Title

Plant zygote development: recent insights and applications to clonal seeds

Authors

Khanday, Imtiyaz
Sundaresan, Venkatesan

Publication Date

2021-02-01

DOI

10.1016/j.pbi.2020.101993

Peer reviewed

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Plant zygote development: recent insights and applications to clonal seeds

Imtiyaz Khanday[1,2] and Venkatesan Sundaresan[1,2,3]

In flowering plants, haploid gametes – an egg cell and a sperm cell fuse to form the first diploid cell – the zygote. The zygote is the progenitor stem cell that gives rise to all the embryonic and post embryonic tissues and organs. Unlike animals, both maternal and paternal gene products participate in the initial development of zygotes in plants. Here, we discuss recent advances in understanding of the zygotic transition and embryo initiation in angiosperms, including the role of parental contributions to gene expression in the zygote. We further discuss utilization of this knowledge in agricultural biotechnology through synthetic apomixis. Parthenogenesis obtained by manipulation of embryogenic factors, combined with mutations that bypass meiosis, enables clonal propagation of hybrid crops through seeds.

Addresses
[1] Department of Plant Biology, University of California, Davis, CA, USA
[2] Innovative Genomics Institute, University of California, Berkeley, CA, USA
[3] Department of Plant Sciences, University of California, Davis, CA, USA

Corresponding author: Sundaresan, Venkatesan (sundar@ucdavis.edu)

Current Opinion in Plant Biology 2021, **59**:101993

This review comes from a themed issue on **Growth and development**

Edited by **Courtney Hollender** and **Zhongchi Liu**

For a complete overview see the Issue and the Editorial

Available online 7th January 2021

https://doi.org/10.1016/j.pbi.2020.101993

Introduction

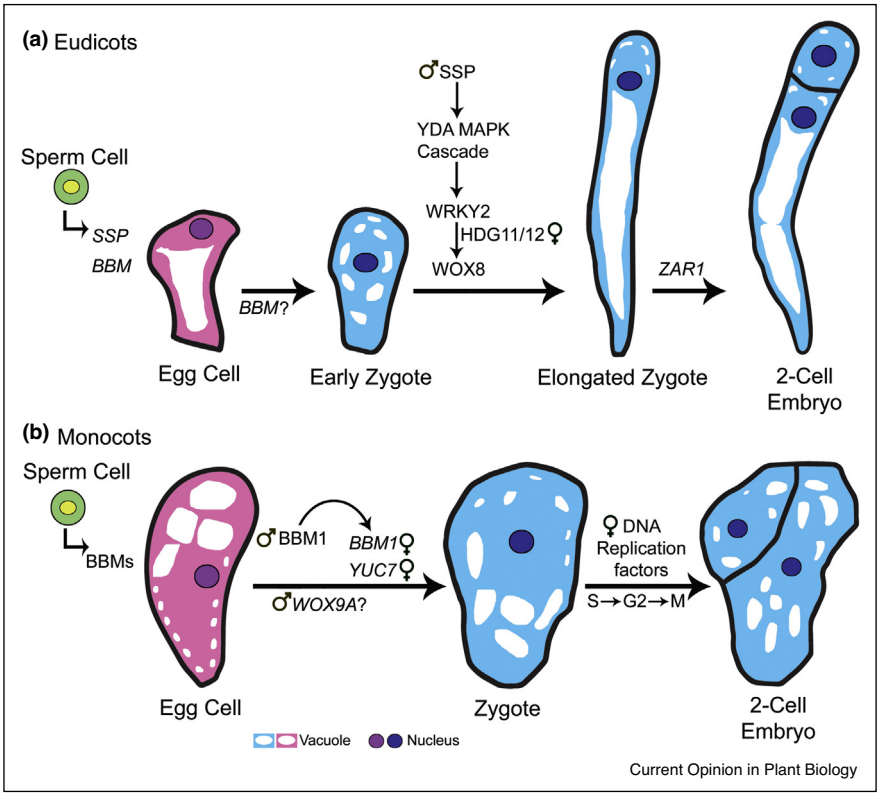
The life cycle of plants alternates between a diploid sporophytic and a haploid gametophytic generation. In flowering plants, the sporophyte is initiated with the fusion of haploid gametes during double fertilization. Two male gametes or sperm cells are borne on male gametophyte or pollen grains inside anthers and female gametes, an egg cell and a homo-diploid central cell are produced inside female gametophyte or embryo sac within an ovule. One of the sperm cells fuses with the egg cell and forms the zygote that ultimately gives rise to the embryo. The second sperm cell fertilizes the central cell, resulting in triploid endosperm — the nutritive tissue for nourishment of developing embryo, and also germinating seedlings in monocots [1]. The zygote undergoes an asymmetric first division and gives rise to a smaller apical cell and a larger basal cell. Typically, the apical cell gives rise to the embryo, while the basal cell forms the extra-embryonic suspensor [2,3]. In this review, we focus on the molecular genetic control of zygote development, including activation of the zygotic genome after fertilization and parent-of-origin gene expression, and discuss the utilization of this knowledge for the clonal propagation of hybrid crops through seed.

Plant zygote development after fertilization

After fertilization, zygotes in dicots undergo a period of growth and elongation before the first asymmetric zygote division (Figure 1a). The earliest stages of embryogenesis involve cellular polarization, and determination of the apical-basal axis [4•]. Egg cells in dicots are polarized with nucleus located at the apical (chalazal pole) and a large vacuole occupies the basal end (micopylar pole) [5] (Figure 1a). This polarity is transiently lost in fertilized egg cell when nucleus assumes a central position with vacuoles dispersed throughout, and then reestablished before the first zygotic division [2,4•,6,7] (Figure 1a). The dynamic rearrangement of cytoskeletal components, microtubules and F-actin after fertilization play a pivotal role in nuclear migration and polarity establishment in zygotes [4•,7]. In *Brassicaceae* and other dicot families, (e.g. Arabidopsis and tobacco), the first zygotic division gives rise to a cytoplasm-rich smaller apical cell and a vacuolated large basal cell. The apical cell gives rise to the embryo proper and the basal cell forms the suspensor and the hypophysis, the uppermost cell of suspensor that contributes to the root apical meristem [3]. However, variations on this stereotypic theme are found in other eudicots where apical cell can contribute to suspensor (*Caryophyllaceae*) and basal cell to proembryos (members of *Asteraceae*) [8,9].

In monocot grass species, large vacuoles are found on the apical side of egg cells [10,11] (Figure 1b). There appears to be no growth of the zygote before the first division in rice, maize and wheat [10–12] (Figure 1b). Compared to eudicots, polarity in grass zygotes is less obvious. The vacuolar redistribution and nuclear migration results in asymmetric appearance with cytoplasm rich apical pole and vacuolated basal (micropylar) pole [11,13]. The first zygotic division results in relatively smaller, denser apical cell and a larger, vacuolated basal cell (Figure 1b) [10,11]. Unlike dicots, in monocot grasses both apical and basal cells divide in multiple planes to give rise to a pear-shaped proembryo before cell fate determination for organ formation.

Figure 1



Schematic illustration of zygote development in eudicots and monocots, accenting parent-specific gene functions. **(a)** Egg cell in dicots (Arabidopsis) with basal vacuole and apically located nucleus. Zygote elongation and asymmetric division gives rise to a large vacuolar basal cell and a smaller cytoplasm-rich apical cell [3,5]. Sperm cells express *SSP* and *BBM* transcripts. Activation of the YDA MAPK cascade in the zygote by SSP results in expression of WRK2, which together with maternally expressed HDG11/12 upregulates *WOX8*, promoting zygote elongation and polarity [50,51••]. **(b)** Egg cell in monocots with large vacuoles at the apical pole. The zygote undergoes division without growth, and gives rise to a relatively larger and more vacuolated basal cell, and a smaller, denser apical cell. Paternal BBM transcription factors initiate embryogenesis [47••]. Paternal BBM1 upregulates maternal *BBM1*, as well as maternal allele of auxin biosynthesis gene *YUCCA7*. The arrows between different cell types denote developmental progression and the arrows between protein/gene names signify regulation. *SSP*, *SHORT SUSPENSOR*; *BBM*, *BABY BBOM*; *WOX*, *WUSCHEL-related homeobox; HDG*, *HOMEODOMAIN GLABROUS*, YDA, YODA; MAPK, mitogen-activated protein kinase, *ZAR1*, *ZYGOTIC ARREST 1*.

Maternal-to-zygotic transition in plants

In animals, RNAs and proteins stored in the egg cell before fertilization have been shown to drive early embryogenesis, while the zygotic genome remains transcriptionally quiescent [14,15], although contributions from male transcripts have also emerged recently [16,17]. This maternal control of embryogenesis can last from hours up to a day and these maternal gene products can support different number of initial cell divisions depending on species [14]. However, the control of embryogenesis is later taken over by the *de novo* synthesized zygotic gene products during a transition period known as maternal-to-zygotic transition (MZT) [15]. The MZT in animals involves two interlinked steps (1) onset of transcription from zygotic genome, also known as zygotic genome activation (ZGA), and (2) removal of maternal transcripts [15]. The understanding of MZT in plants has advanced considerably in the last decade. As in animals, blocking gene expression in zygotes with transcriptional inhibitors in either tobacco [6] or Arabidopsis [18••], results in cell cycle arrest, indicating that *de novo* transcription from zygotic genome is required for the first zygotic division. Initial studies involving the expression of small sets of genes in zygotes in maize [19–21], tobacco [22] and Arabidopsis [23], or genetic studies of zygote development mutants in Arabidopsis [24] indicated that zygotic gene transcription occurs before first zygotic division. However, genetic studies in Arabidopsis suggested that on a larger scale many genes in the early embryo were expressed only from the maternal genome, and that activation of the paternal genome was delayed [25]. Such maternal-specific expression could arise either from parent-of-origin effects on new transcription, or from carryover of transcripts from the unfertilized egg cell, that

is, both genomes might be silent initially. From recent genome wide transcriptomic studies, a consensus has emerged that ZGA in flowering plants occurs before the first zygotic division, as discussed below.

Zygotic genome activation in monocots and dicots

In cereals, the gametes are arrested at the G1 phase of the cell cycle. Studies in rice and maize have provided a detailed picture of the sequence of ZGA in cereals [26••,27•]. A time-staged transcriptomic study in unicellular rice zygotes identified 499 genes upregulated at 2.5 hours after pollination (hap) (karyogamy stage), 181 of which had *de novo* expression in the zygote, compared to egg cells [26••,28]. The number of upregulated genes increased to 1981 at 5 hap (nucleolar fusion) and reached to 2485 genes at 9 hap (G2 phase). The majority of the genes expressed at these different stages of rice zygote development had a maternally biased expression [26••]. In maize, relative to egg cells, the expression of 3489 and 3247 genes was upregulated in zygotes at 12 hap (about 4 hours after fertilization) and 24 hap (corresponding to completion of S-phase), respectively [27•]. Although the parental contributions to the zygotic transcriptome were not investigated in maize, the maize and rice studies are in general agreement on the timing and scale of ZGA.

Most of the studies on ZGA in dicots have been carried out in Arabidopsis. Studies on DNA content of sperm cells suggest that Arabidopsis gametes may be in S phase at the time of fertilization [29]; however, expression of the RBR gene in Arabidopsis zygotes suggests that the egg cell may be arrested at the point of entry into S phase [30]. The timing of ZGA and parental gene expression contributions in Arabidopsis embryogenesis has been controversial, with two contrasting models supported by different studies [18••,31,32,33••,34]. Transcriptomic analysis of Arabidopsis 2–4 cell proembryos supported the concept of overall maternal dominance [31]. This conclusion was further supported by studies with embryo defective mutations and reporter gene assays, in which different paternal alleles are activated at different time points during early stages of embryogenesis [32,33••]. Thus, these studies support a model in which paternal genome activation occurs gradually from fertilized egg cell to globular stage embryos in Arabidopsis, and perhaps reflective of slow ZGA. In contrast, a study of 1–2-cell Arabidopsis proembryos found that zygotic transcription begins early, and the two parental genomes contribute equally [34]. This latter conclusion is supported by a recent study carried out in time-staged single cell zygotes in Arabidopsis which showed 2625 genes upregulated in 14 hap zygotes (spherical zygotes) compared to egg cells and the number of upregulated genes increased to 2951 at 24 hap (elongated zygotes) [18••]. Although ~66% reads were from maternal alleles at 14 hap, almost equal proportion of reads were observed from maternal and paternal genomes at 24 hap [18••], indicating rapid and complete ZGA is achieved in Arabidopsis before the first zygotic division. However, another recent study of the Arabidopsis embryo transcriptomes has reported a predominantly maternal bias for most genes in the earliest embryo stages including zygotes [33••]. It has been hypothesized that the differences in results between these studies might arise from differences in the ecotype combinations used to make the necessary hybrids for parent-of-origin determination in the transcriptomic analyses [32,33••]. Extensive studies on other dicot plants have not been undertaken; however, in tobacco zygotes, out of 1589 detected cDNAs, 1234 were not expressed in egg cells [6], supporting rapid activation of the zygotic genome after fertilization in tobacco.

Maternal transcript removal in plant zygotes

Because of rapid ZGA followed by loss of egg cell differentiation and embryonic cell divisions, maternal transcripts can be diluted out without requiring an active process for their removal as in animals. In the rice zygote study, extensive downregulation of egg cell-expressed transcripts was observed during ZGA, with 2898 genes that were downregulated by late G2 [26••]. However, as many maternally expressed transcripts for basic cellular processes were present in the egg cell and were persistent or upregulated during ZGA, it was concluded that the downregulated transcripts in zygotes represented inactivation of genes corresponding to egg cell fate, and that there was no evidence for an active process promoting widespread removal of maternal transcripts [26••]. In Arabidopsis, downregulation of 2956 egg cell transcripts was observed in 24 hap zygotes, paralleling the rice study, but here the kinetics of the downregulation were interpreted as indicative of active removal of maternal transcripts [18••]. These contrasting conclusions might reflect differences in ZGA between rice and Arabidopsis zygotes. It is worth noting that rice zygotes enter mitosis much more rapidly than Arabidopsis zygotes; in fact, the last time point in the rice study was chronologically earlier than the first time point in the Arabidopsis study. Therefore, a larger number of organisms will need to be surveyed to arrive at general conclusions on maternal transcript removal for the plant MZT.

Epigenetic contributions to plant zygote development

It was pointed out by McClintock that formation of the gametes and establishment of the zygote would require reprogramming of the genome [35]. Such reprogramming is likely to involve mechanisms that erase and reset epigenetic marks from the parental generation. Evidence for large-scale epigenetic modifications mediated by siRNAs in the sperm cell has been implicated in the silencing of transposable elements, a topic reviewed in detail previously [36]. Recent studies on small RNA transcriptomes of rice gametes find that the distribution of 24 nt

siRNA loci is reprogrammed in both the egg cell and the sperm cell, followed by a return to a canonical 24 nt siRNA pattern that is initiated in the zygote [37,38•]. These large-scale changes in siRNAs might be expected to be associated with chromatin modifications in the gametes that are transmitted to the zygotes. Consistent with this concept, genome-wide removal of the Polycomb complex-directed H3K27me3 repressive epigenetic mark in the sperm cell has been found in Arabidopsis, due in part to replacement of canonical histones by histone variants [39•]. These studies point to the importance of more research on epigenetic modifications of gametes and zygotes at the whole-genome level to reveal epigenetic mechanisms operating on expression of individual genes and pathways during zygote development.

Parent-of-origin dependent gene expression and interplay between parental genomes during zygote development

Locus-specific gene imprinting — that is, gene expression from only one parental allele has been well documented in the endosperm [40]; however, it is relatively uncommon in plant embryos. Embryo imprinting was first reported for a maize gene *MATERNALLY EXPRESSED IN EMBRYO 1* (*MEE1*), which is expressed only from the maternal allele in embryos as well as in endosperm [41]. Subsequently, maternal-allele specific expression has been reported for several genes in Arabidopsis and maize embryos [32,33••,42–45]. Paternal-allele specific expression of individual loci is also widely prevalent in the endosperm [46] but not in embryos. Recently, the role of a paternally expressed AP2 family transcription factor *BABY BOOM1* (*BBM1*) was described in the initiation of embryogenesis in rice [47••]. *BBM1* is expressed in the sperm cell and is absent in the egg cell, and continues to be expressed only from the male allele after fertilization, when it triggers embryo development [26••,47••]. Three *BBM* genes (*BBM1*, *BBM2* and *BBM3*) are redundantly required for embryogenesis in rice [47••]. Ectopic expression of *BBM1* in the egg cell leads to embryo formation without fertilization (parthenogenesis), and consequently to haploid progeny [47••]. The paternal expression of *BBM1* has been independently confirmed with *in vitro* fertilized rice zygotes [48]. Expression of the orthologous *BBM* gene in Arabidopsis has also been shown to be present in sperm cells and absent in egg cells [39•], although a detailed characterization of expression and function in the zygote has yet to be performed. In rice, BBM1 has been shown to activate auxin biosynthesis for induction of somatic embryogenesis [49]. The understanding of regulation of embryo initiation by *BBM1* was utilized to develop a synthetic apomixis system for clonal seed propagation in rice (see following section).

Although there is as yet no consensus on contributions of maternal and paternal gene expression at a genome-wide scale in Arabidopsis zygotes [18••,31,33••,34], co-ordination between maternal and paternal factors has been demonstrated in zygote development. The sperm cell transmitted *SHORT SUSPENSOR (SSP)* product activates the YODA mitogen-activated protein kinase (MAPK) pathway, leading to phosphorylation of the transcription factor WRKY2 [50,51••] (Figure 1a). The phosphorylated WRKY2 then activates its target gene *WOX8*, which promotes asymmetric zygotic division and suspensor formation. However, this activation of *WOX8* expression additionally requires the female-expressed class IV HOMEODOMAIN GLABROUS (HDG) transcription factors HDG11/12 (Figure 1a) [51••,52]. Thus, male and female-expressed factors co-operate within the zygote to promote an essential step in embryogenesis. The asymmetric division of the zygote additionally requires the function of ZYGOTIC ARREST 1 (ZAR1), a kinase of RLK/Pelle family [53] (Figure 1a). Since *SSP* is a *Brassicaceae*-specific gene and is not found outside this family [54], alternative mechanisms for MAPK activation and/or pathways other than MAPK may be involved in apical-basal axis formation in monocots. Several other genes, either predicted to regulate or involved in zygote development in various plant species have been reviewed in Zhao *et al.* [13] and are not further discussed here.

In rice zygotes, although most genes have maternal expression, the BBM1 transcription factor demonstrated to act as an embryogenic trigger in rice is paternally expressed [26••,47••,48]. One of the BBM1 target genes expressed *de novo* in zygotes is *YUCCA7*, suggesting that auxin biosynthesis might have a key role in embryogenesis initiation [49]. However, expression of *YUCCA7* is maternal-allele specific, implying that parental genome-specific gene expression is coordinated to promote zygote development [26••]. The initial male allelic expression of *BBM1* is followed by the expression of the female *BBM1* allele before the zygote divides. The female allele of *BBM1* is likely to be a target of male-expressed BBM1, since BBM1 is capable of auto-activation (Figure 1b) [47••]. Another transcription factor with paternal expression in rice zygotes is *WOX9A* [26••], an ortholog of Arabidopsis *WOX8* (see above); however, the role of *WOX9A* in rice zygotic development has not been determined (Figure 1b). To summarize: unfertilized egg cells in rice are arrested at G1 phase. Fertilization by the sperm cell is followed by karyogamy, and activation of zygotic development, marked by replication of the zygotic genome (S-phase) [55]. The replication factors and general cellular metabolism genes required for zygotic DNA replication, as well as for biosynthesis of auxin, are maternally expressed [26••]. Thus, the initiation of the zygote development program involves a paternally expressed factor (BBM1) that then activates a maternally expressed machinery for auxin biosynthesis, cell cycle progression, and other basic cellular processes (Figure 1b).

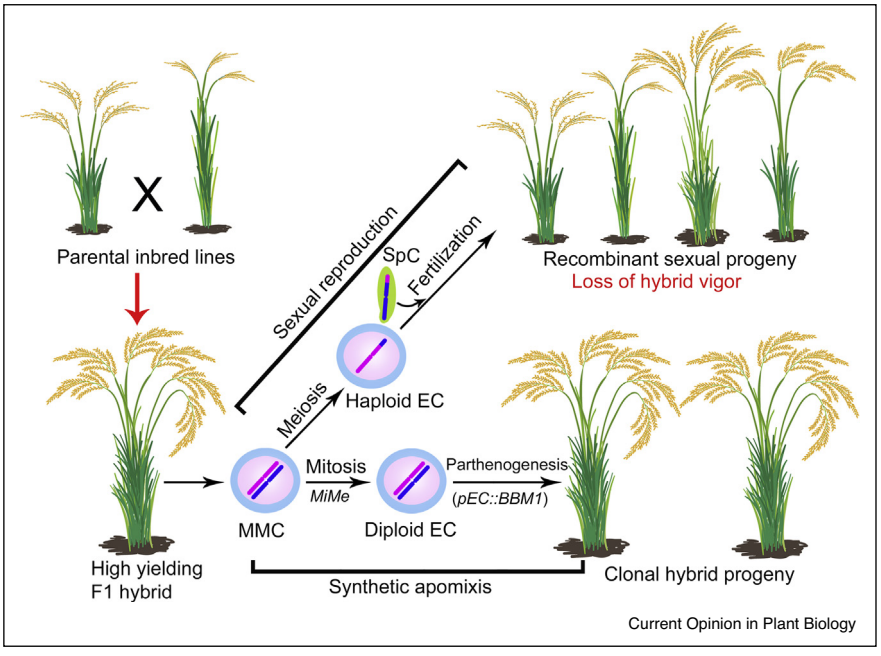
Translating embryo initiation mechanisms into agricultural applications

Crossing specific combinations of inbred lines leads to 'hybrid vigor' or 'heterosis' [56], with the enhancement of several quantitative characteristics of agronomic importance like yield, biomass, abiotic stress, environmental adaptability etc. in the F1 progeny. Crop yields have substantially increased by the use of high-yielding hybrids, but these hybrids cannot be maintained through seed propagation due to genetic segregation. Apomixis is an asexual mode of seed formation known to occur naturally in ~400 angiosperm species [57]. However, except for a few fruit crops and forage grasses, apomixis is rare in crop plants [57,58]. Apomixis is a complex process and can be achieved through several different mechanisms in nature [57]. The common underlying theme in all these different forms of apomixis is bypassing the two fundamental processes involved in sexual reproduction — meiosis and fertilization. Thus, the offspring produced are genetically identical or clones of the mother plant. Although several genes regulating different components of apomixis have been identified and progress has been made in deciphering their role in inducing apomixis, our understanding of natural apomixis at the molecular level is still limited [59–63]. Introduction of apomixis into crop plants for the clonal propagation of hybrids has been described as 'the holy grail' of agricultural biotechnology [64]. Besides enabling fixation of hybrid vigor, apomixis in crop plants has several other benefits for agriculture [57].

Synthetic apomixis: combining ameiotic *MiMe* with parthenogenetic *BBM1*

A conventional strategy to introduce apomixis into sexual crop plants is the introgression of apomictic traits from wild relatives. However, this strategy has had limited success. The alternative approach is synthetic apomixis — using biotechnology to assemble different components of apomixis or modify sexual reproduction to create an apomictic mode of propagation. As mentioned above, the expression of paternally expressed *BBM1* in the egg cell induces parthenogenesis in rice [47••]. This observation was used to devise a procedure of molecular engineering for synthetic apomixis in crop plants. The strategy parallels a type of natural apomixis known as diplospory, in which the avoidance of meiosis (called 'apomeiosis') is combined with embryo formation by the unfertilized egg cell, that is, parthenogenesis [65]. Apomeiosis was achieved by substitution of mitosis for meiosis, also known as'*mitosis instead of meiosis*' or *MiMe* genotype [66], and parthenogenesis by egg cell expression of BBM1 [47••] (Figure 2). *MiMe* is a combination of three mutations, disabling different steps involved in meiosis and converting it into a mitotic like division. Mutations in

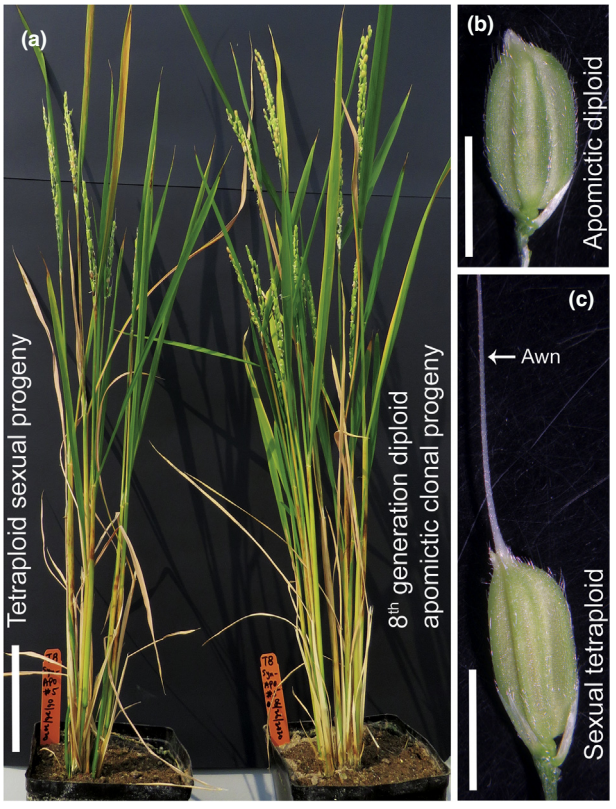
Figure 2



Schematic illustration of clonal propagation of hybrid crop plants with synthetic apomixis. Crossing specific inbred lines results in hybrid vigor in F1 progeny. During sexual reproduction of F1 hybrids, meiosis leads to recombination and reduction, and fertilization creates genetic variation by bringing in new combinations of gametes that leads to loss of vigor in the F2 progeny. In synthetic apomixis, *MiMe* produces diploid egg cells without recombination [72]. With ectopic BBM1 expression, the diploid unreduced egg cells will develop parthenogenetically into embryos and produce clonal progeny [47••]. While sexual reproduction results in seeds with genetic segregation of traits, synthetic apomixis leads to production of clonal seeds that retain the hybrid heterozygosity. MMC, megaspore mother cell; Spc, sperm cell; EC, egg cell; *MiMe*, mitosis instead of meiosis, *BBM1*, *BABY BOOM1*.

SPO11 orthologs in Arabidopsis [67] and rice [68] or *HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS1* (*PAIR1*) of rice [69] disrupt chromosome pairing and meiotic recombination. *REC8* is essential for maintaining centromere cohesion at anaphase I and also controls monopolar orientation of kinetochores during the first meiotic division [70,71]. Mutations in *OMISSION OF SECOND DIVISION 1* (*OSD1*) skip the second meiotic division and result in diploid gametes [66]. A combination of mutations in *SPO11*, *REC8* and *OSD1* in Arabidopsis [66] and *PAIR1*, *REC8* and *OSD1* in rice [72] created *MiMe* genotype and produced diploid gametes without reduction or recombination. However, fertilization of unreduced gametes will double the genome ploidy every generation. Therefore, the embryo triggering capacity of BBM1 in the absence of fertilization was combined with genome edited *MiMe* genes to enable clonal propagation through seeds [47••]. *MiMe* results in unrecombined and unreduced egg cells which parthenogenetically develop into embryos with BBM1 expression (Figure 2). Diploid clonal seeds were observed at 15–29% frequency while as the rest of the seeds were sexual tetraploids (Figure 3). In this synthetic apomixis system, central cell fertilization is still required for endosperm formation and viable seeds [47••]. Such a combination of asexual embryo and sexual endosperm mimics the majority of natural apomicts which undergo pseudogamy [73]. In particular, a natural apomict, *Pennisetum squamulatum* which undergoes pseudogamous apomixis also contains multiple copies of a *BBM*-like gene in its apospory-specific locus which express in the egg cell before fertilization to produce parthenogenic embryos but with fertilized endosperm [60,61]. It may be possible in the future to utilize autonomous endosperm formation to generate asexual endosperm as well, but some additional hurdles will need to be overcome. A maternal-to-paternal genome ratio of 2:1 has been shown to be essential for viable endosperm formation in most angiosperms including major crop plants, and mutants that form autonomous asexual endosperm result in seed abortion [74]. Thus, a synthetic apomixis system with autonomous endosperm formation will require additional complexities of design to overcome the ploidy barrier. Since the sexual endosperm is still hybrid and does not contribute to the genotype of the subsequent generation, the formation of endosperm by fertilization should not be a limiting factor for clonal seed propagation (Additional details are discussed in Khanday *et al.* [47••]).

Figure 3

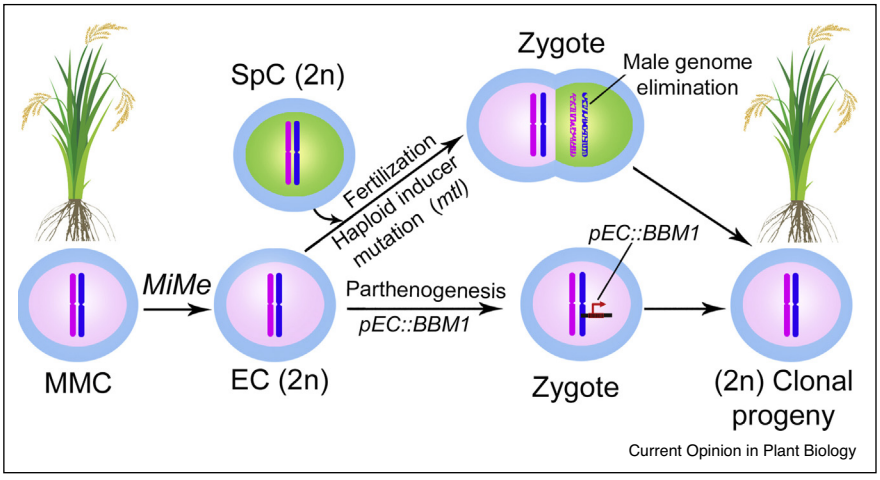


Clonal and sexual progeny from synthetic apomixis plants. **(a)** An eighth-generation clonal diploid progeny (right) from a seventh-generation synthetic apomixis plant is compared with a tetraploid sexual progeny (left) [47••]. **(b)** and **(c)** phenotypic differences in floral morphology between diploid and tetraploid progeny. Tetraploid progeny (c) are sterile and develop an awn. Awns are suppressed in diploid florets (b). Scale bars, 10 cm in (a), and 0.5 cm in (b) and (c).

Other strategies for clonal seed formation

Alternative strategies to synthetic apomixis have used genome elimination. These strategies also require introduction of apomeiosis, typically by *MiMe* (Figure 2); however, instead of parthenogenesis, one of the two parental genomes is eliminated either during or after fertilization (Figure 4). In Arabidopsis, genome elimination can be induced by altered centromere-specific histone 3 (CENH3) [75]. When *cenh3* mutants are crossed to wildtype, mutant chromosomes are eliminated. *MiMe* plants were crossed with *cenh3* mutant plants, resulting in clonal seeds [76] (Figure 4). Although clonal seeds were produced, there is significant sterility due to aneuploidy from incomplete genome eliminations. A more serious limitation is that it is not a self-replicating system, and cross-pollinations are required to produce the clonal seeds. This limitation was recently overcome in rice by using the haploid inducer capability of mutations in a phospholipase A gene *MATRILINEAL (MTL)* [77] (also called *NOT LIKE DAD* [78] or *PHOSPHOLIPASE A1* [79]) first described in maize. The simultaneous knockout of *MiMe* genes and *MTL* was shown to achieve synthetic apomixis in rice at 5–9.5% frequency [80••] (Figure 4). However, widespread seed sterility was also observed with this strategy, reducing the overall clonal seed

Figure 4



Strategies for clonal seed production by synthetic apomixis in crops. *MiMe* results in diploid gametes without reduction and recombination [72]. The diploid egg cell can develop into parthenogenetic embryo with *BBM1* expression [47••] or alternately male genome can be eliminated with *mtl* mutations [77,80••]. All these strategies result in diploid clonal progeny. MMC, megaspore mother cell; Spc, sperm cell; EC, egg cell; *MiMe*, mitosis instead of meiosis; *BBM1*, *BABY BOOM1; mtl*, *matrilineal.*

production to 0.19–0.41% [80••], most likely due to aneuploidy caused by chromosome fragmentation and incomplete genome elimination [81]. Substantial improvements in this frequency could enable this approach to be used for practical applications in the field.

Conclusions and future directions

The successful demonstration of clonal propagation through seeds using the synthetic apomixis approach has the potential to revolutionize agriculture in the future. Both *BBM* and *MiMe* homologous genes are found in other plant species, so this technology could be extendable to other cereals, and perhaps other crops as well. It would enable propagation of high yielding, disease-resistant and climate-resistant hybrid crops as clones without losing these traits through successive generations, and thus also enabling farmers to plant seeds from their own hybrid crops every planting season. New elite genotypes resulting from breeding procedures would be ready for performance testing without the need for progeny genetic stability testing, and thus would significantly reduce the cost and time for releasing new hybrid seeds for breeding programs and commercial seed companies. The cost effectiveness of clonal hybrid seeds would also encourage farmers in the developing world to adopt hybrid crop cultivation. A theoretical concern for this technology is the long-term sustainability of propagation of seeds exclusively through the female germline. It has been hypothesized that epigenetic interactions between parental genomes are important for heterosis, which would imply that genetic fixation by apomixis alone may be not be sufficient to preserve hybrid vigor [82,83]. However, studies in *Hieracium pilosella* shows that hybrids can be stably propagated across generations with apomixis without changes in quantitative traits [64]. Moreover, we have now clonally propagated progeny from our apomictic rice lines for eight generations without any visible phenotypic consequences (Figure 3). Thus, it appears that the male genome is dispensable for several generations, even in a sexually reproducing plant like rice.

One of the limitations on bringing this technology to the farmer's field is the frequency of clonal seed formation, which at present is 15–30% in rice [47••] and represents the frequency of seeds developing parthenogenetically. However, this limitation could be overcome in the future by building better parthenogenesis systems with promoters and other *cis* elements having stronger expression in egg cells. Improved parthenogenesis systems might also be developed by utilizing additional embryogenic genes derived from investigation of reproduction in both sexual and apomictic plants. Alternatively, this technology could be utilized at the current efficiency for hybrid seed production if an efficient procedure for distinguishing diploid clonal seeds from tetraploid sexual seeds could be developed, such that the clonal seeds could be selectively used for field planting.

In summary, based on current evidence from multiple species, the zygotic genome in plants is activated and *de novo* transcripts are synthesized before the first zygotic division. Although the timing of complete paternal genome activation after fertilization is still debated,

particularly in Arabidopsis, various studies show parent-specific activation of genes can occur from zygote to early embryos. An interplay of maternal and paternal factors, acting through transmission of mRNA or parent-of-origin dependent expression, is required for zygotic development. Knowledge of the mechanism of embryo initiation in rice has been utilized to develop a method of synthetic apomixis which can be utilized for preservation of heterosis in hybrid crops.

Conflict of interest statement

Nothing declared.

Acknowledgements

Research on zygote development and synthetic apomixis in our laboratory is supported by the National Science Foundation (IOS-1547760), the Innovative Genomics Institute, and the U.S. Department of Agriculture (USDA) Agricultural Experiment Station (CA-D-XXX-6973-H).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dresselhaus T, Sprunck S, Wessel GM: **Fertilization mechanisms in flowering plants**. *Curr Biol* 2016, **26**:R125-R139.

2. Radoeva T, Vaddepalli P, Zhang Z, Weijers D: **Evolution, initiation, and diversity in early plant embryogenesis**. *Dev Cell* 2019, **50**:533-543.

3. Jurgens G: **Apical-basal pattern formation in Arabidopsis embryogenesis**. *EMBO J* 2001, **20**:3609-3616.

4. • Kimata Y, Kato T, Higaki T, Kurihara D, Yamada T, Segami S, Morita MT, Maeshima M, Hasezawa S, Higashiyama T *et al.*: **Polar vacuolar distribution is essential for accurate asymmetric division of Arabidopsis zygotes**. *Proc Natl Acad Sci U S A* 2019, **116**:2338-2343.
The authors monitored vacuolar dynamics during Arabidopsis zygote polarization and show tubular strands of vacuoles surround apically migrating nucleus first and then they accumulate at the basal region, and this asymmetric vacuolar distribution is essential for apical-basal axis determination.

5. Zhang Z, Laux T: **The asymmetric division of the Arabidopsis zygote: from cell polarity to an embryo axis**. *Sex Plant Reprod* 2011, **24**:161-169.

6. Zhao J, Xin H, Qu L, Ning J, Peng X, Yan T, Ma L, Li S, Sun MX: **Dynamic changes of transcript profiles after fertilization are associated with de novo transcription and maternal elimination in tobacco zygote, and mark the onset of the maternal-to-zygotic transition**. *Plant J* 2011, **65**:131-145.

7. Kimata Y, Higaki T, Kawashima T, Kurihara D, Sato Y, Yamada T, Hasezawa S, Berger F, Higashiyama T, Ueda M: **Cytoskeleton dynamics control the first asymmetric cell division in Arabidopsis zygote**. *Proc Natl Acad Sci U S A* 2016, **113**:14157-14162.

8. Maheshwari P: *An Introduction to the Embryology of Angiosperms*. New York: McGraw-Hill; 1950.

9. Wang K, Chen H, Miao Y, Bayer M: **Square one: zygote polarity and early embryogenesis in flowering plants**. *Curr Opin Plant Biol* 2020, **53**:128-133.

10. Sato A, Toyooka K, Okamoto T: **Asymmetric cell division of rice zygotes located in embryo sac and produced by in vitro fertilization**. *Sex Plant Reprod* 2010, **23**:211-217.

11. Chen J, Kalinowska K, Muller B, Mergner J, Deutzmann R, Schwechheimer C, Hammes UZ, Dresselhaus T: **DiSUMO-LIKE interacts with RNA-binding proteins and affects cell-cycle progression during maize embryogenesis**. *Curr Biol* 2018, **28**:1548-1560.e45.

12. Bakos F, Szabo L, Olmedilla A, Barnabas B: **Histological comparison between wheat embryos developing in vitro from isolated zygotes and those developing in vivo**. *Sex Plant Reprod* 2009, **22**:15-25.

13. Zhao P, Begcy K, Dresselhaus T, Sun MX: **Does early embryogenesis in eudicots and monocots involve the same mechanism and molecular players?** *Plant Physiol* 2017, **173**:130-142.

14. Jukam D, Shariati SAM, Skotheim JM: **Zygotic genome activation in vertebrates**. *Dev Cell* 2017, **42**:316-332.

15. Vastenhouw NL, Cao WX, Lipshitz HD: **The maternal-to-zygotic transition revisited**. *Development* 2019, **146**.

16. Stoeckius M, Grun D, Rajewsky N: **Paternal RNA contributions in the *Caenorhabditis elegans* zygote**. *EMBO J* 2014, **33**:1740-1750.

17. Guo L, Chao SB, Xiao L, Wang ZB, Meng TG, Li YY, Han ZM, Ouyang YC, Hou Y, Sun QY *et al.*: **Sperm-carried RNAs play critical roles in mouse embryonic development**. *Oncotarget* 2017, **8**:67394-67405.

18. •• Zhao P, Zhou X, Shen K, Liu Z, Cheng T, Liu D, Cheng Y, Peng X, Sun MX: **Two-step maternal-to-zygotic transition with two-phase parental genome contributions**. *Dev Cell* 2019, **49**:882-893.e5.
Analysis of gene expression and parental contributions in Arabidopsis zygotes. The authors show initiation of ZGA in zygotes, provide evidence for equiparental contribution to zygotic gene expression and for large-scale clearing of maternal transcripts before first zygotic division.

19. Dresselhaus T, Cordts S, Heuer S, Sauter M, Lorz H, Kranz E: **Novel ribosomal genes from maize are differentially expressed in the zygotic and somatic cell cycles**. *Mol Gen Genet* 1999, **261**:416-427.

20. Sauter M, von Wiegen P, Lörz H, Kranz E: **Cell cycle regulatory genes from maize are differentially controlled during fertilization and first embryonic cell division**. *Sexual Plant Reprod* 1998, **11**:41-48.

21. Okamoto T, Scholten S, Lorz H, Kranz E: **Identification of genes that are up- or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygote development by a cell manipulation- and PCR-based approach**. *Plant Cell Physiol* 2005, **46**:332-338.

22. Ning J, Peng XB, Qu LH, Xin HP, Yan TT, Sun M: **Differential gene expression in egg cells and zygotes suggests that the transcriptome is restructed before the first zygotic division in tobacco**. *FEBS Lett* 2006, **580**:1747-1752.

23. Baroux C, Blanvillain R, Gallois P: **Paternally inherited transgenes are down-regulated but retain low activity during early embryogenesis in Arabidopsis**. *FEBS Lett* 2001, **509**:11-16.

24. Ronceret A, Gadea-Vacas J, Guilleminot J, Lincker F, Delorme V, Lahmy S, Pelletier G, Chaboute ME, Devic M: **The first zygotic division in Arabidopsis requires de novo transcription of thymidylate kinase**. *Plant J* 2008, **53**:776-789.

25. Vielle-Calzada JP, Baskar R, Grossniklaus U: **Delayed activation of the paternal genome during seed development**. *Nature* 2000, **404**:91-94.

26. •• Anderson SN, Johnson CS, Chesnut J, Jones DS, Khanday I, Woodhouse M, Li C, Conrad LJ, Russell SD, Sundaresan V: **The zygotic transition is initiated in unicellular plant zygotes with asymmetric activation of parental genomes**. *Dev Cell* 2017, **43**:349-358.e4.
Characterization of genome wide parental gene expression in unicellular plant zygotes using rice. This study shows ZGA occurs soon after fertilization and that zygotic gene expression has overall maternal bias, but with paternal expression for some genes including pluripotency factors of the BBM family.

27. • Chen J, Strieder N, Krohn NG, Cyprys P, Sprunck S, Engelmann JC, Dresselhaus T: **Zygotic genome activation**

occurs shortly after fertilization in maize. *Plant Cell* 2017, **29**:2106-2125.
The authors investigated gene expression in maize gametes and zygotes, and show ZGA occurs before the first zygotic division in maize.

28. Anderson SN, Johnson CS, Jones DS, Conrad LJ, Gou X, Russell SD, Sundaresan V: **Transcriptomes of isolated *Oryza sativa* gametes characterized by deep sequencing: evidence for distinct sex-dependent chromatin and epigenetic states before fertilization**. *Plant J* 2013, **76**:729-741.

29. Friedman WE: **Expression of the cell cycle in sperm of Arabidopsis: implications for understanding patterns of gametogenesis and fertilization in plants and other eukaryotes**. *Development* 1999, **126**:1065-1075.

30. Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F: **Distinct dynamics of HISTONE3 variants between the two fertilization products in plants**. *Curr Biol* 2007, **17**:1032-1037.

31. Autran D, Baroux C, Raissig MT, Lenormand T, Wittig M, Grob S, Steimer A, Barann M, Klostermeier UC, Leblanc O *et al.*: **Maternal epigenetic pathways control parental contributions to Arabidopsis early embryogenesis**. *Cell* 2011, **145**:707-719.

32. Del Toro-De Leon G, Garcia-Aguilar M, Gillmor CS: **Non-equivalent contributions of maternal and paternal genomes to early plant embryogenesis**. *Nature* 2014, **514**:624-627.

33. •• Alaniz-Fabián J, Xiang D, Toro-De León GD, Orozco-Nieto A, Gao P, Sharpe A, Kochian LV, Selvaraj G, Springer N, Abreu-Goodger C *et al.*: **Maternal genome dominance in early plant embryogenesis**. *bioRxiv* 2020, **2020** http://dx.doi.org/10.1101/2020.01.14.905992.
These authors report that in crosses between closely related ecotypes of Arabidopsis, activation of the paternal genome is delayed, consistent with genetic studies within the same ecotype.

34. Nodine MD, Bartel DP: **Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos**. *Nature* 2012, **482**:94-97.

35. McClintock B: **The significance of responses of the genome to challenge**. *Science* 1984, **226**:792-801.

36. Calarco JP, Martienssen RA: **Genome reprogramming and small interfering RNA in the Arabidopsis germline**. *Curr Opin Genet Dev* 2011, **21**:134-139.

37. Li C, Gent JI, Xu H, Fu H, Russell SD, Sundaresan V: **Resetting of 24-nt siRNA landscape is initiated in the unicellular zygote in rice**. *bioRxiv* 2020, **2020** http://dx.doi.org/10.1101/2020.08.31.275958.

38. • Li C, Xu H, Fu FF, Russell SD, Sundaresan V, Gent JI: **Genome-wide redistribution of 24-nt siRNAs in rice gametes**. *Genome Res* 2020, **30**:173-184.
The article reports how 24nt siRNAs in rice gametes are reprogrammed before fertilization. In gametes, 24nt siRNAs are depleted from heterochromatin boundaries, and are redistributed across heterochromatic regions in sperm cells, and concentrated at a smaller number of heterochromatic loci in egg cells.

39. • Borg M, Jacob Y, Susaki D, LeBlanc C, Buendia D, Axelsson E, Kawashima T, Voigt P, Boavida L, Becker J *et al.*: **Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin**. *Nat Cell Biol* 2020, **22**:621-629.
The authors report a genome-wide resetting of H3K27me3 repressive epigenetic mark in Arabidopsis sperm cells that primes developmentally regulated genes for expression in the next generation.

40. Batista RA, Kohler C: **Genomic imprinting in plants-revisiting existing models**. *Genes Dev* 2020, **34**:24-36.

41. Jahnke S, Scholten S: **Epigenetic resetting of a gene imprinted in plant embryos**. *Curr Biol* 2009, **19**:1677-1681.

42. Raissig MT, Bemer M, Baroux C, Grossniklaus U: **Genomic imprinting in the Arabidopsis embryo is partly regulated by PRC2**. *PLoS Genet* 2013, **9**:e1003862.

43. He S, Sun Y, Yang Q, Zhang X, Huang Q, Zhao P, Sun M, Liu J, Qian W, Qin G *et al.*: **A novel imprinted gene NUWA controls mitochondrial function in early seed development in Arabidopsis**. *PLoS Genet* 2017, **13**:e1006553.

44. Wu JJ, Peng XB, Li WW, He R, Xin HP, Sun MX: **Mitochondrial GCD1 dysfunction reveals reciprocal cell-to-cell signaling during the maturation of Arabidopsis female gametes**. *Dev Cell* 2012, **23**:1043-1058.

45. Meng D, Zhao J, Zhao C, Luo H, Xie M, Liu R, Lai J, Zhang X, Jin W: **Sequential gene activation and gene imprinting during early embryo development in maize**. *Plant J* 2018, **93**:445-459.

46. Gehring M, Satyaki PR: **Endosperm and imprinting, inextricably linked**. *Plant Physiol* 2017, **173**:143-154.

47. •• Khanday I, Skinner D, Yang B, Mercier R, Sundaresan V: **A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds**. *Nature* 2019, **565**:91-95.
Authors show paternal expression of *BBM1* is required for embryo initiation in rice. The authors used *BBM1* egg cell expression with *MiMe* to develop a self-propagating synthetic apomixis system that can be utilized for preservation of hybrid vigor in sexual crop plants.

48. Rahman MH, Toda E, Kobayashi M, Kudo T, Koshimizu S, Takahara M, Iwami M, Watanabe Y, Sekimoto H, Yano K *et al.*: **Expression of genes from paternal alleles in rice zygotes and involvement of osasgr-bbml1 in initiation of zygotic development**. *Plant Cell Physiol* 2019, **60**:725-737.

49. Khanday I, Santos-Medellín C, Sundaresan V: **Rice embryogenic trigger BABY BOOM1 promotes somatic embryogenesis by upregulation of auxin biosynthesis genes**. *bioRxiv* 2020, **2020** http://dx.doi.org/10.1101/2020.08.24.265025.

50. Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W: **Paternal control of embryonic patterning in *Arabidopsis thaliana***. *Science* 2009, **323**:1485-1488.

51. •• Ueda M, Aichinger E, Gong W, Groot E, Verstraeten I, Vu LD, De Smet I, Higashiyama T, Umeda M, Laux T: **Transcriptional integration of paternal and maternal factors in the Arabidopsis zygote**. *Genes Dev* 2017, **31**:617-627.
The authors show interaction of paternal SSP-activated YODA MAPK signalling with maternal HDG11/12 transcription factors for *WOX8* transcriptional activation and regulation of zygote asymmetric division.

52. Ueda M, Zhang Z, Laux T: **Transcriptional activation of Arabidopsis axis patterning genes WOX8/9 links zygote polarity to embryo development**. *Dev Cell* 2011, **20**:264-270.

53. Yu TY, Shi DQ, Jia PF, Tang J, Li HJ, Liu J, Yang WC: **The Arabidopsis receptor kinase ZAR1 is required for zygote asymmetric division and its daughter cell fate**. *PLoS Genet* 2016, **12**:e1005933.

54. Liu SL, Adams KL: **Dramatic change in function and expression pattern of a gene duplicated by polyploidy created a paternal effect gene in the *Brassicaceae***. *Mol Biol Evol* 2010, **27**:2817-2828.

55. Sukawa Y, Okamoto T: **Cell cycle in egg cell and its progression during zygotic development in rice**. *Plant Reprod* 2018, **31**:107-116.

56. Shull GH: **What is "Heterosis"?** *Genetics* 1948, **33**:439-446.

57. Spillane C, Curtis MD, Grossniklaus U: **Apomixis technology development-virgin births in farmers' fields?** *Nat Biotechnol* 2004, **22**:687-691.

58. Wang X, Xu Y, Zhang S, Cao L, Huang Y, Cheng J, Wu G, Tian S, Chen C, Liu Y *et al.*: **Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction**. *Nat Genet* 2017, **49**:765-772.

59. Scheben A, Hojsgaard D: **Can we use gene-editing to induce apomixis in sexual plants?** *Genes (Basel)* 2020, **11**.

60. Conner JA, Mookkan M, Huo H, Chae K, Ozias-Akins P: **A parthenogenesis gene of apomict origin elicits embryo formation from unfertilized eggs in a sexual plant**. *Proc Natl Acad Sci U S A* 2015, **112**:11205-11210.

61. Akiyama Y, Goel S, Conner JA, Hanna WW, Yamada-Akiyama H, Ozias-Akins P: **Evolution of the apomixis transmitting chromosome in *Pennisetum***. *BMC Evol Biol* 2011, **11**:289.

62. Brukhin V, Baskar R: **A brief note on genes that trigger components of apomixis**. *J Biosci* 2019, **44**.

63. Hojsgaard D: **Apomixis technology: separating the wheat from the chaff**. *Genes (Basel)* 2020, **11**.

64. Sailer C, Schmid B, Grossniklaus U: **Apomixis allows the transgenerational fixation of phenotypes in hybrid plants**. *Curr Biol* 2016, **26**:331-337.

65. Hand ML, Koltunow AM: **The genetic control of apomixis: asexual seed formation**. *Genetics* 2014, **197**:441-450.

66. d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R: **Turning meiosis into mitosis**. *PLoS Biol* 2009, **7**: e1000124.

67. Grelon M, Vezon D, Gendrot G, Pelletier G: **AtSPO11-1 is necessary for efficient meiotic recombination in plants**. *EMBO J* 2001, **20**:589-600.

68. Yu H, Wang M, Tang D, Wang K, Chen F, Gong Z, Gu M, Cheng Z: **OsSPO11-1 is essential for both homologous chromosome pairing and crossover formation in rice**. *Chromosoma* 2010, **119**:625-636.

69. Miao C, Tang D, Zhang H, Wang M, Li Y, Tang S, Yu H, Gu M, Cheng Z: **Central region component1, a novel synaptonemal complex component, is essential for meiotic recombination initiation in rice**. *Plant Cell* 2013, **25**:2998-3009.

70. Chelysheva L, Diallo S, Vezon D, Gendrot G, Vrielynck N, Belcram K, Rocques N, Marquez-Lema A, Bhatt AM, Horlow C *et al.*: **AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis**. *J Cell Sci* 2005, **118**:4621-4632.

71. Shao T, Tang D, Wang K, Wang M, Che L, Qin B, Yu H, Li M, Gu M, Cheng Z: **OsREC8 is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis**. *Plant Physiol* 2011, **156**:1386-1396.

72. Mieulet D, Jolivet S, Rivard M, Cromer L, Vernet A, Mayonove P, Pereira L, Droc G, Courtois B, Guiderdoni E *et al.*: **Turning rice meiosis into mitosis**. *Cell Res* 2016, **26**:1242-1254.

73. Horandl E, Cosendai AC, Temsch EM: **Understanding the geographic distributions of apomictic plants: a case for a pluralistic approach**. *Plant Ecol Divers* 2008, **1**:309-320.

74. Lafon-Placette C, Kohler C: **Endosperm-based postzygotic hybridization barriers: developmental mechanisms and evolutionary drivers**. *Mol Ecol* 2016, **25**:2620-2629.

75. Ravi M, Chan SW: **Haploid plants produced by centromere-mediated genome elimination**. *Nature* 2010, **464**:615-618.

76. Marimuthu MP, Jolivet S, Ravi M, Pereira L, Davda JN, Cromer L, Wang L, Nogue F, Chan SW, Siddiqi I *et al.*: **Synthetic clonal reproduction through seeds**. *Science* 2011, **331**:876.

77. Kelliher T, Starr D, Richbourg L, Chintamanani S, Delzer B, Nuccio ML, Green J, Chen Z, McCuiston J, Wang W *et al.*: **MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction**. *Nature* 2017, **542**:105-109.

78. Gilles LM, Khaled A, Laffaire JB, Chaignon S, Gendrot G, Laplaige J, Berges H, Beydon G, Bayle V, Barret P *et al.*: **Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize**. *EMBO J* 2017, **36**:707-717.

79. Liu C, Li X, Meng D, Zhong Y, Chen C, Dong X, Xu X, Chen B, Li W, Li L *et al.*: **A 4-bp insertion at ZmPLA1 encoding a putative phospholipase a generates haploid induction in maize**. *Mol Plant* 2017, **10**:520-522.

80. •• Wang C, Liu Q, Shen Y, Hua Y, Wang J, Lin J, Wu M, Sun T, Cheng Z, Mercier R *et al.*: **Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes**. *Nat Biotechnol* 2019, **37**:283-286.
The authors used gene editing to combined *MiMe* with *matrilineal* mutation to develop an alternate procedure for synthetic apomixis that can be used for heterosis fixation in hybrid crops.

81. Li X, Meng D, Chen S, Luo H, Zhang Q, Jin W, Yan J: **Single nucleus sequencing reveals spermatid chromosome fragmentation as a possible cause of maize haploid induction**. *Nat Commun* 2017, **8**:991.

82. Greaves IK, Groszmann M, Ying H, Taylor JM, Peacock WJ, Dennis ES: **Trans chromosomal methylation in Arabidopsis hybrids**. *Proc Natl Acad Sci U S A* 2012, **109**:3570-3575.

83. Chen ZJ: **Genomic and epigenetic insights into the molecular bases of heterosis**. *Nat Rev Genet* 2013, **14**:471-482.