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PERSPECTIVE

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Exploitation of genes affecting meiotic non-reduction and nuclear restitution: Arabidopsis as a model?

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Abstract Meiosis is a fascinating and complex phenomenon with intriguing practical potential. Some deviations in meiotic reduction are crucial for plant evolution and extremely important for plant breeding. In particular, the production of gametes with unreduced chromosome number and the diplosporic pathway of apomixis hold great promise for the genetic improvement of cultivated plants. The use of meiotic mutations is hampered by the fact that they do not occur widely in crop plants, and traditional breeding approaches to transfer these characters are difficult or impossible. For their exploitation, genetic engineering has been suggested as a promising and more direct way. Since molecular advances for isolating meiotic genes in flowering plants have been pursued mostly in *Arabidopsis thaliana*, mutants of this plant with alterations in meiotic reduction or meiotic nuclear restitution are the main focus of this review. Emphasis has been given to the cytological description of mutated phenotypes, their effect on male/female fertility, and to gene function. Perspectives for using meiotic genes of *Arabidopsis* to manipulate whole genomes through 2*n* gametes and for the exploitation of apomixis are also discussed.

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Meiotic mutants and their use in plant breeding

Meiosis is a complex multistep process that includes chromosome pairing, synaptonemal complex formation and crossing over, recombination and disjunction of homologous chromosomes, and cytokinesis. Together with the unique circumstance of a single round of DNA replication combined with two successive nuclear divisions, it leads to chromosome reduction, accurate chromosome transmission and genetic recombination.

The complexity of such events suggests that many genes, usually present in a dominant state, are involved. Expression of such genes is stage-, site- and time-specific, and tightly regulated to ensure the success of each meiotic step. The normal pattern of meiosis can be drastically modified by inherited variations (i.e. meiotic mutations), which may operate at each stage, starting from the initiation of premeiotic DNA synthesis. These meiotic defects can be detrimental for gamete viability and plant fertility. However, some deviations from normal meiosis are crucial for plant evolution and are extremely important for plant breeding. Indeed, they may contribute to solving certain problems in improving crop species, and to the transfer of useful genes and allelic diversity from one species to another.

A number of examples where breeding progress has been made through meiotic mutations can be considered. The occurrence of a mutation at the *Ph* locus that controls chromosome pairing is one of the best known examples of meiotic mutation noteworthy for practical purposes. *Ph* ensures a diploid-like behaviour in disomic polyploids, such as wheat, which possess more than one diploid set of similar (homeologous) chromosomes. Mutations at the *Ph* locus are necessary in sexual hybridisation between polyploids and their wild relatives to ensure intergenomic exchanges between homeologous chromosomes, and thus to introduce desirable genes from exotic germplasms.

Several disease resistance loci were transferred from wild relatives into wheat by recombining their chromosomes (reviewed in Moore 2002). Currently, molecular markers linked to the *Ph1* locus are being developed to aid gene transfer between wheat and its wild relatives (Wang et al. 2002).

An important group of genes that can be exploited for breeding purposes is that related to meiotic non-reduction that can give rise to gametes with unreduced chromosome number (2*n* gametes) or to the apomictic mechanism of diplospory. 2*n* gametes play a crucial role in the evolution of polyploids, being the basis for sexual polyploidisation events. Due to the genetic consequences associated with 2*n* gametes, high variability, fitness, and heterozygosity are expected in progenies deriving from these events (Carputo et al. 2003). 2*n* gametes have been extensively studied in a number of species, including *Solanum*, *Manihot*, *Malus*, *Arachis*, *Lolium*, *Agropyrum* (Bretagnolle and Thompson 1995), and have been generally attributed to single recessive genes (Peloquin et al. 1999). The genetic significance of each meiotic mutation is that they are essentially first division (FDR) and second division (SDR) restitution mechanisms (i.e. mechanisms that omit first and second meiotic divisions and lead to unreduced nuclei carrying non-sister and sister chromatids, respectively) and transmit high levels of intra- and inter-locus interactions.

From the breeding standpoint, 2*n* gametes have revolutionised strategies to improve important polysomic polyploid crops such as potato and alfalfa. The incorporation of noteworthy traits from diploid wild relatives into tetraploid crops via 2*n* gametes is well documented. In potato, 2*n* gametes allowed the transfer of resistance to biotic stresses from diploid species *Solanum vernei*, *Solanum tarijense*, and *Solanum chacoense* to the cultivated tetraploid gene pool (Ortiz et al. 1997; Carputo et al. 2000; Capo et al. 2002). In alfalfa, introgression of useful genes from diploid *Medicago sativa* ssp. *coerulea*

Table 1 Meiotic mutant genes reported in *Arabidopsis thaliana* and their function. *EMS* ethyl methane sulfonate, *RMS* radial microtubule systems

Mutant	Allele	Gene cloned	Mutated line	Function	Reference
swi1/dyad	*swi1-1*	Yes	T-DNA	Candidate protein required for the establishment of meiotic sister chromatid cohesion	Motamayor et al. 2000
	swi1-2		EMS		Mercier et al. 2001
	dyad		EMS		Agashe et al. 2002
syn1/dif1	*syn1*	Yes	T-DNA	*REC8/RAD21* homologue; required for meiosis sister chromatid cohesion	Bai et al. 1999
	dif1-5		Ac/Ds		Bhatt et al. 1999
asy1		Yes	T-DNA	*HOP1* homologue; required for homologous chromosome synapsis	Ross et al. 1997; Caryl et al. 2000
dsy1		Nr[a]	T-DNA	Required for synapsis maintenance	Ross et al. 1997
dmc1		Yes	T-DNA	*RECA* homologue; required for interhomolog recombination; essential for bivalent stabilization and chiasma formation	Klimyuk and Jones 1997; Couteau et al. 1999
spo11	*spo11-1-1*	Yes	T-DNA	*SPO11* homologue; essential for meiotic recombination and synapsis	Grelon et al. 2001
	spo11-1-2		EMS		
ms4		Nr[a]	EMS	Required for meiosis I progression	Chaudhury et al. 1994
sap		Yes	En/Spm-I/d spm	Novel protein predicted to act as a trascriptional regulator essential for the initiation of meiotic division in megasporogenesis	Byzova et al. 1999
ask1		Yes	Ac/Ds	*SKP1* homologue; putative role in homolog separation in meiosis I, and in cell cycle progression	Yang et al. 1999
sds		Yes	Ac/Ds	Novel cyclin-like protein; required for normal homolog synapsis and recombination	Azumi et al. 2002
tam		Nr[a]	EMS	Required for cell cycle regulation	Magnard et al. 2001
std/tes	*std1-3*	Yes	EMS	Kinesin required for establishment or maintenance of RMS	Hülskamp et al. 1997; Spielman et al. 1997
			EMS		
			γ-irradiation		
	tes1-4		Fast neutron		
			EMS		
			EMS		
			T-DNA		

[a]Corresponding gene not yet reported

and *M. sativa* ssp. *falcata* made possible the selection of hybrids with dry matter content significantly higher than the average value of cultivated genotypes (Motzo et al. 1994). In *Arachis*, Singsit et al. (1995) developed a hexaploid population from 3× × 3× crosses showing a high degree of resistance to *Meloidogyne arenaria* and *Mycosphaerella berkeleyi*.

The other group of genes that is relevant in this context is that involved in the genetic control of diplospory. Diplospory results in the formation of a megagametophytic structure without meiotic reduction as a consequence of aberrant meiosis or failure of meiotic commitment (Koltunow 1993). Diplospory, and apomixis in general, represents a unique reproductive system for the generation of genetically identical seeds without fertilisation and for the maintenance of allelic interactions useful for the production of hybrid seeds. In addition, crop reproduction efficiency is increased in that losses caused by the negative effect of environment factors on sexual reproduction events (such as pollination and fertilisation) are avoided. For a review of the potential benefits of diplospory and other apomixis mechanisms in agriculture, see Spillane et al. (2001).

Despite its occurrence in over 400 species of Angiosperms, apomixis is found in few species of agricultural importance: some forages, *Citrus*, apple, mango and orchids. Sexual hybridisation has been performed between pearl millet and its apomictic relatives to transfer apomixis. Apomictic plants displayed a high degree of seed abortion and high frequency of additional chromosomes (reviewed in Savidan 2001).

The new molecular tools developed in recent years strongly suggest that breeding efforts can be aided by direct gene transfer, and several genes of interest have been isolated and expressed in cultivated genotypes. Many meiotic genes have been cloned from yeast, *Drosophila*, *Caenorhabditis elegans*, and humans (Schwarzacher 2003). In contrast, relatively little knowledge is available in higher plants. Molecular advances for isolating meiotic genes in flowering plants have been pursued mostly using *Arabidopsis thaliana* as a sexual model system and by strategies based on insertional mutagenesis or the homology of meiotic genes in the other above-mentioned organisms.

This paper places emphasis on the class of mutations, so far identified in *A. thaliana*, affecting meiotic reduction, resulting in 2*n* spores. Although the development of such spores is generally arrested in *A. thaliana*, understanding the mechanisms involved may be particularly important and pertinent to plant improvement. Other mutations related to nuclear restitution will also be considered. The question is posed as to whether it is rational to seek meiotic mutations crucial for polyploid evolution and for plant breeding in *A. thaliana*, which is a diploid (2*n*=2×=10) sexual species.

A. thaliana mutants with alterations in meiotic reduction

This section details the cytological events that characterise mutations directly involved in meiotic reduction, or other events that have consequences for reduction (Table 1).

Cohesion-affected mutants

Meiotic reduction in chromosome number depends on a distinctive attachment of chromosomes to the spindle as well as precise regulation of the cohesion between sister chromatids (Moore and Orr-Weaver 1998). At least two *A. thaliana* meiotic mutants are affected in sister chromatid cohesion: *switch1/dyad*, and *syn1/dif1*. Attention will be focussed mainly on the *SWI1/DYAD* gene since the mutated phenotype is of potential utility for apomixis and 2*n* gamete technology.

Three mutant alleles have been isolated in the *SWI1/DYAD* locus: *swi1-1* (Motamayor et al. 2000), *swi1-2* (Mercier et al. 2001) and *dyad* (Agashe et al. 2002). All three alleles cause similar defects in the female meiosis pathway, whereas aberrations in microsporogenesis are reported only for *swi1-2*. The majority of megaspore mother cells (MMCs) perform a mitotic-like division instead of meiosis I, exiting meiosis with an unreduced chromosome number. At *swi1/dyad* metaphase I, ten univalents are observed and the chromosome segregation at anaphase I is equational, with separation of sister chromatids in two equal daughter cells of a dyad. The MMCs are not committed to a mitotic programme but to a meiotic cycle. This conclusion is supported by the expression in these cells of a β-glucuronidase marker under the meiotic-specific promoter *AtDMC1* (Agashe et al. 2002). Subsequently, daughter cells in the dyad perform a second cell cycle, which is either a novel mitosis-like division or an aberrant one with unequal chromosome segregation. Finally, degenerated cells are observed instead of the embryo sac. The mutated cytological phenotype is explained by a loss of synapsis, and loss of centromere cohesion at anaphase I. Mercier et al (2001) suggested a change in centromere configuration leading to a bipolar, instead of monopolar, attachment. This could explain the balanced segregation of chromatids. As stated above, aberrations occur in microsporogenesis only for the *swi1-2* mutant allele, which causes male sterility with severe defects in chromosome behaviour: 20 separated chromatids are observed at metaphase I, and their random segregation in the first and second division leads to a variable number of microspores with unbalanced nuclei. The phenotype observed during male meiosis in the *swi1-2* mutant is explained by the release of chromatid arms and centromere cohesion before metaphase I.

The different phenotype observed in male and female meiocytes indicates a distinct role for SWI1 or, alternatively, a different effect of the loss of SWI1 function in both processes. The differences between cytological behaviour in *swi1-1/dyad* and *swi1-2* are consistent with the molecular characterisation of *swi1* alleles. The *swi1-1*

allele is caused by a T-DNA insertion in the 5′ untranslated region of the gene. A very low level of normal SWI1 protein probably occurs since the mutation is not fully penetrant. The *dyad* allele depends on a frameshift mutation that causes a premature truncation of the protein. The presence of some fertility suggests that some biological activities are retained. The stronger *swi1-2* allele, generated by ethyl methane sulfonate, has a single base pair substitution that causes a stop codon, preventing the production of correct SWI1 protein.

The SWI1 protein, which has no similarity to known proteins, has been localised in the nucleus, in association with chromosomes, exclusively during the pre-leptotene interphase (Mercier et al. 2003). It is proposed that the SWI1 protein promotes the establishment of sister chromatid cohesion, but it is not responsible for its maintenance. A more detailed study of SWI1 function revealed that it is also required for the axial element, a component of the synaptonemal complex (SC), and recombination initiation (Mercier et al. 2003).

In the other mutant defective in sister chromatid cohesion, *syn1/dif1*, no change from reductional to equational division has been described. The effect of the *syn1* mutation on chromosome behaviour results in a complex phenotype that causes both male and female sterility (Bhatt et al. 1999; Bai et al. 1999). The first cytological defect is reported during early leptotene and zygotene of microsporogenesis, with an irregular chromosome condensation and appearance. At prometaphase I, Bai et al. (1999) described 15 univalents. Extensive chromosome fragmentation is clearly observed at anaphase I. The *syn1/dif1* cytological phenotype has been explained by Cai et al. (2003) with a lack of sister chromatid cohesion, and a failure of homolog pairing. The *SYN1/DIF1* gene has been cloned, and shows similarities to the RAD21/REC8 cohesin family. However, the *rec8* mutant of *Schizosaccharomyces pombe* behaves differently from *syn1/dif1*, showing an equational chromosome division at meiosis I.

Synapsis/recombination-affected mutants

Besides sister chromatid cohesion, meiotic reduction can be perturbed by altered synapsis and crossing over. Although, these events per se do not generally lead to 2*n* spore production, they result in a lack of recombination, which is important for diplospory.

As regards synapsis, two mutants characterised by male and female low fertility are defective in homolog synapsis (*asy1*) and in synapsis maintenance (*dsy1*) (Ross et al. 1997; Caryl et al. 2000). In male meiosis, fully synapsed bivalents are never observed at pachytene in the *asy1* mutant. In late diplotene, chromosomes are mostly separated and unconnected to their homologs. In the *dsy1* mutant, an apparently normal pachytene stage is observed. However, in early diplotene, homologous chromosomes are seen loosely aligned rather than associated by chiasmata into bivalents. In both mutants a high number of univalents is observed at metaphase I, and lack of synapsis results in an aberrant segregation of chromosomes during both anaphase I and II division of meiosis. Polyads containing uneven numbers of chromosomes are originated at the end of the meiotic cycle. An examination of female meiosis revealed that chromosome behaviour in MMCs corresponds closely to that of pollen mother cells (PMCs) (Armstrong and Jones 2001). The *ASY1* gene encodes a protein that is first detected in meiotic interphase at G2 (Armstrong et al. 2003) and has significant homology to the yeast HOP1 protein, which is essential for SC assembly and normal synapsis (Caryl et al. 2000). In particular, ASY1 localisation suggests that it is required for SC morphogenesis rather than as a structural component of the SC itself (Armstrong et al. 2002). The *DSY1* gene maps to chromosome 3, between genes GL-1 and HY-2, but it has not yet been isolated.

As discussed above, the lack of chromosome association causes formation of univalents that segregate randomly or divide equationally at meiosis I. It has been demonstrated that univalents in mutants *asy1* and *dsy1* show both reductional and equational divisions during meiosis I, presumably depending on the orientation of sister kinetochores that may or may not be towards opposite poles of the cell. In contrast, the univalents determined by the absence of crossing over and synapsis in *dmc1* and *spo11* mutants exhibit random segregation at meiosis I (Couteau et al. 1999; Grelon et al. 2001). This behaviour indicates that monopolar attachment of centromeres is retained in both mutants. It is obvious that, from a breeding standpoint, mutant genes resulting in equational division of univalents are preferable to those giving random segregation. The *A. thaliana* genes *DMC1* and *SPO11* are homologous to the corresponding genes in other eukaryotes (Hartung and Puchta 2000; Klimyuk and Jones 1997). In budding yeast, SPO11 protein is the endonuclease responsible for induction of double-strand breaks (DSBs) (Keeney et al. 1997) and DMC1 is involved in the first step of DSB repair by promoting the invasion of the single strand into a homologous duplex (Bishop et al. 1992).

Like *spo11*, the *solo dancer* (*sds*) mutant has defects in homolog synapsis and bivalent formation (Azumi et al. 2002). It has reduced fertility and a low frequency of dyads (1.1%). Univalents segregate randomly, leading to unbalanced 2–8 spores in microsporogenesis. Female meiosis is defective in a way similar to male meiosis, but to a lesser extent. The SDS gene encodes a novel cyclin-like protein.

Meiosis-arrested mutants

The formation of 2*n* meiotic products can also be a consequence of meiotic arrest. In the male-sterile *ms4* mutant, dyads occur at the end of meiosis I. They persist until late in the development of the anther and then degenerate (Chaudhury et al. 1994). In another mutant, male and female *sterile apetala* (*sap*), which also results in

the production of dyads, megasporogenesis is arrested after the first meiotic division (Byzova et al. 1999). The dyad cells subsequently degenerate without forming any embryo sac. The role of *SAP* in microsporogenesis is unknown since anthers degenerate prematurely in the *sap* mutant. The *SAP* gene produces a protein with no significant similarity to any other sequence in the database. SAP protein contains a serine/glycine-rich domain often found in eukaryotic transcriptional regulators. The mutation in the *SAP* locus causes not only meiosis arrest but also inflorescence and floral development abnormalities. A detailed cytological examination of meiosis has not been reported either in *ms4* or in *sap* mutants, so it is unknown whether or not dyads carry a balanced chromosome number.

Cell-cycle-affected mutants

Meiotic reduction can be influenced also by defects in cell cycle progression during meiosis. Most of these defects result in dyad production, which is an essential element of diplospory and of 2*n* gamete recovery. Yang et al. (1999) reported a male-sterile *ask1-1* mutant that exhibits 26% of dyads. Although, in this latter paper prophase I and metaphase I of *ask1-1* were observed to be similar to wild type, Wang et al. (2004) show that chromosomes mostly fail to synapse at pachytene in *ask1-1* male meiosis. Moreover, a prolonged presence of leptotene-like nuclei is also described. However, at late prophase I, five bivalents displaying unusual morphology occur in *ask1-1*. Chromosome segregation at anaphase I is aberrant, with no separation of some bivalents, and with some chromosomes stretched along the spindle. Abnormal distribution of chromosomes in meiosis I and II leads to a variable number of chromosomal groups and to the formation of microspores of different sizes. The *ASK1* gene has homology to yeast *SKP1*, which is essential for regulation of the mitotic cell cycle (SKP1 in yeast has been found to regulate both the G1/S and G2/M transitions), targeting specific proteins for ubiquitin-mediated proteolysis (Connelly and Hieter 1996; Bai et al. 1996). The *ASK1* gene is suggested to play a role in the control of homolog separation by degrading/removing a protein required for homolog association in meiosis I (Yang et al. 1999). Alternatively, a defect in cell cycle progression could also explain the *ask1* phenotype (Magnard et al. 2001). The *ASK1* gene is part of a large family with at least nine members in *A. thaliana* (Yang et al. 1999).

As a consequence of altered cell cycle progression during meiosis, the *tardy asynchronous meiosis* (*tam*) mutant forms dyads in male meiosis (Magnard et al. 2001). In this case, dyads are not caused by defects of meiotic reduction (as seen in the mutants described above) but are formed by cytokinesis occurring at the end of meiosis I. Normally, *A. thaliana*, like most dicots, has simultaneous cytokinesis at the end of the two nuclear divisions. *Tam* meiosis mimics monocot-type cytokinesis, with the formation of dyads after meiosis I. Interestingly, dyad formation is related to temperature conditions. Analysis of a *tam/quartet1* double mutant, in which the meiotic products are maintained together after callose wall degradation, showed that most of the dyads evolve into tetrads of four cells and produce functional pollen. The *TAM* gene maps to chromosome 1, between SSLP, nF22K20 and CAPS agp64, but has not yet been isolated. However, it is proposed that the TAM protein positively regulates cell cycle progression, perhaps promoting the G2/M transition, and that TAM has a role in coupling the normal pace of cell cycle progression with the synchrony of cell division during male meiosis.

A. thaliana mutants with nuclear restitution

The last step of meiosis is cytokinesis, the process during which the four haploid nuclei are partitioned by cell walls into four spores. As mentioned above, in *A. thaliana* both meiotic nuclear divisions are followed by a single cytokinesis event. The *stud* (*std*)/*tetraspore* (*tes*) mutant shows normal meiotic divisions but altered male meiosis cytokinesis (Hülskamp et al. 1997; Spielman et al. 1997). This mutation leads to reduced male fertility and to larger pollen than in the wild-type due to the tetranucleate coenocytic microspores. Four *tes* and three *std* mutant alleles have been isolated, and in all these mutants the post-meiotic development of microspore proceeds relatively normally. The nuclei are capable of independently undergoing the complete mitotic cell divisions leading to pollen grains with a variable number of sperm nuclei. Some microspore nuclei can fuse before the first mitotic division, leading to polyploid instead of the haploid sperm nuclei, giving rise to 3× and 4× progenies. However, polyploid eggs are excluded by the *tes* × 2× and *tes* × 4× crosses, which result in diploid or triploid progenies, respectively (Spielman et al. 1997). *TES* has been cloned (Yang et al. 2003) and is predicted to encode a kinesin. Kinesins perform cell division-related functions, including spindle stability and elongation, vesicle transport to the site of division, and chromosome movement. In the *tes* mutant, tubulin immunofluorescence has shown that radial microtubule systems (RMSs) are disorganised and are thus unable to mark the boundaries of the microspore cytoplasts and determine the planes of division (Yang et al. 2003). Therefore, it is suggested that TES protein is required for establishment or maintenance of the RMS involved in male cytokinesis.

Perspectives for using *A. thaliana* meiotic genes for plant breeding

In recent years there has been a considerable renewal of interest in studying mutations affecting the meiotic process and isolating the relevant genes. This area of research may be particularly valuable and pertinent in applying reproductive and/or chromosome manipulations to crop improvement. In particular, attention has focussed on 2*n*

gamete production and apomixis. The meiotic modifications involved in chromosome pairing, frequency and position of crossing over, and chromosome separation can serve to overcome incompatibility barriers in interspecific/interploidy crosses, synthesise highly heterozygous polyploids in plants that are strictly diploid, in F_1 hybrid production, and in clonal propagation of sexually reproducing species. Some practical advantages of diplospory and 2*n* gametes in relation to crop improvement are shown in Figs. 1 and 2.

Despite its great potential, use of diplospory and 2*n* gametes is hampered by the fact that (1) several crops, especially diploid species, do not have the meiotic pathways leading to 2*n* gamete formation or diplospory; (2) traditional breeding approaches to transfer these characters to a wide range of crops are difficult or impossible; and (3) inheritance of these traits (especially apomixis) is still controversial. Genetic engineering has been suggested as a more promising and direct route towards their exploitation (Koltunow et al. 1995; Grossniklaus et al. 1998).

The discovery of *A. thaliana* genes resembling apomixis events is ongoing, and it is likely that *A. thaliana* genes may be used in the future to transfer apomixis elements to crop plants. However, apomixis is a very complex aspect of plant reproduction. Its manipulation requires not only the identification of meiotic genes, but also the elucidation of several other aspects, such as those related to the genetic and molecular mechanisms involved in autonomous embryo and endosperm development. Particularly interesting in this context are mutations in

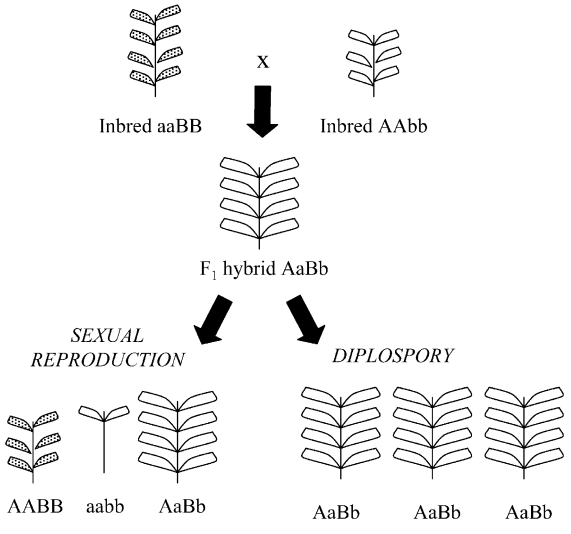

Fig. 1 Comparison of sexual reproduction and diplospory in the fixation of F_1 hybrid heterosis. The cross between two genetically different inbred lines produces heterozygous F_1 hybrids with increased size, yield, and vigour (heterotic effect). Sexual reproduction of F_1 hybrids produces a genetically heterogeneous progeny (three possible genotypes at two loci are shown), as a result of segregation and recombination. Thus, F_1 hybrid performances are lost. In contrast, diplosporic apomixis of F_1 hybrids avoids segregation and recombination, resulting in genetically homogeneous offspring, with fixed hybrid vigour and stable field performances

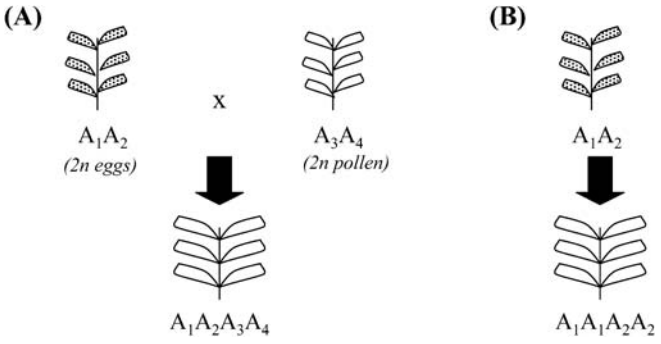

Fig. 2 Comparison of sexual polyploidisation (**A**) and somatic polyploidisation (**B**) in maximising allelic interactions. Heterozygosity is very important in polysomic polyploids, in that with maximum heterozygosity maximum heterosis for polygenic traits is expected. Heterozygosity refers to the possibility of more than two alleles per locus. **A** The cross between two heterozygous diploid parents producing 2*n* gametes gives tetrallelic loci ($A_1A_2A_3A_4$) and maximum heterozygosity, with six first order, four second order, and one third order interaction. In contrast, **B** transmits all the parental heterozygosity, but when a highly heterozygous diploid individual A_1A_2 is doubled, a balanced diallelic tetraploid is formed, which has only one first order interaction, as its progenitor

any of the three genes, *FIS1*/ *MEA*, *FIS2* and *FIS3*/*FIE*, that allow partial endosperm development to occur in the absence of fertilisation (Ohad et al. 1996, 1999; Grossniklaus and Schneitz 1998; Kiyosue et al. 1999; Luo et al. 1999; Vielle-Calzada et al. 1999). As discussed above, *A. thaliana* meiotic genes (i.e. *SWI1/DYAD*), also have mutated phenotypes resembling elements of diplospory. A transfer of genes regulating synapsis and recombination, nuclear restitution, and cytokinesis in a single genotype may induce synthetic diplosporic apomixis in a sexual crop species. Despite the fact that mutations in meiotic genes often cause spore degeneration, it is possible that a combination of different mutations may restore fertility. In a potato synaptic mutant, a second mutation leading to nuclear meiotic restitution in the second division ensures a symmetric incorporation of chromosomes in meiotic products (Johnston et al. 1986).

Some authors (Koltunow 1993; Grossniklaus 2001) have suggested that apomixis might rely more on a global deregulation of sexual reproductive development than on truly new functions, i.e. genes that normally function in sexual reproduction are ectopically and/or prematurely expressed in apomictic reproduction. A duplicate-gene asynchrony hypothesis was proposed on the evidence that apomictic species are generally polyploid or paleopolyploid and, therefore, possess duplicate genes for female development (Carman 1997). However, the fact that mutations in some genes, as discussed above, result in elements of apomixis demonstrated that the mutant-gene hypothesis must not be ruled out. The two hypotheses are not mutually exclusive, and thus the mutagenesis approach in *A. thaliana* is still crucial in allowing the genetic dissection of the meiotic process and the discovery of major genes involved in the diplosporic process.

To better utilise *A. thaliana* as a source of genes for diplospory, it may well be necessary to analyse not only

induced mutations but also natural genetic variants in the *Arabidopsis* genus and its close relatives. Besides the null mutant strategy, which is unable to identify essential single genes involved in reproductive processes, activation tagging mutagenesis can also be used to gain further insights.

The isolation of the genes involved in 2*n* gamete production and their transfer to crops of interest represents the possibility of synthesising new polyploids, performing interploidy crosses, and exploiting the positive genetic consequences associated to FDR and SDR 2*n* gametes. This approach can be hampered by the fact that the genes involved in 2*n* gamete production remain almost unknown, that their inheritance is sometimes controversial, and that the environmental influence on meiotic nuclear restitution is very strong. Mok and Peloquin (1975) suggested that the mutations responsible for 2*n* pollen formation in potato were mono-Mendelian and recessive. In contrast, in alfalfa, Tavoletti et al. (2000) suggested that this trait is under polygenic control. In particular, these authors found that three QTLs were associated to 2*n*, 3*n* and 4*n* pollen in the *jumbo pollen* mutant characterised by failure of post-meiotic cytokinesis.

Although 2*n* gamete formation can be considered a complex trait, we suggest that *A. thaliana* may provide a model system to identify mutated/deregulated genes resulting in phenotypes that mimic 2*n* gamete formation as a consequence of meiotic non-reduction or nuclear restitution. There is much evidence to support this hypothesis. In the *swi1* mutant, most macrospore mother cells exit meiosis with an unreduced chromosome number, and in the *tes* mutant cytokinesis fails and nuclear restitution gives rise to polyploid pollen grains. In addition, phenotypes showing dyads of 2*n* meiotic products are often observed in *A. thaliana* mutants as a consequence of various meiotic defects. Dyads, as terminal or intermediate phenotypes, occur after a meiotic arrest (*ms4*, *sap*) or when the timing of male meiosis progression is altered (*ask1*, *tam*). In the case of the *tam* mutant, delay and asynchrony in the male meiotic cycle cause ectopic cytokinesis at the end of meiosis I. In most cases, the dyads formed in the mutants are not viable. For this reason, as suggested above for diplospory engineering, a combination of different mutations may be required to restore gamete fertility.

We believe that new insights can be obtained in *A. thaliana* by isolating mutants altered in cytoskeleton dynamics during meiosis. In plant species where the cytoskeleton has been analysed during meiosis, there is evidence that a transient actin ring normally occurs at the end of meiosis I in the mid-zone of the male meiocyte, with the putative function of marking the future division plane (Genualdo et al. 1998). Therefore, it is likely that this transient event could become stable under certain conditions. If this is true, it may be relatively easy to obtain premature cytokinesis and SDR 2*n* gametes, as can occur in mutants of *Solanum*. In this genus, meiotic mutations responsible for 2*n* pollen are associated with cytoskeletal abnormalities mainly involving metaphase II spindles, interzonal microtubules and the cytokinetic apparatus (Conicella et al. 2003).

In addition to induced mutations, naturally occurring variations in *A. thaliana* and its relatives offer the possibility of identifying new genes/alleles involved in 2*n* gamete formation. Indeed, *A. thaliana* has been demonstrated to be a paleopolyploid species with many duplicated loci resulting from two polyploidisation events (*Arabidopsis* Genome Initiative 2000). The tools for this evolutionary pathway may well have been 2*n* gametes. Evidence for 2*n* gametes in *A. thaliana* may come from the interspecific hybridisation between *Arabidopsis arenosa* (2*n*=4×=32) and the diploid *A. thaliana* (2*n*=2×=10) that originated the tetraploid *A. suecica* (2*n*=4×=26). Natural *Arabidopsis suecica* lines are likely to be formed through pollination of *Arabidopsis arenaria* with 2*n* gametes from *A. thaliana* (Lee and Chen 2001).

Conclusions

Meiosis is a fascinating phenomenon with intriguing practical potential. Given the high frequency with which new meiotic mutants are being isolated in *A. thaliana*, this plant probably offers the best model system for studies aimed at elucidating the molecular mechanisms involved in meiosis. Among meiotic modifications, those accounting for diplospory and 2*n* gamete formation are particularly important from a practical standpoint, i.e. plant breeding. Although apomixis and 2*n* gametes can be complex characters, *A. thaliana* will help in understanding these phenomena not only through the analysis of meiotic mutations resembling them, but also through other strategies based on natural variation and polyploidy exploitation.

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