

Functional embryo sac formation in *Arabidopsis* without meiosis – one step towards asexual seed formation (apomixis) in crops?

The recent significant food shortages and growing concerns about global food security underscore the continuing need for breeders to develop seeds that deliver high yielding, disease and stress resistant plants suited to a range of soils and environmental conditions. Furthermore, economical breeding tools are required that enable the rapid combination of multiple traits and, if possible, the fixation of hybrid vigour. Most plants reproduce sexually. This process requires the formation of male and female gametes via meiosis and their subsequent fusion during fertilization to form a seed. Sexual reproduction is both a friend and a foe in plant breeding because it allows required traits and hybrid vigour to be combined in a plant following a cross, but also facilitates their separation in subsequent seed generations due to meiosis and recombination.

By contrast, apomixis is a remarkable reproductive process in which the meiotic events of gamete formation and also fertilization are bypassed, resulting in the production of asexual seeds in plants (figure 1). Seeds formed via apomixis thus retain the genotype of the mother plant, a feature that essentially results in clonal propagation via seed. If apomixis could be used as a technology or tool in plant breeding it would provide a tremendous opportunity to maintain hybrid vigour over successive seed generations, and also accelerate the breeding process by enabling the stable combination of many traits. Thus a workable apomixis technology, combined with crop improvement programs using a wider germplasm pool, may aid in the increase of food yields and also assist the improvement and development of more locally adapted plant varieties that cope with changing climate pressures (Koltunow *et al* 1995; Spillane *et al* 2001).

Apomixis is rare in food crops and mainly exists in non-agronomic plants. However, the stable introgression of apomixis to crops by breeding has failed to date. The identification of apomixis genes in natural apomictic species has also been hampered by their association with large genomic regions where recombination is often repressed (Ozias-Akins *et al* 1998). The major components of apomixis include the avoidance of meiosis during female gamete formation, the formation of an embryo and, in some species, endosperm in the absence of fertilization (figure 1). Determining the molecular identity of genes governing these events remains an important step toward the engineering of an apomictic program in crop plants.

Analysis of apomixis in *Hieracium*, a daisy like plant, has shown that sexual and apomictic gene expression programs share common elements once each of the key steps of apomixis are initiated (Tucker *et al* 2003). This, together with the data from other species has led to the suggestion that apomixis results from a deregulation of the sexual program (Koltunow and Grossniklaus 2003). If that is the case, another strategy for engineering an apomictic program in crops might be to switch the sexual pathway onto an apomictic one by changing the function of key genes involved in the sexual process. The recent work of Ravi *et al* (2008) has now provided evidence that it may indeed be possible to do this. They have shown that a mutation in one sexual gene in *Arabidopsis* can lead to the avoidance of meiosis in female gamete formation and production of viable, unreduced gametes.

The *Arabidopsis* gene *DYAD/SWITCH1 (SWI1)* is required for sister chromatid cohesion and centromere organization during meiosis. In *swi1* mutants, a single equational division of the female gamete progenitor cell called the megaspore mother cell occurs in place of normal female meiosis (figure 1), followed by an arrest of subsequent gamete development. Male gamete formation is also affected as the plants are male sterile. The analysis by Ravi *et al* (2008) of a different *swi1* allele called *dyad* where female meiosis is similar to that in *swi1* but male gamete formation is normal (Siddiqi *et al* 2000), led to the interesting observation that while equational division and meiotic abortion occurred in many of the ovules, some produced functional female gametes that had not undergone meiotic reduction

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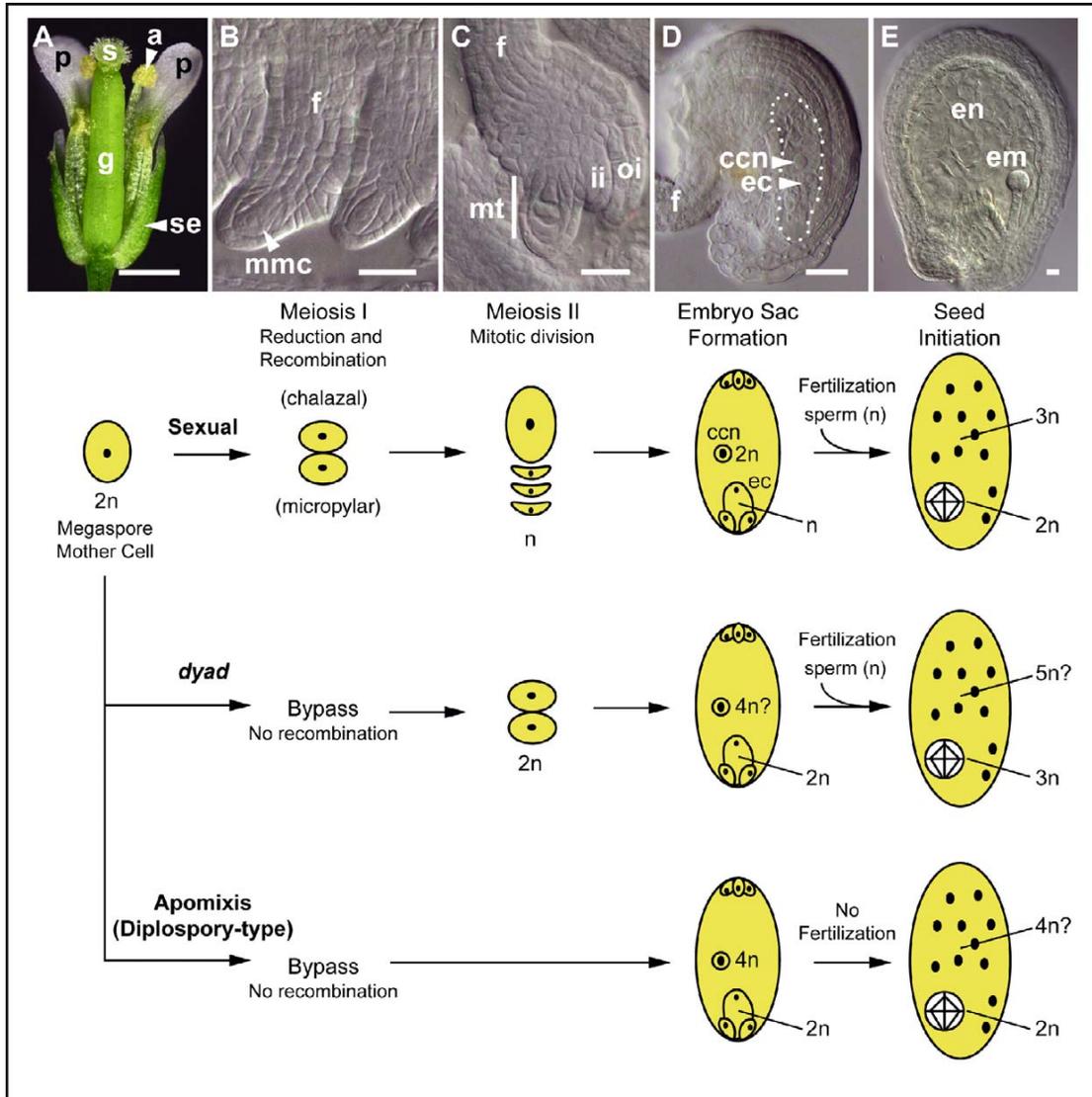


Figure 1. Seed development in sexual and some apomictic plants compared to the *dyad* mutant. (A) *Arabidopsis* flower at anthesis, showing the sepals (se) petals (p), anthers (a), stigmatic papillae (s) and gynoecium (g) that houses the ovules. (B) Developing ovules at megaspore mother cell (mmc) stage are connected to maternal tissues by the funiculus (f). (C) Ovule after the completion of meiosis, showing the meiotic tetrad (mt) of reduced megaspores, outer integument (oi) and inner integument (ii). (D) Ovule at anthesis, containing an egg cell (ec) and fused central cell nucleus (ccn), indicated with arrows. The embryo sac is outlined with a dotted line. (E) Developing seed containing an embryo (em) and endosperm (en). Scale bars = 0.5 mm in A and 20 μ m in B-E. The diagrams below show stages of female gamete and seed development in a sexual plant compared to most diplosporous apomicts and the *Arabidopsis dyad* mutant. In sexual plants such as *Arabidopsis*, the diploid (2n) megaspore mother cell undergoes meiotic reduction and only one of the four reduced megaspores survives while the other three degrade. The selected megaspore undergoes three rounds of mitosis to produce a seven-celled gamete structure also called an embryo sac. This contains a haploid (n) egg cell and a central cell containing a 2n nucleus resulting from the fusion of two of the eight haploid nuclei. Embryo and endosperm initiation occurs after double fertilization, when one haploid sperm cell fuses with the egg and another with the central cell, respectively. In *Arabidopsis dyad* mutants, meiotic reduction and recombination are bypassed, and an equational division occurs, similar to what happens in some diplosporous apomicts. One of these unreduced dyad cells gives rise to the diploid unreduced female gametes. Embryos arising after fertilization are thus triploid (3n), although it is unclear if the endosperm is derived from fertilization of fused or unfused central cell nuclei in *dyad*. In diplosporous apomictic plants, the embryo, and sometimes the endosperm, develops independently of fertilization.

(figure 1). This was first evident when they found triploid plants amongst the selfed *dyad* mutants and further cytological and molecular analysis confirmed this was indeed the case. Furthermore, the plants with unreduced female gametes retained their parental heterozygosity in subsequent generations, indicating an absence of recombination during unreduced gamete formation. The likely origin of the unreduced embryo sac was considered to be one of the diploid "dyad" cells formed during the equational division. Using a marker gene, the authors found that the dyad cell positioned closest to the chalazal end of the ovule (figure 1), similar to the reduced functional megaspore in sexual plants, was the most likely progenitor of the unreduced female gametes.

The bypassing of meiosis in *dyad* appears to resemble in some respects a mechanism called diplospory found in some natural apomicts (Koltunow *et al* 1995; figure 1). In most apomictic plants that form female gametes by diplospory, the megaspore mother cell directly forms an embryo sac by mitosis, completely bypassing meiosis. Fertilization is not required for embryo formation but may or may not be required for endosperm initiation. During sexual reproduction in most species, the two nuclei present in the central cell fuse prior to fertilization and have double the ploidy of the egg (figure 1). In apomicts, the two nuclei may or may not fuse prior to either fertilization-independent or dependent endosperm formation. Therefore the final ploidy of the endosperm in an apomict is dependent upon central cell nuclear fusion, the ploidy level of the maternal plant and also incoming sperm cells if fertilization is required for endosperm initiation. In some diplosporous apomicts, however, the megaspore mother cell undergoes an equational division and one of these cells gives rise to the unreduced embryo sac, much as has now been described by Ravi *et al* (2008) in *dyad* mutants (figure 1). However, the frequency of functional unreduced gamete formation was low in *dyad* when compared to the high frequency of unreduced female gamete formation in natural apomict species. Ravi *et al* (2008) reasoned that this might relate to a coincident triggering of checkpoint mechanisms that block progression through meiosis when key sequential events have not occurred.

It remains to be determined whether DYAD/SWII function is altered in diplosporous apomict plants. Nevertheless, the findings of Ravi *et al* (2008) clearly demonstrate that an alteration in a gene that is directly involved in meiotic chromosome organization can lead to the formation of viable unreduced gametes. Further studies in conjunction with the genetic and molecular analysis of natural apomicts might provide the capacity to develop a functional apomixis technology for breeding.

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