, Vol.153, No.4, (December 1999), pp.1493-1499, ISSN 0016-6731.
- Pichot, C. & El Maâtaoui, M. (2000). Unreduced diploid nuclei in *Cupressus dupreziana* A. Camus pollen. *Theoretical and Applied Genetics*, Vol.101, No.4, (September 2000), pp.574-579, ISSN 0040-5752
- Pikaard, C. (1999). Nucleolar dominance and silencing of transcription. *Trends in Plant Science*, Vol.4, No.12, (December 1999), pp.478-483, ISSN 1360-1385.
- Qu, L. & Hancock, JF. (1995) Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. *Theoretical and Applied Genetics*, Vol.91, No.8, (December 1995), pp.1309-1315, ISSN 0040-5752
- Ramanna, MS. (1983). First division restitution gametes through fertile desynaptic mutants of potato. *Euphytica*, Vol.32, No.2, (June 1983), pp.337-350, ISSN 0014-2336

- Ramanna, MS. & Jacobsen, E. (2003). Relevance of sexual polyploidization for crop improvement-a review. *Euphytica*, Vol.133, No.1, (July 2003), pp.3-18, ISSN 0014-2336
- Ramanna, MS.; Kuipers, AGJ. & Jacobsen, E. (2003). Occurrence of numerically unreduced (2n) gametes in *Alstroemeria* interspecific hybrids and their significance for sexual polyploidization. *Euphytica*, Vol.133, No.1, (July 2003), pp.95-106, ISSN 0014-2336
- Ramsey, J. (2007). Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity*, Vol.98, No.3, (March 2007), pp.143-150, ISSN 0018-067X
- Ramsey, J. & Schemske, DW. (1998). Pathways, mechanisms and rates of polyploidy formation in the flowering plants. *Annual Review of Ecology and Systematics*, Vol.29, pp.267-501, ISSN 0066-4162
- Rhoades, MM. & Dempsey, E. (1966). Induction of chromosome doubling by the elongate gene in maize. *Genetics*, Vol.54, No.2, (August 1966), pp.505-522, ISSN 0016-6731
- Roberts, AV. (2007). The use of bead beating to prepare suspensions of nuclei for flow cytometry from fresh leaves, herbarium leaves, petals and pollen. *Cytometry*, Vol.71A, No.12, (December 2007), pp.1039-1044, ISSN 1552-4930
- Roberts, MA.; Reader, SM.; Dalgliesh, C.; Miller, TE.; Foote, TN.; Fish, LJ.; Snape, JW. & Moore, G. (1999). Induction and characterization of *Ph1* mutants. *Genetics*, Vol.153, No.4, (December 1999), pp.1909-1918, ISSN 0016-6731
- Russel, SD. (1991). Isolation and characterization of sperm cells in flowering plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol.42, pp.189-204, (June 1991), ISSN 1040-2519
- Sanford, JC. (1983). Ploidy manipulations. In: *Methods in fruit breeding*, Janick, J. & Moore, J.N. (eds.), 100-123, Perdue University Press, ISSN 9780911198638, West Lafayette
- Sato, T.; Miyoshi, K. & Okazaki, K. (2010). Induction of 2n gametes and 4n embryo in *Lilium* (*Lilium* × *formolongi* Hort.) by nitrous oxide gas treatment. *Acta Horticulturae*, Vol.855, (February 2010), pp.243-248, ISSN 0567-7572
- Shu-Ye, J. & Srinivasan, R. (2010) Natural and artificial mutants as valuable resources for functional genomics and molecular breeding *International journal of biologicam sciences*. Vol. 6, No. 3 pp. 228-251, ISSN 1449-2288
- Singhal, VK. & Kumar, P. (2008). Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeate* Royle). *Journal of Biosciences*, Vol.33, No.3, (September 2008), pp.371-380, ISSN 0250-5991.
- Sugiura, A.; Ohkuma, T.; Choi, YA. & Tao R. (2000). Production of nonaploid (2n = 9x) japanese persimmons (*Diospyros kaki*) by pollination with unreduced (2n = 6x) pollen and embryo rescue culture. *Journal of the American Society for Horticultural Science*, Vol.125, No.5, (September 2000), pp.609-614, ISSN 0003-1062
- Simioni, C.; Schifino-Wittmann, MT. & Dall'Agnol, M. (2006). Sexual polyploidization in red clover. *Scientia Agricola*, Vol.63, No.1, (January 2006), pp.26-31, ISSN 0103-9016
- Simon, CJ. & Sanford, JC. (1990). Separation of 2n potato pollen from a heterogeneous pollen mixture by velocity sedimentation. *Hortscience*, Vol.25, No.3, (March 1990), pp.342-344, ISSN 0018-5345
- Skiebe, K. (1958). Die bedeutung von unreduzierten gameten für die polyploidiezüchtung bei der Fliederprimeln (*Primula malacoides* Franchet). *Theoretical and Applied Genetics*, Vol.28, No.8, (August 1958), pp.353-359, ISSN 0040-5752

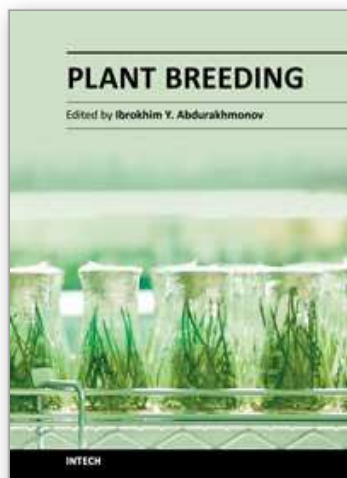
- Soltis, PS. & Soltis, DE. (2000). The role of genetic and genomic attributes in the success of polyploids. *PNAS*, Vol.97, No.13, (June 2000), pp.7051-7057, ISSN 0027-8424
- Ssebuliba, RN.; Tenkouano, A. & Pillay, M. (2008). Male fertility and occurrence of 2n gametes in East African Highland bananas (*Musa spp.*). *Euphytica*, Vol.164, No.1, (November 2008), ISSN 0014-2336
- Stebbins, G. (1971). Chromosomal evolution of higher plants. Edward Arnold Ltd, ISBN 0713122870, London, UK
- Stephens, LC. (1998). Formation of unreduced pollen by an *Impatiens hawkeri* x *Platypetala* interspecific hybrid. *Hereditas*, Vol.128, No.3, (August 1998), pp.251-255, ISSN 0018-0661
- Takamura, T. & Miyajima, I. (2002). Origin of tetraploid progenies in 4x x 2x crosses of Cyclamen (*Cyclamen persicum* Mill.). *Journal of the Japanese Society for Horticultural Science*, Vol.71, No.5, pp.632-637, ISSN 0013-7626
- Takayashi, C.; Leitch, IJ.; Ryan, A., Bennett, MD. & Brandham, PE. (1997). The use of genomic in situ hybridization (GISH) of recombinant chromosomes by a partially fertile bigeneric hybrid, *Gasteria lutzii* x *Aloe aristata* (Aloaceae), to its progeny. *Chromosoma*, Vol.104, No.6, (April 1997), pp.342-348, ISSN 0009-5915
- Taschetto, OM. & Pagliarini, MS. (2003). Occurrence of 2n and jumbo pollen in the Brazilian ginseng (*Pfaffia glomerata* and *P. tuberosa*). *Euphytica*, Vol.133, No.1, (July 2003), pp.139-145, ISSN 0014-2336
- Tavoletti, S., Mariani, A. & Veronesi, F. (1991a). Cytological analysis of macro- and microsporogenesis of a diploid alfalfa clone producing male and female 2n gametes. *Crop Science*, Vol. 31, No.5, (September 1991), pp.1258-1263, ISSN 0011-183X
- Tavoletti, S.; Mariani, A. & Veronesi, F. (1991b). Phenotypic recurrent selection for 2n pollen and 2n egg production in diploid alfalfa. *Euphytica*, Vol.57, No.2, (September 1991), pp.97-102. ISSN 0014-2336
- Tel-Zur, N.; Abbo, S.; Bar-Zvi, D.; Mizrahi, Y. (2003). Chromosome Doubling in Vine Cacti Hybrids. *Journal of Heredity*, Vol.94, No.4, (July 2003), pp.329-333, ISSN 0022-1503
- Thomas, H. (1993). Chromosome manipulation and polyploidy. In: *Plant Breeding Principles and Prospects*, Hayward, MD.; Bosemark, NO.; Rosmagosa, I. (eds.), 79-92, Chapman and Hall, ISBN 0412433907, New York.
- Thompson, JN.; Nuismer, SL. & Merg, K. (2004). Plant polyploidy and the evolutionary ecology of plant/animal interactions. *Biological Journal of the Linnean Society*, Vol.82, No.4, (August 2004), pp.511-519, ISSN 0024-4066
- Trojak-Goluch, A. & Berbeć, A. (2003). Cytological investigations of the interspecific hybrids of *Nicotiana tabacum* x *N. glauca* Grah. *Journal of Applied Genetics*, Vol.44, No.1, pp.45-54, ISSN 1234-1983
- Van Huylenbroeck, J.; Leus, L. & Van Bockstaele, E. (2005). Interploidy crosses in roses: use of triploids. *Acta Horticulturae*, Vol.690, (September 2005), pp.109-112, ISSN 0567-7572
- Van Huylenbroeck, JM.; De Riek, J. & De Loose, M. (2000). Genetic relationships among *Hibiscus syriacus*, *Hibiscus sinosyriacus* and *Hibiscus paramutabilis* revealed by AFLP, morphology and ploidy analysis. *Genetic Resources and Crop Evolution*, Vol. 47, No.3, (June 2000), pp.335-343, ISSN 0925-9864

- Van Laere, K.; Dewitte, A.; Van Huylenbroeck, J. & Van Bockstaele, E. (2009). Evidence for the occurrence of unreduced gametes in interspecific hybrids of *Hibiscus*. *The Journal of Horticultural Science and Biotechnology*, Vol.84:, No.2, (March 2009), pp.240-247, ISSN 1462-0316
- Van Laere, K.; França, SC.; Vansteenkiste, H.; Van Huylenbroeck, J.; Steppe, K. & Van Labeke, M.-C. (2011). Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. *Acta Physiologiae Plantarum*, Vol.33, No.4, pp.1149-1156, ISSN 0137-5881
- Van Tuyl, JM.; de Vries, JN.; Bino, RJ. & Kwakkenbos, AM. (1989). Identification of 2n pollen producing interspecific hybrids of *Lilium* using Flow Cytometry. *Cytologia*, Vol.54, No.4, pp.737-745, ISSN 0011-4545
- Vandenhout, H.; Ortiz, R.; Vuylsteke, D.; Swennen, R.; Bai, KV. (1995). Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica*, Vol.83, No.2, (June 1995), pp.117-122, ISSN 0014-2336
- Veilleux, R. (1985). Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. In: *Plant Breeding reviews* (Vol.3), Janick, J. (ed.), 253-288, AVI Publishing Company, ISBN 0870554875, Westport, USA
- Veilleux, RE. & Lauer, FI. (1981). Variation for 2n pollen production in clones of *Solanum phureja* Juz. and Buk. *Theoretical and Applied Genetics*, Vol.59, No.2, (March 1981), pp.95-100, ISSN 0040-5752
- Veilleux, RE.; McHale, NA. & Lauer, FI. (1982). Unreduced gametes in diploid *Solanum*. Frequencies and types of spindle abnormalities. *Canadian Journal of Genetics and Cytology*, Vol.24, No.3, (June 1982), pp.301-314, ISSN 0008-4093
- Veronesi, F.; Mariani, A. & Bingham, ET. (1986). Unreduced gametes in diploid *Medicago* and their importance in alfalfa breeding. *Theoretical and Applied Genetics*, Vol.72, No.1, (April 1986), pp.37-41, ISSN 0040-5752
- Vorsa, N. & Rowland, LJ. (1997). Estimation of 2n gametophyte heterozygosity in a diploid blueberry (*Vaccinium darrowi* camp) clone using RAPDs. *Journal of Heredity*, Vol.88, No.5, (September 1997), pp.423-426, ISSN 0022-1503
- Wang, W.; Guo, Q.; Xiang, S.; Xiaolin, L. & Guolu, L. (2003). Study on the effect of hot shock treatment on the occurrence frequency of 2n pollen of loquat trees. *Journal of Fruit Science*, Vol.4, pp.284-286, ISSN 1009-9980
- Wang, J.; Kang, X. & Zhu, Q. (2010). Variation in pollen formation and its cytological mechanism in an allotriploid white poplar. *Tree Genetics and Genomes*, Vol.6, No.2, (February 2010), pp.281-290, ISSN 1614-2942
- Watanabe, K. & Peloquin, SJ. (1989). Occurrence of 2n pollen and *ps* gene frequencies in cultivated groups and their related wild species in tuber bearing Solanums. *Theoretical and Applied Genetics*, Vol.78, No.3, (September 1989), pp.329-336, ISSN 0040-5752
- Werner, JE. & Peloquin, SJ. (1987). Frequency and mechanisms of 2n egg formation in haploid tuberosum -wild species F1 hybrids. *American Journal of Potato Research*, Vol.64, No.12, pp.641-654, ISSN 1099-209X
- Werner, JE. & Peloquin, SJ. (1991a). Yield and tuber characteristics of 4x progeny from 2x x 2x crosses. *Potato Research*, Vol.34, No.3, (September 1991), pp.261-267, ISSN 0014-3065

- Werner, JE. & Peloquin, SJ. (1991b). Significance of allelic diversity and 2n gametes for approaching maximum heterozygosity in 4x potatoes. *Euphytica*, Vol.58, No.1, (October 1991), pp.21-29, ISSN 0014-2336
- Wu, H.; Zheng, S.; He, Y.; Yan, G.; Bi, Y. & Zhu, Y. (2007). Diploid female gametes induced by colchicines in oriental lilies. *Scientia Horticulturae*, Vol.114, No.1, (September 2007), pp.50-53, ISSN 0304-4238.
- Xiao, Y-Q.; Zheng, S-X.; Long, C-L.; Zheng, L.; Guan, W-L. & Zhao, Y. (2007). Initial study on 2n-gametes induction of *Strelitzia reginae*. *Journal of Yunnan Agricultural University*, Vol.22, No.4, pp.475-479, ISSN 1004-390X
- Xu, H.; Weterings, K.; Vriezen, W.; Feron, R.; Xue, Y.; Derksen, J. & Mariani, C. (2002). Isolation and characterization of male-germ-cell transcripts in *Nicotiana tabacum*. *Sexual Plant Reproduction*, Vol.14, No.6, pp.339-346, ISSN 0934-0882
- Xu, SJ. & Joppa, LR. (1995). Mechanism and inheritance of first division restitution in hybrids of wheat, rye and *Aegilops squarrosa*. *Genome*, Vol.38, No.3, (June 1995), pp.607-615, ISSN 0831-2796
- Xu, SJ. & Joppa, LR. (2000). First-division restitution in hybrids of Langdon durum disomic substitution lines with rye and *Aegilops squarrosa*. *Plant Breeding*, Vol.119, No.3, (June 2000), pp.233-241, ISSN 0179-9541
- Xu, L-Q.; Zhang, Q-L. & Luo, Z-R. (2008). Occurrence and cytological mechanism of 2n pollen formation in Chinese *Diospyros* spp. (Ebenaceae) staminate germplasm. *The Journal of Horticultural Science and Biotechnology*, Vol.83, No.5, (September 2008), pp.668-672, ISSN 1462-0316
- Xue, Z.; Liu, P. & Liu, M. (2011). Cytological mechanism of 2n pollen formation in Chinese jujube (*Ziziphus jujube* Mill. 'Linglingzao'). *Euphytica*, doi: 10.1007/s10681-011-0461-7, , ISSN 0014-2336
- Yan, G.; Ross Ferguson, A.; McNeilage, MA. & Murray, BG. (1997). Numerically unreduced (2n) gametes and sexual polyploidization in *Actinidia*. *Euphytica*, Vol.96, No.2, (July 1997), pp.267-272, ISSN 0014-2336
- Yan, L.; Zhang, X. & Liu, G. (2000). Occurrence of unreduced gametes and ploidy restoration in haploid *Capsicum annuum* L. *Journal of Horticultural Science and Biotechnology*, Vol.75, No.2, (March 2000), pp.195-197, ISSN 1462-0316
- Yang, M.; Hu, Y.; Lohdi, M.; McCombie, WR. & Ma, H. (1999). The *Arabidopsis* SKP1-LIKE1 gene is essential for male meiosis and can control homologue separation. *PNAS*, Vol.96, No.20, (September 1999), pp.11416-11421, ISSN 0027-8424
- Ye, Z-w.; Du, J-h; Su, M-s.; Li, L-l. & Zhang, S-l. (2010). Effects of high temperature on the microsporogenesis and pollen development of peach. *Acta Horticulturae Sinica*, Vol.3, pp.355-362, ISSN: 0513-353X
- Zhang, G.; Campenot, MK.; McGann, LE. & Cass, DD. (1992). Flow cytometric characteristics of sperm cells isolated from pollen of *Zea Mays* L. *Plant Physiology*, Vol.99, No.1, (May 1992), pp.54-59, ISSN 0032-0889
- Zhang, X. & Liu, G. (1998). Induction of 2n pollen in *Prunus* by heat shock. *Acta Horticulturae Sinica*, Vol.3, pp.392-293, ISSN 0513-353X
- Zhang, Z. & Kang, X. (2010). Cytological characteristics of numerically unreduced pollen production in *Populus tomentosa* Carr. *Euphytica*, Vol.173, No.2, (May 2010), pp. 151-159, ISSN 0014-2336

- Zhang, L.-Q.; Liu, D-C.; Zheng, Y-L.; Yan, Z-H.; Dai, S-F.; Li, Y-F.; Jiang, Q.; Ye, Y-Q. & Yen, Y. (2010). Frequent occurrence of unreduced gametes in *Triticum turgidum*-*Aegilops tauschii* hybrids. *Euphytica*, Vol.172, No.2, (March 2010), pp.285-294, ISSN 0014-2336
- Zhang, L.-Q.; Yen, Y.; Zheng, Y-L. & Liu, D-C. (2007a). Meiotic restriction in emmer wheat is controlled by one or more nuclear genes that continue to function in derived lines. *Sexual Plant Reproduction*, Vol.20, No.3, (September 2007), pp.159-166, ISSN 0934-0882
- Zhang, Z.; Kang, X., Zhang, P.; Li, Y. & Wang, J. (2007b). Incidence and molecular markers of 2n pollen in *Populus tomentosa* Carr. *Euphytica*, Vol. 154, pp.145-152, ISSN 0014-2336
- Zhong, C.; Zhang, S-n.; Yu, X-h.; Li, Y.; Hou, X-l. & Li, S-j. (2010). Studies on the Induction of 2n Gamete in Chinese Cabbage and the Production of Tetraploid by Sexual Polyploidization. *Acta Horticulturae Sinica*, Vol.37, No.11, pp.1789-1795, ISSN 0513-353X
- Zhou, S.; Ramanna, MS.; Visser, RGF. & Van Tuyl, JM. (2008). Analysis of the meiosis in the F1 hybrids of Longiflorum x Asiatic (LA) of lilies (*Lilium*) using genomic in situ hybridisation. *Journal of Genetics and Genomics*, Vol.35, No.11, (November 2008), pp.687-695, ISSN 1673-8527
- Zlesak, DC. (2009). Pollen diameter and guard cell length as predictor of ploidy in diverse rose cultivars, species, and breeding lines. In: *Floriculture and Ornamental Biotechnology* (Vol.3, special issue 1 'Roses'), Zlesak, DC. (ed.), 53-70, Globalsciencebooks, ISBN 9784903313283, Middlesex, UK

IntechOpen



Plant Breeding

Edited by Dr. Ibrokhim Abdurakhmonov

ISBN 978-953-307-932-5

Hard cover, 352 pages

Publisher InTech

Published online 11, January, 2012

Published in print edition January, 2012

Modern plant breeding is considered a discipline originating from the science of genetics. It is a complex subject, involving the use of many interdisciplinary modern sciences and technologies that became art, science and business. Revolutionary developments in plant genetics and genomics and coupling plant "omics" achievements with advances on computer science and informatics, as well as laboratory robotics further resulted in unprecedented developments in modern plant breeding, enriching the traditional breeding practices with precise, fast, efficient and cost-effective breeding tools and approaches. The objective of this Plant Breeding book is to present some of the recent advances of 21st century plant breeding, exemplifying novel views, approaches, research efforts, achievements, challenges and perspectives in breeding of some crop species. The book chapters have presented the latest advances and comprehensive information on selected topics that will enhance the reader's knowledge of contemporary plant breeding.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

A. Dewitte, K. Van Laere and J. Van Huylenbroeck (2012). Use of 2n Gametes in Plant Breeding, Plant Breeding, Dr. Ibrokhim Abdurakhmonov (Ed.), ISBN: 978-953-307-932-5, InTech, Available from: <http://www.intechopen.com/books/plant-breeding/use-of-2n-gametes-in-plant-breeding>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen