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Mechanisms and prospects

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REVIEW PAPER

Unreduced gamete formation in plants: mechanisms and prospects

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Abstract

Polyploids, organisms with more than two sets of chromosomes, are widespread in flowering plants, including many important crop species. Increases in ploidy level are believed to arise commonly through the production of gametes that have not had their ploidy level reduced during meiosis. Although there have been cytological descriptions of unreduced gamete formation in a number of plants, until recently none of the underlying genes or molecular mechanisms involved in unreduced gamete production have been described. The recent discovery of several genes in which mutations give rise to a high frequency of unreduced gametes in the model plant Arabidopsis thaliana opens the door to the elucidation of this important event and its manipulation in crop species. Here this recent progress is reviewed and the identified genes and the mechanism by which the loss of protein function leads to the formation of unreduced gametes are discussed. The potential to use the knowledge gained from Arabidopsis mutants to design tools and develop techniques to engineer unreduced gamete production in important crop species for use in plant breeding is also discussed.

Key words: *Arabidopsis*, meiosis, plant breeding, polyploid, unreduced gamete.

Introduction

Polyploidy, the presence of more than two sets of chromosomes, is an important and widespread phenomenon across numerous eukaryotic taxa, with yeasts, insects, amphibians, reptiles, and fish all containing polyploid members. In flowering plants, polyploidy is especially widespread and is believed to be a major mechanism of adaptation and speciation. It is estimated that up to 70% of angiosperm species are polyploid, and this number is even higher if ancient polyploidization events are taken into account (for reviews, see Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998; Otto and Whitton, 2000; Adams and Wendel, 2005; Otto, 2007). Not only are polyploid plants common in natural ecosystems, but many important crop species including potato, coffee, banana, peanut, tobacco, wheat, oats, sugarcane, and many fruits are also polyploid (Bretagnolle and Thompson, 1995; Stebbins, 1950; Udall and Wendel, 2006). Despite the ecological and agricultural significance of polyploid plants, the molecular mechanisms underlying their formation and adaptation are as yet poorly understood. In natural systems, polyploids are believed to arise commonly through the production of gametes that have not had the somatic chromosome number reduced, and are hence termed unreduced gametes.

Unreduced gametes most commonly arise through meiotic defects. Meiosis is a specialized cell division that is essential for sexual reproduction. It involves a single round of DNA replication followed by two rounds of chromosome division to produce cells with half the chromosome number of the mother cell (Fig. 1A). During meiotic prophase I the meiosis-specific events of pairing and recombination between homologous chromosomes occur. These processes are important not only for generating genetic variability in the

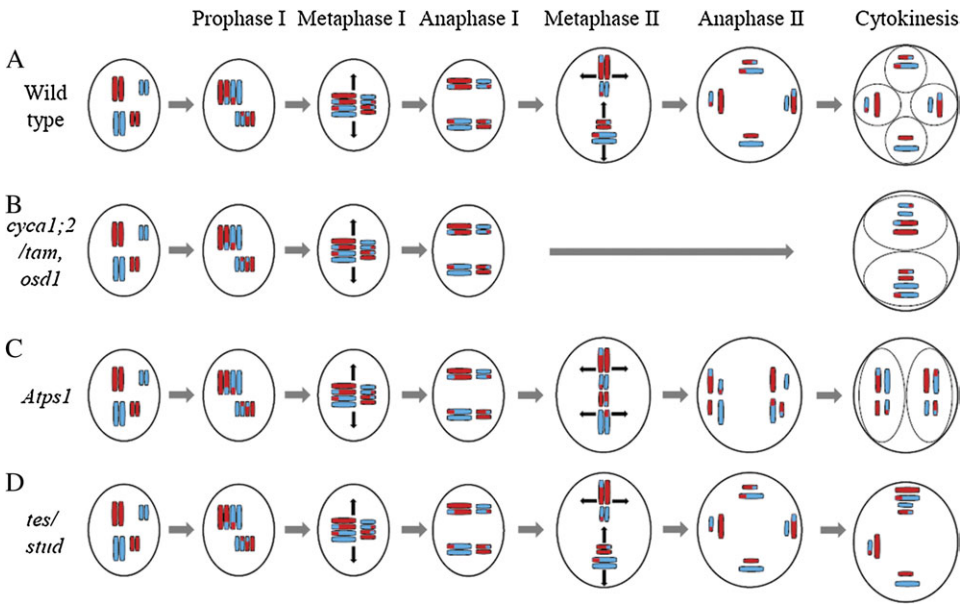

Fig. 1. Schematic diagram of male meiosis in the wild type and mutants that produce a high frequency of unreduced gametes in *Arabidopsis*. (A) Wild-type meiosis. A diploid cell containing two sets of homologous chromosomes (shown as large and small) completes S-phase to produce sister chromatids (connected on the left-hand side). During prophase I the homologous chromosomes pair and recombine, exchanging genetic information in the chromosome arms. At metaphase I, bivalent structures align and homologous chromosomes are separated at anaphase I. At metaphase II the two groups of sister chromatids align on two perpendicular metaphase II plates. Sister chromatids are separated during anaphase II to give four groups of well separated chromosomes. Cytokinesis then occurs, producing a tetrad of four haploid cells. (B) In the *cyca1;2/tam* and *osd1* mutants, the second division does not occur, leading to a dyad of diploid cells containing sister chromosomes at the completion of cytokinesis. (C) In the *Atps1* mutant, the orientation and positioning of the spindles in meiosis II are disturbed, often being parallel (shown) or fused. This results in chromosomes that were separated in the first division being in close physical proximity at the completion of anaphase II and subsequently being contained in a single cell, producing a dyad of diploid cells containing non-sister chromosomes. (D) Cytokinesis is disturbed in the *tes/stud* mutant, resulting in multiple nuclei in a common cytoplasm, some of which can fuse during development.

offspring but also for establishing the attachments between chromosomes required for the subsequent divisions. In the first meiotic division, meiosis I, the homologous chromosomes are separated in what is referred to as a reductional division. Meiosis II resembles mitosis in that it involves the separation of sister chromatids and is referred to as an equational division. In plants, meiotic cytokinesis is either successive, occurring after each round of chromosome separation, or simultaneous, occurring only after the completion of the second chromosome separation. Successive cytokinesis is found in male meiocytes of many monocots and female meiocytes in monocots and some dicots, while simultaneous cytokinesis occurs in most male and some female meiocytes in the dicots. The product of male meiosis in plants is a tetrad of four haploid microspores that are temporarily joined by a callosic wall. After release from the tetrad each microspore undergoes two mitotic divisions to produce a pollen grain containing the two sperm cells required for double fertilization (McCormick, 2004). In most angiosperms, female meiosis produces a linear array of meiotic products, three of which degenerate while the fourth develops into the seven-celled female gametophyte containing the egg and central cells awaiting fertilization (Yang *et al.*, 2010).

While disruption to the meiotic programme often has severe effects and leads to the abortion of the meiocytes or the developing gametophytes and thus sterility, a number of meiotic mutants that produce viable, unreduced gametes have been described in a range of plants (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). Such meiotic defects include the omission of the first or second meiotic division, abnormal spindle morphology in the second division, or disturbed cytokinesis (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). While some cytological descriptions of the mutants have been made, the underlying genes have not been identified.

Many of the recent advances in elucidating the molecular mechanisms involved in plant meiosis have focused on the model dicot plant *Arabidopsis thaliana*. This has been aided by advances in cytological procedures for *Arabidopsis* despite its small chromosome size (Ross *et al.*, 1996, 1997; Caryl *et al.*, 2003) and development of molecular tools enabling both forward and reverse genetics approaches to the identification of meiotic mutants (Mercier and Grelon, 2008). The first genes in which mutations result in the production of viable, unreduced gametes were recently identified in *Arabidopsis* (Ravi *et al.*, 2008; d'Erfurth *et al.*, 2008, 2009, 2010; Erilova *et al.*, 2009; Wang *et al.*, 2010)

and these mutants provide the basis for this review. The cytological events that give rise to unreduced gametes and the role the identified proteins play are described, and the potential of using unreduced gametes for plant breeding and crop improvement is discussed. For reviews on other meiotic processes, especially those involving the events of pairing and recombination, the reader is referred to the recent literature (Petronczki *et al.*, 2003; Ma, 2006; Liu and Qu, 2008; Mercier and Grelon, 2008).

Unreduced gamete production in Arabidopsis

Defects in early meiotic events

Meiotic prophase I is characterized by chromosome cohesion, pairing, and recombination (Ma, 2006). At the end of meiotic prophase I in wild-type diploid *Arabidopsis* five bivalents are present. These bivalents consist of highly condensed paired homologous chromosomes joined at chiasmata, which are the physical sites of crossover between homologous chromosomes and are only established if pairing and recombination occur normally. There are many mutations affecting meiotic prophase I that often result in 10 univalents (paired sister chromatids) rather than bivalents (Ross *et al.*, 1997; Bai *et al.*, 1999; Bhatt *et al.*, 1999; Couteau *et al.*, 1999; Caryl *et al.*, 2000; Grelon *et al.*, 2001; De Muyt *et al.*, 2009). This can result in either a random unbalanced segregation of univalents in meiosis I followed by an equal second division, or, if sister chromatid cohesion is also lost prematurely, separation of sister chromatids in meiosis I followed by either a halt in meiotic progression or an unequal second division. In most cases the cells produced are aneuploid and abort during development. However, in some mutants, a small number of functional gametes are produced and there is a low level of seed set (Couteau *et al.*, 1999; Azumi *et al.*, 2002; Ravi *et al.*, 2008).

One early meiotic mutant that produces a low level of viable seeds is *dyad* (Ravi *et al.*, 2008). The *dyad* allele is one of several mutations in the *SWITCH1* (*SWI1*)/*DYAD* gene, some of which result in defects only in female meiosis (*swi1-1* and *dyad*; Motamayor *et al.*, 2000; Siddiqi *et al.*, 2000), while others display defects in both female and male meiosis (*swi1-2*; Mercier *et al.*, 2001, 2003). The SWI1/DYAD protein is required in prophase I where it has roles in sister chromatid cohesion and recombination (Mercier *et al.*, 2001, 2003; Agashe *et al.*, 2002; Boateng *et al.*, 2008). A lack of SWI1/DYAD can result in an equational division involving the separation of sister chromatids at meiosis I and no further progression in female meiosis (Mercier *et al.*, 2001; Agashe *et al.*, 2002). While most female gametophytes are not functional in *dyad*, a small number of viable female gametes are produced with typically 1–10 seeds produced per *dyad* plant (Ravi *et al.*, 2008). Interestingly, ~60% of these seeds are triploid and result from the fertilization of an unreduced (diploid) female gamete by a reduced (haploid) male gamete. Thus, the *dyad* allele of the *SWI1*/*DYAD* gene produces unreduced female gametes at low frequency, with each unreduced female gamete containing non-sister chromosomes due to the separation of sister chromatids in the single division.

Cell cycle defects

Progress through the cell cycle, mitotic or meiotic, relies upon cyclin-dependent kinase (CDK) activity. In *Arabidopsis*, CDKA;1 appears to be the main kinase involved in both mitotic and meiotic progression, as knockout mutants are embryo lethal while a weak *cdka;1* allele displays meiotic defects (Dissmeyer *et al.*, 2007). Interestingly, mitosis is not disturbed in plants containing the weak *cdka;1* allele, suggesting that a higher level of CDKA;1 activity is required for meiosis than for mitosis. The level of CDK activity and thus the rate of cell cycle progression is controlled by a number of binding proteins that either promote or inhibit CDK activity (Inzé and De Veylder, 2006; Francis, 2007). Cyclins are one of the main activators that bind to, activate, and provide substrate specificity to the CDKs. *Arabidopsis* contains up to 50 putative cyclins from 10 different groups (Wang *et al.*, 2004), and members of different cyclin groups interact with CDKs at different parts of the cell cycle to promote specific stages in mitosis and meiosis (Menges *et al.*, 2005; Inzé and De Veylder, 2006; Francis, 2007).

One of the key differences between the mitotic and meiotic cell cycles is that in meiosis there are two rounds of chromosome separation without any intervening DNA replication. This is likely to require fine adjustment of the cell cycle machinery. Entry into mitosis or meiosis (the G_2/M transition) requires a high level of CDK activity, which in *Arabidopsis* is achieved by cyclins of the A and B groups (Menges *et al.*, 2005; Inzé and De Veylder, 2006; Francis, 2007). Once chromosomes are correctly oriented on the metaphase spindle the E3 ubiquitin ligase activity of the anaphase-promoting complex (APC) becomes active and targets the mitotic cyclins for proteolytic degradation. This switch from high to low CDK activity is essential for coordinating chromosome movement and also for exit from the mitotic programme and subsequent entry into G_1 and S phases. As the meiotic programme requires exit from meiosis I and then entry into meiosis II without intervening DNA synthesis, the level of CDK activity must be reduced without becoming too low to enable exit of meiosis I without promoting entry into S phase. As the meiotic cell cycle requires such tight control, even relatively small modifications of the cell cycle during meiosis I or II may offer the potential to create diploid gametes.

Accordingly, two proteins that are required for meiotic cell cycle progression and in which mutations lead to the production of viable unreduced gametes have recently been identified in *Arabidopsis* (Fig. 1B). One is CYCA1;2, a member of the cyclin A family that is also known as TAM (TARDY ASYNCHRONOUS MEIOSIS; Magnard *et al.*, 2001; Wang *et al.*, 2004, 2010; d'Erfurth *et al.*, 2010). In plants homozygous for null alleles of *CYCA1;2*/*TAM*,

the majority of male meiocytes and ~30% of female meiocytes complete the first meiotic division but fail to enter meiosis II and thus produce a dyad of two diploid cells rather than a tetrad of four haploid cells (Fig. 1B; d'Erfurth *et al.*, 2010; Wang *et al.*, 2010). As meiosis II does not occur in *CYCA1;2/TAM*, sister chromatids are not separated so each diploid cell is predicted to contain sister chromatids. The unreduced gametes are functional, giving rise to polyploid progeny (d'Erfurth *et al.*, 2010; Wang *et al.*, 2010). A single amino acid substitution in CYCA1;2/TAM (*tam-1*; threonine to isoleucine at position 283) results in a partially active protein which is temperature sensitive, causing a delay in cell cycle progression in male meiocytes rather than a complete failure, such that haploid gametes are still produced (Magnard *et al.*, 2001; Wang *et al.*, 2004). These mutants show that the meiosis I to meiosis II transition relies upon CYCA1;2/TAM, which presumably activates CDKA;1 for meiosis II entry. Interestingly, none of the other *Arabidopsis* cyclin A proteins can compensate for CYCA1;2/TAM. Whether this is due to specific expression of *CYCA1;2* at this stage or due to a specialized function is as yet unclear. Control of meiotic progress by specific sets of cyclins may be a general phenomenon, as in mammals one of the two A-type cyclins, cyclin A1, appears to have a specific role in male meiosis, with mutant meiocytes failing to progress after late prophase (Wolgemuth and Roberts, 2010).

The second identified protein required for meiosis II entry in *Arabidopsis* is OMISSION OF SECOND DIVISION 1 (OSD1; d'Erfurth *et al.*, 2009). Like meiocytes lacking CYCA1;2/TAM, *osd1* mutants complete the first meiotic division and then fail to enter the second meiotic division and produce dyads rather than tetrads (Fig. 1B). In *osd1* plants there is a high proportion of unreduced gametes produced in both male (100%) and female (85%) meiosis and, as in *cycA1;2/tam*, these gametes are viable and produce polyploid offspring. OSD1 is also known as UVI4-LIKE due to its similarity to UVI4 that is believed to have a role in maintenance of the mitotic state (Hase *et al.*, 2006). OSD1 and UVI4 are plant-specific proteins without any obvious conserved domains of known function, and the precise role of OSD1 in meiotic progression is unknown but it is likely to have a role in directly or indirectly modifying CDK activity.

It may be expected that a double mutant for both *cyca1;2/tam* and *osd1* would display a similar phenotype to the two single mutants; failure to enter the second meiotic division. While this prediction holds true for female meiosis, this is surprisingly not the case for male meiosis. Male meiocytes lacking both CYCA1;2/TAM and OSD1 fail to enter the first meiotic division (d'Erfurth *et al.*, 2010). It has been proposed that OSD1 may inhibit the activity of the APC, which would promote CDK activity (d'Erfurth *et al.*, 2009), while CYCA1;2/TAM might directly modulate CDKA activity (Wang *et al.*, 2004; d'Erfurth *et al.*, 2010). The difference between the single and double mutants may therefore relate to the degree to which CDK activity is affected. The meiosis I to meiosis II transition may be easily disturbed due to the fine degree of regulation of CDK activity required to ensure exit from meiosis I and the subsequent entry into meiosis II. Thus a moderate decrease in CDK activity due to the loss of either CYCA1;2/TAM or OSD1 may prevent entry into meiosis II without impairing the prophase to meiosis I transition. However, loss of both CYCA1;2/TAM and OSD1 may cause a loss of CDK activity sufficient to impair entry into meiosis I. These differences highlight the essential and tight regulation of CDK activity required during meiosis.

Nuclear restitution by defects in spindle orientation

Another route that can give rise to functional unreduced gametes in plants is nuclear restitution through the regrouping of chromosomes in the second division that had previously been separated in meiosis I. One mechanism that has frequently been observed in species other than *Arabidopsis* that results in such restitution relates to the orientation of the two meiotic spindles during the second meiotic division (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). In meiocytes that undergo simultaneous cytokinesis the sets of chromosomes separated at the first division remain in a common cytoplasm throughout the second division. Thus, in the second division, the organization and orientation of the two spindles must be tightly coordinated to prevent interference or interaction between the two spindles. Male meiosis in *Arabidopsis* involves simultaneous cytokinesis and the two spindles are generally arranged perpendicular to each other and are physically separated (Fig. 1A; d'Erfurth *et al.*, 2008). Such organization ensures that the four chromosome groups are physically separated at the end of the second meiotic division and subsequently each separated group is contained within a single cell. This spindle arrangement in *Arabidopsis* produces tetrads with a characteristic tetrahedral shape.

Disruption of this spindle orientation in meiosis II causes nuclear restitution in the *Arabidopsis parallel spindle1* (*Atps1*) mutant (d'Erfurth *et al.*, 2008). In *Atps1* the products of male meiosis are a mix of dyads and triads (containing two haploid and one diploid cell) as well as some tetrads. Analysis of microtubules during meiosis II revealed the presence of parallel, fused, and tripolar spindles (d'Erfurth *et al.*, 2008). Such arrangements mean that chromosomes associated with different spindles are in close physical proximity at the end of anaphase II and are subsequently contained within one cell during cytokinesis (Fig. 1C). As this mechanism involves the regrouping of chromosomes separated in the first division, each diploid cell is predicted to contain non-sister chromosomes. Female meiosis is not notably altered in *Atps1* mutants, with all female gametes being reduced. The difference between male and female meiosis in *Atps1* is likely to relate to the three-dimensional organization of the meiotic products. In *Arabidopsis* the male meiotic product is generally tetrahedral shaped, whereas female meiosis produces a linear or multiplanar array of meiotic products (Schneitz *et al.*, 1995).

Thus an alteration in spindle orientation may not significantly alter the final meiotic products of *Arabidopsis* female meiosis.

AtPS1 encodes a protein that is highly conserved in the plant kingdom, but is not present in yeast or animals (d'Erfurth *et al.*, 2008). AtPS1 contains an N-terminal fork head-associated (FHA) domain that could be involved in protein–protein interactions (Durocher and Jackson, 2002) and its C-terminal region shows similarity with PINc domains that bind RNA (Clissold and Ponting, 2000). The functional role of AtPS1 during meiosis II, and more specifically how its loss impacts spindle orientation, is as yet unknown. Interestingly, AtPS1 homologues are found throughout the plant kingdom including monocots and other species that have successive rather than simultaneous cytokinesis. It may be that the orientation of the meiosis II spindle also needs to be controlled in species with simultaneous cytokinesis to ensure that the sister chromatids can be adequately separated and that the plane of the subsequent second cytokinesis is correctly oriented in relation to the chromosome groups or perhaps to an, as yet unknown, predetermined plane of the second division.

Similar to *Atps1*, the *jason* mutant in *Arabidopsis* also produces a mixture of dyads, triads, and tetrads at the end of meiosis and subsequently a high frequency of viable unreduced male gametes, while all female gametes are haploid (Erilova *et al.*, 2009). JASON is also a plant-specific protein that lacks any known functional motifs. The role of JASON in male meiosis and the mechanism by which mutations in *JASON* result in unreduced gametes are yet to be fully determined, although it involves a meiotic defect.

Nuclear restitution by defects in cytokinesis

Defects in cytokinesis after normal nuclear divisions can also lead to nuclear restitution and the formation of unreduced gametes. The cytokinetic mechanism in plant meiocytes differs from the bulk of plant cytokinetic events in that the plane of division is not marked before nuclear division by the pre-prophase band of microtubules (Brown and Lemmon, 1988; 2001). Rather meiotic cytokinesis involves a radial microtubule system (RMS), in which microtubules are rearranged from the spindle to form a ball-like structure (the RMS) around each newly forming nucleus. This RMS defines a cytoplasmic domain for each new cell and the new wall is deposited centripetally from the periphery of the meiocyte along the planes marked by interaction of microtubules from opposing RMSs (Brown and Lemmon 1988; 2001). Disturbances in this process can lead to multiple nuclei being present in a single cell.

Male meiotic cytokinesis is disturbed and unreduced male gametes are produced in the *tetraspore* (*tes*)/*stud* mutant in *Arabidopsis* (Spielman *et al.*, 1997; Hülskamp *et al.*, 1997; Yang *et al.*, 2003). The TES/STUD protein is a predicted kinesin with homology to the tobacco NACK proteins that positively regulate cell plate expansion via phosphorylation of mitogen-activated protein (MAP) kinase cascade components in sporophytic tissues (Nishihama *et al.*, 2002; Takahashi *et al.*, 2004). In *tes*/*stud* mutants, the microtubules are disorganized after the second meiotic division as the RMS is not correctly formed and, subsequently, cytokinesis does not occur (Yang *et al.*, 2003). This results in four nuclei in a common cytoplasm and some of these nuclei fuse before the first mitotic division (Fig. 1D). Interestingly, the mitotic divisions occur normally in the common cytoplasm in *tes*/*stud* mutants during pollen development, including an asymmetric first division, leading to the formation of some functional sperm cells that are capable of fertilization. Due to the post-meiotic fusion of nuclei, *tes*/*stud* pollen grains contain nuclei that are diploid, triploid, or even tetraploid, producing offspring of different ploidy levels (Spielman *et al.*, 1997). Female cytokinesis following meiosis appears to be undisturbed in *tes*/*stud* (Spielman *et al.*, 1997), which could be related to the redundant nature of TES/STUD with its closest homologue in *Arabidopsis*, HINKEL (Tanaka *et al.*, 2004). The genetics of the polyploid offspring of *tes*/*stud* have not been determined, but as nuclear fusion events do not appear to be specific to nuclei containing sister or non-sister chromatids it is likely that the polyploid offspring contain either sister or non-sister chromatids from the male parent.

Using unreduced gametes for plant breeding

Mutants that produce unreduced gametes in crop plants have been exploited by plant breeders to engineer sexual polyploidization in a number of species (Ramanna and Jacobsen, 2003; Consiglio *et al.*, 2004). In particular unreduced gametes have proved useful in enabling crosses between plants of different ploidy levels which often fail due to unbalanced parental contributions in the developing seed (Barcaccia *et al.*, 2003; Carputo *et al.*, 2003; Köhler *et al.*, 2010). If the plant of the lower ploidy level can be induced to produce unreduced gametes, such limitations can be rapidly overcome, and such strategies have been successful. For example, in potato, the use of unreduced gametes has enabled the transfer of biotic stress resistance from the diploid species *Solanum vernei*, *Solanum tarijense*, and *Solanum chacoense* to tetraploid cultivated species (Ortiz *et al.*, 1997; Carputo *et al.*, 2000; Capo *et al.*, 2002). Unreduced gametes have also been used to introduce lower cyanide content and disease and pest resistance in *Manihot* (Ogburia *et al.*, 2002) and in alfalfa breeding (Barcaccia *et al.*, 2003). Unreduced gametes could also be used in the generation of new polyploid species, either autopolyploids, where both chromosomes derive from a single species, or allopolyploids, where the chromosomes sets derive from different species. The creation of plants with higher ploidy levels could be of immeasurable breeding value in certain crop species because of the potential enhancement of genetic diversity and heterosis (Ogburia *et al.*, 2002; Ramanna and Jacobsen, 2003; Consiglio *et al.*, 2004).

To date, plant breeders have relied on the presence of mutations that produce unreduced gametes being present in their breeding stock. Depending on the mutation, the

number of unreduced gametes can be quite low and variable. Unreduced gametes can also be generated by treating plants with chemicals such as colchicine that disturb mitosis in somatic tissues, resulting in the formation of polyploid sectors. Floral tissues generated from these sectors will produce unreduced gametes. However, such processes are labour intensive, time-consuming, and often inefficient (Sato *et al.*, 2005), and polyploids created through somatic doubling often display greater variability, and lower fitness and heterozygosity than those produced by sexual polyploidization through mutations leading to unreduced gamete formation. For example, alfalfa sexual polyploids are more productive than somatic ones (McCoy and Rowe, 1986).

Using the knowledge of the molecular mechanisms underlying unreduced gamete formation and the genes involved in the model plant *Arabidopsis*, it is now possible to develop strategies to induce unreduced gamete formation in desirable crop species through the targeted knockdown of specific proteins. Techniques that involve knockdown of RNA levels, such as RNA interference (RNAi), virus-induced gene silencing (VIGS; Kusaba, 2004; Robertson, 2004; Galun, 2005; Angaji *et al.*, 2010), or mutagenesis of the encoding gene by techniques such as site-directed mutagenesis through zinc finger nucleases (Townsend *et al.*, 2009; Shukla *et al.*, 2009) or tilling (Stemple, 2004; Barkley and Wang, 2008), could be used to knock down the level of the selected protein. Depending on the breeding aim, different mutants can be employed. Mutants such as *Atps1* and *jason* (d'Erfurth *et al.*, 2008; Erilova *et al.*, 2009) only affect male meiosis, while others such as *osd1* and *cyca1;2/tam* (d'Erfurth *et al.*, 2009, 2010; Wang *et al.*, 2010) affect both male and female meiosis and so could be used to generate tetraploid plants directly. Another consideration concerns differences in meiotic programmes in different plant species. Meiotic cytokinesis varies from being successive in some plants to simultaneous in others. These differences are likely to influence nuclear restitution mechanisms. In the *Atps1* mutant, nuclear restitution occurs due to the regrouping of homologous chromosomes that were separated in the first meiotic division but have remained in a common cytoplasm as *Arabidopsis* male meiosis displays simultaneous cytokinesis. In male meiosis of many monocot species successive cytokinesis occurs. This most probably prevents nuclear restitution occurring through an *Atps1*-like mechanism as the two meiosis II spindles are not contained in a common cytoplasm and thus the homologous chromosomes cannot be regrouped.

Restitution mechanisms

Another consideration is the genetic outcome due to the mechanism of unreduced gamete formation. The two chromosomes in unreduced gametes can be either non-sister chromatids, which is referred to as first division restitution (FDR), as it is equivalent to the first division not occurring, or sister chromatids, which is referred to as second division restitution (SDR), as it is equivalent to the second division not occurring. With FDR the non-sister chromatids are heterozygous from the centromere to the first crossover point, and hence the gametes retain much of the heterozygosity of the parent. With SDR the two sister chromatids are homozygous between the centromere and the first crossover point, and the resultant gametes have reduced levels of heterozygosity compared with the parent (Bretagnolle and Thompson, 1995). Therefore, the choice of the mechanism to produce unreduced gametes may depend upon the desired outcome. If a high level of heterozygosity is desired, for example in the generation of a new hybrid species, then a mechanism that provides FDR should be used. In alfalfa breeding it has been shown that 2*n* gametes of the FDR type are more advantageous than those of the SDR type for transferring parental heterozygosity and retaining epistatic interactions (Barcaccia *et al.*, 2003). If heterozygosity needs to be minimized, then an SDR mechanism should be used.

As the diploid gametes produced by both *cycA1;2/tam* and *osd1* mutants result from failure to enter the second meiotic division, an SDR mechanism, each diploid cell is expected to contain sister chromatids (Fig. 1B; d'Erfurth *et al.*, 2009, 2010). The regrouping in the second division of chromosomes separated in the first division in the *Atps1* mutant is predicted to be an FDR mechanism and, as such, gametes are predicted to contain non-sister chromatids. Two techniques have been developed in *Arabidopsis* to verify such predictions and confirm if an FDR or SDR mechanism has occurred. The first involves using heterozygous molecular markers. There is a range of *Arabidopsis* accessions, and alleles of *cycA1;2/tam, osd1* and *Atps1* are present in different accessions. Crosses between these plants carrying different alleles produce plants containing both mutant alleles of *cycA1;2/tam, osd1* or *Atps1* (with the mutant phenotype) but with differences in the DNA sequence between the homologous chromosomes. Mutant gametes can then be used to fertilize a wild-type third accession and molecular marker analysis used on triploid progeny (derived from a diploid gamete) to determine if diploid gametes contain the marker for one or both accessions. The presence of only one marker indicates that the diploid gamete contained sister chromatids (homozygous for the DNA region), whereas the presence of both markers indicates that the diploid gamete contained non-sister chromatids (heterozygous for the DNA region). Such marker analysis must be conducted close to the centromeres to avoid segregation of markers through recombination. Consistent with the prediction, marker analysis for both *cycA1;2/tam* and *osd1* revealed that unreduced gametes contain sister chromatids and that *Atps1* unreduced gametes contain non-sister chromatids (d'Erfurth *et al.*, 2008, 2009, 2010).

The second method utilizes the fluorescent-tagged lines (FTLs) tetrad system in *Arabidopsis* developed to provide genetic information of pollen grains (Berchowitz and Copenhaver, 2008). The FTL system is a visual system based on reporter constructs encoding fluorescent proteins located in different regions of the chromosomes and the

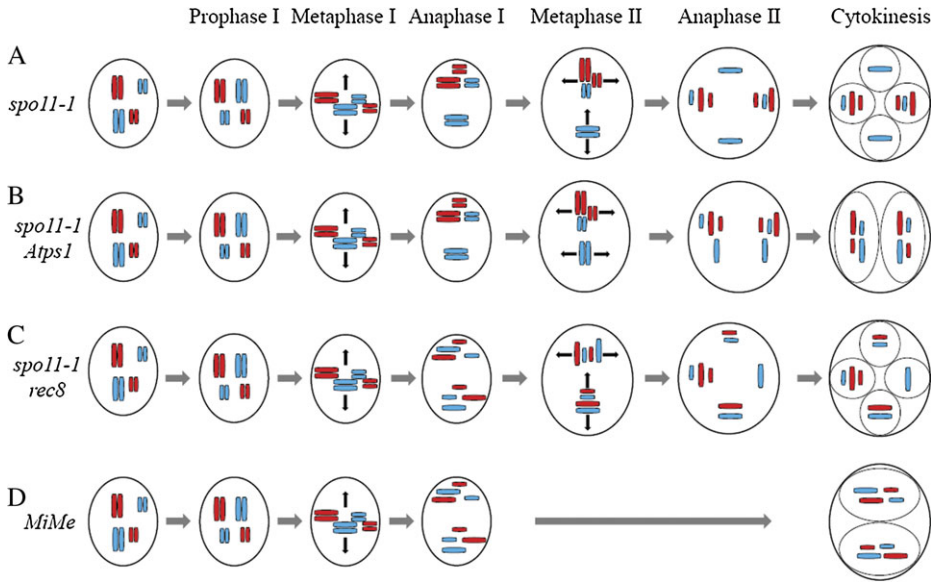

Fig. 2. Combinations of meiotic mutants can produce unreduced gametes in the absence of recombination. (A) In the absence of SPO11-1, pairing and recombination do not occur, leading to univalents at the end of prophase I. Random segregation of univalents at meiosis I leads to unbalanced meiotic products after meiosis II. (B) Combining the *spo11-1* mutation with the *Atps1* mutation leads to balanced dyad formation, as chromosomes separated in the first division are regrouped in the second division. (C) In *spo11-1 rec8* double mutants, univalents are present at the end of prophase I and sister chromatids separate in the first division, producing a mitosis-like division. Random segregation in the second division leads to unbalanced products. (D) In *MiMe* mutants, *spo11-1 rec8* are combined with either *cyca1;2/tam* or *osd1*, which prevents the second division occurring, producing viable diploid gametes from a mitosis-like division.

quartet (*qrt*) mutant in which the male meiotic products remain physically associated throughout pollen development (Preuss *et al.*, 1994; Copenhaver *et al.*, 2000). These reporter constructs and the *qrt* mutation can be introduced into a mutant background such that plants are heterozygous for one or two reporter constructs, enabling sister chromatids (both carrying the same reporter) to be distinguished from non-sister chromatids (carrying different reporters) based on the fluorescence of pollen grains. As with the polymorphic molecular markers, the reporter constructs need to be located close to centromeric regions of the chromosomes to avoid analysing regions that have undergone recombination. Such analysis has confirmed that sister chromatids are present in both *cycA1;2/tam* and *osd1* unreduced gametes (d'Erfurth *et al.*, 2009, 2010).

Controlling recombination—the complete design

The mutants discussed are all homozygous or heterozygous only from the centromere to the first crossover point due to recombination. However, for breeding purposes, gametes with complete homozygosity or heterozygosity in unreduced meiotic products are most desirable. This can be achieved by employing mutants that prevent recombination. In most cases such mutations lead to unbalanced products that are not viable. However, if combined with FDR or SDR mutants they produce viable offspring. The *spo11-1* mutant fails to make double-stranded breaks, preventing recombination, and forms univalents that segregate randomly during meiosis I (Fig. 2A; Grelon *et al.*, 2001), leading to the formation of unbalanced products after the second meiotic division. However, if the *spo11-1* mutation is combined with the *Atps1* mutation, where the products of the first division are regrouped in the second division, balanced dyads are formed as each dyad contains one of the sister chromatids separated in the second division (Fig. 2B; d'Erfurth *et al.*, 2008). Unreduced gametes formed by the *spo11-1 Atps1* double mutant should retain the full level of heterozygosity observed in the parent plant. The same result can be achieved by combining SDR mutants with mutations in *rec8* and *spo11-1*. The *rec8* mutant loses sister chromatid cohesion prematurely (Chelysheva *et al.*, 2005) and, when combined with the *spo11-1* mutant, meiocytes undergo a mitosis-like division in meiosis I with univalents forming and sister chromatids being drawn to opposite poles (Fig. 2C; Chelysheva *et al.*, 2005). The second division then produces unbalanced products. If the *spo11-1 rec8* double mutant is combined with either *cyca1;2/tam* or *osd1* the second division does not occur and meiosis is replaced with a mitotic-like division (Fig. 2D; d'Erfurth *et al.*, 2009, 2010). Such triple mutants have been named *MiMe-1* (*osd1*) and *MiMe-2* (*cyca1;2/tam*) for mitosis instead of meiosis. Again, the result is an unreduced gamete containing non-sister chromatids that have not undergone recombination and so maintain parental heterozygosity (d'Erfurth *et al.*, 2009, 2010). Currently there are no mutant combinations that produce unreduced gametes containing sister chromatids that have not undergone recombination and so are homozygous at all alleles, although there is desire to create such gametes to enable the genetic outcome of crosses to be

predictable and consistent in the production of polyploid F_1 hybrids. One approach to generate predictable F_1 hybrids has classically been achieved by double haploid production, a labour-intensive process involving the culturing of gametophytes followed by somatic doubling (Maluszynski *et al.*, 2003). The production of double haploids has been greatly advanced by a recent technology that allows the production of haploids by manipulating the centromere-specific histone CENH3. Mutant chromosomes are eliminated in the zygote, producing haploid progeny that can be spontaneously converted into fertile diploids through meiotic non-reduction, allowing their genotype to be perpetuated (Ravi and Chan, 2010).

Conclusion

The recent discoveries of the underlying genetic mechanisms leading to the formation of unreduced gametes in *Arabidopsis* open an exciting avenue to translate this knowledge into practical benefits for plant breeding. Targeted manipulation of gamete ploidy and level of heterozygosity holds immense promises for plant breeding and crop improvement. With the available techniques of targeted gene manipulation, the generation of crops producing designed gametes is becoming a realistic vision. Furthermore, the newly gained knowledge on sexual polyploidization is an important cornerstone for our understanding of the evolution and speciation of flowering plants that is tightly interconnected with the recurrent phenomenon of polyploidization.

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