*Plant Archives* Vol. 26, No. 2, 2026 pp. 2119-2132 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.291

# CLONAL SEED PRODUCTION THROUGH SYNTHETIC APOMIXIS: A NEW ERA FOR HYBRID CROPS

**Atheetha Lal[1] and Minimol J.S.[2*]**

[1]Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala 680656, India.

[2]Plant Breeding and Genetics, Cocoa Research Centre, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala 680656, India

*Corresponding author E-mail: minimol.js@kau.in

(Date of Receiving-23-07-2026; Date of Revision-19-09-2026; Date of Acceptance-30-09-2026)

**ABSTRACT**

Hybrid crop varieties form the backbone of modern agriculture, however the hybrid vigour present in the first filial generation is lost thereafter, forcing farmers to buy new seeds every season and limiting the adoption of improved varieties by small-scale growers. Hybrid vigour can be stabilized in subsequent generations if plants are able to reproduce asexually by apomixis, that is, by forming seeds containing a clone of the maternal genotype. However, apomixis naturally occurs in less than 0.1 percent of flowering plant species, and is entirely absent in the world's major grain crops. Efforts to introduce apomixis from natural sources into economically important species have so far proved unsuccessful, due to the genetic complexity of the process and difficulties in obtaining fertile hybrids due to ploidy differences. As a result, researchers have begun to pursue the more viable option of engineering apomixis genes de novo. The process of engineering clonal reproduction would entail suppression of meiosis to produce unreduced gametes, activation of embryogenesis without fertilisation and suppression of imprinting in the endosperm, which is known to abort hybrid seeds due to the loss of genomic dosage compensation. In hybrid rice plants these three processes have recently been combined into single systems, which have shown remarkable success in producing fully fertile seeds with a clonal maternal genotype. However, the suppression of natural reproductive controls comes with a cost, as these plants exhibit a range of negative side effects including incomplete penetrance of engineered traits and abnormal ploidy levels of the resultant seeds. We hypothesize that further research into alternative or complementary mechanisms will be necessary in order to fully engineer apomixis and that these issues, alongside regulatory concerns regarding transgene-free edited lines, will determine if and how rapidly such a system could become a practical tool for stabilising hybrid vigour in subsequent generations of crop plants.

***Key words*:** Synthetic apomixis, Hybrid vigour, MiMe, Parthenogenesis, Genome editing

## Introduction

Ensuring global food security has emerged as a critical challenge in the 21st century, as agriculture wrestles with the combined challenge of exploding demographics, shrinking arable lands, as well as changing climates (Molotoks *et al.*, 2020). Hybrid varieties have been a major boost to increase agricultural production. Hybrids have proven to be high yielding, resistant to pests and diseases and able to tolerate a wide range of environments compared to traditional land races or varieties (Birchler *et al.*, 2010; Schnable and Springer, 2013). The primary constraint in maintaining new hybrid varieties is that of genetic segregation in the F2 generation and beyond. The gene complex which confers heterosis is lost in subsequent generations and hence hybrid vigour is not realized (Fiaz *et al.*, 2021; Underwood and Mercier, 2022). Since most desirable traits are not stably expressed in the subsequent

generations, farmers are compelled to buy new hybrid seeds every year. This poses a challenge to both farmers and manufacturers since the former are faced with additional costs while the latter must bear the additional production costs of producing certified seeds. This additional monetary burden is a major constraint to hybrid crop production especially in the developing world (Reed *et al*., 2025).

On the contrary, apomixis, a type of asexual reproduction through seeds, bypasses genetic segregation and allows the indefinite preservation of hybrid vigour in subsequent generations (Xiong *et al*., 2023). Through the permanent fixation of elite heterozygous genotype, this mechanism ensures absolute uniformity of phenotype and stability of desired traits without the genetic reshuffling of sexual reproduction. As a result, the process of cultivar development is simplified and accelerated, since the need for time-consuming hybridization and stabilization of hybrids is eliminated. In addition, apomixis allows the creation of high yield and stress resistant crops and the conservation of valuable germplasm with unique allelic combinations. Ultimately, integrating this reproductive strategy into breeding programs provides a streamlined, cost-effective pathway toward predictable and sustainable crop production (Mahlandt *et al*., 2023). The contrast between segregation in conventionally propagated hybrids and the permanent fixation of the maternal genotype under apomixis is represented schematically in Fig. 1.

To introduce apomictic traits into the elite staple crops, the research has been traditionally devoted to either introgression of naturally occurring apomictic loci from the related wild species or the de novo engineering of synthetic apomixis (Heidemann *et al*., 2025). The majority of the traditional wide hybridization and backcross breeding attempts, like the transfer of characters from *Tripsacum dactyloides* into *Zea mays* have been hindered due to the reproductive isolation and genetic incompatibility with limited success (Sokolov *et al*., 2024). Therefore, the attention has turned towards synthetic apomixis, a revolutionary bioengineering approach that bypasses traditional meiosis to generate unrecombined, unreduced diploid egg cells while replacing egg fertilization with parthenogenesis, whereas endosperm formation remains pseudogamous (Qu *et al*., 2024).

## Natural Apomixis

### Nature's blueprint for clonal seed production:

Natural apomixis serves as the essential biological blueprint for developing modern clonal seed technologies. This naturally occurring reproductive anomaly allows plants to bypass both meiosis and fertilization, yielding offspring that are genetically identical to the maternal plant (Xue *et al*., 2024). In wild populations of taxa such as *Taraxacum* (dandelion) and *Pilosella* (hawkweed), this trait operates as a powerful engine for rapid geographical colonization, enabling well-adapted hybrids to lock in heterosis without succumbing to inbreeding depression (Bicknell *et al*., 2023). While this highly desirable trait is documented across more than 400 angiosperm species, it remains conspicuously absent in the world's major staple crops, a paradox that has driven intense interest in decoding its underlying genomic architecture (Xu *et al*., 2022). Recent comparative phylogenomic studies emphasize that apomixis has evolved independently across diverse botanical lineages, suggesting that searching for a singular "apomixis gene" paradigm may be fundamentally flawed; instead, capturing these natural systems in their broader evolutionary context is vital for successfully engineering the trait into commercial cultivars (Goeckeritz *et al*., 2024).

Deconstructing the architecture of these natural systems requires untangling the specific developmental pathways that sidestep standard embryogenesis. Nature predominantly utilizes two distinct structural frameworks:

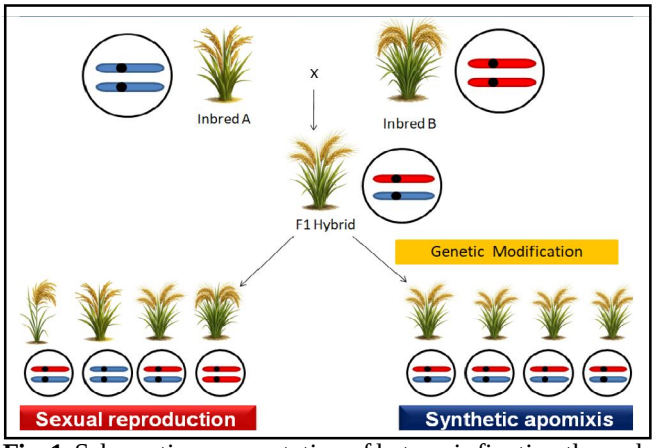



**Fig. 1:** Schematic representation of heterosis fixation through synthetic apomixis. Crossing two homozygous inbred lines (A and B) generates a heterozygous F1 hybrid. During natural sexual reproduction (left), meiotic recombination and segregation cause the F1 plant to produce genetically diverse offspring, leading to the breakdown of hybrid vigour. Conversely, applying targeted genetic modifications to induce synthetic apomixes (right) allows the F1 hybrid to bypass meiosis and fertilization. This results in the production of clonal seeds that perfectly retain the maternal heterozygous genotype, effectively locking in hybrid vigour for all subsequent generations.

gametophytic and sporophytic apomixis. During gametophytic apomixis, an unreduced embryo sac emerges either directly from the megaspore mother cell by bypassing meiosis- a process known as diplospory or from adjacent somatic nucellar cells through apospory, ultimately yielding clonal egg cells capable of parthenogenetic development (Xue *et al.*, 2024). Sporophytic apomixis, often termed adventitious embryony, circumvents the gametophytic phase entirely. Under this paradigm, multiple clonal embryos bud directly from the sporophytic tissues enveloping the sexual embryo sac, heavily leveraging the endosperm for nutritional support. The developmental divergence between amphimixis, diplospory, apospory and adventitious embryony is compared in Fig. 2. While these divergent mechanisms evolved independently across lineages, they share a unified molecular imperative: decoupling embryo initiation from fertilization. Continued structural and genomic analysis of these natural apomicts provides the mechanistic blueprints necessary to isolate the master regulatory nodes orchestrating reproductive autonomy (Goeckeritz *et al.*, 2024).

Despite its obvious ecological utility in fixing advantageous hybrid states, apomixis remains remarkably absent from the major agronomic staple crops that sustain the global food supply. This phylogenetic gap creates a monumental vulnerability for agriculture, leaving modern crop breeding wholly tethered to the costly and labour-intensive annual production of F1 hybrid seeds (Underwood and Mercier, 2022; Xiong *et al.*, 2023). The innate reproductive barriers preventing the natural introgression of apomictic traits from wild relatives into cultivated cereals likely stem from the highly complex, multi-genic nature of the trait and pervasive ploidy mismatches between species (Ozias-Akins and van Dijk, 2007; Hand and Koltunow, 2014; Conner *et al.*, 2013). With the realization of the hopelessness of wide crosses, research has shifted towards to the development of new synthetic ways of engineering apomixis pathways directly into the genome of choice crops. This synthetic effort is necessitated by the pressing need to produce one-line hybrid crops that are genetically engineered to retain their heterozygous state forever (Liang and Gao, 2024).

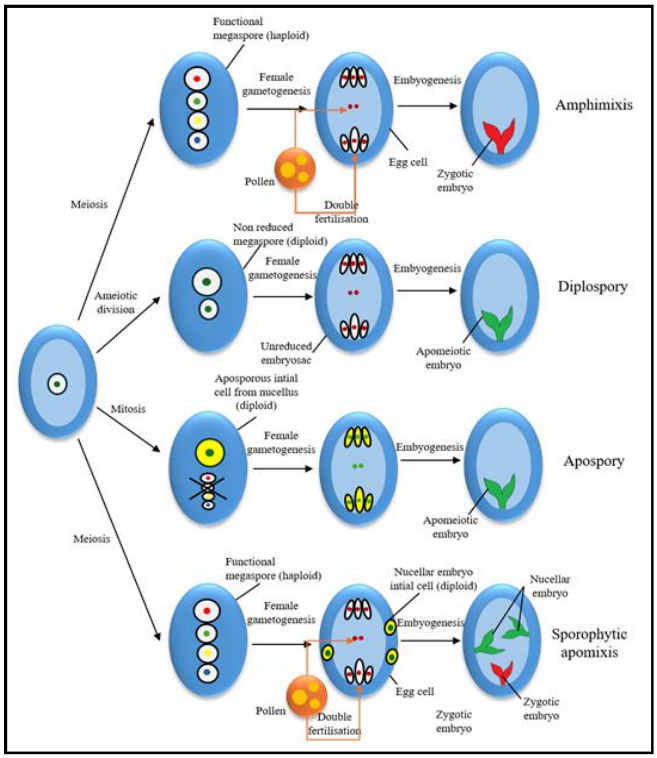



**Fig. 2:** A schematic comparison of sexual reproduction (amphimixis) and three naturally occurring pathways of asexual seed formation (apomixis) in angiosperms.

Amphimixis (Sexual Reproduction): The classical pathway begins with a megaspore mother cell undergoing meiosis to produce a functional haploid megaspore. This cell progresses through female gametogenesis to form a typical reduced embryo sac. Successful reproduction relies on double fertilization, where sperm cells from pollen fuse with both the egg cell and the central cell to generate a zygotic embryo and endosperm, respectively. Diplospory: In this form of gametophytic apomixis, the meiotic division of the megaspore mother cell is completely bypassed or severely modified (ameiosis). This results in a non-reduced diploid megaspore. Gametogenesis proceeds from this unreduced cell, yielding an embryo sac where the diploid egg cell initiates embryogenesis autonomously (parthenogenesis) without the need for fertilization. Apospory: Alternatively, apospory involves the spontaneous differentiation of a somatic cell from the nucellus into an aposporous initial cell. While the legitimate meiotically derived megaspores typically abort or degenerate, this diploid somatic cell undergoes mitotic divisions to form an unreduced embryo sac. Similar to diplospory, the resulting apomictic embryo develops parthenogenetically from the unreduced egg cell. Sporophytic Apomixis (Adventitious Embryony): Unlike gametophytic apomixis, this pathway does not bypass normal gametogenesis. A standard meiotic division occurs, forming a haploid embryo sac, and double fertilisation can proceed normally to form a zygotic embryo. Concurrently, unreduced somatic cells surrounding the embryo sac (nucellar embryo initials) undergo direct embryogenesis. These somatic cells intrude into the embryo sac cavity, ultimately developing into multiple clonal (nucellar) embryos that mature alongside or competitively replace the zygotic embryo.

With the discovery of these natural blueprints for apomixis, recent biological advancements have reached

a new level of success in creating artificial apomixis in cereals. By sequentially targeting and silencing endogenous meiotic genes, researchers successfully force the production of diploid clonal gametes. The critical subsequent step—inducing parthenogenesis—has rapidly advanced through the targeted expression of master regulatory genes. Triggering the expression of a dandelion-derived *PAR* gene alongside meiotic silencing cascades in hybrid rice has recently yielded high-frequency clonal propagation coupled with robust fertility and improved agronomic performance (Song *et al.*, 2024b). Venturing still further into the possibilities of genetic engineering, it was found that expression of the natural *OsWUS* gene in unfertilized eggs is enough for inducing parthenogenesis without resorting to genes from wild apomicts (Huang *et al*., 2025). These milestones validate the concept that nature's intricate apomictic machinery can be elegantly reconstructed to launch a highly resilient new era of self-reproducing hybrid agriculture.

## Dissecting the reproductive cascade

### Bypassing sexual milestones:

Apomeiosis represents the fundamental prerequisite for synthetic clonal reproduction, reprogramming the meiotic cascade towards a mitotic-like division to yield unreduced (2n) megaspores. By specifically targeting and simultaneously abrogating critical meiotic check points including homologous chromosome pairing, sister chromatid cohesion and the second meiotic division, researchers have successfully generated the so-called "Mitosis instead of Meiosis" (MiMe) genotype (d'Erfurth *et al*., 2009). This multiplexed mutational approach completely suppresses meiotic reduction and recombination, securing the highly heterozygous maternal genome within the megagametophyte. The resulting cytological outcome of this genomic engineering is the generation of a functional diploid embryo sac template, which can be utilized for the asexual production of seeds, effectively bypassing the Mendelian segregation that normally reduces hybrid vigour across generations (Vernet *et al*., 2022).

After the circumvention of meiosis, the reproductive pathway needs to skip syngamy through parthenogenesis, enabling fertilisation-independent morphogenesis of the unreduced egg cell. In artificial apomictic systems, this process is mainly facilitated by the genetic regulation of embryo master regulators, especially the ectopic activation of the AP2-domain transcription factor called *BABY BOOM 1* (*BBM1*) solely in the egg cell before anthesis (Li *et al*., 2023). By artificially mimicking the transcriptional surge that canonically follows gamete fusion, *BBM1* activation effectively initiates autonomous zygotic cleavage. Alternatively, parthenogenetic haploid induction can be catalysed through the disruption of sperm-specific paternal factors. For instance, inducing severe oxidative stress within the pollen tube via reactive oxygen species (ROS) bursts or mutating specific phospholipases precipitates male genome elimination, thereby permitting solely maternal embryonic proliferation without chromosomal introgression (Jiang *et al*., 2022).

The final, and arguably most stringent, bottleneck in the synthetic apomictic cascade occurs during endospermogenesis, which is governed by deeply conserved genomic imprinting mechanisms that tightly monitor the maternal-to-paternal genome ratio. In canonical sexual reproduction, viable endosperm strictly requires a 2:1 maternal-to-paternal ratio for normal seed development, deviations from this stoichiometric balance, typically induced when apomeiotic (2n) central cells fuse with reduced (1n) sperm, precipitate severe endosperm abortion and seed lethality, a phenomenon termed the triploid block (Bellucci *et al*., 2023). Present day high-yielding synthetic apomictic systems depend upon the pseudogamous synchronization, in which fertilization of the central cell by the male gamete results in endosperm formation, even though the egg is not fertilized. Achieving entirely autonomous endospermogenesis in staple crops remains a critical frontier, necessitating the identification of epigenetic master switches capable of uncoupling central cell proliferation from paternal genomic triggers (Bellucci *et al*., 2023; Heidemann *et al*., 2025).

## Reprogramming Meiosis into Mitosis

### The MiMe paradigm:

The fundamental conversion of meiosis to a mitotic-like division requires the precise dismantling of highly conserved meiotic checkpoints, achieved by engineering of the "Mitosis instead of Meiosis" (MiMe) genotype. This synthetic cassette simultaneously inactivates three critical loci to collapse the canonical meiotic program. The disruption of *SPO11*, a topoisomerase-like protein required for generating DNA double-strand breaks, entirely eliminates homologous recombination and bivalent formation (Li *et al*., 2023). Suppression of *REC8*, a meiosis-specific subunit of the cohesin complex, abolishes proper sister chromatid cohesion and forces premature chromatid segregation (Xue *et al*., 2024). Knocking out *OMISSION OF SECOND DIVISION 1* (*OSD1*), a critical inhibitor of the Anaphase-Promoting Complex/Cyclosome (APC/C), prevents the cell from entering the second meiotic division (Vernet *et al*., 2022). By ablating these three regulatory nodes, the megaspore mother cell is effectively forced to bypass reductive division and

undergo a strictly equational, apomeiotic division.

Translating the theoretical MiMe framework into agriculturally relevant taxa has been dramatically accelerated by multiplexed CRISPR-Cas9 genome editing. Because apomeiosis requires the concurrent disruption of multiple independent loci, traditional breeding or sequential mutagenesis is largely unfeasible due to accumulated linkage drag and protracted generation times. However, the deployment of polycistronic CRISPR arrays now permits the simultaneous, targeted ablation of *SPO11*, *REC8*, and *OSD1* within a single transformation event (Heidemann *et al*., 2025). The cytological validation of this multiplexed intervention reveals a profound phenotypic shift. Cytogenetic profiling and chromosome spreading techniques demonstrate an explicit absence of meiotic cross-overs during prophase I, followed by the extrusion of unreduced (2n) dyads in lieu of the canonical haploid tetrads (Wang *et al*., 2019). Ultimately, this targeted chromosomal intervention successfully intercepts the gametophytic trajectory before genetic reduction occurs, establishing a stable diploid megagametophyte that perfectly mirrors the maternal genomic architecture. The cytological consequences of these three lesions, set against wild-type meiosis, are summarised in Fig. 3, and the full set of meiotic and gametophytic genes so far implicated in apomeiosis across species is compiled in Table 1.

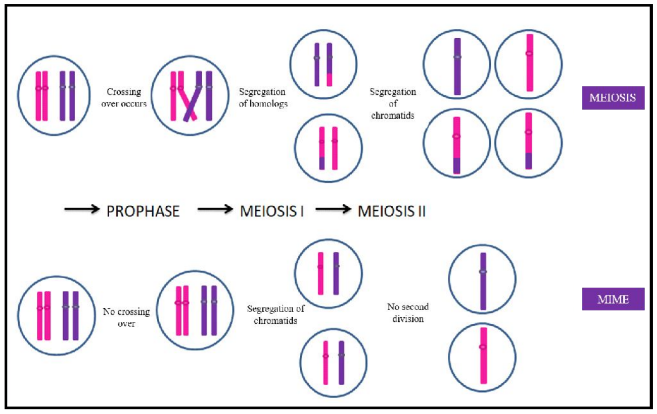



**Fig. 3:** Cytological comparison between wild-type meiosis and the engineered pathway. During standard gametogenesis (top), homologous recombination occurs in prophase, followed by two sequential segregation events: the reductional segregation of homologs (meiosis I) and the equational segregation of sister chromatids (Meiosis II), ultimately yielding four recombinant haploid cells. In the MiMe phenotype (bottom), three critical modifications induce apomeiosis: the suppression of crossing over, the premature equational segregation of sister chromatids in place of homolog segregation, and the complete omission of the second meiotic division. These combined alterations functionally convert meiosis into a mitotic-like division, resulting the formation of unreduced, genetically identical diploid gametes.

## Inducing Parthenogenesis

### The Master Regulatory Role of *BABY BOOM*

The precise spatiotemporal control of this mechanism is of utmost importance, as the constitutive *BBM1* expression leads to severe pleiotropic defects, including the advent of somatic embryogenesis on vegetative tissues. A strategy to restrict its activity employs highly egg-cell-specific promoters such as pAtDD45 or endogenous cereal equivalents to drive *BBM1* expression only in the egg cell just before anthesis. When synchronized with meiotic avoidance (*MiMe*), this targeted ectopic expression has successfully yielded clonal seed induction rates exceeding 98 percent in optimized hybrid rice (Dan *et al*., 2024) and is actively being adapted for other crops like sorghum (Simon *et al*., 2026).

While *BBM1* initiates embryogenesis directly, male-genome elimination provides an additional route to generate haploid. This strategy takes advantage of mutations in the centromere-specific histone H3 (*CENH3*). When an egg is fertilised by pollen carrying mutated *CENH3*, the paternal chromosomes fail to attach properly to the spindle and are degraded post-zygotically, yielding a maternal haploid (Xue *et al*., 2024). However, *CENH3*-derived haploids are typically sterile and require chemical chromosome doubling. In contrast, combining *BBM1* expression with MiMe directly generates fully fertile diploid clones, offering a vastly superior, single-step architecture for fixing hybrid vigour without requiring downstream ploidy manipulation (Ji *et al*., 2025; Xue *et al*., 2024).

## Overcoming the triploid block

### Endosperm development and pseudogamy:

Viability of the endosperm in flowering plants requires the strict 2:1 ratio of maternal to paternal genetic material, and this balance is determined by tightly coordinated parent imprinting. Engineering clonal seed production requires uncoupling this reproductive mechanism by combining apomeiosis, the formation of unreduced gametes with parthenogenesis. However, when unreduced central cells undergo fertilization, the resultant ploidy imbalance precipitates a severe post-zygotic barrier known as the triploid block. This disruption triggers abnormal endosperm cellularization, inevitably culminating in the abortion of the associated embryos (Palumbo *et al*., 2024). Because synthetic apomictic systems must seamlessly integrate unreduced gamete formation with viable nutrient tissue development, overcoming the triploid block remains

**Table1:** Candidate genes for apomixis and their biological roles.

| Genes | Species | Gene product/ Function | Specific functions in apomixis | References |
|---|---|---|---|---|
| **Apomeiosis** | | | | |
| *SPO11-1* | *A. thaliana* | Sporulation11-1 | Lack of recombination | Grelon *et al*., 2001, d'Erfurth *et al*.,2009; Mieulet *et al*., 2016 |
| *SPO11-1* | *O. sativa* | Sporulation11-1 | Lack of recombination | Mieulet *et al*., 2016; d'Erfurth *et al.*, 2010; Xie *et al*., 2019 |
| *SPO11-1* | *S. lycopersicum* | Sporulation11-1 | Lack of recombination | Wang *et al*., 2024 |
| *SPO11-2* | *A. thaliana* | Sporulation11-2 | Lack of recombination | Stacey *et al*., 2006 |
| *SPO11-2* | *O. sativa* | Sporulation11-2 | Lack of recombination | Fayos *et al*., 2020 |
| *SPO11-2* | *Z. mays* | Sporulation11-2 | Lack of recombination | Li *et al*., 2022 |
| *MTOPVIB* | *A. thaliana* | Meiotic topoisomerase VI B-like | Lack of recombination | Chen *et al*., 2024 |
| *MTOPVIB* | *O. sativa* | Meiotic topoisomerase VI B-like | Lack of recombination | Xue *et al*., 2019 |
| *MTOPVIB* | *Z. mays* | Meiotic topoisomerase VI B-like | Lack of recombination | Jing *et al*., 2020 |
| *MTOPVIB* | *H. vulgare* | Meiotic topoisomerase VI B-like | Lack of recombination | Steckenborn *et al*., 2023 |
| *PAIR 1* | *O. sativa* | Homologous pairing aberration in rice meiosis 1 | Lack of recombination | Nonomura *et al*., 2004; Wang *et al*., 2019; Vernet *et al*., 2022; Khanday *et al*., 2019 |
| *PRD 1* | *A. thaliana* | Putative recombination initiation defect 1 | Lack of recombination | Mieulet *et al*., 2016 |
| *PRD 1* | *O. sativa* | Putative recombination initiation defect 1 | Lack of recombination | Shi *et al*., 2021 |
| *PRD 1* | *Z. mays* | Putative recombination initiation defect 1 | Lack of recombination | Wang *et al*., 2022 |
| *PRD 2* | *A. thaliana* | Putative recombination initiation defect 2 | Lack of recombination | De Muyt *et al*., 2009, Mieulet *et al*., 2016 |
| *PRD 2* | *O. sativa* | Putative recombination initiation defect 2 | Lack of recombination | Wang *et al*., 2023 |
| *PRD 3* | *A. thaliana* | Putative recombination initiation defect 3 | Lack of recombination | De Muyt *et al*., 2009, Mieulet *et al*., 2016 |
| *PRD 3* | *Z. mays* | Putative recombination initiation defect 3 | Lack of recombination | Wang *et al*., 2023 |
| *SDS* | *O. sativa* | Synaptonemal complex defective in sporulation 1 | Lack of recombination | Wu *et al*., 2015 |
| *RDR* | *O. sativa* | RNA-dependent RNA polymerase 6 | Lack of recombination | Zhan, 2020 |
| *DFO* | *A. thaliana* | DSB formation | Lack of recombination | Zhang *et al*., 2012 |
| *DYAD/ SWI1* | *A. thaliana* | DYAD/Switch 1 | Single-gene-induced clonal diploid gamete production | Ravi *et al*., 2008; Marimuthu *et al*, 2011 |
| *CRC1* | *O. sativa* | Central region component 1 | Lack of recombination | Miao *et al*., 2013 |
| *BVF1* | *O. sativa* | Bivalent formation 1 | Lack of recombination | Zhou *et al*., 2017, Ji *et al*., 2016 |
| *REC 8* | *A. thaliana* | Meiotic recombination protein 8 | Loss of co-segregation of sister chromatids | d'Erfurth *et al*., 2009 |
| *REC 8* | *O. sativa* | Meiotic recombination protein 8 | Loss of co-segregation of sister chromatids | Shao *et al*., 2011; Wang *et al*., 2019; Khanday *et al*., 2019; Wei *et al*., 2023 |
| *REC 8* | *S. lycopersicum* | Meiotic recombination protein 8 | Loss of co-segregation of sister chromatids | Wang *et al*., 2024 |
| *OSD1* | *A. thaliana* | Omission of second division | Skipping of second meiotic division | d'Erfurth *et al*., 2009; Cifuentes *et al*., 2016; Marimuthu *et al*., 2011 |

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| | | | | |
|---|---|---|---|---|
| *OSD1* | *O. sativa* | Omission of second division | Skipping of second meiotic division | Mieulet *et al*., 2016; Wang *et al*., 2019; Song *et al*., 2024a; |
| *TAM/CYAC 1;2* | *A. thaliana* | Tardy asynchronous meiosis/cyclin A1; A2 | Skipping of second meiotic division | d'Erfurth *et al*., 2010; Cifuentes *et al*., 2016 |
| *TAM/CYAC 1;2* | *S. lycopersicum* | Tardy asynchronous meiosis/cyclin A1; A2 | Skipping of second meiotic division | Wang *et al*., 2024 |
| *TDM* | *A. thaliana* | Three division mutant 1 | Skipping of second meiotic division | Cifuentes *et al*., 2016; Ross *et al*, 1997 |
| **Abnormal fertillisation** | | | | |
| *CENH3* | *A. thaliana* | Centromeric histone H3 | Paternal gene elimination in haploid induction and apomixis | Marimuthu *et al*, 2011; Ravi *et al*., 2010 |
| *CENH3* | *T. aestivum* | Centromeric histone H3 | Paternal gene elimination in haploid induction and apomixis | Lv *et al*., 2020 |
| *CENH3* | *Z. mays* | Centromeric histone H3 | Paternal gene elimination in haploid induction and apomixis | Wang *et al*., 2021 |
| *MTL/PLA1/ NLD* | *O. sativa* | Matrilineal/Phospholipase A1/Not like dad | Paternal gene elimination in haploid induction and apomixis | Wang *et al*., 2019; Yao *et al*., 2018; Xie *et al*., 2019 |
| *MTL/PLA1/ NLD* | *Z. mays* | Matrilineal/Phospholipase A1/Not like dad | Paternal gene elimination in haploid induction and apomixis | Gilles *et al*., 2017; Kelliher *et al*., 2017; Liu *et al*., 2017 |
| *PLD3* | *Z. mays* | Phospholipase D3 | Paternal gene elimination in haploid induction | Li *et al*., 2021 |
| *POD65* | *Z. mays* | Peroxidase 65 | Paternal gene elimination in haploid induction | Jiang *et al*., 2022 |
| *pPLAIIγ* | *A. thaliana* | Gynoecium-expressed phospholipase AII | Expressed in maternal tissue influencing fertilization | Jang *et al*., 2023 |
| *GEX1* | *Z. mays* | Gamete expressed protein 1 | Expressed in maternal tissue influencing fertilization | Sun *et al*., 2025 |
| **Parthenogenesis** | | | | |
| *ECS* | *A. thaliana* | Egg cell- specific aspartic endopeptidase | Induced parthenogenesis in haploid induction | Mao *et al*., 2023; Zhang *et al*., 2023 |
| *ECS* | *O. sativa* | Egg cell- specific aspartic endopeptidase | Induced parthenogenesis in haploid induction | Zhang *et al*., 2023 |
| *ASGR-BBML* | *Pennisetum squamulatum* | Apospory-specific-genomic region-baby boom like 1 | Induced parthenogenesis in haploid induction | Conner *et al*., 2015; Conner *et al*., 2017; Zhang *et al*., 2020 |
| *BBM1* | *O. sativa* | Baby boom 1 | Induced parthenogenesis in haploid induction and apomixis | Vernet *et al*., 2022; Khanday *et al*., 2019; Song *et al*., 2024a |
| *BBM1* | *Z. mays* | Baby boom 1 | Induced parthenogenesis in haploid induction and apomixis | Skinner *et al*., 2023 |
| *BBM2* | *O. sativa* | Baby boom 2 | Induced parthenogenesis in haploid induction and apomixis | Khanday *et al*., 2019 |
| *BBM3* | *O. sativa* | Baby boom 3 | Induced parthenogenesis in haploid induction and apomixis | Khanday *et al*., 2019; Wei et al., 2023 |
| *BBM4* | *O. sativa* | Baby boom 4 | Induced parthenogenesis in haploid induction and apomixis | Khanday *et al*., 2019; Wei *et al*., 2023 |
| *PAR* | *Taraxacum officinale* | Parthenogenesis | Induced parthenogenesis in haploid induction and apomixis | Underwood *et al*., 2022; Huang *et al.*, 2024; Song *et al*., 2024b |
| *WUS/ MOC3* | *O. sativa* | Wuschel/Monoculm 3 | Induced parthenogenesis in apomixis | Huang *et al*., 2025 |

arguably the most formidable molecular bottleneck in agricultural engineering (Qian *et al*., 2024). Successfully navigating the triploid block is the most critical step because it determines whether hybrid vigour will be established or genomic imprinting will lead to embryo abortion (Heidemann *et al*., 2025).

Natural apomicts offer evolutionary blueprints for dismantling this reproductive barrier, frequently relying on pseudogamy to secure endosperm development. Pseudogamy involves fertilization of the central cell by pollen without a contribution of pollen DNA to the diploid egg, resulting in massive deviations from the canonical genomic ratio. To sustain endosperm proliferation under conditions of profound maternal excess, apomicts implement specialized genetic and epigenetic recalibrations. Specific regulatory factors, such as variant alleles of the origin of recognition complex subunit 3, are constitutively upregulated in apomictic endosperm to bypass normal imprinting constraints and support aberrant ploidy ratios (Bellucci *et al*., 2023). This distinct epigenetic plasticity enables unreduced gametophytes to accommodate extreme genomic imbalances, a phenomenon frequently reflected in significant variations in endosperm ploidy and final seed mass among wild apomictic populations (Paczesniak *et al*., 2022). Unraveling these complex, highly reticulate genetic adaptations clarifies how plants recurrently evolved mechanisms to tolerate severely skewed genomic dosages during early reproductive development (Hörandl *et al*., 2024).

Eliminating the reliance on male gametes entirely through autonomous endosperm formation represents the ultimate trajectory for synthetic clonal crops. True fertilization-independent endosperm development circumvents the triploid block by completely silencing the requirement for paternal imprinting signatures. Recent mechanistic studies demonstrate that manipulating specific epigenetic networks or localized hormone signalling, particularly auxin-dependent pathways, can artificially initiate autonomous endosperm proliferation in otherwise sexual models (Rojek and Ohad, 2023). Bridging the developmental gap between facultative natural apomicts and major agronomic crops hinges on identifying the precise molecular switches that decouple endosperm cellularization from fertilization cues. While engineering a fully penetrant, fertilization-independent endosperm remains technically demanding, targeted genetic manipulation of these autonomous pathways holds the key to establishing stable, self-sustaining clonal seed production systems (Xue *et al*., 2024). Genes whose loss of function permits fertilisation-independent endosperm initiation, most of them components of the FIS-class Polycomb repressive complex, are listed in Table 2.

**Milestones in crop engineering:**

**Translating theory into *Oryza sativa*:**

The establishment of synthetic apomixis in hybrid rice has gone through multiple key stages that have led to a stable clonal reproduction from seeds. The first successful approach utilized multiplex genome editing to induce the disruption of meiosis, which formed the MiMe system. In combination with haploid-inducing genes, such as *OsMTL*, *OsPLDá2*, and *OspPLAIIê*, the system produced clonal diploid gametes, but its limited level of clonal efficiency restricted its practical application. The following strategy also relied on the MiMe system but incorporated the genes that drive the parthenogenetic development of egg cells to the level of a fully developed plant, namely OsBBM1, OsBBM4, and OsWUS. The optimized version of OsBBM1 demonstrated outstanding performance, reaching up to 95 percent of clonal efficiency or higher, however it was accompanied by a significant decrease in seed fertility and variable penetrance between different lines. The most recent advances in the study utilize the sperm-specific transcription factor, HUAXU (OsRKD8). Ectopic expression of HUAXU in egg cells supplemented with the MiMe system and optimized through the use of an attenuated promoter established a universal system that reached a near 100 percent level of clonal seed production in multiple cultivars. Moreover, the system allows sustaining seed yield comparable to typical F1 hybrids, effectively making it suitable for producing self-perpetuating commercial hybrids (Chen *et al.*, 2026).

One of the key milestones attained during this progression was confirming stable transmission over several generations with high efficiency levels in clonal reproduction above 99.7% demonstrated by numerous T2 field populations. Moreover, researchers successfully extended this near-complete penetrance across multiple distinct elite hybrid rice cultivars, proving that the mechanism remains universally robust regardless of the underlying genetic background. Large-scale field trials also represented another milestone where it was confirmed that this technology ensured full F1 heterosis, same panicle structure and the same grain yield levels even under varying environmental conditions, soil types and water regimes (Chen *et al*., 2026). It should be noted that as of the middle of 2026, there are no synthetic apomicts that have been marketed for farmers use on a commercial scale. Nevertheless, this technology is just about to cross all the thresholds needed for

**Table 2:** Candidate genes for autonomous endosperm formation.

| Genes | Species | Effects of mutants on endosperm development | Reference |
|---|---|---|---|
| *FIS1/MEA* | *A. thaliana* | In the *FIS1* (mea) and *FIS2* mutant, autonomous diploid endosperm development progresses to the cellularised stage | Grossniklaus *et al*., 1998; Chaudhury *et al*., 1997 |
| *FIS2* | *A. thaliana* | In the *FIS1* (mea) and *FIS*2 mutant, autonomous diploid endosperm development progresses to the cellularised stage | Chaudhury *et al*., 1997 |
| *FIS3/FIE* | *A. thaliana* | Mutation acts in the female gametophyte to initiate endosperm development | Ohad *et al*., 1999; Chaudhury *et al*., 1997 |
| *MSI1* | *A. thaliana* | Mutation displays autonomous endosperm development | Guitton *et al*., 2004 |
| *OsFIE2* | *O. sativa* | RNAi lines can trigger endosperm formation without fertilization | Li *et al*., 2014 |
| *EMF2a* | *O. sativa* | Mutation causes an autonomous endosperm phenotype in the absence of fertilization | Tonosaki *et al*., 2021 |
| *NF-YB1* | *O. sativa* | Repression of expressing caused abnormal seeds with defected embryo and endosperm | Sun *et al*., 2014 |
| *YUC11* | *O. sativa* | Mutation led to endosperm-filling defects | Xu *et al*., 2021 |
| *YUC1/DE18* | Z. *mays* | Mutation showed reduced level of endoreduplication in the endosperm | Bernardi *et al*., 2012 |

commercialization, laying the groundwork for potential future market deployment.

## Biological bottlenecks

### Ploidy instability and penetrance deficits:

Artificially engineering apomixis, the holy grail of clonal reproduction through seeds, is often challenged by the inherent stochastic behavior of reproductive reprogramming, seen most commonly as incomplete penetrance. Even when reduction division is prevented through genetic modifications for the formation of unreduced gametes, external factors and stochastic epigenetics may cause reversion back to the sexual pathway (Niccolò *et al*., 2023). Such developmental flexibility implies that the engineered strains do not usually display apomictic exclusivity, leaving some residual fertilization with the formation of highly mosaic offspring. The direct result of the aforementioned molecular stochasticity is the considerable agronomic threat of ploidy increase. When unreduced (2n) eggs unite with haploid (1n) or unreduced (2n) sperm as a result of the insufficient effectiveness of pre-zygotic barriers, the resulting zygotes become triploid or tetraploid unstable progenies (Cornaro *et al*., 2024). Not only do such polyploid offspring differ from the desired genotype, but they are also genomically unstable and lose their hybrid vigour, which was the aim of synthetic apomixis.

In addition to the effects of cytological instability, by avoiding regular reproduction, plants pay a high price in terms of metabolism and pleiotropic consequences that greatly reduce their productivity. The ectopic activation of embryonic development factors places a strain on the energy resources of the plant and disrupts the natural course of organogenesis. Reprogramming these developmental pathways affects the plant's morphology and further leads to the development of asynchronous endosperms that cause seed abortion (He *et al*., 2023; Li and Yu, 2022). The proper maturation of the seed depends on the exact ratio of maternal and paternal genomes in the endosperm, which is disrupted by avoiding fertilization, and leads to the inability of the endosperm to develop normally and results in the production of deformed and small-sized seeds. Modern methods of haploid induction and apomeiosis accidentally activate these lethal pathways due to the intrinsic evolutionary conflict between artificially introduced clonality and survival strategies (Liu *et al*., 2025). The inability of the plant to produce nutrients for the clonal embryo without the sexual endosperm contributes directly to yield loss.

## Agronomic horizons

### The socioeconomic landscape of self-cloning crops:

Engineering self-perpetuating hybrid crops through synthetic apomixis fundamentally reconfigures the global agricultural economy by decentralizing seed production. Smallholder farmers, historically tethered to the financial burden of repurchasing heterozygous F1 seeds annually, stand to gain unprecedented yield security and seed sovereignty as clonal propagation permanently locks in hybrid vigour (Ringeisen *et al*., 2024; Ronceret and Bolaños-Villegas, 2024). Driving this paradigm shift are advanced transgene-free genome editing platforms, which allow researchers to meticulously alter reproductive pathways without leaving residual foreign DNA in the final progeny (Gajardo *et al*., 2023). Yet, scaling this technology from the laboratory to commercial acreage collides with fragmented, geopolitical regulatory barriers.

Because researchers frequently deploy CRISPR-Cas9 transiently to induce apomeiosis or parthenogenesis, the resulting crops occupy an ambiguous legal territory; they lack transgenes but still face intense scrutiny under rigid, legacy GMO frameworks designed for older recombinant technologies (Hernandes-Lopes *et al*., 2023). Navigating this legislative bottleneck will dictate whether apomictic hybrids remain a strictly academic triumph or evolve into a globally accessible tool for climate-resilient agriculture.

Addressing these regulatory and agronomic hurdles will involve fine-tuning the genetic mechanisms governing apomixis for optimal penetrance and fertility levels. Clonal seed production using earlier approaches has been highly dependent on the use of complicated multiplex edits, such as the MiMe mutants, in conjunction with the ectopic expression of master embryogenic genes like BBM1 to circumvent the need for fertilization (Fiaz *et al*., 2021; Xue *et al*., 2024). Although groundbreaking, multiplex gene cassettes have often resulted in yield penalties and varying intergenerational stability. Advances in the underlying mechanics of the process now suggest that a single-gene master regulators can trigger high-efficiency parthenogenesis while maintaining near-normal seed set. Beyond promoter engineering, accumulating evidence shows that clonal seed penetrance and fertility outcomes are strongly genotype- and environment-dependent, with the same synthetic construct performing inconsistently across different hybrid backgrounds, growing seasons and field sites (Chen *et al*., 2026), underscoring that multi-generational, multi-location field validation is as critical to commercial translation as the underlying molecular design. Encouragingly, synthetic apomixis is no longer confined to rice: its recent extension to sorghum demonstrates that these engineering principles can be transferred across cereal species, broadening the crop base to which the technology may eventually be applied (Simon *et al*., 2026). Future research vectors must prioritize discovering and validating alternative single-gene targets, such as distinct *PAR* alleles or sperm-specific transcription factors, which possess the precision necessary to uncouple gametogenesis from fertilization natively (Heidemann *et al*., 2025). Isolating these novel molecular targets will simplify the bioengineering pipeline, yielding apomictic crops that satisfy stringent regulatory demands and the biological imperatives of high-yield farming.

## Conclusion

### Toward the democratization of hybrid vigour

For decades, the natural occurrence of apomixis remained an evolutionary oddity best studied in isolation in one or two wild species. However, the recent developments described in this article demonstrate how scientists are now approaching the problem from the opposite direction and successfully engineering apomixis in various crops. Modern biologists are now targeting genes responsible for the process of apomixis and modifying them in order to achieve the desired outcome, namely, triggering the development of viable seeds without fertilization. The mechanisms described by researchers allow for the introduction of apomixis into major food plants and lead to the production of hybrids with desirable characteristics that would be impossible to obtain through conventional breeding techniques.

Translating these molecular triumphs into broad-scale agronomic reality, however, is an ongoing endeavor. The main challenges, including the unpredictability of clonal penetrance, can be overcome by improved understanding of biological processes, such as ploidy increases due to accidental fertilisation or epigenetic constraints to spontaneous endosperm formation. However, the trend in the field suggests that the problem is bound to be solved soon due to the combined efforts of scientists, who actively work on the improvement of such genetic techniques as multiplex genome editing and discovery of key regulatory genes. It is crystallizing into an imminent agricultural technology, one that holds the unprecedented potential to permanently democratize heterosis, empower seed sovereignty, and fundamentally stabilize global food security networks for future generations.

## Acknowledgement

The authors acknowledge the use of ChatGPT (OpenAI) for assistance in language editing and improving the clarity of the manuscript; all scientific content and interpretations are the authors' responsibility.

## References

Bellucci, M., Caceres M.E., Paolocci F., Vega J.M., Ortiz J.P.A., Ceccarelli M., De Marchis F. and Pupilli F. (2023). ORIGIN OF RECOGNITION COMPLEX 3 controls the development of maternal excess endosperm in the Paspalum simplex agamic complex (Poaceae). *J. Exp. Bot.*, **74(10)**, 3074-3093.

Bernardi, J., Lanubile A., Li Q.B., Kumar D., Kladnik A., Cook S.D., Ross J.J., Marocco A. and Chourey P.S. (2012). Impaired auxin biosynthesis in the defective endosperm18 mutant is due to mutational loss of expression in the *ZmYuc1* gene encoding endosperm-specific YUCCA1 protein in maize. *Plant Physiol.*, **160(3)**, 1318-1328.

Bicknell, R., Gaillard M., Catanach A., McGee R., Erasmuson S., Fulton B. and Winefield C. (2023). Genetic mapping of the LOSS OF PARTHENOGENESIS locus in *Pilosella*

*piloselloides* and the evolution of apomixis in the Lactuceae. *Front. Plant Sci.*, **14**, 1239191**.**

Birchler, J.A., Yao H., Chudalayandi S., Vaiman D. and Veitia R. A. (2010). Heterosis. *Plant Cell*, **22(7)**, 2105-2112.

Chaudhury, A.M., Ming L., Miller C., Craig S., Dennis E.S. and Peacock W.J. (1997). Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci.*, **94(8)**, 4223-4228.

Chen, W.Q., Liu C., Lu H., Yang S., Ma J., Huang Y., Xiong J., Huang D., Tao Z., Lin J. and Wang K. (2026). Fixing hybrid vigor in rice: achieving near-100% clonal seed production with normal seed set. *Vita*. (https://doi.org/10.15302/vita.2026.07.0053).

Chen, H.W., Yeh H.Y., Chang C.C., Kuo W.C., Lin S.W., Vrielynck N., Grelon M., Chan N.L. and Chi P. (2024). Biochemical characterization of the meiosis-essential yet evolutionarily divergent topoisomerase VIB-like protein MTOPVIB from *Arabidopsis thaliana*. *Nucleic Acids Res.*, **52(8)**, 4541-4555.

Cifuentes, M., Jolivet S., Cromer L., Harashima H., Bulankova P., Renne C., Crismani W., Nomura Y., Nakagami H., Sugimoto K., Schnittger A., Riha K. and Mercier R. (2016). *TDM1* regulation determines the number of meiotic divisions. *PLoS Genet.*, **12(2)**, e1005856.

Conner, J.A., Gunawan G. and Ozias-Akins P. (2013). Recombination within the apospory specific genomic region leads to the uncoupling of apomixis components in *Cenchrus ciliaris*. *Planta*, **238(1)**, 51-63.

Conner, J.A., Mookkan M., Huo H., Chae K. and Ozias-Akins P. (2015). A parthenogenesis gene of apomict origin elicits embryo formation from unfertilized eggs in a sexual plant. *Proc. Natl. Acad. Sci.*, **112(36)**, 11205-11210.

Conner, J.A., Podio M. and Ozias-Akins P. (2017). Haploid embryo production in rice and maize induced by *PsASGR-BBML* transgenes. *Plant Reprod.*, **30(1)**, 41-52.

Cornaro, L., Banfi C., Cavalleri A., van Dijk P.J., Radoeva T., Cucinotta M. and Colombo L. (2024). Apomixis at high resolution: unravelling diplospory in Asteraceae. *J. Exp. Bot.*, **76(6)**, 1644-1657.

Dan, J., Xia Y., Wang Y., Zhan Y., Tian J., Tang N., Deng H. and Cao M. (2024). One-line hybrid rice with high-efficiency synthetic apomixis and near-normal fertility. *Plant Cell Rep.*, **43(3)**, 79.

d'Erfurth, I., Cromer L., Jolivet S., Girard C., Horlow C., Sun Y., To J.P.C., Berchowitz L.E., Copenhaver J.P. and Mercier R. (2010). The cyclin-A CYCA1; 2/TAM is required for the meiosis I to meiosis II transition and cooperates with *OSD1* for the prophase to first meiotic division transition. *PLoS Genet.*, **6(6)**, e1000989.

d'Erfurth, I., Jolivet S., Froger N., Catrice O., Novatchkova M. and Mercier R. (2009). Turning meiosis into mitosis. *PLoS Biol.*, **7(6)**, e1000124.

De Muyt, A., Pereira L., Vezon D., Chelysheva L., Gendrot G., Chambon A., Laine-Choinard S., Pelletier G., Mercier R., Nogue F. and Grelon M. (2009). A high throughput genetic screen identifies new early meiotic recombination functions in *Arabidopsis thaliana*. *PLoS Genet.*, **5(9)**, e1000654.

Fayos, I., Meunier A.C., Vernet A., Navarro-Sanz S., Portefaix M., Lartaud M., Bastianelli G., Perin C., Nicolas A. and Guiderdoni E. (2020). Assessment of the roles of SPO11-2 and SPO11-4 in meiosis in rice using CRISPR/Cas9 mutagenesis. *J. Exp. Bot.*, **71(22)**, 7046-7058.

Fiaz, S., Wang X., Younas A., Alharthi B., Riaz A. and Ali H. (2021). Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations. *GM Crops Food*, **12(1)**, 57-70.

Gajardo, H.A., Gómez-Espinoza O., Boscariol Ferreira P., Carrer H. and Bravo L.A. (2023). The potential of CRISPR/Cas technology to enhance crop performance on adverse soil conditions. *Plants*, **12(9)**, 1892.

Gilles, L.M., Khaled A., Laffaire J.B., Chaignon S, Gendrot G., Laplaige J., Bergès H., Beydon G., Bayle V., Barret P., Comadran J., Martinant J.M., Rogowsky P. M. and Widiez T. (2017). Loss of pollen specific phospholipase NOT LIKE DAD triggers gynogenesis in maize. *The EMBO J.*, **36(6)**, 707-717.

Goeckeritz, C.Z., Zheng X., Harkess A. and Dresselhaus T. (2024). Widespread application of apomixis in agriculture requires further study of natural apomicts. *IScience*, **27(9)**.

Grelon, M., Vezon D., Gendrot G. and Pelletier G. (2001). *AtSPO11 1* is necessary for efficient meiotic recombination in plants. *EMBO J.*, **20(3)**, 589-600.

Grossniklaus, U., Vielle-Calzada J.P., Hoeppner M.A. and Gagliano W.B. (1998). Maternal control of embryogenesis by *MEDEA*, a polycomb group gene in Arabidopsis. *Science*, **280(5362)**, 446-450.

Guitton, A.E., Page D.R., Chambrier P., Lionnet C., Faure J.E., Grossniklaus U. and Berger F. (2004). Identification of new members of Fertilisation Independent Seed Polycomb Group pathway involved in the control of seed development in *Arabidopsis thaliana*. *Development*, **131(12)**, 2971-2981.

Hand, M.L. and Koltunow A.M. (2014). The genetic control of apomixis: asexual seed formation. *Genetics*, **197(2)**, 441-450

He, H., Shiragaki K. and Tezuka T. (2023). Understanding and overcoming hybrid lethality in seed and seedling stages as barriers to hybridization and gene flow. *Front. Plant Sci.*, **14**, 1219417.

Heidemann, B., Primetis E., Zahn I.E. and Underwood C.J. (2025). To infinity and beyond: recent progress, bottlenecks, and potential of clonal seeds by apomixis. *Plant J.*, **121(4)**, e70054.

Hernandes-Lopes, J., Yassitepe J.E.D.C.T., Koltun A., Pauwels L., Silva V.C.H.D., Dante R.A., Gerhardt I.R. and Arruda P. (2023). Genome editing in maize: Toward improving complex traits in a global crop. *Genet. Mol. Biol.*, **46(1)**, e20220217.

Hörandl, E., Hojsgaard D., Caperta A., Conceição S.I., Róis A.S., Dickinson T., Mandakova T., Windham M.D.,

Appelhans M.S., Mraz P. and Chrtek J. (2024). Apomixis in systematics, evolution and phylogenetics of angiosperms: current developments and prospects. *Crit. Rev. Plant Sci.*, **44(4)**, 218-260.

Huang, Y., Meng X., Rao Y., Xie Y., Sun T., Chen W., Wie X., Xiong J., Yu H., Li J. and Wang K. (2025). OsWUS-driven synthetic apomixis in hybrid rice. *Plant Commun.*, **6(1)**, 101136.

Huang, Y., Liang Y., Xie Y., Rao Y., Xiong J., Liu C., Wang C., Wang X., Qian Q. and Wang K. (2024). Efficient haploid induction via egg cell expression of dandelion PARTHENOGENESIS in foxtail millet (*Setaria italica*). *Plant Biotechnol. J.*, **22(7)**, 1797.

Jang, J.H., Seo H.S., Widiez T. and Lee O.R. (2023). Loss of function of gynoecium expressed phospholipase pPLAIIã triggers maternal haploid induction in Arabidopsis. *New Phytol.*, **238(5)**, 1813-1824.

Ji, J., Tang D., Shen Y., Xue Z., Wang H., Shi W., Zhang C., Du G., Li Y. and Cheng Z. (2016). P31comet, a member of the synaptonemal complex, participates in meiotic DSB formation in rice. *Proc. Natl. Acad. Sci.*, **113(38)**, 10577-10582.

Ji, Y., Xiong J., Qiu X. and Wang K. (2025). Progress on plant parthenogenesis: promoting the application of synthetic apomixis. *Hereditas (Beijing)*, **47(4)**, 448-455.

Jiang, C., Sun J., Li R., Yan S., Chen W., Guo L., Qin G., Wang P., Luo C., Huang W., Zhang Q., Fernie A.R., Jackson D., Li X. and Yan J. (2022). A reactive oxygen species burst causes haploid induction in maize. *Mol. Plant*, **15(6)**, 943-955.

Jing, J.L., Zhang T., Kao Y.H., Huang T.H., Wang C.J.R. and He Y. (2020). *ZmMTOPVIB* enables DNA double-strand break formation and bipolar spindle assembly during maize meiosis. *Plant Physiol.*, **184(4)**, 1811-1822.

Kelliher, T., Starr D., Richbourg L., Chintamanani S., Delzer B., Nuccio M.L., Green J., Chen Z., McCuiston J., Wang W., Liebler T., Bullock P. and Martin B. (2017). Matrilineal, a sperm-specific phospholipase, triggers maize haploid induction. *Nature*, **542(7639)**, 105-109.

Khanday, I., Skinner D., Yang B., Mercier R. and Sundaresan V. (2019). A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*, **565(7737)**, 91-95.

Li, M., Li S., He Y., Wang Y., Zhang T., Li P. and He Y. (2022). ZmSPO11-2 is critical for meiotic recombination in maize. *Chromosome Res.*, **30(4)**, 415-428.

Li, S.Z., Wang J., Jia S.G., Wang K. and Li H.J. (2023). Synthetic apomixis: from genetic basis to agricultural application. *Seed Biol.*, **2(1)**.

Li, S., Zhou B., Peng X., Kuang Q., Huang X., Yao J., Du B. and Sun M.X. (2014). O s FIE 2 plays an essential role in the regulation of rice vegetative and reproductive development. *New Phytol.*, **201(1)**, 66-79**.**

Li, Y., Lin Z., Yue Y., Zhao H., Fei X., E L., Liu C., Chen S., Lai J. and Song W. (2021). Loss-of-function alleles of *ZmPLD3* cause haploid induction in maize. *Nat. Plants*, **7(12)**, 1579-1588.

Li, C. and Yu H. (2022). Parental regulation of seed development. *Seed Biol.*, **1(1)**, 1-12.

Liang, R. and Gao C. (2024). Creating one-line hybrid crops by synthetic apomixis. *Mol. Plant.*, **17(1)**,16-18.

Liu, Y., Elshan M., Li G., Han X., Chen X. and Feng X. (2025). Perspectives of genome editing mediated haploid inducer systems in legumes. *Int. J. Mol. Sci*, **26(3)**, 1154.

Liu, C., Li X., Meng D., Zhong Y., Chen C., Dong X., Xu X., Chen B., Li W., Li L., Tian X., Zhao H., Song W., Luo H., Zhang Q., Lai J., Jin W., Yan J. and Chen S. (2017). A 4-bp insertion at *ZmPLA1* encoding a putative phospholipase A generates haploid induction in maize. *Mol. Plant.*, **10(3)**, 520-522.

Lv, J., Yu K., Wei J., Gui H., Liu C., Liang D., Wang Y., Zhou H., Carlin R., Rich R., Lu T., Que Q., Wang W.C., Zhang X. and Kelliher T. (2020). Generation of paternal haploids in wheat by genome editing of the centromeric histone CENH3. *Nat. Biotechnol.*, **38(12)**, 1397-1401.

Mahlandt, A., Singh D.K. and Mercier R. (2023). Engineering apomixis in crops. *Theor. Appl. Genet.*, **136(6)**, 131.

Mao, Y., Nakel T., Erbasol Serbes I., Joshi S., Tekleyohans D.G., Baum T. and Groß-Hardt R. (2023). ECS1 and ECS2 suppress polyspermy and the formation of haploid plants by promoting double fertilization. *Elife*, **12**, e85832.

Marimuthu, M.P., Jolivet S., Ravi M., Pereira L., Davda J.N., Cromer L., Wang L., Nogue F., Chan S.W.L. and Mercier R. (2011). Synthetic clonal reproduction through seeds. *Science*, **331(6019)**, 876-876.

Miao, C., Tang D., Zhang H., Wang M., Li Y., Tang S., Yu H., Gu M. and Cheng Z. (2013). Central region component1, a novel synaptonemal complex component, is essential for meiotic recombination initiation in rice. *Plant Cell*, **25(8)**, 2998-3009.

Mieulet, D., Jolivet S., Rivard M., Cromer L., Vernet A., Mayonove P., Pereira L., Droc G., Courtois B., Guiderdoni E. and Mercier R. (2016). Turning rice meiosis into mitosis. *Cell Res.*, **26(11)**, 1242-1254.

Molotoks, A., Smith P. and Dawson T.P. (2020). Impacts of land use, population, and climate change on global food security. *Food energy secur.*, **10(1)**, e261.

Niccolò, T., Anderson A.W. and Emidio A. (2023). Apomixis: oh, what a tangled web we have!. *Planta*, **257(5)**, 92.

Nonomura, K.I., Nakano M., Fukuda T., Eiguchi M., Miyao A., Hirochika H. and Kurata N. (2004). The novel gene HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS1 of rice encodes a putative coiled-coil protein required for homologous chromosome pairing in meiosis. *Plant Cell.*, **16(4)**, 1008-1020.

Ohad, N., Yadegari R., Margossian L., Hannon M., Michaeli D., Harada J.J., Goldberg R.B. and Fischer R.L. (1999). Mutations in *FIE*, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell*, **11(3)**, 407-415.

Ozias-Akins, P. and van Dijk P.J. (2007). Mendelian genetics of apomixis in plants. *Annu. Rev. Genet.*, **41(1)**, 509-537.

Paczesniak, D., Pellino M., Goertzen R., Guenter D., Jahnke S., Fischbach A., Lovell J.T. and Sharbel T.F. (2022). Seed size, endosperm and germination variation in sexual and apomictic Boechera. *Front. Plant Sci.*, **13**, 991531.

Palumbo, F., Gabelli G., Pasquali E., Vannozzi A., Farinati S., Draga S., Ravi S., Lucia M.C.D., Bertoldo G. and Barcaccia G. (2024). RNA-seq analyses on gametogenic tissues of alfalfa (*Medicago sativa*) revealed plant reproduction- and ploidy-related genes. *BMC Plant Biol.*, **24(1)**, 826.

Qian, H., Guo J. and Shi H. (2024). Genetic manipulation of the genes for clonal seeds results in sterility in cotton. *BMC Plant Biol.*, **24(1)**, 946.

Qu, Y., Fernie A.R., Liu J. and Yan J. (2024). Doubled haploid technology and synthetic apomixis: Recent advances and applications in future crop breeding. *Mol. Plant*, **17(7)**, 1005–1018.

Ravi, M., Marimuthu M.P. and Siddiqi I. (2008). Gamete formation without meiosis in Arabidopsis. *Nature*, **451(7182)**, 1121-1124.

Ravi, M. and Chan S.W. (2010). Haploid plants produced by centromere-mediated genome elimination. *Nature*, **464(7288)**, 615-618.

Reed, R.C., Iqbal R., Sundaresan V. and Khanday I. (2025). Reinventing inheritance: clonal propagation of crops through seeds with apomixis. *Crit. Rev. Plant Sci.*, **44(5)**, 326-344.

Ringeisen, B.R., de Azevedo Souza C., Njuguna E.W. and Ronald P.C. (2024). We need to act now to ensure global food security, and reduce agricultural greenhouse gas emissions. *Bull. At. Sci.*, **80(3)**, 162-167.

Rojek, J. and Ohad N. (2023). The phenomenon of autonomous endosperm in sexual and apomictic plants. *J. Exp. Bot.*, **74(15)**, 4324-4348.

Ronceret, A. and Bolaños Villegas P. (2024). Plant reproduction research in Latin America: Toward sustainable agriculture in a changing environment. *Plant-Environ. Interact.*, **5(3)**, e10143.

Ross, K.J., Fransz P., Armstrong S.J., Vizir I., Mulligan B., Franklin F.C.H. and Jones G.H. (1997). Cytological characterization of four meiotic mutants of Arabidopsis isolated from T-DNA-transformed lines. *Chromosome Res.*, **5(8)**, 551-559.

Schnable, P.S. and Springer N.M. (2013). Progress toward understanding heterosis in crop plants. *Annu. Rev. Plant Biol.*, **64**, 71-88.

Shao, T., Tang D., Wang K., Wang M., Che L., Qin B., Yu H., Li M., Gu M. and Cheng Z. (2011). *OsREC8* is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis. *Plant Physiol.*, **156(3)**, 1386-1396.

Shi, W., Ji J., Xue Z., Zhang F., Miao Y., Yang H., Tang D., Du G., Li Y., Shen Y. and Cheng Z. (2021). PRD1, a homologous recombination initiation factor, is involved in spindle assembly in rice meiosis. *New Phytol.*, **230(2)**, 585-600.

Simon, M.K., Yuan L., Che P., Day K., Jones T., Godwin I.D., Koltunow A.M.G. and Albertsen M.C. (2026). Induction of synthetic apomixis in two sorghum hybrids enables seed yield and genotype preservation over multiple generations. *Plant Biotechnol. J.*, **24(3)**, 1712-1724.

Skinner, D.J., Mallari M.D., Zafar K., Cho M.J. and Sundaresan V. (2023). Efficient parthenogenesis via egg cell expression of maize BABY BOOM 1: a step toward synthetic apomixis. *Plant Physiol.*, **193(4)**, 2278-2281.

Sokolov, V.A., Panikhin P.A., Plotnikov K.O., Chepurnov G.Y. and Blinov A.G. (2024). Development of apomictic 56-Chromosomal maize–tripsacum hybrids: a potential breakthrough in heterosis fixation. *Plants*, **13(15)**, 2138.

Song, M., Li F., Chen Z., Hou H., Wang Y., Liu H., Li J., Peng T., Zhao Y., Du Y., Sun H., Du C., Zhang J., Zhao Q. and Miao C. (2024a). Engineering high-frequency apomixis with normal seed production in hybrid rice. *Iscience*, **27(12)**, 111479.

Song, M., Wang W., Ji C., Li S., Liu W., Hu X., Anhui F., Shuang R., Du S., Wang H., Dai K., Guo L., Qian Q. and Hu X. (2024b). Simultaneous production of high-frequency synthetic apomixis with high fertility and improved agronomic traits in hybrid rice. *Mol. Plant*, **17(1)**, 4-7.

Stacey, N.J., Kuromori T., Azumi Y., Roberts G., Breuer C., Wada T., Maxwell A., Roberts K. and Sugimoto Shirasu K. (2006). Arabidopsis *SPO11 2* functions with *SPO11 1* in meiotic recombination. *The Plant J.*, **48(2)**, 206-216.

Steckenborn, S., Cuacos M., Ayoub M.A., Feng C., Schubert V., Hoffie I., Hensel G., Kumlehn J. and Heckmann S. (2023). The meiotic topoisomerase VI B subunit (MTOPVIB) is essential for meiotic DNA double-strand break formation in barley (*Hordeum vulgare* L.). *Plant Reprod.*, **36(1)**, 1-15.

Sun, Y., Hou L., Li C., Zhou Z., Wu J. and Yu H. (2025). Maternal haploid induction in maize via mutation of Gamete Expression protein 1. *Plant Biotechnol. J.*, **23(7)**, 2595-2607.

Sun, X., Ling S., Lu Z., Ouyang Y.D., Liu S. and Yao J. (2014). *OsNF-YB1*, a rice endosperm-specific gene, is essential for cell proliferation in endosperm development. *Gene*, **551(2)**, 214-221.

Tonosaki, K., Ono A., Kunisada M., Nishino M., Nagata H., Sakamoto S., Kijima S.T., Furuumi H., Nonomura K.I., Sato Y., Ohme-Takagi M., Endo M., Comai L., Hatakeyama K., Kawakatsu T. and Kinoshita T. (2021). Mutation of the imprinted gene *OsEMF2a* induces autonomous endosperm development and delayed cellularization in rice. *Plant Cell*, **33(1)**, 85-103.

Underwood, C.J. and Mercier R. (2022). Engineering apomixis: clonal seeds approaching the fields. *Annu. Rev. Plant Biol.*, **73(1)**, 201-225.

Underwood, C.J., Vijverberg K., Rigola D., Okamoto S., Oplaat C., Camp R.H.O.D., Tatyana R., Schauer S.E., Fierens J., Jansen K., Mansveld S., Busscher M., Xiong W., Datema

E., Nijbroek K., Blom E.J., Bicknell R., Catanach A., Erasmuson S., Winefield C., Van Tunen A.J., Prins M., Schranz M.C. and Van Dijk P.J. (2022). A PARTHENOGENESIS allele from apomictic dandelion can induce egg cell division without fertilization in lettuce. *Nat. Genet.*, **54(1)**, 84-93.

Vernet, A., Meynard D., Lian Q., Mieulet D., Gibert O., Bissah M., Rivallan R., Autran D., Leblanc O., Meunier A.C., Frouin J., Taillebois J., Shankle K., Khanday I., Mercier R., Sunderasan V. and Guiderdoni E. (2022). High-frequency synthetic apomixis in hybrid rice. *Nature Commun.*, **13(1)**, 7963.

Wang, C., Liu Q., Shen Y., Hua Y., Wang J., Lin J., Wu M., Sun T., Cheng Z., Mercier R. and Wang K. (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nat. Biotechnol.*, **37(3)**, 283-286.

Wang, C., Qu S., Zhang J., Fu M., Chen X. and Liang W. (2023). OsPRD2 is essential for double-strand break formation, but not spindle assembly during rice meiosis. *Front. Plant Sci.*, **13**, 1122202.

Wang, N., Gent J.I. and Dawe R.K. (2021). Haploid induction by a maize *cenh3* null mutant. *Sci. Adv.*, **7(4)**, eabe2299.

Wang, Y., Fuentes R.R., van Rengs W.M., Effgen S., Zaidan M.W.A.M., Franzen R., Susanto T., Fernandes J.B., Mercier R. and Underwood C.J. (2024). Harnessing clonal gametes in hybrid crops to engineer polyploid genomes. *Nat. Genet.*, **56(6)**, 1075-1079.

Wang, Y., Li S.Y., Wang Y.Z. and He Y. (2023). ZmASY1 interacts with ZmPRD3 and is crucial for meiotic double strand break formation in maize. *New Phytol.*, **237(2)**, 454-470.

Wang, Y., Wang Y., Zang J., Chen H. and He Y. (2022). ZmPRD1 is essential for double-strand break formation, but is not required for bipolar spindle assembly during maize meiosis. *J. Exp. Bot.*, **73(11)**, 3386-3400.

Wei, X., Liu C., Chen X., Lu H., Wang J., Yang S. and Wang K. (2023). Synthetic apomixis with normal hybrid rice seed production. *Mol. Plant*, **16(3)**, 489-492.

Wu, Z., Ji J., Tang D., Wang H., Shen Y., Shi W., Li Y., Tan X., Cheng Z. and Luo Q. (2015). *OsSDS* is essential for DSB formation in rice meiosis. *Front. Plant Sci.*, **6**, 21.

Xie, E., Li Y., Tang D., Lv Y., Shen Y. and Cheng Z. (2019). A strategy for generating rice apomixis by gene editing. *J. Integr. Plant Biol.*, **61(8)**, 911-916.

Xiong, J., Hu F., Ren J., Huang Y., Liu C. and Wang K. (2023). Synthetic apomixis: the beginning of a new era. *Curr. Opin. Biotechnol.*, **79**, 102877.

Xu, Y., Jia H., Tan C., Wu X., Deng X. and Xu Q. (2022). Apomixis: genetic basis and controlling genes. *Hortic. Res.*, **9**, uhac150.

Xu, X., E Z., Zhang D., Yun Q., Zhou Y., Niu B. and Chen C. (2021). *OsYUC11*-mediated auxin biosynthesis is essential for endosperm development of rice. *Plant Physiol.*, **185(3)**, 934-950.

Xue, Z., Liu C., Shi W., Miao Y., Shen Y., Tang D., Li Y., You A., Xu Y., Chong K. and Cheng Z. (2019). *OsMTOPVIB* is required for meiotic bipolar spindle assembly. *Proc. Natl. Acad. Sci.*, **116(32)**, 15967-15972.

Xue, L., Zhang Y., Wei F., Shi G., Tian B., Yuan Y., Jiang W., Zhao M., Hu L., Xie Z. and Gu H. (2024). Recent progress on plant apomixis for genetic improvement. *Int. J. Mol. Sci.*, **25(21)**, 11378.

Yao, L., Zhang Y., Liu C., Liu Y., Wang Y., Liang D., Liu J., Sahoo G. and Kelliher T. (2018). *OsMATL* mutation induces haploid seed formation in indica rice. *Nat. Plants*, **4(8)**, 530-533.

Zhan, J. (2020). *RDR6* is essential for double-Strand break formation during male meiosis in Rice. *Plant Cell*, **32(10)**, 3053.

Zhang, Z., Conner J., Guo Y. and Ozias-Akins P. (2020). Haploidy in tobacco induced by *PsASGR-BBML* transgenes via parthenogenesis. *Genes*, **11(9)**, 1072.

Zhang, X., Shi C., Li S., Zhang B., Luo P., Peng X., Zhao P., Dresselhaus T. and Sun M.X. (2023). A female in vivo haploid-induction system via mutagenesis of egg cell-specific peptidases. *Mol. Plant.*, **16(2)**, 471-480.

Zhang, C., Song Y., Cheng Z.H., Wang Y.X., Zhu J., Ma H., Xu L. and Yang Z.N. (2012). The *Arabidopsis thaliana* DSB formation (*AtDFO*) gene is required for meiotic double strand break formation. *Plant J.*, **72(2)**, 271-281.

Zhou, L., Han J., Chen Y., Wang Y. and Liu Y.G. (2017). Bivalent Formation 1, a plant-conserved gene, encodes an OmpH/coiled-coil motif-containing protein required for meiotic recombination in rice. *J. Exp. Bot.*, **68(9)**, 2163-2174.