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Decoding female sterility in plant crops: genetic insights, limitations, and future research directions

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Abstract

Global food security and the demand for high-yielding crops to support a growing population are critical challenges in modern agriculture. Hybridization, a well-established technique for enhancing crop yield and resilience, often leverages plant sterility, with male sterility being extensively studied. However, female sterility remains underexplored despite its potential in hybrid seed production and evolutionary research. This review examines the types, characteristics, and genetic basis of female sterility in crop plants, with a focus on understanding ovule development and the associated sterile mutants. Genes that disrupt normal ovule development, termed female-sterile mutants, are critical in promoting female sterility. Female-sterile mutants are classified according to the developmental phases of the female gametophyte in plant crops to provide a comprehensive understanding of their functions and disruptions they cause. Moreover, this review highlights key applications, such as cost-effective hybrid seed production, and limitations, including the complexity of detecting female sterility and the difficulty of its commercialization. By addressing gaps in current knowledge, this article advocates for advanced molecular and genetic research to identify novel female-sterile mutants and enhance their utility in hybrid breeding for sustainable agriculture.

Keywords: Female sterility, Ovule development, Female-sterile mutants, S5 sterile gene, Hybrid seed production, Regulatory networks

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Introduction

The global population is expected to reach almost 10 billion by the end of the 21st century[1], and the existing agricultural systems are facing increasing pressure to sustain global food security under limited natural resources and a rapidly growing population[2]. Modern agricultural technologies and crop breeding techniques (such as hybridization, gene editing, marker-assisted selection, speed breeding, etc.) are crucial in combating the challenge of accelerated food insecurity[3]. Among the various modern approaches, hybrid breeding is an emerging strategy to enhance crop productivity, maintain stability, and improve stress tolerance in major crops (rice [*Oryza sativa*], wheat [*Triticum aestivum*], maize [*Zea mays*])[4]. This approach fundamentally relies on the manipulation of plants' reproductive biology to promote cross-pollination and heterosis (hybrid vigor)[5]. Therefore, understanding the regulation of plants' reproductive processes is of great significance, particularly fertility and sterility mechanisms[6,7].

Plant sterility refers to the inability to produce functional gametes or viable seeds because of disruptions in reproductive development[8,9]. Several factors induce sterility in crop plants, which can be environmental or genetic; these can be natural or artificial. These factors will be discussed in later sections of this review[10]. Although both male and female sterility contribute to reproductive failure, their biological basis, mode of development, and applications in the field are significantly different. In the recent past, the concept of male sterility (the failure to produce viable pollen) has been extensively studied and widely utilized in hybrid breeding programs[10,11]. In contrast, female sterility (disruptions in either ovule development or female gametophytes' formation or fertilization) is relatively less explored despite its important biological and agricultural implications[12,13].

According to its genetic characterization, female sterility is controlled by complex regulatory networks involving cellular specification, meiotic progression, hormonal signaling, and small RNA-mediated pathways. These mechanisms are fundamentally distinct from those underlying male sterility, reflecting differences in gametophyte formation, developmental stages, and reproductive biology[14,15]. Consequently, female sterility cannot be interpreted simply as the counterpart of male sterility but requires an independent conceptual and developmental framework.

From a developmental perspective, female reproductive development depends on a series of tightly coordinated processes, including ovule primordium initiation, specification of the megaspore mother cell (MMC), meiosis during megasporogenesis, and embryo sac development during megagametogenesis[16,17]; disruptions at any of these stages can lead to female sterility. Understanding these developmental stages provides a conceptual framework for interpreting the diverse female-sterile mutants reported in different crop species. Unlike male gametogenesis, which produces large numbers of pollen grains, female gametophyte development is restricted to a single functional megaspore per ovule, making defects more difficult to detect and genetically characterize[18].

Beyond its developmental significance, female sterility has important applications in crop improvement and evolutionary biology. It is commonly observed in crop plants like soybean (*Glycine max*), maize, and rice, and it is caused by abnormalities in female organs like inappropriate development of the ovule or abnormal formation and growth of the embryo and embryo sac[19]. Female sterility also contributes to the development of seedless fruits in

certain plant species[14]. Still, there is a potential knowledge gap in studying and understanding female sterility compared with male sterility. It is necessary to exploit female sterility in crop plants to control fertilization, improve hybrid seeds' purity, facilitate the study of the reproductive system's evolution (including transitions from hermaphroditism to dioecy), and crop breeding research. However, its practical utilization remains limited compared with male sterility because of challenges in detection, maintenance, and large-scale implementation.

This review aimed to formulate a clear conceptual framework to integrate the diverse genetic and developmental mechanisms underlying female sterility. In this review, we synthesized current knowledge on female sterility by classifying female-sterile mutants according to developmental stages of female gametophyte formation and linking these disruptions to their underlying molecular mechanisms. Furthermore, we discuss the applications, limitations, and future potential of female sterility in crop breeding and reproductive biology.

Concept and classification of female sterility

The phenomenon of female sterility is attributed to failure of female floral organs to produce a functional megagametophyte as a result of natural (structural or nonstructural defects) or artificially induced (conditions or induced mutation) factors[20]. Female sterility can be divided into three types according to the structural, conditional, and nonstructural abnormalities (Supplementary Fig. S1). This classification focuses on the nature of the defect that leads to female sterility, including structural abnormalities in the reproductive organs, environmentally or artificially induced conditional sterility, and nonstructural reproductive defects that occur despite apparently normal floral structures. Apart from this, female sterility can also be interpreted from a developmental perspective, where reproductive failure occurs at specific stages of female gametophyte development, such as MMC specification, meiosis, or embryo sac formation (discussed in a later section). Therefore, classification based on defect type and the developmental-stage perspective represent complementary approaches for understanding the mechanisms underlying female sterility in crop plants. In this section, different mutants are described as potential examples of these classifications. A given mutant may belong to one of the categories described here while simultaneously affecting a particular stage of female reproductive development. Therefore, it is likely that the same mutants are categorized in different classifications.

Female sterility based on structural deficiencies

The first type is categorized on the basis of the absence of female organs or the incomplete formation of female organs. The female organs of flowers are not differentiated in this type of sterility, or their carpels are transformed into male organs[19]. The second type of female sterility is one where the female organ does not have a normal embryo sac because of retarded development of the embryo to a megasporocyte[19]. The abnormal meiotic process of the megasporocyte leads to this type of sterility. The third type of female sterility exists when there is a normal, mature embryo sac, but the embryo is abnormally developed after pollination[19]. The *MULTIPLE SPOROCYTE 1 (MSP1)* mutant *msp1* has a large number of female gametocytes, and it hinders the normal process of ovule development in rice[21]. It appears at the third stage of ovule development, i.e., division of the integument primordium in rice. The ovule developmental process in flowering plants proceeds through a series of coordinated stages, including ovule primordium initiation, integument formation, and embryo sac development, which together ensure successful fertilization and seed formation. The *msp1* mutant is an example of female sterility because of a structural deficiency in the ovule.

Phenotypic traits of female sterility can be observed at a wide scale, ranging from defects in stigma to the absence of ovules[22]. Apart from crop plants, there are different shrubs or flowering species that possess defects in female organs and lead to female sterility naturally[23]. There are fewer studies on female sterility, and some of them are described below. These are based on structural deficiencies and defects in the female organs of plant crops[20]. The tobacco plant (*Nicotiana glauca*), gram (*Cicer arietinum*), and cactus species *Consolea moniliformis, C. millspaughii, C. nashii, C. picardae, C. rubescens, and C. spinosissima* show defects in nucleus development, and their megagametophytes do not show any development[22]. In the shrubby tree *Rauwolfia sellowii*, female sterility is observed from the functional megaspore to the megagametophyte stage, and it results in sterile plants with either no megagametophytes or partially developed megagametophytes[24].

In some flowers of *Monteverdia obtusifolia*, megagametophytes do not develop, and female sterility can be seen. The integuments are also seen to be collapsed. Sometimes, this species has an onset of female sterility, inticated by the manifestation of sterile ovules with delayed development of the megagametophytes, with the nuclei having multiple gametophytes and degenerated megagametophytes at different developmental stages[25]. *Nicotiana glauca* is another flowering plant where female sterility can be seen naturally, and the megagametophyte starts making a big vacuole, and synergids are not properly developed[26]. Such studies provide a genetic pathway to studying female sterility in the natural population of flowering species and crop plants, but there is much to be explored yet to understand female sterility in detail.

Conditional female sterility

Conditional female sterility represents a distinct category of reproductive failure in plants because it is triggered by environmental conditions rather than permanent genetic defects in ovule development. Unlike constitutive female sterility, which typically results from mutations affecting key developmental processes such as MMC specification, meiosis, or embryo sac formation, conditional female sterility occurs when environmental stresses interfere with otherwise normal reproductive development. Factors such as temperature fluctuations, chemical stress, or other abiotic stresses can alter hormonal signaling, RNA silencing pathways, or cellular metabolism within the ovule, ultimately leading to temporary sterility. Importantly, these environmentally regulated systems are often reversible, allowing fertility to be restored once favorable conditions return. This distinction is biologically significant because stress-induced sterility involves regulatory responses to environmental cues, whereas developmental sterility results from defects in the intrinsic genetic pathways controlling female gametophyte formation. From an applied perspective, conditional female sterility systems are particularly valuable for hybrid breeding programs, as they enable breeders to control fertility through environmental manipulation rather than permanent genetic modifications.

Apart from these, there is another type of female sterility where the desired conditions are induced in female organs to make them sterile to obtain different benefits, like hybridization. Such sterility is categorized as conditional female sterility, where environmental conditions like temperature are changed accordingly. In these cases, reproductive structures may develop normally under standard

growth conditions, but environmental stress can disrupt key physiological or molecular pathways required for successful fertilization. The change in environmental factors can also be natural, and female sterility can be seen; fertility can be restored when favorable conditions are available. Li et al.[27] identified a thermosensitive female sterile mutant (*Tfs1*) that demonstrates complete sterility under high temperature and partial fertile behavior under low temperature as a point mutation in *ARGONAUTE7 (AGO7)*. This mutant illustrates how temperature-dependent regulatory pathways can influence female reproductive development and cause reversible sterility. This is an example of conditional female sterility caused by a change in environmental factors, i.e., temperature.

Another example of conditional female sterility is the demonstration of female sterility in the application of beta-aminobutyric acid (BABA). BABA is used to develop resistance in plants against different pathogens. Significantly, it is applied in *Arabidopsis thaliana* to protect against pathogens that affect callose production and deposition. However, the continuous application of BABA affects female fertility, causing the ovules to become sterile. BABA affects pollen tubes, and callose becomes deposited in the ovules' micropyle. Such stress-induced responses demonstrate how chemical or environmental signals can interfere with fertilization processes without permanently altering the genetic program of ovule development. This study has given rise to conditional female sterility that is dependent on BABA[28].

Nonstructural deficiency-based female sterility

As explained earlier, a female-sterile plant is usually categorized according to the absence of pistils, a shriveled ovary without stigma, or normal stigmas, or an ovary with abnormal internal structures. These types of apparent abnormalities are usually found in all known female-sterile mutants of rice, and it was believed that the female-sterile plants had distorted or abnormal female organs. However, another female-sterile rice mutant was discovered that was different from all three categories[19]. A female-sterile mutant was found in the *indica* rice restorer line 202R. The mutant demonstrated a low seed-setting rate. When a cross was made with a mutant as a pollen donor, normal seed-setting was observed, but no seed was obtained when the mutant was used as a pollen receiver. Mutant florets had six stigmas and one pistil, and resembled the wild-type in this context. The pollen fertility rate was also 87.1%, almost equal to the wild-type. Moreover, 90% of mutant mature embryos had complete inner morphological traits. Egg cells were found to have disintegrated after the pollination stage, and there was no sperm, embryo, or endosperm in the embryo sac. Further investigation showed that the pollen grains usually germinated, but the problem was in the pollen tube, which was seen to be abnormally elongated. Mutant pollen tubes demonstrated different defects in the style, like swollen tips, delayed or slowed or disorganized elongation, and branched tips. The pollen tube's growth stopped in the style and it could not reach the embryo sac, halting double fertilization. It was found that the female organs of the mutant were similar to the 202R variant of rice, and there was no abnormality in the number or structure of the mutant embryo. Therefore, this type of female sterility was defined as 'nonstructural deficiency' sterility, and it was caused by elongation of the pollen tube.

Developmental stage-based classification of female sterility

This section discusses female-sterile mutants according to the developmental phases of the female gametophyte in plant crops. Various mutants in different species of plant crops in the same developmental phase and their expression have a significant impact on the physiology or morphology of plant crops.

Female sterility in crop plants can be more clearly interpreted when examined within the developmental framework of ovule and female gametophyte formation. In flowering plants, female reproductive development proceeds through a series of well-defined stages that act as critical regulatory checkpoints. These stages include (i) specification of the MMC within the nucellus, (ii) meiotic division of the MMC during megasporogenesis to produce haploid megaspores, and (iii) female gametophyte development and maturation during megagametogenesis, ultimately leading to the formation of a functional embryo sac capable of fertilization. Mutations affecting the genes involved in any of these developmental stages may disrupt ovule development, fertilization processes, or embryo sac formation, thereby resulting in female sterility.

Therefore, female-sterile mutants can be interpreted according to the developmental stage at which reproductive failure occurs. In this review, the reported mutants are organized according to these developmental checkpoints, including defects in MMC specification, meiotic progression, female gametophyte development, and ovule integument formation. The female-sterile mutants are classified on the basis of the premeiotic phase, meiotic phase, postmeiotic phase, and integument phase. Rice, as a model plant, has been used below to illustrate the genes and their respective mutants affecting reproduction (Figs 1 and 2; Supplementary Fig. S1).

For better readability, each mutant discussed below is described with reference to three aspects with respect to the available information: (i) The molecular or genetic function of the gene, (ii) the phenotype observed in the corresponding mutant, and (iii) its biological significance or potential implications for plant breeding.

Comparative studies among model and crop species such as *A. thaliana*, rice, and maize have revealed both conserved and species-specific regulatory mechanisms controlling female reproductive development. Whereas fundamental processes such as MMC specification, megasporogenesis, and embryo sac formation are largely conserved across flowering plants, different gene families and regulatory networks have evolved in monocot crops and dicot model systems. A comparative overview of the key regulatory genes involved in female reproductive development is presented in Table 1.

MMC specification (premeiotic phase)

In crop plants such as rice, maize, and wheat, the premeiotic phase encompasses the early stages of ovule development, starting with the emergence of the ovule primordia from the floral meristem, followed by the specification of archesporial cells and their differentiation into MMCs[45–47]. This stage corresponds to the MMC specification phase, which establishes the female germline and determines the cell that will undergo meiosis to initiate female gametophyte development. This phase is crucial for establishing the female germline, where epigenetic and hormonal signals, including auxin gradients, guide cell fate decisions to ensure proper MMC formation[45]. Disruptions here, which are common in cereals, prevent entry into meiosis, leading to absent or malformed embryo sacs and reduced seed yield, as seen in hybrid breeding systems where controlled sterility enhances cross-pollination[48].

Mutants in this phase typically affect early ovule primordia formation, archesporial cell identity, or MMC specification, preventing progression to meiosis[48].

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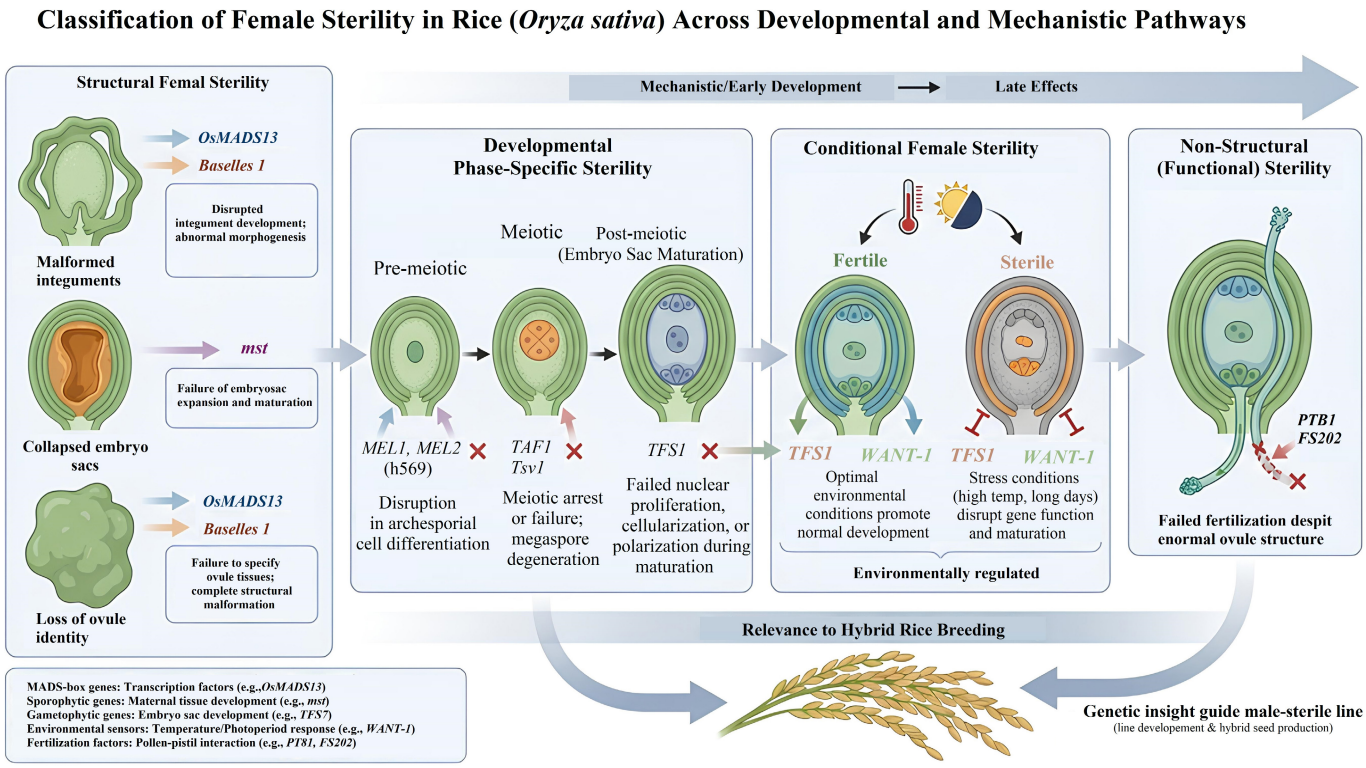

Fig. 1 Classification of female sterility based on the nature of reproductive defects. Female sterility is categorized into structural, conditional, and nonstructural types, reflecting distinct biological mechanisms affecting ovule development and fertilization.

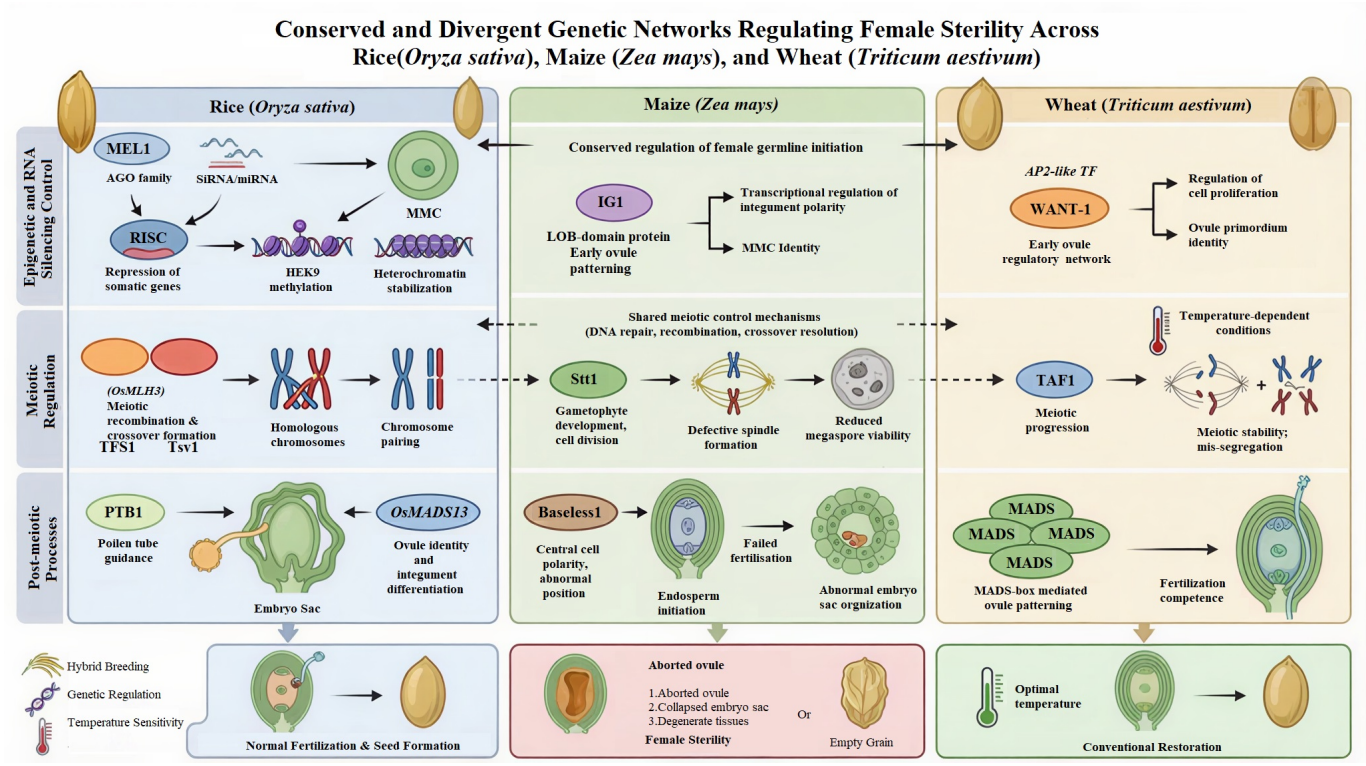

Fig. 2 Overview of the regulatory pathways governing female sterility in crop plants. The diagram integrates genetic, hormonal, and small RNA-mediated pathways that control ovule development, gametophyte formation, and fertilization processes. Disruptions in these interconnected pathways lead to diverse female-sterile phenotypes across rice, maize, and wheat.

Table 1. Comparative regulatory mechanisms controlling female reproductive development in model and crop plants.

Developmental stage	*A. thaliana* (model dicot)	Rice (*O. sativa* L.)	Maize (*Z. mays* L.)	Key biological insight
Megaspore mother cell (MMC) specification	Sporocyteless/nozzle (SPL/NZZ) regulates germline specification and restricts MMC formation[29]	*MEL1* regulates germline identity and small RNA-mediated silencing[30]	IG1 controls female germline proliferation and embryo sac development[32]	Germline specification is conserved but regulated by different gene families in dicots and monocots
Meiosis during megasporogenesis	DMC1 and other meiotic recombination proteins regulate chromosome pairing and recombination[33]	*MEL2* regulates meiotic entry and germ cell cycle progression[31]	MLH3 homologs regulate crossover formation during meiosis[34]	Meiotic regulation is highly conserved across species
Female gametophyte development	Fertilization-independent seed (FIS) complex regulates embryo sac development and fertilization competence[35]	*AGO7* pathway regulates tasiRNA production, affecting embryo sac development[27]	STS1 influences embryo sac maturation and floral organ specification[36,37]	Small RNA pathways and transcription factors regulate embryo sac development
Ovule and integument development	*AINTEGUMENTA (ANT)* controls ovule growth and integument formation[38]	*OsMADS13* regulates ovule identity and female fertility[39,40]	SK1 regulates pistil development and prevents pistil abortion[41]	Ovule morphogenesis involves conserved transcriptional regulators
Fertilization and seed formation	Central cell and endosperm development are controlled by epigenetic regulators[42]	*PTB1* regulates pollen tube guidance and seed-setting rate[43]	BSL1 influences central cell polarity and endosperm development[44]	Fertilization success depends on coordinated gametophyte and saprophytic interactions

MEL1 in rice

Molecular function

The *MEIOSIS ARRESTED AT LEPTOTENE1 (MEL1)* gene in rice encodes an ARGONAUTE family protein involved in small RNA-mediated gene silencing during early germ cell development[30]. *MEL1* participates in epigenetic regulation through small RNA pathways and contributes to chromatin modification processes that ensure proper germline specification. The protein localizes to the cytoplasm of germ cells and regulates histone modifications, including *H3K9* dimethylation at pericentromeric regions, which is essential for maintaining genome stability during early reproductive development[49,50].

Mutant phenotype

In *mel1* mutants, female gametogenesis is arrested at the premeiotic stage. Germ cells fail to properly differentiate into MMCs, resulting in the ovules lacking functional embryo sacs and complete female sterility[21]. Although reproductive development is severely affected, vegetative growth and overall plant morphology remain largely normal. Molecular analyses indicate that disruption of *MEL1* leads to abnormal mRNA processing and defective nucleolar organization in germ cells.

Biological and breeding significance

The *mel1* mutant highlights the critical role of small RNA-mediated epigenetic regulation in establishing germline identity in monocots. Because *MEL1* specifically affects female reproductive development without impairing vegetative growth, this pathway has potential applications in engineering sterility systems for breeding hybrid rice[48].

H569 mutant in rice

Molecular function

The *MEL2* gene in rice encodes a cyclin-like protein that plays an important role in regulating the transition from the premeiotic phase to meiosis in reproductive cells[31]. *MEL2* coordinates with RNA-binding proteins to control the cell cycle's progression during early germline development. In addition to its role in meiotic entry, *MEL2* is also associated with small RNA regulatory pathways similar to those involving *MEL1*, contributing to proper germ cell development and reproductive cell differentiation[31].

Mutant phenotype

The *h569* mutant represents a mutation in the *MEL2* gene identified in rice. In this mutant, germ cells fail to proliferate normally, leading to the disruption of MMC specification and complete female sterility while maintaining normal male fertility[31]. Ovules formed in the *h569* mutant lack functional embryo sacs, although overall plant morphology and vegetative growth remain unaffected. A detailed genetic analysis revealed that the *h569* mutation is caused by a point mutation (A1106G) in the *MEL2* gene (*LOC_Os12g38460*) located on chromosome 12, which encodes an RNA recognition motif (RRM) protein[31]. The mutation results in a severe reduction in the seed-setting rate (approximately 0.03%) caused by a failure of female gametophyte development, although pollen fertility remains normal[31].

Biological and breeding significance

The *h569* mutant has important implications for hybrid rice breeding systems. Hybrid rice seeds are typically produced by crossing a male-sterile maternal line with a fertile paternal line. However, self-pollinated seeds from the paternal line may contaminate the hybrid seed pool, requiring labor-intensive separation during mixed harvesting. The use of female-sterile paternal lines can minimize such contamination and improve hybrid seeds' purity. A study by Wang et al.[31] developed a maintainer system that propagates female-sterile lines (*h569* homozygotes) using transgenic maintainers carrying a DsRed fluorescence marker for sorting nontransgenic sterile seeds. This strategy enables mechanized mixed-seeding and mixed-harvesting systems, achieving hybrid seed purity greater than 99.9% while reducing production costs by approximately 50% compared with traditional three-line hybrid systems.

IG1 mutant in maize

Molecular function

The *Indeterminate gametophyte 1 (IG1)* gene is specific to maize and encodes a lateral organ boundaries (LOB) domain protein that regulates the proliferative phase of the female germline during ovule development. *IG1* plays an important role in MMC specification and contributes to integument initiation during ovule formation[32]. Studies have suggested that *IG1* also helps maintain ancestral regulatory mechanisms coordinating interactions between the gametophytic and sporophytic tissues during reproductive development, and orthologous genes in rice and other grasses appear to influence female germlines' development similarly.

Mutant phenotype

Several mutations have been identified in the *IG1* gene, including the *ig1-O* and *ig1-mum* alleles. These mutations disrupt normal

embryo sac development by delaying nuclear divisions during female gametophyte development. As a result, abnormal embryo sacs are formed with unusually enlarged egg cells, synergids, and polar nuclei. These structural abnormalities impair fertilization and ultimately lead to female sterility[32]. Although ovule primordia are formed in *ig1* mutants, proper polarity is not established during development, which can lead to abnormal embryo structures characterized by unsegmented embryonic regions and the absence of pole cells.

Biological and breeding significance

The *ig1* mutant provides important insights into the regulatory mechanisms controlling female germline development in maize and other grasses. Because orthologous genes exist in related monocot species, *IG1* contributes to our understanding of conserved reproductive pathways among cereal crops[32]. From an applied perspective, *ig1* mutants may have potential applications in crop improvement programs, including the development of seedless varieties and the enhancement of hybrid maize production by manipulating female reproductive development.

WANT-1 in wheat

Molecular function

WANT-1 is a wheat homolog of the *AINTEGUMENTA (ANT)* gene in *A. thaliana* and encodes an AP2 family transcription factor that regulates ovule development[51]. *WANT-1*is expressed in the ovule primordia and controls cell proliferation and proper elongation of integument tissues during the ovule's morphogenesis.

Mutant phenotype

Mutations in *WANT-1* disrupt normal ovule development and lead to abnormal elongation of the integuments, resulting in female sterility[51]. The mutants fail to properly regulate the ovule's morphology and polarity after the initiation of ovule formation, producing abnormal or absent integuments. In some cases, ectopic ovule formation occurs in transformed stamens and pistils under long-day photoperiod conditions[51]. These abnormalities resemble the phenotypic effects observed in mutants of the *A. thaliana ANT* gene.

Biological and breeding significance

WANT-1 can be considered to be a conditional female-sterile mutant, as its expression and phenotypic effects are influenced by environmental conditions such as photoperiod[51]. The study of *WANT-1* and its orthologs across different plant species highlights the evolutionary conservation of ovule developmental pathways[51]. Furthermore, *WANT-1* may be useful in hybrid breeding programs, where controlled environmental conditions could be used to induce female sterility and facilitate the maintenance of female-sterile and restorer lines.

Meiosis during megasporogenesis

The meiotic phase of plant crops includes the divisions of MMCs through meiosis Phases I and II, resulting in tetrad formation of haploid spores; usually only one out of the four tetrad cells is a functional megaspore and leads to the formation of the embryo sac[52–54]. This phase is crucial, as it has a role in preserving genetic diversity through the processes of segregation and recombination, but defects at this stage cause degeneration or defects in tetrads, resulting in severe problems in seed-setting in cereal crops, where fertility is important for higher yield[32]. Mutations of the meiotic phase interrupt the meiosis divisions in MMCs, resulting in defective tetrad formation or even the failure of chromosomes to become segregated and form a tetrad[53].

Taf1 in wheat

Molecular function

The *TAF1* gene has been associated with female reproductive development in wheat and appears to be involved in regulating meiotic processes during megasporogenesis. Genetic and quantitative trait locus (QTL) analyses suggest that *TAF1* may participate in pathways related to DNA repair and recombination during meiotic chromosome pairing[55]. Environmental factors also influence the expression of this gene, indicating that its regulatory function may be linked to temperature-dependent reproductive development. QTL mapping has identified a major locus associated with female sterility, tentatively designated *TAF1*, located on chromosome 2DS[55].

Mutant phenotype

A novel female-sterile wheat line carrying a *TAF1* mutation was identified in China[55]. This mutant exhibit complete female sterility, characterized by the absence of seed set on spikes, whereas male fertility remains normal during autumn sowing conditions. Cytological observations indicate that the *TAF1* mutation disrupts meiotic division, resulting in reduced ovule viability and abortion of megaspores during chromosome pairing[55]. Consequently, seed-setting is significantly reduced or completely absent. Interestingly, both male and female fertility can be restored when plants are grown under late winter sowing conditions, indicating that the mutant displays temperature-dependent or conditional female sterility[55].

Biological and breeding significance

The temperature-sensitive nature of the *taf1* mutant provides valuable opportunities for developing thermosensitive hybrid breeding systems in wheat[55]. Such conditional sterility systems help overcome challenges associated with self-pollination by facilitating controlled cross-pollination for hybrid seed production. Understanding the genetic basis of fertility inheritance in these lines is critical for wheat breeding programs, particularly for restoring fertility in sterile lines. To investigate the genetic basis of this trait, Dou et al.[55] crossed the female-sterile line XND126 with the elite cultivar Gaocheng 8901. Through bulk segregant analysis (BSA) and a recessive allele identification strategy, simple sequence repeat (SSR) markers were used to map the genetic locus. A linkage map was constructed using MapMaker 3.0, and QTL analysis with QTL Network 2.0 identified a major locus controlling female sterility on chromosome 2DS, accounting for 44.99% of the phenotypic variation[55]. Continued investigation of such female-sterile lines will facilitate the identification of additional genetic loci and improve the development of hybrid wheat breeding systems.

Female gametophyte development and maturation

The postmeiotic phase corresponds to female gametophyte development (megagametogenesis), during which the functional megaspore undergoes a series of mitotic divisions to form the mature embryo sac containing the egg cell, central cell, synergids, and antipodals. It involves tetrad formation, functional megaspore selection, and a series of three mitotic divisions to develop a mature embryo sac[49]. The phase regulates gametophytes' maturation and prepares the cell for fertilization. Moreover, RNA silencing and the hormonal signaling pathway assist in cell polarity, playing a direct role in seed development[56]. Mutations of this phase affect the

MMC's specification, mitotic divisions, and embryo sac maturation after meiosis[49].

Tfs1 in rice

Molecular function

Li et al.[27] identified a spontaneous thermosensitive female sterility mutation, *Tfs1*, in rice. The mutation occurs in the *ARGONAUTE7 (AGO7)* gene, which functions in small RNA-mediated gene regulation. *AGO7* interacts with microRNA miR390 to form the RNA-induced silencing complex (RISC). These complex recruits *SUPPRESSOR OF GENE SILENCING 3 (SGS3)* and *RNA-DEPENDENT RNA POLYMERASE 6 (RDR6)* to *TRANS-ACTING3 (TAS3)* loci, promoting the production of *trans*-acting small interfering RNAs (tasiRNAs). The *Tfs1* mutation disrupts tasiRNA biogenesis by impairing the loading of the miR390/miR390 duplex into the RISC complex and preventing the proper release of miR390. A separate miR390 mutation also induces female sterility, confirming that the miR390–*AGO7* regulatory pathway plays an essential role in female fertility in rice[27].

Mutant phenotype

In *Tfs1* mutants, embryo sac development is disrupted after meiosis. The mutation affects small RNA regulation, leading to abnormal RISC formation and elevated auxin response factor (ARF) levels, which interfere with normal female gametophyte development. As a result, the ovules exhibit disintegrated central cells and synergids, and the panicles produced by mutant plants are largely seedless despite normal vegetative growth[27]. The sterile phenotype is temperature-dependent, with complete female sterility observed at high temperatures and partial fertility restored at lower temperatures[27].

Biological and breeding significance

The temperature-sensitive nature of the *Tfs1* mutation makes it a valuable genetic resource for breeding hybrid rice. The *Tfs1* allele has been introduced into multiple rice lines, demonstrating its potential as a controllable sterility system for hybrid seed production[27]. These findings highlight the importance of small RNA-mediated regulatory pathways in controlling female reproductive development in cereal crops.

Fsv1 in rice

Molecular function

Female sterile variant 1 (Fsv1) is a rice mutant affecting embryo sac development. Map-based cloning identified the underlying gene as *OsMLH3*, which encodes MutL-homolog 3, a protein involved in meiotic recombination and crossover formation[27]. *OsMLH3* forms a heterodimer with *OsMLH1*, and this complex plays an essential role in regulating crossover events during meiosis in MMCs. Orthologous proteins of *OsMLH3* are found in other plant species, including *HvMLH3* in barley (*Hordeum vulgare*) and *AtMLH3* in *A. thaliana*, indicating evolutionary conservation of this pathway[27].

Mutant phenotype

The *fsv1* mutant primarily affects the ovule's sporophytic tissues and results in frequent abortion of embryo sacs. Although meiosis initially occurs normally, defects in crossover formation lead to abnormal meiotic progression, causing collapsed central cells and the absence of antipodal cells in developing embryo sacs[27]. Consequently, plants exhibit sterile florets and a significantly reduced seed-setting rate, although the anthers remain morphologically normal.

Biological and breeding significance

The identification of *Fsv1* and its causal gene *OsMLH3* provides important insights into the regulation of meiotic recombination and embryo sac development in rice. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9m (Cas9)-generated knockout lines of *OsMLH3* and *OsMLH1* confirmed the essential role of these genes in crossover formation and female gametophyte development[27]. These mutants provide useful genetic tools for studying meiotic regulation and offer potential resources for developing female-sterile lines in breeding hybrid rice.

Sts1 in maize

Molecular function

The *Sterile tassel silky ear1 (sts1)* mutant in maize is caused by a loss-of-function mutation in *Zmm16*, a MADS-box gene involved in floral organ specification and reproductive development[27]. *STS1* interacts with other regulatory genes, including *gt1*, to coordinate floral growth and organ differentiation during reproductive development[36].

Mutant phenotype

The *sts1* mutant disrupts postmeiotic embryo sac maturation and leads to the abortion of carpels. Plants carrying this mutation display female sterility and altered floral morphology, including abnormal B-class gene expression and disruption of zygomorphic floral primordia development. In addition, the tassels produce an unusual number of silks[32]. Transcriptomic analyses have shown that the mutation also affects jasmonic acid (JA) and ethylene (ET) signaling pathways, leading to reduced pollen viability and delayed anther dehiscence[57].

Biological and breeding significance

The *sts1* mutant provides an important model for studying the genetic regulation of floral organ specification and postmeiotic female gametophyte development in maize. By revealing interactions between MADS-box genes and hormonal signaling pathways, sts1 contributes to a deeper understanding of reproductive development and may provide useful insights for manipulating reproductive traits in maize breeding programs.

Stt1 in maize

Molecular function

The *Stunter1 (Stt1)* gene in maize is a recessive mutation affecting the development of both male and female gametophytes. The gene plays an important role in regulating normal embryo and endosperm development during seed formation[58].

Mutant phenotype

Plants carrying the *Stt1* mutation produce reduced but morphologically normal endosperms and embryos, resulting in impaired seed development. The mutation affects both male and female gametophytes and leads to reduced transmission through both parental lines[58]. In male gametophytes, *Stt1*-containing pollen grains are smaller, show lower germination efficiency, and exhibit reduced pollen tube growth compared with wild-type pollen. In female gametophytes, *Stt1* embryo sacs display developmental abnormalities, including smaller central cells and aberrant antipodal cells, which contribute to female sterility. Additionally, embryos and endosperms carrying the *Stt1* mutation develop more slowly than their wild-type counterparts. Deletion of the *Stt1* allele restores normal reproductive development, allowing maize embryos to complete the globular stage even without endosperm formation[58].

Biological and breeding significance

The *Stt1* mutant provides a useful model for investigating the genetic mechanisms underlying gametophyte development and

seed formation in maize. By revealing how mutations affect both male and female reproductive pathways, *Stt1* contributes to understanding the genetic basis of fertility and has potential implications for improving maize breeding strategies[58].

Baseless (bsl1) in maize

Molecular function

The maize mutant *baseless1 (bsl1)* affects central cells' development within the embryo sac and influences the positioning of the two polar nuclei[44]. The mutation exhibits a maternal effect and plays an important role in regulating early seed development processes.

Mutant phenotype

In *bsl1* mutants, heterozygous plants used as females produce abnormal embryo sacs lacking proper cell polarity. These defects lead to seed abortion and disruptions in cell cycle regulation[44]. Although fertilization can still occur efficiently, the later stages of seed development are severely affected. Detailed analyses have shown that the mutation alters the development of the basal endosperm transfer layer (BETL), an essential tissue for nutrient transfer during seed development. Kernels exhibiting abnormal BETL development correlate with the proportion of embryo sacs affected by the *bsl1* mutation[44].

Biological and breeding significance

The *bsl1* mutant highlights the importance of proper embryo sac polarity and development of the endosperm transfer layer for successful seed formation. Studies of this mutant provide valuable insights into the maternal genetic control of seed development and the coordination between gametophytic and sporophytic tissues during reproduction in maize[44].

Ovule and integument development

Plants undergo an integument development phase involving growth of the inner and outer integument around the nucleus, forming the ovule's curvature. The phase is important for the cell cycle, as it provides protective structures to the embryo sac[45, 59]. This phase works on sporophytic tissues, whereas the MADS-box and the jasmonate signaling pathway regulate the morphology and fertility needed for seed protection[60]. Mutations in this context involve abnormalities in embryo sacs, reduced pollination, and delayed seed-setting[61,62]. These mutants cause abnormalities and disruptions in integument elongation, curvature formation in the ovule, and embryo sac formation.

The PTB1 mutant gene in rice

Molecular function

High rice yield is determined by several key factors, including grain number, panicle seed-setting rate, panicle number, and grain weight. Although genes regulating panicle number and grain weight are well documented, those controlling seed-setting rates remain less understood. Li et al.[43] identified the domestication-related *POLLEN TUBE BLOCKED 1 (PTB1)* gene in rice, which plays a critical role in regulating panicles' seed-setting rate by positively influencing pollen tubes' growth during fertilization.

Mutant phenotype

A natural mutation in the *PTB1* gene disrupts normal pollen tube development, leading to reduced fertilization efficiency and a female-sterile phenotype characterized by a significantly reduced seed-setting rate[43].

Biological and breeding significance

The identification of *PTB1* and its mutant provides important insights into the genetic mechanisms regulating female fertility and seed production in rice. Understanding the role of *PTB1* in pollen tube development provides a potential molecular target for improving seed-setting rates and rice yield, and also supports the development of female-sterile lines that can be utilized in hybrid breeding programs[43].

The mst mutant in rice

Molecular function

The *multiple stigma (mst)* mutant is specific to rice and affects the development of ovule integuments and floral organ identity. The mutation alters developmental regulation along the proximal–distal axis during ovule formation[63].

Mutant phenotype

The *mst* mutation modifies ovule integuments and converts inner floral organs into lemma-like structures, resulting in abnormal integument formation and female sterility[63]. Despite these abnormalities in female reproductive structures, male fertility can remain intact when environmental conditions are favorable.

Biological and breeding significance

The *mst* mutant provides insights into the developmental regulation of ovule morphology and floral organ identity in rice. By affecting integument formation and ovule structure, this mutant helps clarify the genetic mechanisms underlying female reproductive development in cereal crops[63].

OsMADS13 in rice

Molecular function

OsMADS13 is a MADS-box transcription factor in rice that regulates ovule identity and reproductive organ development[39,40]. This gene plays an essential role in maintaining correct ovule specification during floral development.

Mutant phenotype

Mutations in *OsMADS13* result in the conversion of ovules into carpelloid-like structures, leading to female sterility[39,40]. Plants carrying this mutation do not produce seeds and instead develop carpel-like organs in place of ovules, although their overall floral morphology remains largely unaffected[40].

Biological and breeding significance

The *osmads13* mutant highlights the importance of MADS-box transcription factors in regulating ovule identity and reproductive development in rice. Studies of *OsMADS13* contribute to understanding the genetic control of ovule morphogenesis and female fertility in cereal crops[39,40].

Sk1 in maize

Molecular function

Silkless1 (Sk1) in maize encodes a uridine diphosphate (UDP)-glycosyltransferase that is required for proper late floret differentiation and pistil development during reproductive growth[41].

Mutant phenotype

The *Sk-A7110* mutant causes degeneration of the pistils shortly after the initiation of their development, resulting in female sterility and the formation of ears lacking silks. Transcriptomic analyses using RNA sequencing have shown that upregulation of JA biosynthesis genes is associated with pistil apoptosis in these mutants[37].

Biological and breeding significance

The *sk1* mutant provides important insights into the hormonal and molecular regulation of pistil development in maize. Understanding the mechanisms underlying pistil abortion and female sterility may contribute to improved manipulation of reproductive development in maize breeding programs[37, 41].

MADS31 in wheat

Molecular function

MADS31 is a MADS-box gene identified in wheat that participates in regulating ovule development and maintaining a normal ovule morphology[64].

Mutant phenotype

Mutations in *MADS31* disrupt ovule development by disorganizing nuclear cells and causing abnormalities in integument formation. These defects interfere with postfertilization processes and lead to partial female sterility in wheat plants[64].

Biological and breeding significance

The functions of *MADS31* suggest that this gene plays an important role in maintaining proper ovule structure and reproductive development. Studying such genes contributes to our understanding of the molecular regulation of ovule morphogenesis and fertility in wheat and other cereal crops[64].

Applications of female sterility in crop plants

Female sterility in crop plants is a valuable trait with significant applications in plant breeding, hybrid seed production, and reproductive biology research. It provides an effective mechanism for preventing self-fertilization and facilitates controlled hybridization, thereby enabling the development of hybrid cultivars with improved yield, stress tolerance, and vigor. Beyond breeding applications, female sterility also offers insights into the evolutionary transition from gyno-dioecy (the coexistence of female and hermaphroditic plants) to dioecy (separate male and female plants). In addition, female-sterile lines can function as effective pollenizers in hybrid seed production systems, providing both practical and research-oriented benefits (Table 2).

Hybrid seed production

The primary application of female sterility lies in facilitating hybrid seed production, particularly in crops such as rice. Hybridization is a cornerstone of modern agriculture because hybrid varieties often exhibit higher yield, improved stress tolerance, and enhanced vigor compared with inbred cultivars. Although male sterility systems have been widely utilized in hybrid breeding programs, female thermosensitive or conditional sterility represents an underexplored but promising alternative.

Female-sterile lines can reduce some of the labor-intensive processes involved in hybrid seed production, such as manual emasculation or strict spatial isolation of the parental lines. In a typical hybrid rice breeding system, seeds are produced by crossing a sterile line with a fertile parental line. However, seeds produced by the fertile parental line may contaminate the hybrid seed lot during mixed harvesting, thereby reducing hybrid seeds' purity.

The use of female-sterile lines as pollenizers can effectively address this challenge. Because female-sterile plants cannot produce viable seeds, contamination by the pollen donor line is minimized. This approach allows mixed planting and harvesting of parental lines while maintaining high hybrid seed purity.

A recent study by Wang et al.[31] identified the female-sterile mutant *h569*, caused by a mutation (A1106G) in the *MEL2* gene. Although *MEL2* was previously known to promote meiotic entry in both male and female reproductive organs, the *h569* mutation results in female sterility while maintaining male fertility. This system enables the propagation of female-sterile parental lines through mixed seeding and harvesting strategies, significantly reducing labor and production costs in hybrid rice breeding programs.

Evolutionary and genetic research

Female-sterile mutants provide valuable materials for studying the genetic and molecular mechanisms underlying plants' reproductive development. By analyzing these mutants, researchers can

Table 2. Representative female-sterile (FS) genes identified in major crop species and their roles in reproductive development and hybrid breeding.

Gene ID and Ref.	Phenotype(s) in reproductive organs	Spp.	Applications	Notes
Taf1 (wheat)[55]	No seed-setting on spike; female sterility	Wheat	Used as a pollinator/FS line in wheat hybridization	Located on chr 2DS in XND126; interacts with *mre11* in *A. thaliana*
Tfs1/AGO7 (rice)[27]	Female sterility at ~22 °C; blocked pollen tubes; reduced seed-setting; restored at higher temperatures	Rice	Conditional FS system for hybrid rice breeding	Thermosensitive allele in cultivar 4266; useful as a restorer/maintainer line
FS202 (rice)[19]	Low seed-setting; abnormal embryo sac; failed double fertilization	Rice	Research/functional genetics	Spontaneous mutant in the restorer line 202
H569/MEL2 (Os12g38460) (rice)[31]	Female sterility, absence of embryo sacs, no seed-setting; male fertility normal	Rice	Hybrid rice female-sterile systems/breeding	Mutation A1106G; blocks female meiosis, but male meiosis is unaffected
PTB1 (LOC_Os05g05280) (rice)[43]	Female sterility caused by blocked pollen tube growth; low seed-setting	Rice	Hybrid seed production; maintainer system (e.g. *M-fs4A*)	Maternal control of pollen tube growth; used in transgenic systems
Stt1 (*Stunter1*) (maize)[58]	Smaller embryo sacs; abnormal antipodal/synergids; reduced seed-setting (> 50% embryo sacs fail)	Maize	Research/functional genetics	Spontaneous inbred W23 mutation; mapping populations available
Baseless1 (*Bsl1*) (maize)[44]	Abnormal seed-setting; displaced central cell polar nuclei; sometimes in fertilization	Maize	Research/functional genetics	Found in W22; impacts BETL and BETL factors
Fsv1 (rice)[27]	Ovule degeneration; aborted female gametophytes; small/degenerate ovules	Rice	Research/functional genetics; insights into auxin involvement	Differential up-/downregulation of multiple transcription factors and auxin genes (*OsTAA1*, *OsIAA2*, *OsARF6*, *OsPIN1a*)
M-fs4A (transgenic rice system)[65]	Female-sterile seeds in M-fs4A lines; stable 1:1 propagation	Rice	Commercial hybrid seed production (maintainer system)	Described as the *M-fs4A/M-fs4B* system
Glumy (maize)[66]	Glume-like ear florets; sterile organs; disturbed floral development	Maize	Research/inflorescence development	Spontaneous mutant

identify the genes involved in ovule development, embryo sac formation, and female gametophyte function.

For example, the mutation in the *MEL2* gene observed in the *h569* mutant highlights its specific role in female reproductive development. Such mutants serve as important models for investigating the evolutionary transition from hermaphroditism to dioecy in higher plants. Studying female sterility genes, therefore, contributes to understanding sex determination pathways and the evolutionary diversification of plants' reproductive systems.

Advantages of female sterility in breeding systems

The incorporation of female-sterile lines into crop breeding programs offers several advantages that enhance the efficiency and scalability of hybrid seed production.

One major advantage is the reduction in genetic contamination during hybrid seed production. Female-sterile lines are unable to produce viable seeds through self-pollination, ensuring that harvested seeds originate exclusively from the desired pollen donor. This prevents contamination by selfed seeds and improves hybrid seeds' purity and uniformity.

Another important benefit is cost efficiency. Traditional hybrid seed production often relies on labor-intensive practices such as manual emasculation or strict spatial isolation between parental lines. Female-sterile systems reduce or eliminate these requirements by allowing mixed planting of parental lines and mechanical harvesting. As a result, production costs are reduced, and large-scale seed production becomes more economically feasible.

Female sterility also improves scalability and operational flexibility in breeding programs. Many female sterility systems can be controlled through environmental conditions or genetic regulation, allowing breeders to restore fertility under specific conditions for seed multiplication. Such conditional sterility systems provide dynamic control over plants' reproduction and are particularly valuable in mechanized agricultural systems.

When integrated with molecular breeding, genomics, and precision agriculture technologies, female sterility systems represent a powerful tool for improving hybrid seed production and meeting the increasing global demand for high-yielding crop varieties.

Role of S5 female-sterile genes in increasing yield

The S5 locus in rice is a major genetic factor regulating reproductive isolation and hybrid sterility between the *indica* and *japonica* subspecies, primarily affecting female fertility through embryo sac abortion in hybrids[67]. The *S5n* allele (also denoted as *S5-n*) is a neutral or wide-compatibility allele at this locus, rather than a female-sterile mutant itself. It plays a crucial role in overcoming the hybrid sterility barrier by preventing the killer-protector interaction that leads to female gamete abortion in *S5-i/S5-j* heterozygotes (where S5-i is the *indica* allele and *S5-j* is the japonica allele). In inter-subspecific hybridization (*indica* × *japonica*), the *S5n* allele acts as a compatibility factor, restoring spikelet fertility by suppressing the programmed cell death in the embryo sac that occurs in incompatible hybrids. This enables the production of viable seeds with hybrid vigor, addressing the low fertility (often < 50%) typical in such crosses[68]. Breeders pyramid or stack *S5n* with other neutral alleles (e.g., *f5-n, Sa-n*) to develop widely compatible lines, facilitating three-line hybrid rice systems and expanding the genetic pool for improved yield, stress tolerance, and heterosis (Fig. 3). This is particularly valuable in rice breeding programs to break reproductive barriers and create fertile intersubspecies hybrids. The S5 locus encodes three tightly linked open reading frames *(ORF3, ORF4,* and *ORF5)*, where *ORF5* acts as a killer gene, inducing endoplasmic reticulum stress in hybrids. *S5n* features a deletion or mutation that neutralizes this effect, ensuring normal female gametophyte development[68].

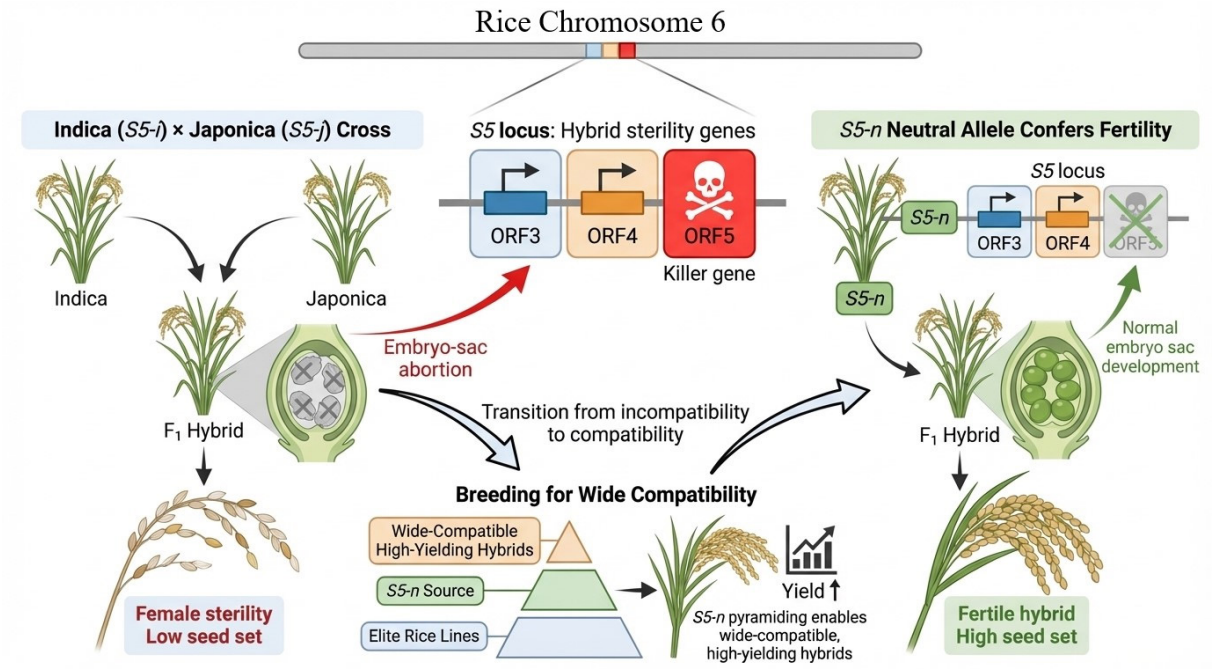

Fig. 3 Functional role of the S5 female-sterile gene in developing hybrid seed production and enhancing yield potential. The *S5-n* allele acts as a compatibility factor that prevents embryo sac abortion in *indica–japonica* hybrids, thereby restoring fertility.

Limitations and challenges

While male sterility has been extensively studied and widely applied in plant breeding, particularly for hybridization to meet global food demands, female sterility not well explored because of several inherent challenges. These limitations stem from biological, technical, and practical constraints, making the identification and application of female-sterile lines more complex than those of male sterility. Below, we discuss the key factors that hinder the study of female sterility in crop plants.

Biological complexity of female reproductive structures

The female reproductive organs (megagametophytes) in plants are less amenable to study compared with male reproductive structures (microgametophytes). Microgametophytes, such as pollen, are more sensitive to environmental and genetic modifications, making male sterility easier to detect and manipulate[69]. In contrast, megagametophytes, which develop into ovules, are less responsive to such changes, complicating the identification of female sterility. The presence of a single ovule in many plant species further limits the ability to detect sterility, as it reduces the number of observable reproductive units compared with the numerous pollen grains available for studying male sterility. This structural constraint requires detailed and labor-intensive analyses to confirm female sterility.

Difficulty in establishing female-sterile lines

Male sterility has been successfully leveraged to develop stable sterile lines for hybrid seed production, but establishing equivalent female-sterile lines is more challenging. The genetic and physiological mechanisms governing female sterility are less understood, and fewer naturally occurring female-sterile mutants are documented compared with male-sterile mutants. This scarcity limits the availability of genetic material for breeding programs. Moreover, maintaining and propagating female-sterile lines is complicated, as these plants cannot produce viable seeds, requiring alternative propagation strategies such as vegetative methods or the use of male-fertile revertants, which add complexity to breeding systems.

Technical challenges in detection and analysis

Detecting female sterility requires a meticulous examination of the female reproductive organs at various developmental stages, which is both time-consuming and technically demanding. Unlike male sterility, which can often be identified through pollen viability assays or visual inspection of the anthers, female sterility requires detailed histological or molecular analyses to assess ovules' development and functionality. The need for such in-depth investigations, coupled with the lack of high-throughput screening methods for female sterility, poses a significant barrier to large-scale studies. Additionally, the subtle phenotypic expression of female sterility compared with male sterility makes it harder to identify mutants without advanced genetic or microscopic tools.

Limited commercial applications

Though male sterility has been extensively exploited for hybrid seed production because of its compatibility with existing breeding systems, female sterility has fewer immediate commercial applications, which reduces the incentive for research investment. Although female-sterile lines offer potential benefits, such as reduced seed contamination in hybrid rice production (as discussed in literature[31]), their practical implementation is still in the early stages. The focus on male sterility for hybridization has overshadowed research into female sterility, limiting the development of protocols and technologies needed to study and apply this trait effectively.

The genetic basis of female sterility is less well-characterized than that of male sterility. Though genes like *MEL2* have been identified as critical for female reproductive development (e.g., in the *h569* mutant[31]), the full spectrum of the genes and pathways involved remains poorly understood. This knowledge gap hinders the ability to manipulate female sterility for breeding purposes or to use it as a tool for studying plants' reproductive evolution. Comprehensive genomic and transcriptomic studies are needed to elucidate these mechanisms, but such research is resource-intensive and requires long-term commitment.

Conclusions

Sterility in crop plants, encompassing both male and female sterility, is a critical area of study for advancing hybrid breeding and addressing global food security. Though male sterility has been extensively characterized, with numerous genes and classifications identified, female sterility remains underexplored, particularly beyond model systems like *A. thaliana*. Recent discoveries of female-sterile mutants, such as *taf1* in wheat and *tfs1* in rice, underscore the potential of female sterility to establish efficient sterile systems for hybrid seed production, enabling higher yields and desirable traits. However, challenges such as the complexity of megagametophyte development and limited genetic insights hinder progress. This review highlights the benefits of female sterility, including reduced labor in hybridization, and its limitations, such as detection difficulties. To meet the demands of a growing population, particularly for staple crops like wheat and rice, advanced molecular and genetic research is essential to identify novel female-sterile genes and develop protocols for their application. Future studies should aim to answer several key research questions.

1. What is the core genetic and regulatory networks controlling MMC specification and female germline establishment in crop plants?

2. How do epigenetic regulation and small RNA pathways contribute to female gametophytes' development and female sterility?

3. What molecular mechanisms underlie environmentally regulated or conditional female sterility systems?

4. How conserved are the regulatory mechanisms of female reproductive development across model plants and major crop species?

5. How can female sterility systems be effectively integrated into modern crop breeding programs?

Author contributions

The authors confirm their contributions to the paper as follows: study conception: Bashir S, Jiang P; draft manuscript preparation: Bashir S, Hassan MA, Raza A; literature review and data collection: Bashir A, Hassan MA. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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