

## Review Article

# Apomixis: Mechanism, Genetic Basis and its Significance in Horticultural Crops

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## A B S T R A C T

Apomixis, which allows seeds to form asexually without undergoing meiosis or fertilization, holds great promise for agricultural biotechnology by facilitating the generation of maternal clones that preserve favorable traits in crops. There are two primary types of apomixis: gametophytic and sporophytic, which are differentiated by whether the embryo originates from the embryo sac or directly from diploid somatic cells. In gametophytic apomixis, embryo sacs are formed via mitosis rather than meiosis (apomeiosis), followed by embryo development without fertilization (parthenogenesis). In contrast, sporophytic apomixis involves the formation of embryos from somatic cells, while still relying on the sexually derived embryo sac for endosperm development. The genetic regulation of apomixis is complex, involving several loci with restricted recombination, often linked to heterochromatic regions. Key aspects of apomixis, such as the avoidance of meiosis, parthenogenesis, and the development of endosperm without fertilization, are controlled by distinct genetic loci. Recent progress has highlighted crucial genetic areas like the Apospory-Specific Genomic Region (ASGR) in Pennisetum that are linked to apomictic processes. Synthetic apomixis, which involves the engineering of pathways for apomeiosis and independent embryo and endosperm development, emerges as a promising avenue for enhancing crop breeding efforts.

**Keywords:** Apomeiosis, Parthenogenesis, Loss of Apomeiosis (LOA) and Loss of Parthenogenesis (LOP) and MiMe (Mitosis instead of Meiosis)

## Introduction

Apomixis, the process of forming seeds asexually, enables plants to bypass meiosis and fertilization, resulting in seed development without male contribution. Consequently, the resulting plant is a maternal clone of the parent. This ability to produce identical maternal offspring offers significant potential for transforming crop breeding, as it allows the rapid stabilization of preferred genotypes. Apomixis could become a valuable breeding technique, enabling the propagation of any genotype, including F1 hybrids, through

seeds, making it an important tool for future agricultural advancements.

The word "apomixis" is derived from the Greek terms "apo," meaning "away from," and "mixis," meaning "mixing," which reflects a type of reproduction that bypasses the fusion of gametes. This reproductive strategy has been documented in more than 300 species across at least 35 different plant families. Some of the most prominent examples can be found in the Asteraceae family (daisies), Gramineae family (grasses), Rosaceae family (roses),

and Rutaceae family (citrus plants). Apomixis allows these plants to reproduce asexually, producing seeds without the genetic reshuffling that occurs during sexual reproduction. This results in offspring that are genetically identical to the parent, preserving desirable traits and creating opportunities for consistent crop production. The first recorded instance of apomixis in citrus dates back to 1719 when Leeuwenhoek noted the development of two plantlets from a single seed. In crops like citrus, apomixis plays a crucial role in fixing value traits and maintaining heterozygosity.

Though most studies have focused on gametophytic apomixis, sporophytic apomixis, which includes nucellar embryony, remains less understood.

### Reproductive Strategies in Plants

In flowering plants (angiosperms), seed formation occurs through either sexual or asexual means. Sexual reproduction promotes genetic variation, whereas apomixis leads to offspring that are exact genetic copies of the parent. This contrast between sexual reproduction, which fosters diversity, and asexual reproduction, which enables cloning, makes apomixis a fascinating subject from both developmental and evolutionary viewpoints. Many naturally apomictic species display facultative reproduction, meaning they are capable of reproducing through both sexual and asexual methods. Understanding the mechanisms behind apomictic seed formation requires examining the detailed processes involved in sexual seed production as a point of comparison.

### Sexual Reproduction and Seed Development in Angiosperms

In angiosperms, seed formation begins when the plant shifts from vegetative to reproductive growth. This transition is signaled by the development of flowers, which contain the male (anther) and female (ovule) reproductive structures. Within these structures, diploid precursor cells of gametes are produced (Figure 1). The anther holds multiple diploid precursor cells for male gametes, while the ovule, which will become the seed, contains a single female precursor cell, known as the megaspore mother cell. Both male and female precursor cells undergo meiosis followed by mitosis, resulting in the formation of mature, multicellular structures that contain the gametes—the pollen grain in males and the embryo sac in females.

Figure 1 illustrates the steps leading to the formation of the most common type of embryo sac, known as the Polygonum type, which is present in more than 70% of angiosperms. The diploid megaspore mother cell undergoes meiosis, producing four haploid megaspores. Of these four, only one is selected as the functional megaspore, while the remaining three degenerate. The haploid functional megaspore then undergoes three mitotic divisions without

cytokinesis, forming a coenocyte with eight haploid nuclei surrounded by a cell wall. These nuclei migrate and undergo cellularization, forming a mature embryo sac composed of seven cells.<sup>1</sup>

The mature embryo sac consists of three antipodal cells, whose role remains unclear, two synergid cells that attract male gametes, a single egg cell that develops into the embryo, and a large central cell with two nuclei that will later fuse to create a diploid precursor nucleus for the endosperm (Figure 1). After double fertilization, seed development begins. The male pollen tube, which carries two sperm cells, penetrates the ovule. One sperm cell fuses with the egg cell, initiating the division and formation of the diploid embryo. The second sperm cell fuses with the diploid central cell nucleus, triggering the development of the triploid endosperm, which supplies essential nutrients to the growing embryo.<sup>2</sup> Eventually, the surrounding ovule tissues mature to form the seed coat.

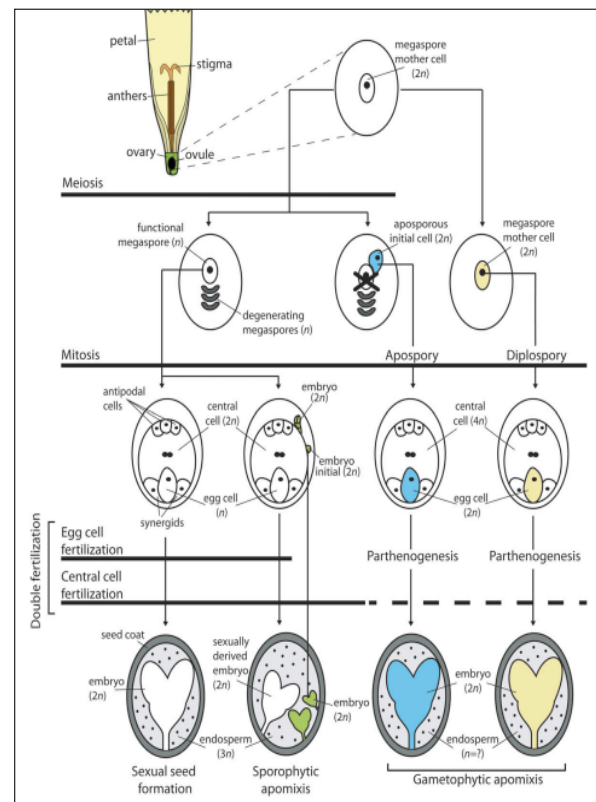


Figure 1. Mechanisms of sexual and apomictic seed development<sup>1</sup>

### Apomixis Mechanism

In contrast to the typical sexual seed development process, apomixis allows seeds to form without undergoing the crucial steps of meiosis and fertilization, thereby bypassing genetic recombination altogether. This process of apomixis comprises three main developmental stages: (i) the avoidance of meiosis during the creation of the embryo sac, also referred to as apomeiosis, (ii) the formation of an embryo

independent of fertilization, a process known as parthenogenesis, and (iii) the development of viable endosperm, which can occur either without fertilization or following fertilization of the central cell with sperm.<sup>3</sup> In apomictic reproduction, the egg originates from a diploid maternal cell that does not undergo meiotic reduction, which enables the embryo to develop autonomously without the need for fertilization. As a result, the progeny produced through apomixis are genetically identical clones of the maternal plant.

Apomixis can be divided into two major categories: gametophytic and sporophytic, depending on the origin of the embryo. In gametophytic apomixis, the embryo develops from the gametophyte (embryo sac), whereas in sporophytic apomixis, it forms directly from diploid somatic (sporophytic) cells within the ovule (as illustrated in Figure 1).

In sporophytic apomixis, the embryo sac follows the standard sexual reproductive pathway observed in angiosperms. However, during the mitotic division of the functional megaspore, neighboring diploid somatic cells begin to take on an embryogenic role, undergoing mitosis to form multiple globular embryos. These embryos can only mature if the sexually derived embryo sac is fertilized, as both the sexual and asexual embryos share the same nutritive endosperm. This process may result in seeds containing multiple embryos, which is a phenomenon commonly observed in citrus species. In such cases, the sexually derived embryo may or may not develop fully or germinate.<sup>4</sup> Although sporophytic apomixis has not been extensively studied at the molecular level, it is recognized for its genetic complexity.<sup>5</sup>

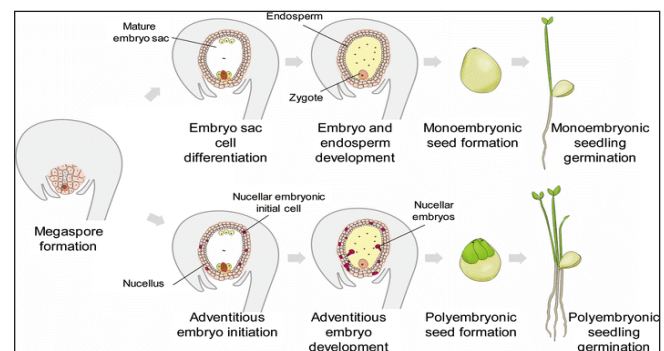
Conversely, gametophytic apomixis involves the formation of an embryo sac via mitosis, completely bypassing the meiotic process, a step known as apomeiosis. In this form of apomixis, the embryo develops without fertilization, though the formation of the endosperm may or may not require fertilization (Figure 1). Gametophytic apomictic embryo sac development is further divided into two types: diplospory and apospory, depending on the origin of the diploid precursor cell responsible for giving rise to the embryo sac. In diplospory, the megaspore mother cell acts as the precursor and either begins meiosis and then aborts, or directly initiates mitosis. Diplospory is observed in some plant species, although it is less commonly relevant to fruit crops.

Apospory, more frequently seen in fruits like citrus, involves the development of an embryo sac from a diploid somatic cell located adjacent to the megaspore mother cell. This cell, called the aposporous initial cell, undergoes mitotic division, eventually leading to cellularization of the nuclei. Although this process does not always result in the formation of a typical seven-nucleate Polygonum-type embryo

sac, it generally produces an egg cell, a central cell, and synergids (Figure 1). In certain species, both sexually derived and aposporous embryo sacs can coexist within the same ovule; however, in fruits such as citrus, the development of the aposporous embryo sac often suppresses or prevents the maturation of the sexually derived embryo sac.

The embryo in both aposporous and diplosporous embryo sacs develops from the diploid egg cell without fertilization, a process called parthenogenesis. While it is rare, endosperm formation in apomictic fruits can sometimes occur without the fertilization of the central cell. In citrus species where fertilization is necessary for endosperm development, disruptions in the contributions of maternal and paternal genomes often occur. For example, when a tetraploid central cell is fertilized, the resulting endosperm may have an unusual maternal-to-paternal genome ratio, such as 4:1 instead of the typical 2:1 ratio found in sexually reproducing species. These plants have evolved various mechanisms to ensure seed viability, carefully balancing both asexual and sexual reproductive pathways, which are crucial for successful apomictic seed formation.<sup>4&5</sup>

### Adventitious Embryony



**Figure 2. Adventitious embryogenesis<sup>6</sup>**

In the figure 2 adventitious embryogenesis, megaspore formation occurs similarly to zygotic embryogenesis. However, instead of following the typical sexual reproduction pathway, certain cells from the nucellus, which is somatic tissue surrounding the embryo sac, begin to divide and differentiate into nucellar embryonic initial cells. These initial cells bypass the need for fertilization and give rise to embryos through adventitious development. As a result, multiple embryos form from the nucellar tissue alongside the sexually produced zygotic embryo.

This phenomenon leads to the formation of polyembryonic seeds, where both zygotic and adventitious embryos are present within the same seed. Upon germination, these polyembryonic seeds produce multiple seedlings, most of which are genetically identical to the parent plant, except for the zygotic embryo. This process is particularly common in citrus and other fruit crops, allowing for the propagation of clonal plants through seeds.

Adventive embryony results in additional embryos formed from cells in nucellar (found in Citrus, Mangifera, and Garcinia) or integuments (found in Spiranthescernua). The result is one sexual and multiple asexual embryos (Polyembryony).

**Classification of apomixis**

**Types of apomixis based on occurrences**

- **Recurrent Apomixis:** An embryo sac develops from the MMC or megaspore mother cell (archesporial cell) where meiosis is disturbed (sporogenesis failed) or from some adjoining cell (in that case MMC disintegrates). Consequently, the egg-cell is diploid. The embryo subsequently develops directly from the diploid egg-cell without fertilization. Somaticapospory, diploid parthenogenesis and diploid apogamy are recurrent apomixis.<sup>7</sup> However, diploid parthenogenesis/apogamy occur only in aposporic (somatic) embryo-sacs. Therefore, it is the somatic or diploid apospory that constitutes the recurrent apomixis. Malus (apple), and Rudbeckia where pollination appears to be necessary, either to stimulate embryo development or to produce viable endosperm.
- **Non-recurrent Apomixis:** An embryo arises directly from a normal egg cell (n) without fertilization. Since an egg-cell is haploid, the resulting embryo will also be haploid.<sup>7</sup> Haploid parthenogenesis and haploid apogamy, and androgamy fall in this category. Such types of apomixis are of rare occurrence.
- **Adventives Embryony:** Embryos arise from a cell or a group of cells either in the nucleus or in the integuments, e.g. in oranges and roses. Since it takes place outside the embryosac, it is not grouped with recurrent apomixis, though this is regenerated with accuracy.<sup>6&7</sup> In addition to such embryos, regular embryos within the embryos may also develop simultaneously, thus giving rise to polyembryony condition, as in Citrus, Opuntia.
- **Vegetative Apomixis:** In some cases, like Poa bulbosa and some Allium, Agave and gas species, vegetative buds or bulbils, instead of flowers are produced in the inflorescence. They can also be reproduced without fertilization.

**Types of apomixis based on frequency**

Some species or individuals produce only apomictic embryos and are known as obligate apomicts;

**Ex: Mangosteen (Garcinia mangostana)**

however, the majority of apomictic species produce both apomictic and sexual embryos on the same plant and are known as facultative apomicts

**Ex: Apple (Malus spp)**

In Table 1, various examples are provided for different forms of apomixis based on their occurrences.

**Table 1. Occurrence of Apomixis in Fruit Crops**

Apomixis	Fruit crop
Obligate Apomixis	Mangosteen
Adventive Embryony	Sweet orange (Citrus sinensis), Lemon (Citrus limon), and Grapefruit (Citrus paradisi), Mango: Olour, Apple, Jamun
Facultative Apomixis	Apple: Indian summer, Snowdrift, Strawberry Parifit
Diplospory	Chokeberry, is a group of deciduous shrubs in the Rosaceae family
Sexual Polyembryony	Almond (Polyembryonic cultivars: Nonpareil, Price, Sonora, Jiml, Carmel, Johlyn and Mission), Citrus and Coconut
Recurrent apomixis	Malus and Raspberry
Gametophytic apomixis	Asteraceae, Rosaceae, and Poaceae

**Genetics and Inheritance of Apomixis**

Apomixis, a reproductive mechanism that enables plants to produce seeds without fertilization, has evolved independently multiple times across various angiosperm families. The genetic control of apomixis has garnered significant attention, with studies indicating that it is typically inherited as a dominant trait. Early research suggested that apomixis was governed by a single dominant locus; however, more recent investigations have revealed a much more complex genetic architecture, often involving multiple loci with suppressed recombination. This complexity poses challenges for genetic analysis. Nevertheless, through the examination of rare recombinants and gamma deletion mutagenesis, it has been identified that the different components of apomixis—such as meiotic avoidance, parthenogenesis, and fertilization-independent endosperm development—are regulated by separate loci.

For instance, in certain species like Taraxacum and Erigeron, diplospory and parthenogenesis are controlled by independent genetic loci. Similar patterns have been observed in species such as Hypericum, Poa, Hieracium, and Cenchrus, where apospory and parthenogenesis are also governed by distinct loci. Notably, in Hieracium, research has revealed that fertilization-independent endosperm formation can segregate separately from other apomictic traits, further highlighting the genetic complexity of this process.

One of the key insights from studies on apomictic species,

particularly in *Hieracium praealtum*, is that sexual reproduction is the default developmental pathway. Mutants lacking specific loci associated with apomixis, such as the LOSS OF APOMEIOSIS (LOA) and LOSS OF PARTHENOGENESIS (LOP), revert to sexual reproduction. For example, in mutants devoid of LOA, diploid embryo sacs fail to form through apospory, leading to a return to normal meiosis. Similarly, the deletion of both LOA and LOP results in the complete restoration of sexual reproduction, confirming that apomixis is layered upon the sexual reproductive pathway. This indicates that apomixis may function by redirecting cells with gametic potential rather than operating as a wholly independent process.

Apomixis can be regarded as an alteration of sexual reproduction, modifying the timing and spatial expression of sexual processes. In diplospory, the megaspore mother cell, which typically undergoes meiosis, either aborts this process or shifts to mitotic divisions, resulting in the formation of a diploid embryo sac. In apospory, an adjacent somatic cell in the ovule takes on this role, bypassing the megaspore mother cell entirely. These mechanisms ultimately lead to the formation of embryos and endosperms without fertilization, a phenomenon referred to as parthenogenesis.

Interestingly, many apomictic species are polyploids, leading to the hypothesis that apomixis may arise as a result of hybridization or genome duplication. However, polyploidy alone does not guarantee apomixis, as numerous polyploid species reproduce sexually. Although apomixis has been perceived as an evolutionary dead end due to its lack of genetic variation, the incomplete penetrance of apomixis allows for the coexistence of a sexual reproductive pathway. This dual pathway facilitates opportunities for genetic variation and diversification through recombination, enabling apomictic populations to adapt to environmental changes.

### Characteristics of Apomixis-Related Loci

Identifying the genetic loci responsible for apomixis through map-based cloning has proven difficult in many apomictic species due to the frequent suppression of recombination around these loci. This suppression of recombination suggests significant divergence of alleles in genomic regions related to apomixis. In some cases, this divergence is linked to hemizygoty, where only one allele is present at a given locus. For instance, studies in *Hieracium*,<sup>8</sup> *Pennisetum*,<sup>9</sup> and *Paspalum*<sup>10</sup> have shown that hemizygoty is a common feature of apomictic loci. Moreover, some apomictic loci are located in regions rich in repetitive sequences and heterochromatin, complicating genetic analysis.

In *Pennisetum squamulatum*, for example, the Apospory-Specific Genomic Region (ASGR) is situated in a heterochromatic, telomeric region of a single chromosome,

which is known for being nonsynaptic during meiosis. Similarly, in *Hieracium*, the LOA locus responsible for apomixis is associated with extensive repetitive sequences and transposon-rich areas. The *Paspalum* apomictic controlling locus (ACL) also shows strong suppression of recombination, with large-scale chromosomal rearrangements caused by transposable elements, when compared to similar regions in rice.<sup>11</sup>

These repetitive and heterochromatic regions have led researchers to propose that such chromosomal structures may play a functional role in apomixis. One hypothesis suggests that the repetitive sequences could sequester factors involved in sexual reproduction, altering their expression and potentially triggering apomictic development.<sup>3</sup> However, recent studies challenge this idea. Research on *Hieracium* indicates that the repetitive sequences associated with the LOA locus are not required for apospory to occur successfully. Progeny from crosses between sexual *H. pilosella* and apomictic *H. praealtum* that lack the extensive repetitive sequences linked to LOA still exhibit apomictic reproduction.<sup>12</sup> These findings suggest that the repetitive chromosomal structures seen in some apomixis loci may not be necessary for apomixis itself. Instead, these features may have evolved as a result of asexual reproduction and the associated suppression of recombination, helping to maintain the genetic elements required for apomixis. The presence of these chromosomal structures could reflect the evolutionary age of apomixis in certain species. Species where apomixis components can still be separated by recombination, such as *Poa*.<sup>13</sup>

### Genes Linked with Apomixis Loci

Despite the common suppression of recombination in apomictic plants, several genes linked to apomixis loci have been identified across various species. Sequencing these loci has uncovered numerous genes that likely play crucial roles in the apomictic process. In *Pennisetum*, the Apospory-Specific Genomic Region (ASGR) was first identified through random amplified polymorphic DNA (RAPD)-based analysis, which revealed apomixis-specific markers defining the ASGR.<sup>14</sup> Follow-up shotgun sequencing of bacterial artificial chromosome (BAC) clones from *Pennisetum* and *Cenchrus* uncovered 40 potential protein-coding genes, two of which showed a strong sequence similarity to the BABY BOOM (BBM) gene from rice. BBM was initially discovered in *Brassica napus* as an AP2-domain transcription factor and was found to induce embryo formation from vegetative tissues when overexpressed in *Arabidopsis*. The ASGR-BBM-like genes identified in both *Pennisetum* and *Cenchrus* are strong candidates for being involved in the induction or maintenance of apomixis.<sup>15</sup>

In *Hypericum*, the HYPERICUM APOSPORY (HAPPY) locus was identified using amplified fragment length polymor-

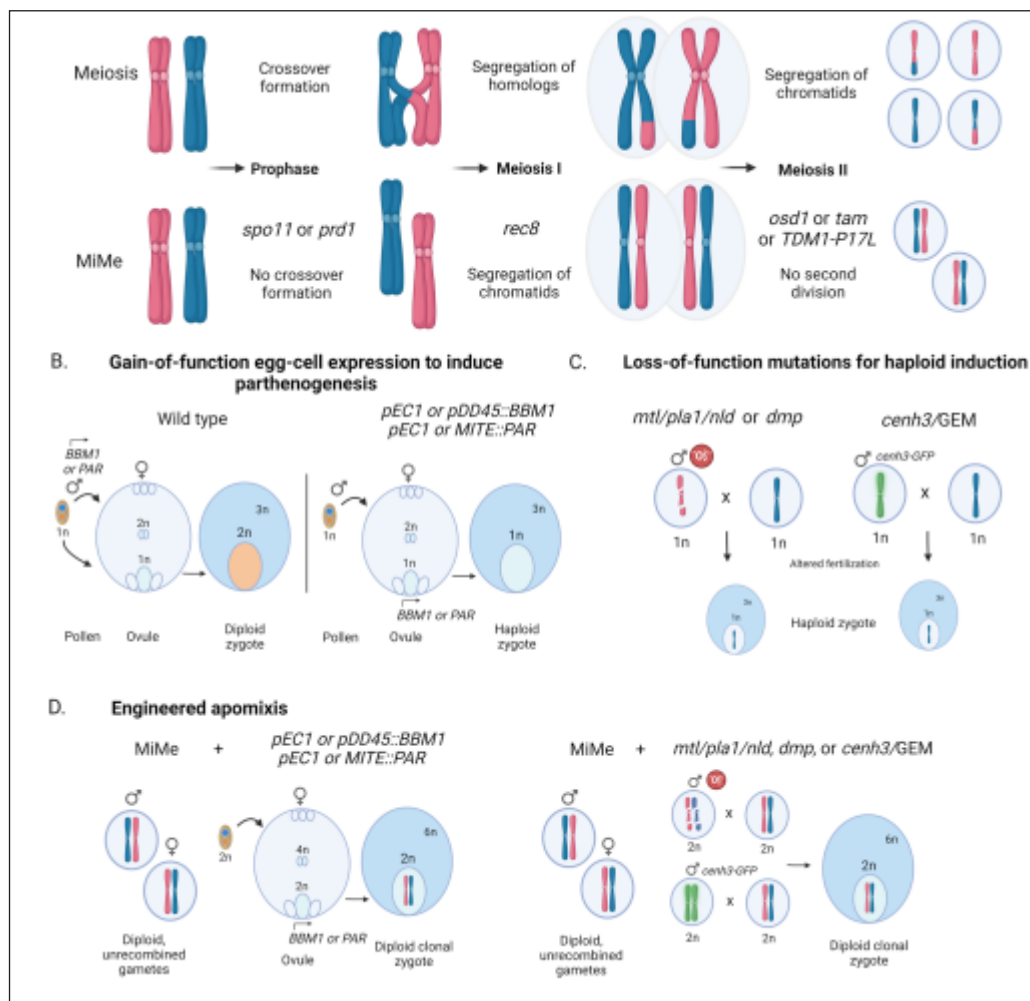
phism (AFLP) screening, comparing apomictic and sexual plants. A marker that perfectly co-segregated with apospory led to the discovery of a clone containing a gene encoding a ubiquitin-mediated E3 ligase, specifically an ARIADNE 7-like E3 ligase (HpARI). This gene is a strong candidate for the HAPPY locus because one of the four HpARI alleles in the tetraploid apomict is truncated, in contrast to the alleles present in sexual plants. It is hypothesized that this truncated HpARI allele might function in a dominant-negative manner by interacting with the three remaining functional alleles. E3 ligases like HpARI are involved in the ubiquitin-mediated degradation of proteins and play an important role in embryo sac development. For instance, the MATH-BTB protein MAB interacts with an E3 ubiquitin ligase component, Cullin 3a, and is crucial for mitotic spindle function and nuclear fate determination in developing embryo sacs of maize (*Zea mays*).<sup>16</sup> Therefore, changes in the expression or function of the HpARI E3 ligase could potentially affect embryo sac development in *Hypericum*, contributing to apomictic reproduction.<sup>17</sup>

Apomixis, although present in many plant species, is notably absent in major crops. Recent advancements in genetic engineering have focused on inducing apomeiosis—modifying meiosis into a mitosis-like division—an essential step toward developing *de novo* apomixis in crops. Three critical differences between meiosis and mitosis need to be addressed to transform meiosis into mitosis: recombination during prophase I of meiosis, co-segregation of sister chromatids during meiosis I, and a second round of division. Eliminating these differences using specific genetic mutations is referred to as MiMe (Mitosis-instead-of-Meiosis).<sup>18</sup>

**Engineering apomixis step I: apomeiosis**

- **Suppression of Recombination:** As depicted in Figure 3, recombination during meiosis is initiated by the formation of DNA double-strand breaks (DSBs), catalyzed by proteins that are conserved across different species. One key complex involved in this process is SPO11, a tetramer consisting of TOPOVIB, SPO11-1, and SPO11-2. Disrupting the SPO11 complex or other recombination initiation genes, such as PRD1, PRD2, and

**Engineered Apomixis**



**Figure 3. Engineering Apomeiosis and Parthenogenesis: Altering Meiotic Genes to Mimic Mitotic Division<sup>18</sup>**

PRD3/PAIR1, can effectively prevent recombination. Mutations in these genes, including *spo11-1* and *prd1*, have successfully generated MiMe mutants, eliminating recombination and making meiosis resemble mitosis.<sup>19</sup>

- **Monopolar Orientation of Sister Chromatids:** During meiosis, homologous chromosomes are aligned with a monopolar orientation at metaphase I, which results in sister chromatids co-segregating to the same pole. In contrast, during mitosis, sister chromatids orient in a bipolar manner and segregate to opposite poles. In plants, the cohesin complex, a four-protein structure, is responsible for this monopolar orientation in meiosis, holding sister chromatids together after replication. The REC8 subunit of cohesin, which is specific to meiosis, plays a crucial role. Combining mutations in *rec8* with the suppression of recombination results in a mitotic-like division during meiosis I, causing the sister chromatids to separate, as in mitosis. However, a second division without crossover can lead to random segregation and sterility. Mutations in other cohesin subunits often prove lethal, limiting their utility for apomeiosis engineering.<sup>19</sup>
- **Two Rounds of Division:** The two consecutive divisions during meiosis further distinguish it from mitosis. In *spo11 rec8* mutants, the first division mimics mitosis; however, the second division still occurs, resulting in meiotic failure. The Arabidopsis mutant *osd1* (omission of second division1) offers a solution to this issue. OSD1, a regulator of the anaphase-promoting complex/cyclosome (APC/C), is responsible for skipping meiosis II and producing diploid spores and gametes. In *osd1* mutants, the frequency of diploid gametes is 100% in males and 85% in females, though these gametes are recombinant due to recombination and chromosome segregation occurring during meiosis I.<sup>20</sup>
- **Combining the Three to Turn Meiosis into Mitosis:** A highly efficient method for inducing apomeiosis, termed MiMe, was developed in Arabidopsis by combining three mutations:
  1. A *spo11* mutation to block recombination,
  2. A *rec8* mutation to prevent monopolar orientation, and
  3. An *osd1* mutation to skip meiosis II.

This combination leads to the production of MiMe gametes, which are maternal cell clones. As a result, the ploidy level doubles in subsequent generations due to the fusion of diploid gametes.<sup>21, 22 & 23</sup>

## Step 2: Engineering Apomixis via Parthenogenesis

**A Fertilization Checkpoint before Embryogenesis:** While apomeiosis can be induced by turning off key meiotic genes, MiMe alone cannot produce clonal progeny because fertilization is still required. Parthenogenesis, which involves embryo formation without fertilization, has been observed

in various plant species, producing either haploid or diploid offspring. Research has shown that parthenogenesis and apospory are regulated by separate dominant loci in gametophytic apomicts. A key gene, PsASGR-BBML, linked to apospory in *Pennisetum squamulatum*, has been found to induce diploid progeny. Transgenic rice and maize lines carrying PsASGR-BBML demonstrated high rates of haploid embryo formation, indicating the potential for BBM-like genes to be applied in monocot crops. The BABY BOOM (BBM) gene, first identified in *Brassica napus*, plays a key role in embryo development. In rice, OsBBM1 has been shown to induce somatic embryos and is exclusively expressed in the male genome before fertilization. When expressed in the female genome, OsBBM1 bypasses the fertilization checkpoint, triggering parthenogenesis.<sup>24, 25</sup>

**Haploid Induction as an Alternative to Fertilization**  
An alternative strategy for inducing apomixis combines apomeiosis with directed genome elimination, which triggers haploid induction by preventing one parental genome from participating in fertilization. In Arabidopsis, mutations in the centromere-specific histone CENH3 eliminate one parental genome, resulting in the production of haploids. This technique has also been successfully applied to crops like maize and wheat, although haploid induction rates remain relatively low. A different mechanism for haploid induction has been linked to mutations in the MATRILINEAL (*ZmMTL*) gene, which plays a critical role in pollen development. *ZmMTL* mutations have been shown to induce haploids in maize, rice, and wheat, with similar results achieved through mutations in the homologous gene *ZmPLD3*. Recent research suggests that reactive oxygen species (ROS) generated in *ZmMTL*-mutant pollen cause chromosome fragmentation, leading to haploid induction. Treatments that induce ROS have successfully produced haploids in diverse genetic backgrounds. Additionally, the *qhir1* locus in maize has uncovered the role of the *ZmDMP* gene in haploid induction. Although induction rates are relatively low, combining *ZmDMP* mutations with *MTL/PLA1/NLD* significantly boosts efficiency. *ZmDMP* is conserved across both monocots and dicots, suggesting its potential for haploid induction in a wider range of crops, including legumes and brassicas. These findings open new possibilities for utilizing genome elimination and ROS induction to improve haploid production across various plant species.<sup>26</sup>

## Applied apomixis

### Engineered apomixis in principle and in hybrid crops

By integrating MiMe-driven apomeiosis with methods to induce parthenogenesis, it is now possible to create clonal diploid gametes that develop into synthetic apomicts. The first successful combination of CENH3-mediated genome elimination (GEM) with MiMe in Arabidopsis demonstrated how the formation of diploid embryos and meiotic recom-

bination could be circumvented, resulting in clonal seeds identical to the maternal parent. While this process faced challenges such as repeated crosses and low seed viability, it provided proof that synthetic clonal propagation, mimicking natural apomixis, is indeed achievable.

This technique was later extended to rice, marking the first instance of synthetic and autonomous clonal reproduction in a major monocot crop. By inducing parthenogenesis via BBM1- or MTL-based pathways, clonal progeny were able to maintain heterozygosity across several generations. Although initial apomictic rates reached 29%, advances in parthenogenetic induction within commercial F1 hybrids have significantly increased these rates, with some cases surpassing 95%. This progress indicates that engineering autonomous endosperm development may not be essential for efficient synthetic apomixis. The increased efficiency in producing clonal seeds is believed to result from enhanced BBM1 expression, facilitated by an all-in-one construct.

However, certain issues persist, particularly regarding fertility. Some of these challenges stem from the incomplete penetrance of the *osd1* mutation, leading to the formation of a small proportion of haploid gametes following female meiosis. Alternative approaches, such as utilizing different regulators of meiotic progression, could help address these fertility problems. Interestingly, expression of *OsBBM1* in egg cells has been observed to induce a second embryo from adjacent synergid cells, although the consequences of this phenomenon remain unclear.

Another strategy pairs null *OsMTL* alleles with MiMe to produce clonal progeny directly in hybrid varieties. However, this method has resulted in significant fertility reductions compared to BBM1-based approaches, potentially due to incomplete genome elimination by the *osmtl* mutation. Despite these limitations, both MTL and BBM1 approaches have shown success in maintaining hybrid vigor in engineered apomictic crops, proving that apomixis can effectively transmit complex traits from hybrids.

Reduced seed viability remains a challenge, stemming from several factors, but efforts to mitigate these issues are ongoing. While synthetic apomixis has yet to be widely applied in major crops, there is optimism given the conservation of key meiotic genes and the presence of BBM, MTL, and DMP-like genes across different species. Furthermore, recent studies in citrus have shed light on specific loci, such as a MITE insertion in the promoter of the *CitRWP* gene, which plays a role in polyembryony, a key feature of sporophytic apomixis.<sup>27</sup> However, the coexistence of both sexual and asexual embryos may present obstacles to the overall efficiency of engineered apomixis.

### Significance of apomixis

Apomixis plays a crucial role in agriculture and horticulture as it allows the propagation of seedlings with identical genotypes to the mother plant. This asexual reproduction process eliminates genetic variability, enabling the immediate “fixing” of desired traits in a cultivar. Despite this advantage, plants produced through apomixis still go through the same juvenile period as those derived from sexual reproduction.<sup>28</sup>

Currently, only a few economically significant food crops demonstrate apomixis, including Citrus, mango (*Mangifera*), and mangosteen (*Garcinia*), all of which exhibit adventitious embryony. These crops have been primarily utilized for producing clonal seedling rootstocks used in grafting and budding. The benefits of these rootstocks include being virus-free, demonstrating seedling vigor, and maintaining uniformity. However, sexual embryos can occasionally be produced, introducing unwanted genetic variability.<sup>28</sup>

To address this, DNA fingerprinting techniques are now being employed to distinguish between sexual and asexual embryos, ensuring that only asexual seedlings are selected for rootstock production. As outlined in Table 2, several examples have been provided to facilitate a clearer understanding of apomixis and its application in these crops.<sup>28</sup>

**Table 2. Significance of apomixis in fruit crop improvement**

Production of clonally uniform planting material	Citrus: Acid lime and Mandarins and apple rootstock
Elimination of Viruses	In citrus, prevent virus spread during grafting. In apples, apomictic rootstocks from <i>Malus sieboldii</i> show natural resistance to Apple Proliferation disease, making them a promising control method.
Identification of hybrid seedling	Helpful for identification, as trifoliolate leaf character in citrus (Trifoliolate orange), is found in apomictic seedlings
Ability to survive under local stresses	Apomictic seedling plants can survive local stresses, such as extreme climates or pathogen pressure, and are valuable for propagation.
Propagation of endangered cultivars	Selecting a superior plant and reproducing it asexually via apomixis helps propagate endangered cultivars, establish collections, and create a clear pedigree for new source clones.



## Conclusion

The advancement of apomixis research, while yielding significant insights and potential, is still in its developmental stages, especially in terms of its practical applications in commercial agriculture. The ability to engineer apomixis using transgenic approaches opens up promising opportunities to modify sexual reproduction in plants, addressing issues such as seed viability and genetic diversity. However, challenges such as endosperm imbalance, especially in cereal crops, still need to be tackled to realize its full potential. Future research must focus on gaining deeper knowledge of the genetic control mechanisms underlying apomixis to ensure its practical deployment. For now, while the benefits to public-sector research are evident, breakthroughs are still required before this technology can be utilized widely in commercial farming, allowing farmers to reap the full advantages of this innovative solution.

## Future thrust

Development of Haplotype-Phased and Subgenome-Resolved Genomes of Natural Apomicts for Comparative Omics.

The creation of haplotype-phased and subgenome-resolved genomic maps of natural apomictic species is essential for in-depth comparative omics studies. These maps enable researchers to discern how apomixis functions at the genetic level by comparing apomictic genomes with those of sexual counterparts. Understanding the variations between apomictic and sexual plants can help identify the genetic mechanisms that control asexual reproduction, providing insights into gene expression, epigenetic regulation, and genomic stability in apomictic species.

Identification of Dominant Apomeiosis-Related Genes in Natural Apomicts to Develop a Single Gene-Controlled Dominant MiMe System.

One of the primary goals of apomixis research is to identify dominant genes that regulate apomeiosis (bypass of meiosis) in natural apomicts. Isolating these genes would allow scientists to create a simplified system where a single gene controls MiMe (Mitosis instead of Meiosis). This system could be engineered into sexual plants, making it possible to induce apomixis in a controlled, reliable manner. A single gene-controlled dominant MiMe system would significantly reduce the complexity of creating synthetic apomicts in crops.

Discovery of Additional Apomixis Genes and Promoters for Engineering Parthenogenesis Across Different Crops.

While several genes related to apomixis have already been identified, more need to be discovered, especially those responsible for parthenogenesis (embryo development without fertilization). Identifying these genes and their

associated promoters will enable researchers to customize apomixis for different crop species, particularly those where current knowledge of the apomixis process is limited. This is important because synthetic apomixis will likely require adaptation to fit the unique genetic makeup of each crop species, ensuring its successful application across diverse agricultural contexts.

Identification of Autonomous Endosperm Genes for Pollen-Sterile Apomictic Plants.

To avoid gene flow and contamination between apomictic and sexually reproducing crops, it's necessary to create pollen-sterile apomictic plants. Identifying genes that regulate autonomous endosperm formation—where the endosperm develops without fertilization—can facilitate this goal. By making apomictic plants pollen-sterile, there would be less risk of cross-pollination and unwanted genetic introgression into sexual crop fields, ensuring the stability of crop traits.

Production of Clonal Seeds Without Compromising Fertility.

A major challenge in synthetic apomixis is producing clonal seeds without reducing plant fertility. In some apomictic systems, fertility issues arise due to improper genome elimination or incomplete suppression of sexual processes. Strategies that focus on balancing apomictic seed production while maintaining or even enhancing fertility are critical for the practical use of apomixis in agriculture. Enhancing fertility while ensuring clonal reproduction will make apomixis viable for commercial crop production.

Development of Synthetic Apomixis That is Stable Over Generations and Resistant to Environmental Fluctuations.

One of the key goals in synthetic apomixis is to ensure that the asexual reproduction process remains stable across multiple generations and under varying environmental conditions. In some cases, apomixis may be influenced by external factors such as temperature or stress, which could disrupt its stability. Achieving a system where synthetic apomixis is consistently maintained, regardless of external influences, is crucial for its long-term use in crop breeding.

Application of Synthetic Apomixis in a Broader Range of Crops, Especially Eudicots and Difficult-to-Hybridize Species.

To maximize the benefits of synthetic apomixis, it needs to be applied to a wider variety of crops, particularly in eudicots and species where traditional hybridization methods are challenging. Eudicots represent a large group of economically important crops, and their successful adaptation to synthetic apomixis would revolutionize breeding programs by enabling the propagation of elite genotypes through seeds, particularly in species where hybrid breeding is difficult or inefficient.

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## References

- Drews, G., & Koltunow, A. M. (2011). The female gametophyte. *The Arabidopsis Book*, 9, e0155.
- Berger, F., Hamamura, Y., Ingouff, M., & Higashiyama, T. (2008). Double fertilization: Caught in the act. *Trends in Plant Science*, 13, 437–443.
- Koltunow, A. M., Grossniklaus, U. (2003). Apomixis: A developmental perspective. *Annual Review of Plant Biology*, 54, 547–574.
- Koltunow, A. M., Hidaka, T., & Robinson, S. P. (1996). Polyembryony in citrus: Accumulation of seed storage proteins in seeds and in embryos cultured in vitro. *Plant Physiology*, 110, 599–609.
- García, R., Asíns, M. J., Forner, J., & Carbonell, E. A. (1999). Genetic analysis of apomixis in citrus and *Poncirus* by molecular markers. *Theoretical and Applied Genetics*, 99, 511–518.
- Zhang, S., Liang, M., Wang, N., Xu, Q., Deng, X., & Chai, L. (2018). Reproduction in woody perennial citrus: An update on nucellar embryony and self-incompatibility. *Plant Reproduction*, 31, 43–57.
- Pupilli, F., & Barcaccia, G. (2012). Cloning plants by seeds: inheritance models and candidate genes to increase fundamental knowledge for engineering apomixis in sexual crops. *Journal of biotechnology*, 159(4), 291–311.
- Okada, T., Ito, K., Johnson, S. D., Oelkers, K., Suzuki, G., & Koltunow, A. M. (2011). Chromosomes carrying meiotic avoidance loci in three apomictic eudicot *Hieracium* subgenus *Pilosella* species share structural features with two monocot apomicts. *Plant Physiology*, 157, 1327–1341.
- Akiyama, Y., Conner, J. A., Goel, S., Morishige, D. T., Mullet, J. E., Hanna, W. W., & Ozias-Akins, P. (2004). High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiology*, 134, 1733–1741.
- Calderini, O., Chang, S. B., De Jong, H., Busti, A., Paoletti, F., & Pessino, S. (2006). Molecular cytogenetics and DNA sequence analysis of an apomixis-linked BAC in *Paspalum simplex* reveal a non-pericentric location and partial microcolinearity with rice. *Theoretical and Applied Genetics*, 112, 1179–1191.
- Akiyama, Y., W. W. Hanna, and P. Ozias-Akins, 2005 High-resolution physical mapping reveals that the apospory-specific genomic region (ASGR) in *Cenchrus ciliaris* is located on a heterochromatic and hemizygous region of a single chromosome. *Theor. Appl. Genet.* 111: 1042–1051.
- Kotani, Y., Henderson, S. T., Suzuki, G., Johnson, S. D., Okada, T., & Koltunow, A. M. (2013). The LOSS OF APOMEIOSIS (LOA) locus in *Hieracium praealtum* can function independently of the associated large-scale repetitive chromosomal structure. *New Phytologist*, 1–15.
- Albertini, E., A. Porceddu, F. Ferranti, L. Reale, G. Barcaccia et al., 2001 Apospory and parthenogenesis may be uncoupled in *Poa pratensis*: a cytological investigation. *Sex. Plant Reprod.* 14: 213–217.
- Ozias-Akins, P., Roche, D., & Hanna, W. W. (1998). Tight clustering and hemizygoty of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 5127–5132.
- Conner, J. A., Goel, S., Gunawan, G., Cordonnier-Pratt, M. M., Johnson, V. E., Liang, C., & Ozias-Akins, P. (2008). Sequence analysis of bacterial artificial chromosome clones from the apospory-specific genomic region of *Pennisetum* and *Cenchrus*. *Plant Physiology*, 147, 1396–1411.
- Juranic, M., Srilunchang, K. O., Krohn, N. G., Leljak-Levanic, D., Sprunck, S., & Dresselhaus, T. (2012). Germline-specific MATH-BTB substrate adaptor MAB1 regulates spindle length and nuclei identity in maize. *The Plant Cell*, 24, 4974–4991.
- Schallau, A., Arzenton, F., Johnston, A. J., Hähnel, U., Koszegi, D., Blattner, F. R., & Grossniklaus, U. (2010). Identification and genetic analysis of the APOS POR1 locus in *Hypericum perforatum* L. *The Plant Journal*, 62, 773–784.
- Mahlandt, A., Singh, D. K., & Mercier, R. (2023). Engineering apomixis in crops. *Theoretical and Applied Genetics*, 136(6), 131.
- Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J* 20(3):589–600.
- d'Erfurth I, Cromer L, Jolivet S, Girard C, Horlow C, Sun Y, To JPC, Berchowicz LE, Copenhaver GP, 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).
- d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R (2009) Turning Meiosis into Mitosis. *PLOS Biol* 7(6).
- Cromer L, Heyman J, Touati S, Harashima H, Araou E, Girard C, Horlow C, Wassmann K, Schnittger A, Veylder LD, Mercier R (2012) OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1;2/TAM. *PLOS Genet* 8(7).
- Mieulet D, Jolivet S, Rivard M, Cromer L, Vernet A, Mayonove P, Pereira L, Droc G, Courtois B, Guiderdoni E, Mercier R (2016) Turning rice meiosis into mitosis.

- Cell Res 26(11).
24. Conner JA, Ozias-Akins P (2017) Apomixis: engineering the ability to harness hybrid vigor in crop plants. In: Schmidt A (ed) *Plant germline development: methods and protocols*. Springer, Berlin, pp 17–34.
  25. Khanday I, Skinner D, Yang B, Mercier R, Sundaresan V (2019) A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature* 565(7737):91.
  26. Wang N, Gent J, Dawe RK (2021) Haploid induction by a maize *cenH3* null mutant. *Sci Adv* 7(4).
  27. Waki T, Hiki T, Watanabe R, Hashimoto T, Nakajima K (2011) The Arabidopsis RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Curr Biol* 21(15):1277–1281.
  28. Hand, M. L., & Koltunow, A. M. (2014). The genetic control of apomixis: Asexual seed formation. *Genetics*, 197(2), 441–450. Curtis, M. D., & Grossniklaus, U. (2008). Molecular control of autonomous embryo and endosperm development. *Sexual Plant Reproduction*, 21, 79–88.
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