Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2025) **23**, pp. 2330–2345 doi: 10.1111/pbi.70057

Review Article

Advancements in hybrid rice production: improvements in male sterility and synthetic apomixis for sustainable agriculture

Sunok Moon[1,†], Yang-Seok Lee[2,†], Jose Gutierrez-Marcos[2] and Ki-Hong Jung[1,3,*]

[1]*Graduate School of Green-Bio Science and Crop Biotech Institute, Kyung Hee University, Yongin, Korea*
[2]*School of Life Sciences, University of Warwick, Coventry, UK*
[3]*Research Center for Plant Plasticity, Seoul National University, Seoul, Korea*

Received 21 November 2024;
revised 11 February 2025;
accepted 5 March 2025.
**Correspondence* (Tel +82 31 201 3474; fax +82 31 204 3178; email khjung2010@khu.ac.kr)
[†]These authors contributed equally to this article.

Keywords: hybrid rice, male-sterile line, apomixis.

Summary

Rice serves as a staple food for approximately half of the world's population, and enhanced yields from hybrid rice play a crucial role in ensuring food security and augmenting incomes. However, the annual purchase and high cost of hybrid seeds hinder widespread hybrid rice adoption. In this review, we discuss hybrid seed production strategies based on molecular mechanisms along with biotechnological techniques employed for production and future prospects. Male-sterile lines are pivotal in hybrid seed production, with ongoing developments markedly advancing this process. Initially, cytoplasmic male-sterile lines facilitated three-line hybrid seed production. Subsequent innovations, including environmentally responsive gene-based and biotechnology-driven male-sterile lines, enabled two-line hybrid rice production. Ongoing research is focusing on implementing a one-line hybrid seed production method using apomixis, driving innovation in hybrid seed production. Overall, advancements in male-sterile lines and synthetic apomixis present promising avenues for improving the efficiency and sustainability of hybrid rice production. These developments highlight the critical need for continued research and concerted efforts to address global food security challenges.

Introduction

Heterosis refers to the phenomenon where hybrids exhibit superior agronomic traits, including yield, compared with their parents (Fu *et al*., 2022). Hybrid seeds are generated through the crossing of genetically distinct inbred parental lines, resulting in a yield advantage typically exceeding 15%–20% compared with inbred rice varieties (Wang *et al*., 2023). Hybrid rice is cultivated in over 40 countries, with half of China's total rice-growing area planted with hybrids in 2019 (Yan *et al*., 2022). Despite its yield advantages, hybrid rice's cultivation area has continuously decreased, primarily due to high seed costs (Yan *et al*., 2022). As rice is strictly self-pollinated, the removal of male organs is essential for hybrid seed production (Cui *et al*., 2020). Initially, to simplify the laborious process of emasculation, male gametocides were employed to induce male sterility; however, they had the potential to exert toxic effects or induce incomplete sterility, leading to impure hybrid seeds (Shimada *et al*., 1992). Using cytosolic male-sterile lines eliminates the need for castration and simplifies hybrid seed production (Xu *et al*., 2023a). As cytosolic male-sterile lines cannot be maintained through self-pollination, the three-line hybrid seed production system was established (Chen *et al*., 2023). Subsequently, self-maintainable, environmentally sensitive male-sterile lines and biotechnology-based male-sterile lines have been developed, leading to the adoption of the two-line hybrid seed production strategy (Xu *et al*., 2023a). The development of these male-sterile mutants has simplified hybrid seed production. Additionally, research on apomixis, an asexual plant reproductive mechanism, may enable one-line hybrid seed production in the near future (Wei *et al*., 2023). In this review, we introduce the strategies employed in hybrid seed production based on molecular mechanisms as well as the biotechnological approaches used in the production process. Through comparisons of each strategy's strengths and weaknesses, we highlight future research directions to optimize hybrid seed production efficiency and sustainability.

Cytoplasmic male sterility in first-generation hybrid rice production

Cytoplasmic male sterility (CMS) occurs naturally in higher plants and has been employed in hybrid rice production since the 1970s (Yuan, 2019). CMS results from nuclear–mitochondrial incompatibility, typically from backcrossing distantly related species (Huang *et al*., 2014). This maternal inheritance is controlled by mitochondrial genes that can rapidly rearrange to form toxic chimeric open reading frames (ORFs), causing male sterility (Li *et al*., 2020; Tanaka *et al*., 2012; Toriyama, 2021). Notably, toxic proteins resulting from the toxic chimeric ORFs cause sterility by disrupting mitochondrial function (Li *et al*., 2020). However, this sterility-causing effect of ORFs can be counteracted by the nuclear restorer of fertility (*Rf*) gene, which restores male fertility despite the presence of CMS-associated genes in the mitochondria (Toriyama, 2021). CMS-based hybrid breeding requires three

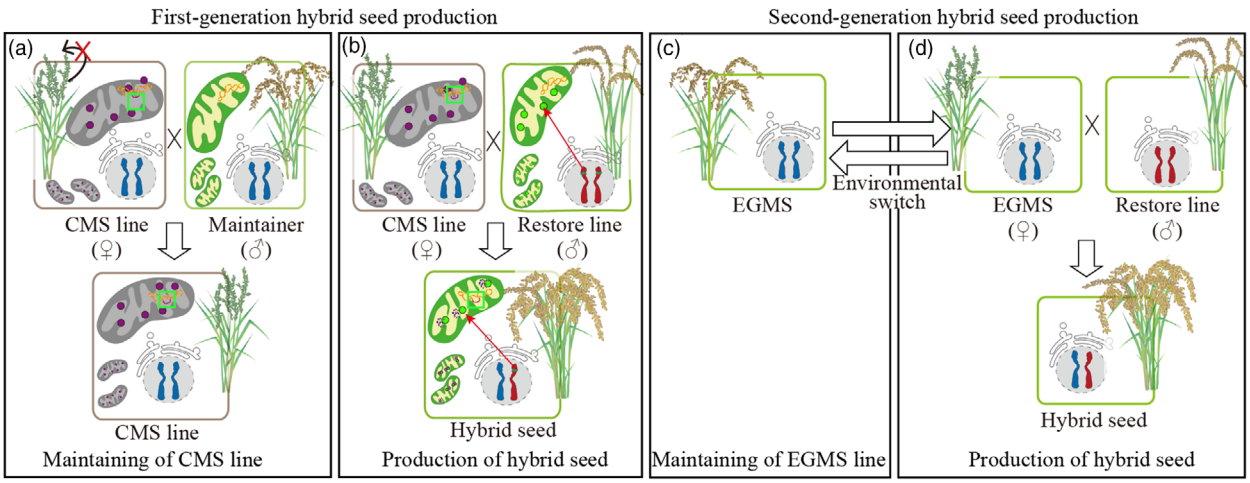

Figure 1 Schematic representation of first-generation (a, b) and second-generation (c, d) hybrid seed production. (a) Maintenance of the cytoplasmic male sterility (CMS) line through crossing with the maintainer line. The CMS-associated gene in the CMS line (green rectangle in mitochondria) leads to nonfunctional mitochondria (grey-coloured mitochondria) due to toxic protein accumulation (purple circles). The maintainer line lacks the CMS-associated gene and serves as a pollen donor. Progeny resulting from this cross inherit the CMS-associated gene and exhibit male sterility. (b) Hybrid seed production through crossing with the restorer line. The restorer line with the *Rf* gene (green lines in the red chromosome) serves as a pollen donor. RF proteins (green circles in mitochondria) prevent toxic protein accumulation in mitochondria, restoring fertility in progeny resulting from this cross. (c, d) Environment-sensitive genic male sterility (EGMS) enables the transition between fertility (c) and sterility (d) based on environmental conditions. (c) Seed production under favourable conditions. (d) Hybrid seed production through EGMS line–restorer line crossing under unfavourable environmental conditions. Red and blue chromosomes represent genetic differences between parental lines.

parental lines: CMS, sterility maintainer and fertility restorer lines (Li *et al.*, 2021a) (Figure 1a,b). The CMS line, which is unable to produce fertile pollen because of its nonfunctional mitochondria, is maintained through crossing with a maintainer line with similar genetic background and functional mitochondria (Figure 1a). Hybrid seeds are produced by crossing the CMS line with a restorer line, which carries the necessary *Rf* genes and exhibits genetic divergence from CMS, providing hybrid vigour (Figure 1b).

Mechanisms linking CMS-associated and *Rf* genes

Since the 1970s, when CMS was first used for hybrid seed production, the molecular mechanisms linking CMS-associated genes and *Rf* genes have been elucidated in several CMS lines (Table 1). *orf79* variants (*orf79*, *orfH79* and *L-orf79*) are CMS-associated genes isolated from Boro-Taichung–type CMS (BT-CMS), Hong Lian–type CMS (HL-CMS) and Lead Rice-type CMS (LD-CMS) lines (Toriyama, 2021) (Figure 2a). Corresponding nuclear *Rf* genes have been characterized in restorer lines for each CMS line: *Rf1a* and *Rf1b* for BT-CMS, *Rf5* (also known as *Rf1a*) and *Rf6* for HL-CMS and *Rf2* for LD-CMS (Akagi *et al.*, 2004; Hu *et al.*, 2012; Huang *et al.*, 2015; Itabashi *et al.*, 2011; Wang *et al.*, 2006) (Figure 2a). The *orf79* gene encodes a chimeric protein with a transmembrane domain containing a sequence from the cytochrome c oxidase subunit 1 (*cox1*) and a sequence of unknown origin (Akagi *et al.*, 1994). This protein binds to the N-terminus of P61, which is a nuclear encoded subunit of the mitochondrial electron transport chain complex III (Wang *et al.*, 2013). orf79–P61 specifically impairs the enzyme activity of electron transport chain complex III, disrupting energy production, increasing oxidative stress and ultimately causing mitochondrial dysfunction (Wang *et al.*, 2013). Variations in orf79 accumulation between BT-CMS and LD-CMS lines influence the severity of pollen development defects, with lower ORF79 protein levels in LD-CMS resulting in milder pollen defects compared with BT-CMS (Kazama *et al.*, 2008, 2016). The *orf79* variants are expressed through bicistronic *ATPase subunit 6* (*atp6*)–*orf79* transcripts, which are likely driven by the *atp6* promoter. The bicistronic *atp6–orf79* transcripts are processed by the fertility restoration factors Rf1a, Rf1b, Rf2 and Rf6 (Huang *et al.*, 2015; Itabashi *et al.*, 2011; Kazama *et al.*, 2008, 2016; Wang *et al.*, 2006). Given that *atp6* encodes a subunit of respiratory complex V, essential for ATP production, its expression is crucial for mitochondrial function. Alongside the *atp6–orf79* bicistronic gene, another Nipponbare *atp6* locus (N–*atp6*) exists in the mitochondria of BT- and HL-CMS but is absent in LD-CMS (Kazama *et al.*, 2016; Luan *et al.*, 2013). However, *atp6* transcripts are expressed in the mitochondria of all three CMS lines, including LD-CMS, where they may originate from the *atp6–orf79* bicistronic locus (Kazama *et al.*, 2016; Luan *et al.*, 2013). Fertility restoration factors reduce toxic orf79 protein levels by processing *atp6–orf79* transcripts without affecting mitochondrial atp6 function (Huang *et al.*, 2015; Itabashi *et al.*, 2011; Kazama *et al.*, 2008, 2016; Wang *et al.*, 2006) (Figure 2a). In BT-CMS, ORF79 accumulates in microspores, not in the anther wall tissue, causing gametophytic male sterility (Wang *et al.*, 2006). He *et al.* (2020) screened *atp6–orf79* structures in *Oryza rufipogon* and *Oryza sativa*, identifying 16 haplotypes, which were more common in wild rice than in cultivated rice, where only five haplotypes were found, suggesting genetic diversity loss during domestication. *Atp6–orf79*-like structures evolved multicentrically in *O. rufipogon*, and CMS genes in cultivated rice originated mainly from wild rice in South and Southeast Asia (He *et al.*, 2020). *Rf2* encodes a small

Table 1 Mechanisms of cytosolic male sterility and fertility restoration

CMS-associated genes			
Types	Variants	CMS mechanisms	Fertility restoration genes and their function
orf79	*orf79* from BT-CMS	Interaction with the N-terminus of P61, a subunit of mitochondrial electron transport chain complex III Gametophytic defects	*Rf1a* (LOC_Os10g35436, PPR protein): processing *atp6–orf79* transcripts *Rf1b* (LOC_Os10g35640, PPR protein): processing *atp6–orf79* transcripts
	orfH79 from HL-CMS		*Rf5*/*Rf1a* (LOC_Os10g35436, PPR protein): processing *atp6–orf79* transcripts
	L-orf79 from LD-CMS		*Rf6* (LOC_Os08g01870, PPR protein): processing *atp6–orf79* transcripts *Rf2* (LOC_Os02g17380, glycine-rich protein): processing *atp6–orf79* transcripts
WA352	*WA352* from WA-CMS	Interaction with COX11 triggers ROS accumulation and Cytc release Premature tapetal PCD	*Rf3* (unknown): reducing WA352 protein levels *Rf4* (LOC_Os10g35240, PPR protein): processing *rpl5–WA352* transcripts *Rf20* (LOC_Os01g58630, PPR protein): interaction with COX11, inhibiting WA352–COX11 binding
	orf352 from RT102-CMS		*Rf102* (unknown): processing *rpl5–WA352* transcripts
CW-orf307 from CW-CMS		Retrograde signalling by CW-orf307 drives *Rf17* expression in CW-CMS Inhibition of starch degradation in mature pollen	*Rf17* (LOC_Os04g40020, acyl-carrier protein synthase): a SNP in the *Rf17* promoter suppresses CW-orf307-induced *Rf17* expression
orf312 from TAA-CMS		Anther dehiscence inhibition	*Rf* (unknown): processing *orf312* transcripts
orf182	*orf182* from D1-CMS *orf182* from FA-CMS	Delayed tapetal PCD	*Rf* (unknown): processing *orf182* transcripts *Rf19* (PPR protein): processing *orf182* transcripts
orf113 from RT98-CMS		Pollen germination defects	*Rf98*: Processing polycistronic *orf113* transcripts

mitochondrial glycine-rich protein, whereas *Rf1a*, *Rf1b* and *Rf6* encode mitochondrial-targeted pentatricopeptide repeat (PPR) proteins (Matsuda *et al*., 2020). The evolution of *Rf* genes in rice involves significant structural variations, including duplications, recombination and selection. Restorer-of-fertility-like (*RFL*) genes in rice cluster primarily on chromosome 10, which contains *Rf1a*, *Rf1b* and *Rf4* (Melonek *et al*., 2016). Duplication and recombination within *RFL* clusters generate functional diversity in proteins, enhancing their ability to bind new mitochondrial RNA targets (Melonek *et al*., 2016). Thus, *Rf* genes have evolved to counteract CMS gene toxicity.

WA352 variants (also known as *orf352*) are CMS-associated genes isolated from Wild Abortive-type CMS (WA-CMS) and RT102-type CMS (RT102-CMS) lines. Their corresponding nuclear *Rf* genes include *Rf3*, *Rf4* and *Rf20* for WA-CMS and *Rf102* for RT102-CMS (Kazama and Toriyama, 2014; Luo *et al*., 2013; Okazaki *et al*., 2013; Tang *et al*., 2014). *WA352* (*WA352c*) encodes a chimeric protein containing segments from *orf284*, *orf224* and *orf288* in the Nipponbare mitochondrial genome along with an unknown sequence (Toriyama, 2021). *WA352* is cotranscribed with the ribosomal protein gene *rpl5* in WA-CMS and RT102-CMS, likely driven by the *rpl5* promoter (Luo *et al*., 2013; Okazaki *et al*., 2013). Additionally, in WA-CMS, *WA352* is transcribed *de novo*, independently of *rpl5* (Luo *et al*., 2013). The accumulated WA352 protein interacts with the cytochrome c oxidase (mitochondrial electron transport chain complex IV) assembly protein COX11, triggering a burst of reactive oxygen species (ROS) and the release of cytochrome c (Luo *et al*., 2013). Evolutionary analysis of WA352-like structures suggests that they arose through multiple mitochondrial genome rearrangements and substoichiometric shifting in *O. rufipogon* (Tang *et al*., 2017). Sequence variations functionalize CMS genes, enabling strong COX11 interactions, a process completed within a short timeframe in *O. rufipogon* (Tang *et al*., 2017). WA352c-based male-sterile lines show significant mitochondrial variation, indicating that domestication enhances genome variability and evolutionary trajectories (Gu *et al*., 2021). Rf102 suppress *WA352*'s function, reducing RNA levels, whereas Rf3 reduces WA352 protein levels without affecting RNA levels (Kazama and Toriyama, 2014; Luo *et al*., 2013) (Figure 2b). *Rf4* and *Rf20* encode PPR proteins, whereas the proteins encoded by *Rf3* and *Rf102* remain unidentified (Kazama and Toriyama, 2014; Luo *et al*., 2013). The *Rf4* locus, originating from wild rice, *O. meyeriana*, with a primitive ancestral sequence, evolved into 69 haplotypes across *Oryza* through sequence and copy number variations (Zhao *et al*., 2023). Eight *Rf4* haplotypes, enriched in modern cultivars through selection, exhibit structural diversity with varying *Rf4* copy and PPR gene numbers (Zhao *et al*., 2023). Varieties with double-copy *Rf4* haplotypes exhibit stronger fertility restoration and predominant in restorer lines (Zhao *et al*., 2023). Rf20 binds to COX11, preventing the WA352–COX11 interaction and maintaining COX11 function despite WA352's presence (Song *et al*., 2024b). *Rf20* is found in all core parental lines of three-line hybrid rice; although, its expression varies with haplotypes and structural variations (Song *et al*., 2024b). *Rf20* expression is a key regulator of fertility restoration in WA-CMS (Song *et al*., 2024b). In WA-CMS lines, WA352 preferentially accumulates in the anther tapetum, causing premature tapetal programmed cell death (PCD) (Luo *et al*., 2013).

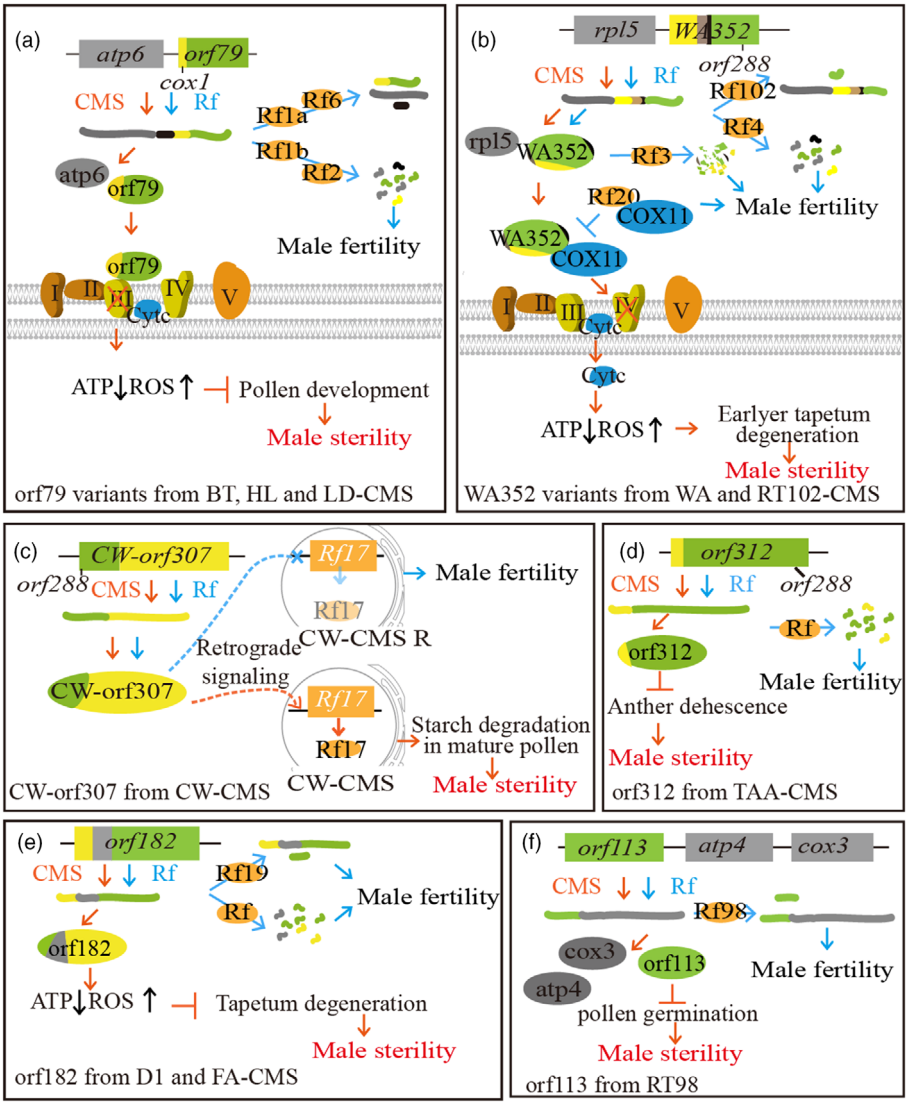

Figure 2 Mechanism of cytoplasmic male sterility (CMS)-associated genes and fertility restorer genes. Orange arrows indicate events in CMS line mitochondria, whereas blue arrows display events in restorer line mitochondria. (a) In BT-CMS, HL-CMS and LD-CMS mitochondrias, orf79 impairs mitochondrial function by interacting with the mitochondrial electron transport chain complex III, causing male sterility. Rf proteins in the revertant line degrade or cleave the bicistronic *atp6-orf79* transcript, restoring fertility. (b) In WA-CMS and RT102-CMS mitochondria, WA352 impairs function of complex IV by interacting with COX11, leading to premature tapetum degradation and male sterility. Rf4 and Rf102 process bicistronic *rpl5-WA352* transcripts, Rf3 reduces WA352 protein levels, and Rf20 blocks the binding between WA352 and COX11 through interaction with COX11. (c) Induced retrograde signals by CW-orf307 trigger *Rf17* expression in the CW-CMS line (orange dotted arrow) but not in the restorer line, due to a SNP in *Rf17* promoter (blue dotted arrow). (d) orf312 blocks anther dehiscence in the TAA-CMS line. Rf processes *orf312* transcripts in the restorer line. (e) orf182 delays tapetum degeneration in the D1-CMS and FA-CMS lines. Rf proteins process *orf182* transcripts in the restore line. (f) orf113 inhibits pollen germination in the RT98-CMS line, whereas polycistronic *orf113-atp4-cox3* transcripts are processed by Rf98 in the restorer line.

Segments of *orf288* are also found in other CMS-associated genes: *CW-orf307* in a Chinese Wild rice-type CMS (CW-CMS) line and *orf312* in a Tadukan-type CMS (TAA-CMS) line (Fujii *et al.*, 2010; Takatsuka *et al.*, 2021). CW-orf307 inhibits starch degradation in mature pollen before anthesis, causing sterility through the induction of expression *Rf17* (also known as *RETROGRADE-REGULATED MALE STERILITY*), an acyl-carrier protein synthase (Fujii *et al.*, 2010). A single nucleotide polymorphism (SNP) in the *Rf17* promoter region is crucial for suppressing its expression in the restorer line (Fujii *et al.*, 2010). The mitochondrial signal induced by CW-orf307 drives the expression of *Rf17* in the nuclei of the CW-CMS line through retrograde signalling, but it remains unrecognized by the mutated promoter region of *Rf17* in the restorer line (Fujii *et al.*, 2010) (Figure 2c). The molecular function of Rf17 in starch degradation remains unclear. *orf312* blocks anther dehiscence in TAA-CMS lines, with an unidentified Rf protein reducing *orf312* transcript levels (Takatsuka *et al.*, 2021) (Figure 2d). CW-CMS and TAA-CMS are gametophytic and sporophytic male-sterile lines, respectively (Huang *et al.*, 2015; Takatsuka *et al.*, 2021).

Another mitochondrial chimeric protein, orf182, has been identified in Dian 1-type CMS (D1-CMS) and Fujian Abortive-type CMS (FA-CMS) lines (Jiang *et al.*, 2022b; Xie *et al.*, 2018). Male sterility in D1-CMS is primarily caused by delayed tapetal PCD and

abnormal microspore PCD, potentially due to increased jasmonic acid levels (Wang *et al*., 2024). Notably, *orf182* expression in *Escherichia coli* inhibits host growth, increases ROS accumulation and reduces ATP levels (Xie *et al*., 2018). *orf182* transcripts are processed by an unidentified Rf protein in D1-CMS, whereas in FA-CMS, Rf19, a PPR protein, processes the transcripts (Jiang *et al*., 2022b; Xie *et al*., 2018) (Figure 2e).

orf113 is a CMS-associated gene isolated from a RT98-type CMS (RT98-CMS) line, a male-sterile mutant with defects in pollen germination (Igarashi *et al*., 2013). *orf113* is transcribed as a polycistronic transcript with ATP synthase subunit 4 (*atp4*) and cytochrome c oxidase subunit 3 (*cox3*) (Igarashi *et al*., 2013). Igarashi *et al*. (2016) identified a fertility restorer gene encoding a PPR protein as a candidate *Rf98* gene; however, the protein cannot process the polycistronic *orf113* transcript (Figure 2f).

Most *Rf* genes restore fertility by reducing toxic CMS-associated protein levels through RNA processing or protein degradation. Their mechanisms have been analysed via RNA and protein gel blot assays, with RNA cleavage sites identified using molecular techniques, including primer extension (Hu *et al*., 2012; Huang *et al*., 2015; Igarashi *et al*., 2016; Jiang *et al*., 2022b; Kazama *et al*., 2016; Kazama and Toriyama, 2014; Okazaki *et al*., 2013; Takatsuka *et al*., 2021; Xie *et al*., 2018). However, Rf17 and Rf20 function differently compared with other Rf proteins, which are transported into the mitochondria to reduce CMS-associated protein levels (Fujii *et al*., 2010; Song *et al*., 2024b). Mitochondrial signal induced by CMS-associated proteins drives *Rf17* expression; however, it remains low in the restorer line (Fujii *et al*., 2010). Rf20 suppresses toxic CMS-associated protein function by competing for its target (Song *et al*., 2024b).

Environment-sensitive genic male sterility used for second-generation hybrid rice production

Environment-sensitive genic male sterility (EGMS) is controlled by nuclear genes, triggering male sterility under specific environmental conditions (Virmani and Ilyas-Ahmed, 2001). EGMS lines facilitate a streamlined two-line hybrid seed production system, switching between sterility to fertility based on environmental factors (Peng *et al*., 2023b) (Figure 1c,d).

Action mechanism of photoperiod-sensitive genic male sterility-inducing genes

Photoperiod-sensitive genic male sterility (PGMS) is regulated by photoperiod, exhibiting day length–dependent levels of transcripts, RNA derivatives and proteins (Peng *et al*., 2023b) (Table 2). Two R2R3 MYB transcription factors, carbon starved anther (*CSA*) and *CSA2*, display differential expression patterns depending on day length (Wang *et al*., 2021a). Both *csa* and *csa*2 mutants confer PGMS traits, with *csa* and *csa2* inducing male sterility under short day (SD) and long day (LD) conditions, respectively (Zhang *et al*., 2013). Impaired anther development associated with *csa* and *csa2* results from defective allocation of sugar from leaves to anthers (Zhang *et al*., 2010, 2013). CSA directly regulates *monosaccharide transporter 8* (*MST8*) expression, an anther-specific monosaccharide transporter gene (Mamun *et al*., 2006; Zhang *et al*., 2010), especially under SD conditions; thus, *csa* mutants are infertile due to sugar deficiency under these conditions (Zhang *et al*., 2013) (Figure 3a,b). CSA's role in *MST8* induction diminishes under LD conditions due to low expression levels and the presence of other MYB transcription factors (MYB5 and MYB8), maintaining fertility (Zhang *et al*., 2013) (Figure 3a, b). *CSA2*, a *CSA* homologue, is highly expressed under LD conditions and regulates genes involved in sugar partitioning, such as sugar transporters and invertases (Wang *et al*., 2021a) (Figure 3c,d). The *csa2* mutant exhibits semi-sterility under LD conditions, given the defective allocation of sugar from leaves to anthers (Wang *et al*., 2021a) (Figure 3d). *CSA2* demonstrates relatively low transcript levels, contrasting with *CSA* (Wang *et al*., 2021a). Moreover, it is expressed at higher levels under LD conditions relative to SD conditions, and *csa2* mutants exhibit normal fertility under SD, similar to the wild type (Wang *et al*., 2021a) (Figure 3c,d). *CSA2*, under control of *CSA* promoter, can rescue the *csa* phenotype under SD, suggesting that CSA and CSA2 share the same regulatory modules during anther development (Wang *et al*., 2021a). The PGMS trait in both *csa* and *csa2* stems from photoperiod-dependent differences in their expression levels (Wang *et al*., 2021a; Zhang *et al*., 2013).

PMS1 and *PMS3* were identified in the Nongken 58S (NK58S) line, which exhibits male sterility under LD condition and fertility under SD conditions (Ding *et al*., 2012a; Fan *et al*., 2016). *PMS1* and *PMS3* encode long noncoding RNAs (lncRNAs) processed into 21-nucleotide (nt) small RNAs (Ding *et al*., 2012a; Fan *et al*., 2016; Zhou *et al*., 2012). *PMS3*, also known as *LDMAR* or *PITMS12-1*, is processed into *osa-smR5864w* (Zhou *et al*., 2012), which plays a role in anther development. In wild-type NK58, *LDMAR* expression is higher under LD conditions than under SD conditions, but in the NK58S line, it remains at SD levels even under LD conditions (Ding *et al*., 2012a) (Figure 3e,f). In the promoter region, CG methylation is higher in NK58S than in NK58, whereas in the transcribed region, the opposite trend is observed (Ding *et al*., 2012a). Hypermethylation of the *LDMAR* promoter in NK58S is regulated through two mechanisms (Ding *et al*., 2012a, 2012b): siRNA from *AK111270* mediates RNA-directed DNA methylation and a spontaneous G-C mutation (SNP) in *LDMAR* alters its secondary structure, leading to increased promoter methylation (Figure 3e,f). This methylation represses transcription, reducing *osa-smR5864* levels and causing male sterility under LD conditions (Ding *et al*., 2012a; Zhou *et al*., 2012). *LDMAR*'s function is highly sensitive to LD conditions, but the precise LD-dependent sterility mechanism remains unclear (Ding *et al*., 2012a). Its photoperiod-specific regulation may involve interactions with circadian rhythm genes or carbon metabolism pathways. *PMS1* (also known as *PMS1T*), a precursor lncRNA, is responsible for producing 21-nt phased secondary small interfering RNAs (phasiRNAs) triggered by *miR2118*, a 22-nt microRNA (Fan *et al*., 2016). A SNP (G to T) near the *miR2118* recognition site reduces *PMS1T* by enhancing the cleavage activity of *miR2118*, leading to 21-nt *PMS1T*-phasiRNA accumulation (derived from the *PMS1T* lncRNA precursor) in NK58S compared with NK58, especially under LD conditions (Fan *et al*., 2016) (Figure 3g,h). The increase in 21-nt *PMS1T*-phasiRNA levels under LD conditions is primarily driven by photoperiod-sensitive interactions between *PMS1T* and *miR2118*. Anther-specific Argonaute (AGO) proteins, AGO1b and AGO1d, are critical for phasiRNA biogenesis through *miR2118* binding and are implicated in temperature-sensitive male sterility (Araki *et al*., 2020; Shi *et al*., 2022, 2024; Tamotsu *et al*., 2023). Warmer periods under LD conditions may influence small RNA pathways, potentially increasing 21-nt *PMS1T*-phasiRNA accumulation via temperature-sensitive regulatory mechanisms involving AGOs. In the NK58S line, the mutation-induced reduction in

Table 2 Mechanism of environment-sensitive genic male sterility and fertility restoration

Type	EGMS genes	EGMS mechanism	Fertility restoration mechanism
PGMS	*CSA* (R2R3 MYB TF) LOC_Os01g16810	Strong expression of *CSA* under SD CSA-dependent *MST8* expression causes SD-dependent sugar allocation defects in *csa*	*MST8* expression induced by MYB5 and MYB8 under LD
	CSA2 (R2R3 MYB TF) Os05g049060	Higher *CSA2* levels under LD than under SD CSA2-dependent sugar partitioning gene expression causes LD-dependent sugar allocation defects in *csa2*	Sugar partitioning gene expression induced by unknown proteins under SD
	PMS1 (lncRNA) AK242308	Accumulation of 21-nt *PMS1T*-phasiRNAs by the SNP near the *miR2118* recognition site in *PMS1T* under LD conditions	21-nt *PMS1T*-phasiRNAs do not accumulate under SD, resulting in fertility
	PMS3/LDMAR/T/PMS12-1 (lncRNA) LOC_Os12g36030	Mutation-induced reduction in *LDMAR* levels, relative to those in NK58, leads to a decrease in *osa-smR5864* levels under LD conditions due to methylation changes	Hypermethylation of the LDMAR promoter independent of day length Similar *osa-smR5864* levels between NK58 and NK58S under SD
TGMS	*MS1/TMS9-1/PTC1* (Histone binding protein) LOC_Os09g27620	L301P mutant (MS1^{wenmin1}) cannot induce *EAT1* expression owing to its incomplete nuclear localization and reduced protein levels under HT	MS1^{wenmin1} interacts with TDR, enhancing its binding to the EAT1 promoter despite incomplete nuclear localization under LT
	Ugp1 (UDP-glucose pyrophosphorylase 1) LOC_Os09g38030	Overexpression of *Ugp1* under *Ubi1* promoter control produces intron-containing *Ugp1* transcripts, which suppress native *Ugp1* expression	Proper *Ubi1* intron splicing, along with intron-containing *Ugp1* transcript, produces UGPase at LT
	TMS5 (RNase Z^{S1}) LOC_Os02g12290	Strong expression of Ub_{L40} at HT requires RNase Z^{S1} for fertility RNase Z^{S1} mutation leads to Ub_{L40} accumulation at HT	RNase Z^{S1} mutant is fertile under LT owing to low-Ub_{L40} mRNA levels
	AGO1d (Argonaute) LOC_Os06g51310	Substantial reduction in phasiRNA biogenesis and AGO1d-mediated gene silencing in *ago1d* under LT Up-regulation of fertility-related and cold tolerance –associated genes under LT	*Ago1d* mutant exhibits normal fertility under standard temperatures (~28°C), given the functional redundancy of *Ago1b*
	TMS10 (LRR-RLK) LOC_Os02g18320	Male sterility under HT	Complementation of *tms10* through high expression of *TMS10L* under LT
	TMS15 (LRR-RLK) LOC_Os01g68870	Reduced interaction between the V487E mutant and OsTDL1A under HT Reduced *TMS15* expression under HT	Interaction and expression are restored under LT
	OsTMS18/NP1 (Glucose-methanol-choline oxidoreductase) LOC_Os10g38050	G61S mutant exhibiting exine-like structures on the surface of its microspores Flawed pollen wall cannot protect the microspore from HT	Flawed pollen wall protects the microspore from LT
	TMS19 (PPR protein) LOC_Os02g21580	V207A mutation causes ROS accumulation at HT	Effective scavenging of ROS occurs under LT
HGMS	*OsGL1-4/OsCER1* (Member of the Glossy) LOC_Os02g40784 *OsOSC12/OsPTS1* (Bicyclic triterpene synthase) Os08g0223900 *HMS1* (β-ketoacyl-CoA synthase) LOC_Os03g12030	Functions in pollen coat formation Mutants exhibit rapid dehydration or pollen-to-stigma adhesion defects	High humidity allows defective pollen to reach the stigma and germinate without viability loss

LDMAR levels compared with those in NK58 (resulting in lower *osa-smR5864* levels), and elevated 21-nt *PMS1T*-phasiRNA levels (due to enhanced *miR2118* cleavage) cause male sterility under LD conditions (Ding *et al*., 2012a; Fan *et al*., 2016; Zhou *et al*., 2012).

Action mechanism of thermosensitive genic male sterility-inducing genes

In thermosensitive genic male sterility (TGMS) lines, fertility conversion is regulated by temperature. Unlike PGMS, genes causing TGMS have diverse molecular functions, including transcriptional regulation, RNA processing, protein ubiquitination, signal transduction and pollen wall synthesis (Peng *et al*., 2023b) (Table 2). *MALE STERILITY 1* (*MS1*)/*THERMOSENSITIVE MALE STERILITY 9–1* encodes a histone binding protein and regulates tapetal PCD and pollen exine formation in rice (Wu *et al*., 2022; Yang *et al*., 2019). A null mutant of *MS1* displays complete male sterility with delayed tapetal PCD, whereas an L301P mutant of *MS1* (MS1^{wenmin1}) exhibits TGMS, being sterile and fertile at high temperatures (HT) and low temperatures (LT), respectively (Wu *et al*., 2022) (Figure 4a,b). MS1 interacts with tapetum degeneration retardation (TDR), enhancing its binding to the *EARLY ANTHER TAPETAL CELL DEATH 1* (*EAT1*) promoter (Wu *et al*., 2022) (Figure 4a). At HT, reduced MS1 levels remain sufficient for TDR interaction and resultant *EAT1* expression (Wu *et al*., 2022) (Figure 4a). Although partially mislocalized in the cytosol, MS1^{wenmin1} can still induce *EAT1* expression at LT (Wu *et al*., 2022) (Figure 4b). However, MS1^{wenmin1} cannot sufficiently induce *EAT1* expression due to its incomplete nuclear localization and protein level reduction (Wu *et al*., 2022) (Figure 4b).

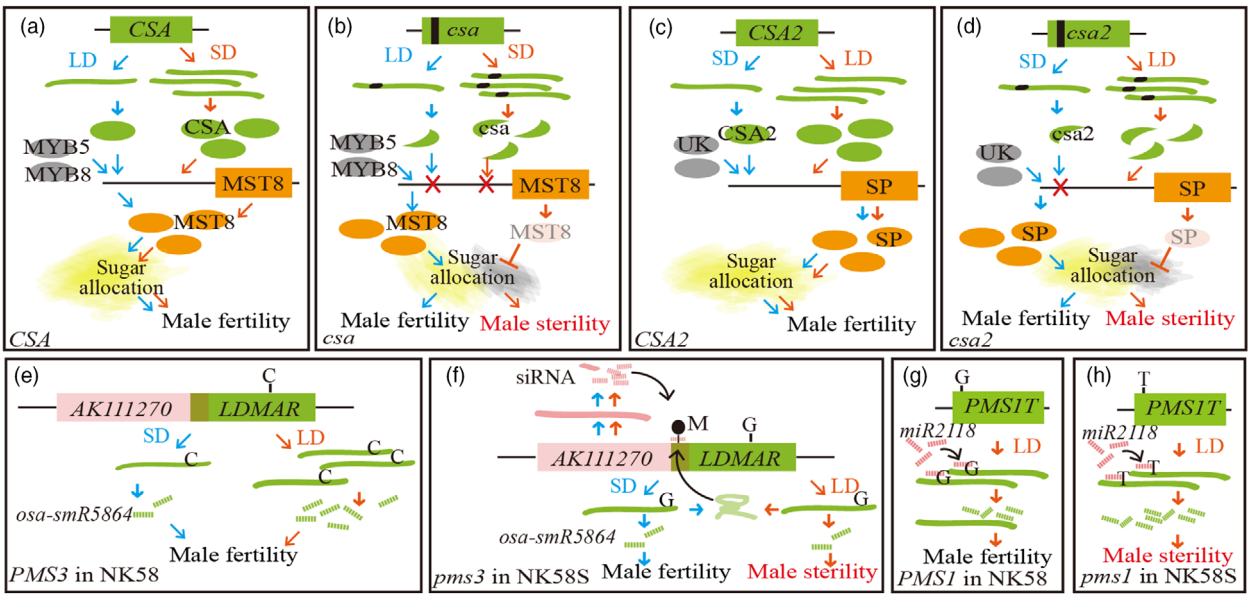

Figure 3 Working mechanism of photoperiod-sensitive genic male sterility-inducing genes. Orange and blue arrows represent events occurring under restrictive and permissive conditions, respectively. (a, b) Short day (SD)-dependent male sterility caused by *csa*. (a) Induction of *MST8* expression by CSA under long day (LD) and SD conditions and by MYB5 and MYB8 under LD conditions. (b) In *csa* under SD conditions, insufficient MST8 results in male sterility. (c, d) LD-dependent male sterility caused by *cas2*. Induction of sugar partitioning (*SP*) gene expression is driven by CSA2 independently of day length, and by unknown proteins under SD conditions. In *csa2* under LD, insufficient SP protein causes male sterility (d). (e, f) LD-dependent male sterility caused by *pms3* (LDMAR, a lncRNA processed into *osa-smR5864*). (e) Induction of LDMAR expression in NK58 under LD conditions, leading abundant *osa-smR5864*. (f) Reduced expression of LDMAR in NK58S due to promoter methylation. Both siRNAs originated from *AK111270* (pink lines) and the altered secondary structure of *LDMAR*, caused by a SNP (C–G conversion), leads to *LDMAR* hypermethylation (marked as M). Lower LDMAR levels, compared to NK58, leads to a reduction in *osa-smR5864*, causing male sterility under LD (f). (g, h) LD-dependent male sterility of *pms1*. (g) *PMS1T* lncRNA is processed into 21-nt *PMS1T*-phasiRNAs by *miR2118*. (h) A SNP (G to T) near the *miR2118* recognition site leads to *PMS1T*-phasiRNA accumulation in NK58S under LD conditions due to enhanced miR2118 cleavage.

UDP-glucose pyrophosphorylase 1 (Ugp1) is crucial for callose deposition during pollen mother cell meiosis. Thus, suppressing *Ugp1* causes male sterility (Chen *et al.*, 2007). Overexpressing *Ugp1* under *Ubi1* promoter control can trigger cosuppression by siRNAs derived from abnormal RNAs containing an unspliced intron in the 5′-untranslated region of the *Ubi1* promoter (Chen *et al.*, 2007). In the *Ugp1*-OX line, this intron-containing *Ugp1* suppresses native *Ugp1* expression (Chen *et al.*, 2007) (Figure 4c). Cosuppression occurs regardless of temperature; however, proper *Ubi1* intron splicing at LT allows UGPase production and facilitates fertility restoration (Chen *et al.*, 2007) (Figure 4c).

TMS5, from the *indica* TGMS line Zhu1S (frequently employed as a female parent in two-line hybrid rice breeding), encodes a short RNase Z (RNase Z^{S1}) that processes ubiquitin fusion ribosomal protein L40 (Ub_{L40}) mRNAs (Zhou *et al.*, 2014) (Figure 4d). Three Ub_{L40} genes, highly expressed in microspore mother cells, and particularly induced by HT, cause male sterility when overexpressed (Zhou *et al.*, 2014) (Figure 4d). RNase Z^{S1} regulates Ub_{L40} mRNA levels by processing them (Figure 4d); however, in RNase Z^{S1} mutants, increased expression of Ub_{L40} at HT leads to the accumulation of unprocessed Ub_{L40} and male sterility (Zhou *et al.*, 2014) (Figure 4e). Excessive accumulation of Ub_{L40} mRNAs can lead to an overabundance of ubiquitin, promoting the degradation of unstable proteins, including those essential for anther development, especially under HT conditions (Peng *et al.*, 2023a, 2024).

In recent years, studies have highlighted the potential of *tms5* for stable two-line hybrid seed production. The critical sterility-inducing temperature (CSIT) of *tms5*-based TGMS lines varies across rice varieties, limiting their application. Mutations in *CSIT1* and *CSIT2* raise the CSIT of *tms5*, requiring higher temperatures to induce sterility (Peng *et al.*, 2023a, 2024). *CSIT1* and *CIST2*, encoding E3 ubiquitin ligases, regulate the degradation of unstable proteins through ribosome-associated quality control (Peng *et al.*, 2023a, 2024). Under high Ub_{L40} mRNA levels, CIST1 and CIST2 excessively ubiquitinate unstable anther-related proteins, including catalases, leading to their degradation (Peng *et al.*, 2023a, 2024). This protein depletion along with ROS accumulation in *tms5*, disrupts anther development (Peng *et al.*, 2023a, 2024). However, mutations in *CSIT1* and *CSIT2* prevent this degradation, allowing proteins to refold into active forms, mitigating ROS accumulation and increasing the CSIT in *tms5*-based TGMS lines (Peng *et al.*, 2023a, 2024).

TMS15, also known as *MULTIPLE SPOROCYTE1* (*MSP1*), encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) that interacts with its ligand OsTDL1A via the LRR domain to initiate tapetum development in rice (Han *et al.*, 2023) (Figure 4f). Knockout mutants of *MSP1* exhibit male sterility due to the absence of the tapetal layer, whereas a V487E mutant in the LRR domain (*mOsTMS15*) results in TGMS (Han *et al.*, 2023). Reduced interaction between mOsTMS15 and OsTDL1A, coupled with decreased *OsTMS15* expression in *mOsTMS15* under HT, leads to male sterility (Han *et al.*, 2023) (Figure 4g). However, at LT, the interaction and expression are restored; thereby, recovering fertility (Han *et al.*, 2023).

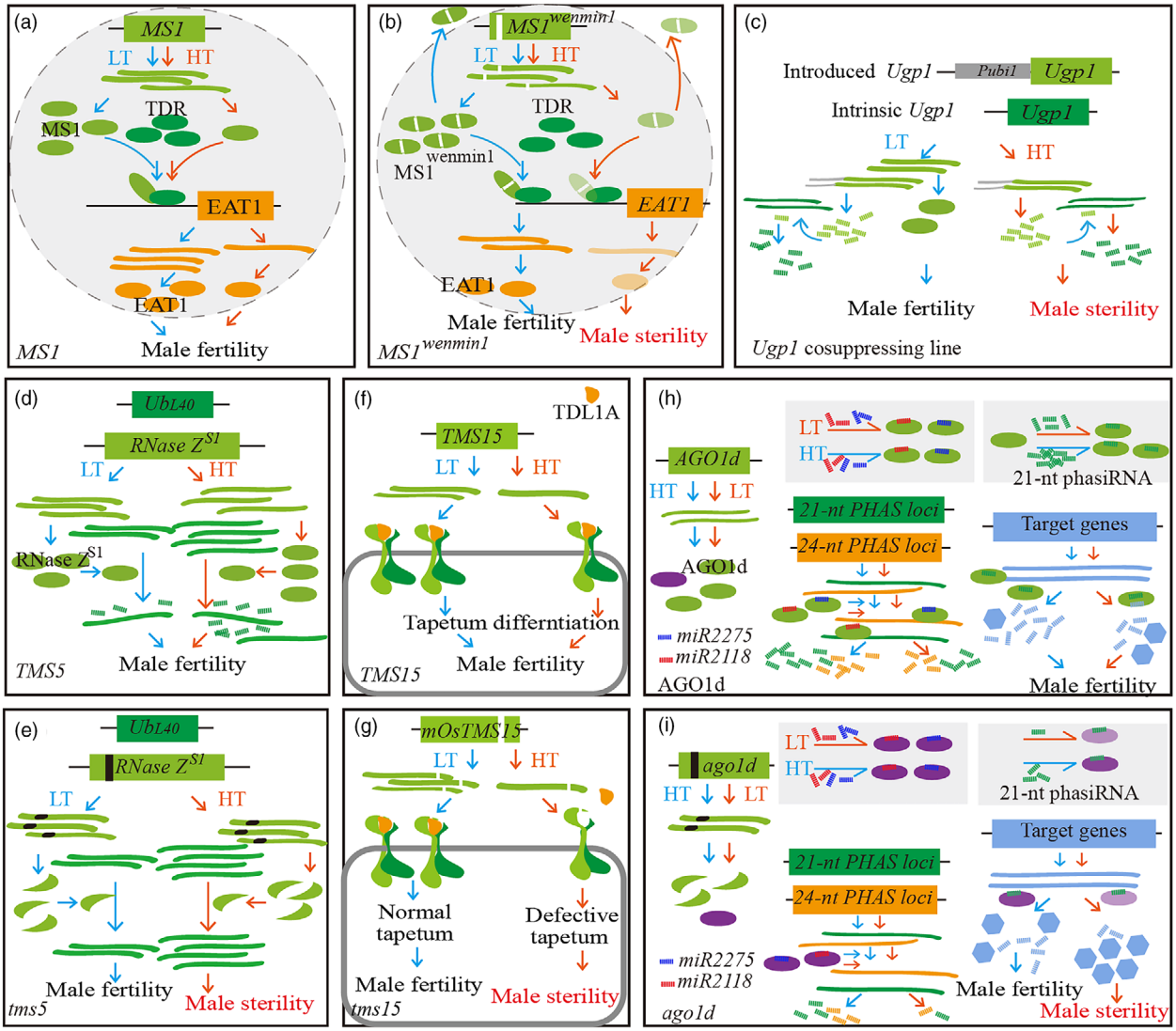

Figure 4 Mechanism of thermosensitive genic male sterility-inducing genes. Orange and blue arrows represent events occurring under restrictive and permissive conditions, respectively. (a, b) HT-dependent male sterility by MS1^{wenmin1} (L301P). (a) MS1 interacts with TDR, enhancing *EAT1* promoter binding. (b) MS1^{wenmin1}, localized in the nucleus and cytosol, can induce *EAT1* expression at low temperatures (LT) but fails due to incomplete nuclear localization and reduced MS1^{wenmin1} at high temperatures (HT). (c) In the *Ugp1*-OX line, *Ugp1* transcript containing the unprocessed *Ubi1* intron suppresses native *Ugp1* expression. LT-dependent UGPase production through correct splicing. (d, e) Mechanism of HT-dependent male sterility caused by RNase Z^{S1}. (d) HT-induced *Ub$_{L40}$* mRNAs are processed by RNase Z^{S1} (e) Mutation in RNase Z^{S1} prevents *Ub$_{L40}$* mRNA processing, leading to accumulation of *Ub$_{L40}$* mRNA at HT. (f, g) HT-dependent male sterility caused by *tms15*. (f) TMS15 interacts with OsTDL1A, initiating tapetum development. (g) Decreased *mOsTMS15* expression and reduced *mOsTMS15*–OsTDL1A interaction leads to male sterility under HT. However, interaction and expression are restored under LT. (h, i) LT-dependent male sterility caused by AGO1d. (h) AGO1d binds with *miR2118* and *miR2275* to generate 21- and 24-nt phasiRNA, respectively. 21-nt phasiRNAs induce target gene silencing via AGO1d. (i) Reduced phasiRNA biogenesis and gene silencing in *ago1d*, especially under LT.

TMS19 encodes a mitochondrial-targeted PPR protein, and a V207A mutation induces male sterility under HT or LD conditions (P/TGMS) because of excessive ROS accumulation (Zhou *et al.*, 2024). However, under LT or SD conditions, ROS is effectively scavenged in mutant anthers, restoring fertility (Zhou *et al.*, 2024).

pms3, which is processed into 21-nt phasiRNA, causes PGMS in *japonica* Nongken58S and confers TGMS in *indica* Peiai64 (Liu *et al.*, 2023a). 21- and 24-nt phasiRNAs are essential for male fertility (Jiang *et al.*, 2020; Zhai *et al.*, 2015). The anther-specific Argonaute *AGO1d* preferentially binds to *miR2118* and *miR2275*, producing 21- and 24-nt phasiRNAs, respectively (Shi *et al.*, 2022) (Figure 4h). *miR2118*-derived 21-nt phasiRNAs silence target genes when loaded into *AGO1d* (Shi *et al.*, 2022, 2024) (Figure 4h). However, LT slightly reduce 21-nt phasiRNA biogenesis and target gene silencing (Shi *et al.*, 2024) (Figure 4h). In *AGO1d* mutants, phasiRNA levels are decreased and AGO1d-mediated gene silencing is moderately reduced; nevertheless, the mutants exhibit male fertility at HT (Shi *et al.*, 2022) (Figure 4i). However, under LT, these mutants exhibit severe reductions in phasiRNA biogenesis alongside up-regulation of fertility-related and cold tolerance–associated genes. This suggests that male sterility is due to reduced silencing by 21-nt phasiRNAs or AGO1d (Shi *et al.*, 2024) (Figure 4i).

Additionally, *AGO1b* may be a TGMS target, as *ago1b–AGO1d* double mutants show more severe anther defects relative to the respective single mutants. Given that AGO1b and AGO1d share similar 21-nt phasiRNA clusters in rice, *ago1b* mutations combined with *AGO1d* could be used for TGMS in hybrid rice breeding (Tamotsu *et al*., 2023).

Beyond AGO1b and AGO1d, the 19 AGOs annotated in the rice genome (Zhai *et al*., 2014) are considered putative TGMS candidates. In maize, knockdowns of *MALE-ASSOCIATED ARGONAUTE PROTEINS 1* and *2* (*MAGO1/2*) show temperature-sensitive male sterility. Specifically, knockdown mutants exhibit partial defects at 28°C but severe malfunctions at 35°C or under typical summer conditions (Lee *et al*., 2021). MAGO1/2 in maize controls retrotransposon activity in male meiocytes via temperature–induced phasiRNAs. Phosphorylation of surface-located serine residues modulates Argonaute activity and interactions with retrotransposon RNA targets, enabling RNA-guided surveillance for male fertility under environmental stress (Lee *et al*., 2021). In rice, MEIOSIS ARRESTED AT LEPTOTENE1 (MEL1), a homologue of MAGO1/2, binds *miR2118* and 21-nt phasiRNAs (Komiya *et al*., 2014), The *mel1* mutant exhibits male sterility at 30°C or under typical summer conditions due to meiotic arrest in pollen mother cells (Nonomura *et al*., 2007). Although untested for TGMS, *mel1* mutants may exhibit partial or full fertility under LT, similar to other TGMS rice lines. Notably, grasses, including rice and maize, possess numerous phasiRNA-generating loci (PHAS loci), with the phasiRNAs preferentially accumulating in male reproductive organs (Johnson *et al*., 2009; Xia *et al*., 2019; Zhai *et al*., 2015). This highlights phasiRNAs' vital role in male reproductive organ development and its potential involvement in stress responses. Although rice TGMS research on phasiRNAs remains limited, related studies in other species have linked TGMS to siRNA regulation, including phasiRNAs.

PhasiRNA biogenesis relies on DICER-LIKE (DCL) proteins for precise double-stranded RNA cleavage. DCL4 and DCL5 produce 21- and 24-nt phasiRNAs in monocots, respectively (Margis *et al*., 2006; Song *et al*., 2012a, 2012b). Maize *dcl5* mutants lack an abundance of 24-nt phasiRNAs and exhibit male sterility at HT or under typical summer conditions (Teng *et al*., 2020). Specifically, *dcl5* mutants are sterile at 28°C, partially fertile at 26°C and fully fertile at 23°C (Teng *et al*., 2020). This suggests that DCL5 and 24-nt phasiRNAs are crucial for maize TGMS. Similarly, knocking down OsDCL3b, a rice DCL5 homologue, affects 21- and 24-nt small RNA biogenesis, including phasiRNAs, while reducing pollen fertility and seed-setting rate (Liao *et al*., 2019; Song *et al*., 2012a). Thus, *OsDCL3b* mutants could be putative TGMS lines for rice hybrid production.

The rice LRR-RLK *TMS10* and *TMS10L* redundantly regulate male fertility under LT (Yu *et al*., 2017). *TMS10L*'s higher expression at LT likely complements *tms10* (Yu *et al*., 2017). The *tms10* mutant is sterile at HT, but fertile at LT due to high *TMS10L* expression (Yu *et al*., 2017).

OsTMS18/NP1, which encodes a glucose-methanol-choline oxidoreductase, plays a crucial role in rice anther cuticle and pollen exine formation (Liu *et al*., 2017b). *OsTMS18* null mutants show complete male sterility, whereas a point mutant (*mOsTMS18/G61S*) exhibits TGMS (Zhang *et al*., 2022b). The mOsTMS18/G61S protein may retain some catalytic activity for sporopollenin synthesis, allowing exine-like structures to form on the surface of *mOsTMS18* microspores (Zhang *et al*., 2022b). The flawed pollen wall in *mOsTMS18* is sufficient to protect its microspores at LT but not HT, causing TGMS (Zhang *et al*., 2022b).

Action mechanism of humidity-sensitive genic male sterility-inducing genes

Humidity-sensitive genic male sterility (HGMS) rice lines exhibit sterility under low humidity. Rice pollen has relatively high-water content at shedding and is highly sensitive to desiccation (Moon and Jung, 2020). Once shed, pollen must reach the stigma and germinate before losing too much water. The pollen wall, particularly the coat, plays a crucial role in preventing dehydration and ensuring adhesion to the stigma (Moon and Jung, 2020). All identified HGMS-inducing genes in rice, including *GLOSSY1-4* (*OsGL1-4*, also known as *ECERIFERUM1*), *HUMIDITY-SENSITIVE GENETIC MALE STERILITY1* (*HMS1*) and *OXIDOSQUALENE CYCLASES12/POACEATAPETOL SYNTHASE1* (*OsOSC12/OsPTS1*), are involved in pollen coat formation. Mutations in these genes lead to rapid dehydration or impaired pollen adhesion due to pollen wall defects (Chen *et al*., 2020; Ni *et al*., 2021; Xue *et al*., 2018; Yu *et al*., 2019). Under high humidity, defective pollen retains viability long enough to land on the stigma and germinate, restoring fertility (Moon and Jung, 2020).

Biotechnology-driven male sterility in third-generation hybrid rice production

Advances in biotechnology have revolutionized the maintenance of stable male sterility lines through self-pollination. DuPont-Pioneer introduced seed production technology (SPT) that uses an SPT maintainer to produce maize hybrid seeds (Wu *et al*., 2016). Specifically, an SPT maintainer is a transformed homozygous recessive male-sterile mutant (*ms/ms*) containing an SPT construct (Wu *et al*., 2016) (Figure 5a). The SPT construct has three components: a wild-type coding gene (*MS*) associated with sterility, controlled by its native promoter; an α-amylase gene (*ZmAA1*) under the control of a pollen-specific promoter that prevents transmission of the SPT cassette through pollen by inducing defects in the carrying pollen; and a colour marker gene under the control of seed-specific promoter (Wu *et al*., 2016) (Figure 5a). Through transformation, the SPT maintainer acquires functional *MS* genes, enabling its propagation via self-pollination. All haploid gametophytes in the SPT maintainer carry the *ms* genotype, with or without an SPT cassette insertion (Figure 5b). The expression of the pollen-killing gene inhibits pollen tube growth in pollen carrying the SPT cassette, ensuring that SPT maintainer progeny consist of two groups: one half carrying a heterozygous SPT cassette, functioning as maintainers and the other half lacking the SPT cassette, resulting in male sterility (Figure 5c,d). The progenies of SPT maintainers can be sorted based on colour: those without a red fluorescent protein (RFP) signal are male-sterile lines for hybrid rice production, whereas those with an RFP signal serve as maintainer lines (Figure 5c,d). Similar strategies have been adopted in rice. SPT maintainers have been developed using male sterility genes (*CYP704B2*, *OsNP1*, *CYP703A3* and *OsMS1*), pollen-killing genes (α-amylase and *orfH79*) and seed sorting genes [*RFP* and ADP-glucose pyrophosphorylase (*AGP*)] (Chang *et al*., 2016; Song *et al*., 2021; Wu *et al*., 2021). *AGP* enables seed sorting by weight, given its role in endosperm starch biosynthesis (Wu *et al*., 2021).

The application of CRISPR/Cas9 technology has accelerated the development of STP maintainers in maize. Qi *et al*. (2020) shortened the STP maintainer generation period by transforming wild-type embryos using a CRISPR/Cas9 vector targeting a

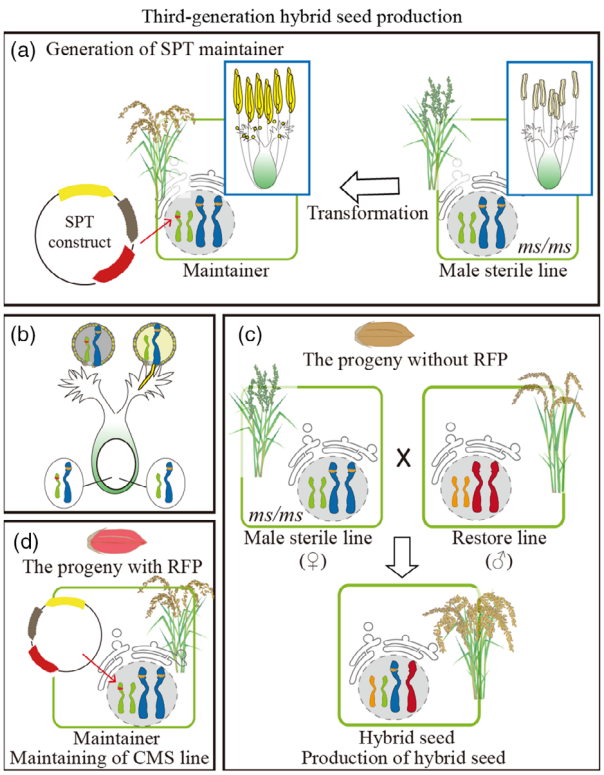

Figure 5 Schematic representation of third-generation hybrid seed production using biotechnology-based male sterility. (a) The seed production technology (SPT) construct (circular vector, red line in the green chromosome) containing a fertility restorer gene (MS: yellow), pollen killer (brown) and RFP (red) is introduced into the male-sterile line. Mutations in a fertility-regulating gene are represented by orange lines in the blue chromosome. (b) During self-pollination, pollen carrying the SPT cassette exhibits defects, leading to progeny of SPT maintainers, with one half inheriting a heterozygous SPT cassette and the other half lacking it. (c, d) Sorting of maintainer progeny by colour: seeds without the SPT cassette are used for hybrid seed production (c), whereas seeds with the SPT cassette (red) are used for maintenance (d).

fertility-regulating gene, alongside an STP construct containing a modified restoration gene lacking the target site.

Synthetic apomixis in hybrid rice production

Apomixis bypasses the conventional separation events in sexual reproduction, enabling the production of one-line hybrid seeds (Tucker and Koltunow, 2009). Given that hybrid seeds segregate in the next generation, heterosis is not inherited, necessitating the continual renewal of seeds for hybrid rice cultivation (Figure 6a). Apomixis is a plant reproductive mechanism that generates seeds without meiosis and fertilization (Underwood and Mercier, 2022). This results in genetically identical offspring with parents, bypassing the need for pollination and gamete fusion; thereby, enabling clonal reproduction (Figure 6b). Natural apomixis occurs in several hundred plant species, although it remains relatively rare in major crops, including rice, maize and wheat.

Efforts to introduce diplospory, a form of apomixis, into rice are ongoing (Mieulet *et al.*, 2016; Yin *et al.*, 2022a). During diplospory, embryos develop from unreduced megaspores. The first step in engineering apomixis is inducing mitosis instead of meiosis (MiMe) (d'Erfurth *et al.*, 2009; Mieulet *et al.*, 2016). Mutations in genes involved in three key meiotic processes switch meiosis to mitosis, as observed in *pair1 rec8 osd1* and *spo11-1 rec8 osd1* mutants (Mieulet *et al.*, 2016; Xie *et al.*, 2019) (Figure 6b). Proteins encoded by *PAIR1* and *SPO11-1* catalyse double-strand breaks, participating in recombination (Grelon *et al.*, 2001; Nonomura *et al.*, 2004), whereas those of *REC8* and *OSD1* mediate sister chromatid cohesion and secondary meiotic division, respectively (Mieulet *et al.*, 2016; Shao *et al.*, 2011). MiMe generates diploid gametes, which fertilize normally and produce tetraploid offspring through self-fertilization (Mieulet *et al.*, 2016).

To prevent MiMe-induced genome doubling in subsequent generations, strategies like haploid induction or parthenogenesis are commonly used (Conner *et al.*, 2017). *MATRILINEAL* (*MATL*), also known as *PHOSPHOLIPASE A* or *NOT LIKE DAD*, is a key quantitative trait locus for haploid production in maize (Gilles *et al.*, 2017; Kelliher *et al.*, 2017; Liu *et al.*, 2017a). *MATL* induces maternal haploids through genome elimination, with a frequency of ~6.7% observed in maize mutants lacking *MATL* (Kelliher *et al.*, 2017). Rice *OsMATL*, a pollen-specific phospholipase A, exhibits pollen-specific expression, and its mutant, *osmatl*, shows reduced seed set and induces haploids at a rate of 2%–6% (Singh *et al.*, 2012; Yao *et al.*, 2018) (Figure 6b). MATL localizes in the endoplasmic membrane surrounding the sperm cells in the pollen grain (Gilles *et al.*, 2021; Li *et al.*, 2021b), with this localization resulting from lipid anchoring and electrostatic interactions (Gilles *et al.*, 2021; Li *et al.*, 2021b). MATL's function appears related to maintaining communication between pollen vegetative cells and enclosed sperm cells (Gilles *et al.*, 2021; Li *et al.*, 2021b). Elevated ROS levels linked to *MATL* mutations during late pollen development cause DNA damage and chromosomal fragmentation, which are critical for paternal genome elimination (Jiang *et al.*, 2022a; Li *et al.*, 2017; Sun *et al.*, 2022). In rice, the *OsMATL* mutation has been integrated with the MiMe system to generate clonal progeny (Wang *et al.*, 2019). The quadruple mutants (named '*Fix*', for Fixation of hybrids) produce 6.2% diploid clonal progeny (Wang *et al.*, 2019). Additionally, the mutation of *OsMATL2* induces up to 9.4% haploid formation (Jang *et al.*, 2023). In *Arabidopsis*, Marimuthu *et al.* (2011) showed that combining a MiMe line with a centromeric histone H3 (*CENH3*)-induced genome elimination line resulted in 34% diploid progeny. Manipulating *CENH3* in crop species, such as maize, wheat and rice, has produced haploid plants (Den Camp *et al.*, 2017; Kalinowska *et al.*, 2019; Kelliher *et al.*, 2016; Lv *et al.*, 2020; Wang *et al.*, 2021b). However, this method is unsuitable for rice apomixis because *CENH3*-induced genome elimination occurs only when crossed with the wild type (Kalinowska *et al.*, 2019).

Parthenogenesis, where eggs develop into embryos without pollen fertilization, offers another approach. The *PsASGR-BABY BOOM-like* (*PsASGR-BBML*) gene from the apomictic grass *Pennisetum squamulatum* is largely responsible for the parthenogenesis trait (Conner *et al.*, 2015). When expressed in rice and maize egg cells, the AP2 transcription factor PsASGR-BBML induces parthenogenesis, yielding haploid offspring (Conner *et al.*, 2017). In rice, *BABY BOOM1* (*BBM1*) is expressed in sperm cells and triggers embryo development postfertilization (Khanday *et al.*, 2019). When *BBM1* and *BBM4* are specifically expressed in egg cells, they produce parthenogenic embryos without fertilization (Khanday *et al.*, 2019; Wei *et al.*, 2023). Khanday *et al.* (2019) revealed that combining MiMe with the egg cell-

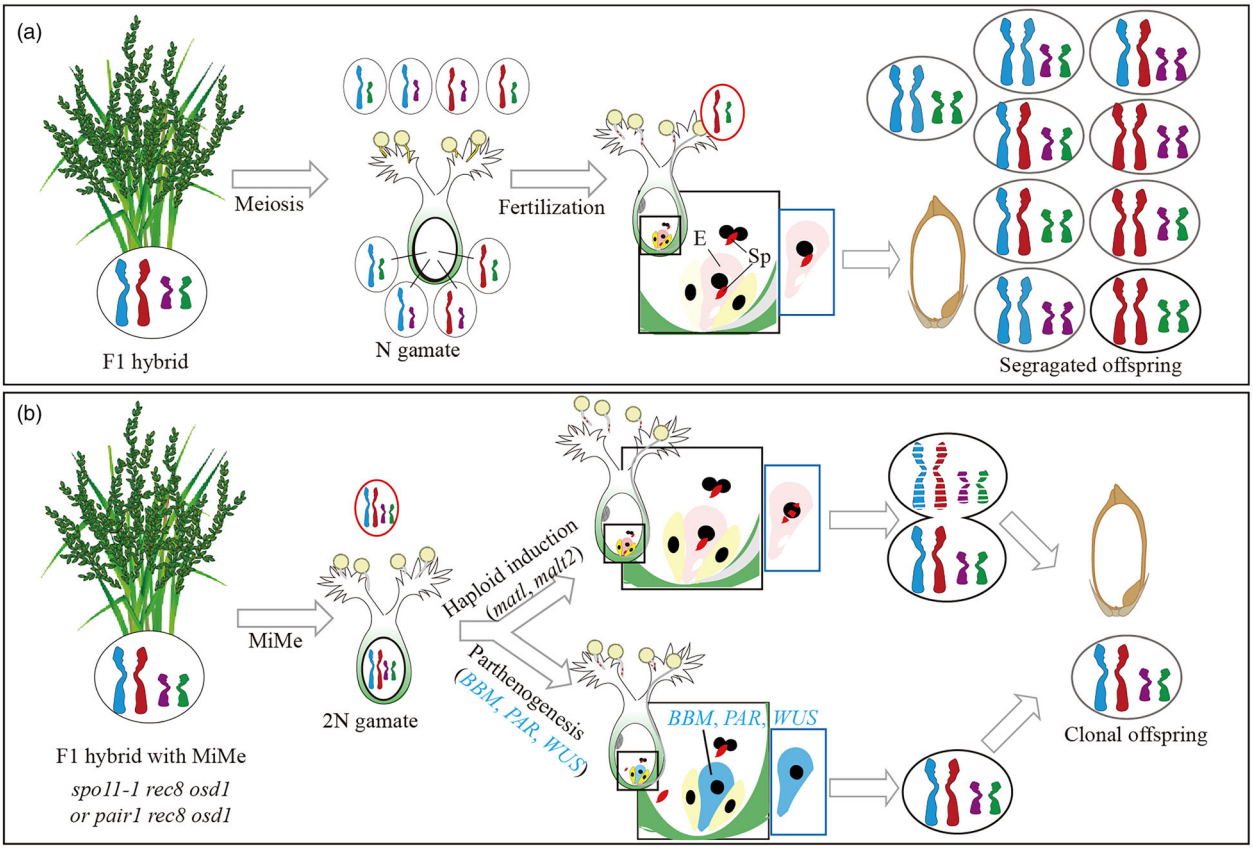

Figure 6 Schematic comparing sexual reproduction and synthetic apomixis. (a) In sexual reproduction, haploid gametes form through meiosis, followed by fertilization, resulting in segregated progeny. (b) In synthetic apomixis, mitosis replaces meiosis (MiMe) through CRISPR/Cas9-mediated mutagenesis targeting meiotic genes, such as *spo11-1 rec8 osd1* and *pair1 rec8 osd1*, producing diploid gametes. Under MiMe, haploid induction is achieved via paternal genome elimination through CRISPR mutagenesis targeting genes such as *matl* and *malt2*. Alternatively, parthenogenesis can be induced by the ectopic expression of parthenogenic genes like *BBM*, *PAR* and *WUS* in egg cells, resulting in the production of clonal progenies. The schematic diagram shows $2n = 4$. E, egg cell; Sp, sperm cell. Cells in blue boxes develop to seed.

specific expression of *BBM1* resulted in up to 29% clonal diploid offspring (Figure 6b). Moreover, Vernet *et al*. (2022) reported a clonal seed generation frequency of >95% using MiMe and BBM1.

RWP-RK DOMAIN CONTAINING PROTEIN (RKD) transcription factors are also promising candidates for inducing parthenogenesis. *Arabidopsis RKD4* (*AtRKD4*) is pivotal in early embryogenesis, with its ectopic expression triggering somatic embryogenesis without the need for external growth regulators (Waki *et al*., 2011). Similarly, the citrus RKD *CitRWP* is associated with citrus polyembryony and regulates sporophytic apomixis (Wang *et al*., 2017). Its expression levels are higher in the ovules of polyembryonic cultivars, which feature a miniature inverted-repeat transposable element insertion in the promoter region, typical of citrus varieties like sweet orange, grapefruit and lemon (Wang *et al*., 2017). Furthermore, *CitRKD1* knockdown in sweet orange abolishes polyembryonic seed production, underscoring its vital role, akin to *AtRKD4*, in somatic embryogenesis (Shimada *et al*., 2018). In rice, overexpression of the *AtRKD4* homologue *OsRKD3* promotes somatic embryo formation in the Indonesian black rice landrace Cempo Ireng, which is resistant to somatic embryogenesis (Purwestri *et al*., 2023). Although the precise relationship between *RKD* genes and parthenogenesis remains unclear, RKD proteins, which aid in embryogenesis and germ cell differentiation, are highly conserved across diverse plant species (Chardin *et al*., 2014; Koi *et al*., 2016; Koszegi *et al*., 2011; Rovekamp *et al*., 2016). Thus, combining MiMe with ectopic expression of *OsRKD3* in the ovule represents a promising strategy for synthetic apomixis in rice. Notably, in MiMe-combined transgenic rice plants, egg cell-specific expression of dandelion-originated *PARTHENOGENESIS* (*PAR*) and *OsWUSCHEL* (*OsWUS*) produced clonal diploid offspring at rates reaching 62.5% and 21.7%, respectively (Huang *et al*., 2024; Song *et al*., 2024a).

Conclusions and future perspectives

Hybrid seeds derive from crosses between two genetically distinct inbred parental lines. Rice is strictly self-pollinated; thus, male-sterile lines have been used as maternal parents for hybrid seed purity. Various types of male-sterile lines, including cytosolic, environmentally sensitive and biotechnology-based lines, have been employed for hybrid seed production.

To maximize hybrid vigour, breeders typically select parental lines with broad genetic divergence (Awad-Allah *et al*., 2022). However, germplasm resources for CMS-based hybrid breeding remain limited. For example, in China, CMS lines can be generated from only 1% of rice germplasms, and in Southeast Asia, CMS-restorer genes are found in only 5% of *indica* rice germplasms, restricting optimal parental line combinations and

high hybrid vigour (Deng *et al*., 2013). Genetic engineering has enabled the creation of male-sterile and restorer lines with diverse genetic backgrounds through the expression of mitochondria-targeted CMS-associated and Rf genes (Jiang *et al*., 2022b; Kazama and Toriyama, 2014). CRISPR/Cas9 technology efficiently edits nuclear genomes, facilitating Rf gene mutation (Jiang *et al*., 2022b); nonetheless, CRISPR/Cas9-based editing of CMS-associated genes in the mitochondrial genome remains challenging owing to inefficient gRNA targeting (Yin *et al*., 2022b). *Arabidopsis* mesophyll cells contains 300–600 mitochondria per cell on average (Preuten *et al*., 2010), and the rice mitochondrial genome spans approximately 490 kb and may include additional circular plasmid-like DNA (Kanazawa *et al*., 1998; Notsu *et al*., 2002). Therefore, editing most CMS-associated genes within cells is critical for achieving a sterile-to-fertile phenotype conversion. In recent years, advances have enabled the knockout of CMS-associated genes in rice using mitochondria-targeted transcription activator–like effector nucleases (mitoTALEN) (Forner *et al*., 2022; Kazama *et al*., 2019; Omukai *et al*., 2021; Takatsuka *et al*., 2022). Biotechnological advancements have facilitated the creation of CMS and *Rf* lines with broader genetic diversity. However, the fertility restoration mechanism for CMS lines remains complex. Molecular markers and a quantitative trait loci (QTL) analysis identified QTLs on chromosomes 2, 3, 4, 5, 7, 11 and 12 in various WA-CMS-restorer lines, along with two major genes, *Rf3* and *Rf4* (Bazrkar *et al*., 2008). The minor *Rf* genes *Rf18(t)*, *Rf19(t)* and *Rf20(t)* have also been identified (Liu *et al*., 2023b; Xu *et al*., 2023b; Zhang *et al*., 2022a). As *Rf* gene identification is incomplete, gene editing technology has limitations in increasing CMS germplasm resources. Moreover, hybrid seed production with CMS lines is labor-intensive, owing to the separate maintenance of CMS lines for three-line hybrid seed production.

As EGMS is caused by null or point mutations in nuclear genes, EGMS lines can be generated using CRISPR/Cas9 for hybrid seed production (Barman *et al*., 2019). The availability of EGMS lines with diverse genetic backgrounds optimizes parental combinations for superior hybrid seed production (Peng *et al*., 2023b). However, controlling environmental factors that trigger fertility and sterility transitions is challenging. Climate change may affect EGMS-based hybrid seed purity through incomplete sterility arising under unintended conditions (Chen *et al*., 2023). Furthermore, infertility may fluctuate depending on variety, causing instability in sterility-inducing conditions (Chen *et al*., 2023). For instance, the CSIT of *tms5* in AnnongS-1 is approximately 26°C; however, some *tms5*-based TGMS lines have CSIT as high as 32°C, limiting two-line hybrid rice development (Peng *et al*., 2023a). Studying *CSIT1* and *CSIT2*, Peng *et al*. (2024) suggested that CSIT in *tms5*-based TGMS lines can be controlled by regulating the genes' expression levels. Therefore, understanding the EGMS control mechanism and conducting related research is essential for advancing hybrid seed production using EGMS, as it depends on genetic background and environmental factors. Although biotechnology-based male-sterile lines are stable and easy to maintain, seed sorting equipment is required to select male-sterile lines effectively. Despite these challenges, current male-sterile mutations are effectively used for hybrid seed production, and biotechnology enables the rapid generation of male-sterile lines with diverse genetic backgrounds.

Apomixis, the asexual reproductive mechanism of plants, represents an innovative alternative for hybrid seed production. The combination of MiMe and parthenogenesis holds considerable promise; however, the continuous duplication of genetic material in non-parthenogenetic seeds as well as the fertility of hybrid seeds with apomixis, requires refinement for applying apomixis to crops. Despite its low efficiency, haploid induction avoids transgene introduction, using only knockouts. Technological innovations, such as knock-in using the CRISPR/Cas9 system, will make transgene-free apomixis possible through natural promoter insertion in parthenogenesis-inducing genes. Therefore, continued research into male sterility, parthenogenesis, embryogenesis and technological innovations will be crucial for maximizing the utility of male sterility and apomixis techniques for efficient hybrid seed production in crop species.

Acknowledgements

This work was supported by the grant received from the New Breeding Technologies Development Program (RS-2024-00322278 to SM), the Rural Development Administration of the Republic of Korea and a grant from the National Research Foundation of Korea (RS-2021-NR060084 to JKH and RS-2020-NR048152 to SM).

Conflict of interest

The authors declare that they have no conflict of interests.

Author contributions

S.M. and Y.S.L. wrote the article. J.G.M. and K.H.J. finalized the manuscript.

Data availability statement

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

References

Akagi, H., Nakamura, A., Yokozeki-Misono, Y., Inagaki, A., Takahashi, H., Mori, K. and Fujimura, T. (2004) Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. *Theor. Appl. Genet.* **108**, 1449–1457.

Akagi, H., Sakamoto, M., Shinjyo, C., Shimada, H. and Fujimura, T. (1994) A unique sequence located downstream from the rice mitochondrial atp6 may cause male sterility. *Curr. Genet.* **25**, 52–58.

Araki, S., Le, N.T., Koizumi, K., Villar-Briones, A., Nonomura, K.I., Endo, M., Inoue, H. *et al*. (2020) miR2118-dependent U-rich phasiRNA production in rice anther wall development. *Nat. Commun.* **11**, 3115.

Awad-Allah, M.M.A., Shafie, W.W.M., Alsubeie, M.S., Alatawi, A., Safhi, F.A., SM, A.L., Albalawi, D.A. *et al*. (2022) Utilization of genetic resources, genetic diversity and genetic variability for selecting new restorer lines of rice (*Oryza sativa* L.). *Genes* **13**(12), 2227.

Barman, H.N., Sheng, Z., Fiaz, S., Zhong, M., Wu, Y., Cai, Y., Wang, W. *et al*. (2019) Generation of a new thermo-sensitive genic male sterile rice line by targeted mutagenesis of TMS5 gene through CRISPR/Cas9 system. *BMC Plant Biol.* **19**, 109.

Bazrkar, L., Ali, A.J., Babaeian, N.A., Ebadi, A.A., Allahgholipour, M., Kazemitabar, K. and Nematzadeh, G. (2008) Tagging of four fertility restorer loci for wild abortive–cytoplasmic male sterility system in rice (*Oryza sativa* L.) using microsatellite markers. *Euphytica* **164**, 669–677.

Chang, Z., Chen, Z., Wang, N., Xie, G., Lu, J., Yan, W., Zhou, J. *et al*. (2016) Construction of a male sterility system for hybrid rice breeding and seed production using a nuclear male sterility gene. *Proc. Natl. Acad. Sci. USA* **113**, 14145–14150.

Chardin, C., Girin, T., Roudier, F., Meyer, C. and Krapp, A. (2014) The plant RWP-RK transcription factors: key regulators of nitrogen responses and of gametophyte development. *J. Exp. Bot.* **65**, 5577–5587.

Chen, H., Zhang, Z., Ni, E., Lin, J., Peng, G., Huang, J., Zhu, L. *et al*. (2020) HMS1 interacts with HMS1I to regulate very-long-chain fatty acid biosynthesis and the humidity-sensitive genic male sterility in rice (*Oryza sativa*). *New Phytol.* **225**, 2077–2093.

Chen, R., Zhao, X., Shao, Z., Wei, Z., Wang, Y., Zhu, L., Zhao, J. *et al*. (2007) Rice UDP-glucose pyrophosphorylase1 is essential for pollen callose deposition and its cosuppression results in a new type of thermosensitive genic male sterility. *Plant Cell* **19**, 847–861.

Chen, Z., Wu, J., Deng, X.W. and Tang, X. (2023) Establishment and advances of third-generation hybrid rice technology: a review. *Rice* **16**, 56.

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

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

Cui, Y., Li, R., Li, G., Zhang, F., Zhu, T., Zhang, Q., Ali, J. *et al*. (2020) Hybrid breeding of rice via genomic selection. *Plant Biotechnol. J.* **18**, 57–67.

Den Camp, R.H.M.O., Van Dijk, P.J. and Gallard, A. (2017) *Method for the production of haploid and subsequent doubled haploid plants*. In: *WO2017200386A1*. International patent.

Deng, X., Wang, H., Tang, X., Zhou, J., Chen, H., He, G., Chen, L. *et al*. (2013) Hybrid rice breeding welcomes a new era of molecular crop design. *Sci. Sin. Vitae.* **43**(10), 864–868.

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

Ding, J., Lu, Q., Ouyang, Y., Mao, H., Zhang, P., Yao, J., Xu, C. *et al*. (2012a) A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. *Proc. Natl. Acad. Sci. USA* **109**, 2654–2659.

Ding, J., Shen, J., Mao, H., Xie, W., Li, X. and Zhang, Q. (2012b) RNA-directed DNA methylation is involved in regulating photoperiod-sensitive male sterility in rice. *Mol. Plant* **5**, 1210–1216.

Fan, Y., Yang, J., Mathioni, S.M., Yu, J., Shen, J., Yang, X., Wang, L. *et al*. (2016) PMS1T, producing phased small-interfering RNAs, regulates photoperiod-sensitive male sterility in rice. *Proc. Natl. Acad. Sci. USA* **113**, 15144–15149.

Forner, J., Kleinschmidt, D., Meyer, E.H., Fischer, A., Morbitzer, R., Lahaye, T., Schottler, M.A. *et al*. (2022) Targeted introduction of heritable point mutations into the plant mitochondrial genome. *Nat. Plants* **8**, 245–256.

Fu, J., Zhang, Y., Yan, T., Li, Y., Jiang, N., Zhou, Y., Zhou, Q. *et al*. (2022) Transcriptome profiling of two super hybrid rice provides insights into the genetic basis of heterosis. *BMC Plant Biol.* **22**, 314.

Fujii, S., Kazama, T., Yamada, M. and Toriyama, K. (2010) Discovery of global genomic re-organization based on comparison of two newly sequenced rice mitochondrial genomes with cytoplasmic male sterility-related genes. *BMC Genomics* **11**, 209.

Gilles, L.M., Calhau, A.R.M., La Padula, V., Jacquier, N.M.A., Lionnet, C., Martinant, J.P., Rogowsky, P.M. *et al*. (2021) Lipid anchoring and electrostatic interactions target NOT-LIKE-DAD to pollen endo-plasma membrane. *J. Cell Biol.* **220**, e202010077.

Gilles, L.M., Khaled, A., Laffaire, J.B., Chaignon, S., Gendrot, G., Laplaige, J., Berges, H. *et al*. (2017) Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize. *EMBO J.* **36**, 707–717.

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

Gu, Z., Zhu, Z., Li, Z., Zhan, Q., Feng, Q., Zhou, C., Zhao, Q. *et al*. (2021) Cytoplasmic and nuclear genome variations of rice hybrids and their parents inform the trajectory and strategy of hybrid rice breeding. *Mol. Plant* **14**, 2056–2071.

Han, Y., Jiang, S.Z., Zhong, X., Chen, X., Ma, C.K., Yang, Y.M., Mao, Y.C. *et al*. (2023) Low temperature compensates for defective tapetum initiation to restore the fertility of the novel TGMS line ostms15. *Plant Biotechnol. J.* **21**, 1659–1670.

He, W., Chen, C., Adedze, Y.M.N., Dong, X., Xi, K., Sun, Y., Dang, T. *et al*. (2020) Multicentric origin and diversification of atp6-orf79-like structures reveal mitochondrial gene flows in *Oryza rufipogon* and *Oryza sativa*. *Evol. Appl.* **13**, 2284–2299.

Hu, J., Wang, K., Huang, W., Liu, G., Gao, Y., Wang, J., Huang, Q. *et al*. (2012) The rice pentatricopeptide repeat protein RF5 restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein GRP162. *Plant Cell* **24**, 109–122.

Huang, J.Z., E, Z.G., Zhang, H.L. and Shu, Q.Y. (2014) Workable male sterility systems for hybrid rice: Genetics, biochemistry, molecular biology, and utilization. *Rice* **7**, 13.

Huang, W., Yu, C., Hu, J., Wang, L., Dan, Z., Zhou, W., He, C. *et al*. (2015) Pentatricopeptide-repeat family protein RF6 functions with hexokinase 6 to rescue rice cytoplasmic male sterility. *Proc. Natl. Acad. Sci. USA* **112**, 14984–14989.

Huang, Y., Meng, X., Rao, Y., Xie, Y., Sun, T., Chen, W., Wei, X. *et al*. (2024) OsWUS-driven synthetic apomixis in hybrid rice. *Plant Commun* **6**, 101136.

Igarashi, K., Kazama, T., Motomura, K. and Toriyama, K. (2013) Whole genomic sequencing of RT98 mitochondria derived from *Oryza rufipogon* and northern blot analysis to uncover a cytoplasmic male sterility-associated gene. *Plant Cell Physiol.* **54**, 237–243.

Igarashi, K., Kazama, T. and Toriyama, K. (2016) A gene encoding pentatricopeptide repeat protein partially restores fertility in RT98-type cytoplasmic male-sterile rice. *Plant Cell Physiol.* **57**, 2187–2193.

Itabashi, E., Iwata, N., Fujii, S., Kazama, T. and Toriyama, K. (2011) The fertility restorer gene, Rf2, for Lead Rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. *Plant J.* **65**, 359–367.

Jang, J.H., Noh, G., Seo, H.S., Jung, K.H., Kim, Y.J. and Lee, O.R. (2023) Loss of function of pollen-expressed phospholipase OsMATL2 triggers haploid induction in *japonica* rice. *Plant Physiol.* **193**, 1749–1752.

Jiang, C., Sun, J., Li, R., Yan, S., Chen, W., Guo, L., Qin, G. *et al*. (2022a) A reactive oxygen species burst causes haploid induction in maize. *Mol. Plant* **15**, 943–955.

Jiang, H., Lu, Q., Qiu, S., Yu, H., Wang, Z., Yu, Z., Lu, Y. *et al*. (2022b) Fujian cytoplasmic male sterility and the fertility restorer gene OsRf19 provide a promising breeding system for hybrid rice. *Proc. Natl. Acad. Sci. USA* **119**, e2208759119.

Jiang, P., Lian, B., Liu, C., Fu, Z., Shen, Y., Cheng, Z. and Qi, Y. (2020) 21-nt phasiRNAs direct target mRNA cleavage in rice male germ cells. *Nat. Commun.* **11**, 5191.

Johnson, C., Kasprzewska, A., Tennessen, K., Fernandes, J., Nan, G.L., Walbot, V., Sundaresan, V. *et al*. (2009) Clusters and superclusters of phased small RNAs in the developing inflorescence of rice. *Genome Res.* **19**, 1429–1440.

Kalinowska, K., Chamas, S., Unkel, K., Demidov, D., Lermontova, I., Dresselhaus, T., Kumlehn, J. *et al*. (2019) State-of-the-art and novel developments of in vivo haploid technologies. *Theor. Appl. Genet.* **132**, 593–605.

Kanazawa, A., Tsutsumi, N. and Hirai, A. (1998) Differential changes in copy numbers of rice mitochondrial plasmid-like DNAs and main mitochondrial genomic DNAs that depend on temperature. *Curr. Genet.* **33**, 437–444.

Kazama, T., Itabashi, E., Fujii, S., Nakamura, T. and Toriyama, K. (2016) Mitochondrial ORF79 levels determine pollen abortion in cytoplasmic male sterile rice. *Plant J.* **85**, 707–716.

Kazama, T., Nakamura, T., Watanabe, M., Sugita, M. and Toriyama, K. (2008) Suppression mechanism of mitochondrial ORF79 accumulation by Rf1 protein in BT-type cytoplasmic male sterile rice. *Plant J.* **55**, 619–628.

Kazama, T., Okuno, M., Watari, Y., Yanase, S., Koizuka, C., Tsuruta, Y., Sugaya, H. *et al*. (2019) Curing cytoplasmic male sterility via TALEN-mediated mitochondrial genome editing. *Nat. Plants* **5**, 722–730.

Kazama, T. and Toriyama, K. (2014) A fertility restorer gene, Rf4, widely used for hybrid rice breeding encodes a pentatricopeptide repeat protein. *Rice (N. Y.)* **7**, 28.

Kelliher, T., Starr, D., Richbourg, L., Chintamanani, S., Delzer, B., Nuccio, M.L., Green, J. *et al*. (2017) MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. *Nature* **542**, 105–109.

Kelliher, T., Starr, D., Wang, W., McCuiston, J., Zhong, H., Nuccio, M.L. and Martin, B. (2016) Maternal haploids are preferentially induced by CENH3-tailswap transgenic complementation in Maize. *Front. Plant Sci.* **7**, 414.

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

Koi, S., Hisanaga, T., Sato, K., Shimamura, M., Yamato, K.T., Ishizaki, K., Kohchi, T. *et al*. (2016) An evolutionarily conserved plant RKD factor controls germ cell differentiation. *Curr. Biol.* **26**, 1775–1781.

Komiya, R., Ohyanagi, H., Niihama, M., Watanabe, T., Nakano, M., Kurata, N. and Nonomura, K. (2014) Rice germline-specific Argonaute MEL1 protein binds to phasiRNAs generated from more than 700 lincRNAs. *Plant J.* **78**, 385–397.

Koszegi, D., Johnston, A.J., Rutten, T., Czihal, A., Altschmied, L., Kumlehn, J., Wust, S.E. *et al*. (2011) Members of the RKD transcription factor family induce an egg cell-like gene expression program. *Plant J.* **67**(2), 280–291.

Lee, Y.S., Maple, R., Durr, J., Dawson, A., Tamim, S., Del Genio, C., Papareddy, R. *et al*. (2021) A transposon surveillance mechanism that safeguards plant male fertility during stress. *Nat. Plants* **7**, 34–41.

Li, M., Chen, L., Tang, D., Liao, X., Kong, X., Li, B., You, J. *et al*. (2020) Discovery of four novel ORFs responsible for cytoplasmic male sterility (CMS) in cotton (*Gossypium hirsutum* L.) through comparative analysis of the mitochondrial genomes of four isoplasmic lines. *Agronomy* **10**, 765.

Li, W., Guo, X., Wu, W., Yu, W., Li, S., Luo, D., Wang, T. *et al*. (2021a) Construction of a novel female sterility system for hybrid Rice. *Front. Plant Sci.* **12**, 815401.

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

Li, Y., Lin, Z., Yue, Y., Zhao, H., Fei, X., E, L., Liu, C. *et al*. (2021b) Loss-of-function alleles of ZmPLD3 cause haploid induction in maize. *Nat. Plants.* **7**, 1579–1588.

Liao, P.F., Ouyang, J.X., Zhang, J.J., Yang, L., Wang, X., Peng, X.J., Wang, D. *et al*. (2019) OsDCL3b affects grain yield and quality in rice. *Plant Mol. Biol.* **99**, 193–204.

Liu, C., Li, X., Meng, D., Zhong, Y., Chen, C., Dong, X., Xu, X. *et al*. (2017a) A 4-bp insertion at ZmPLA1 encoding a putative phospholipase A generates haploid induction in Maize. *Mol. Plant* **10**, 520–522.

Liu, D., Shi, J., Liang, W. and Zhang, D. (2023a) Molecular mechanisms underlying plant environment-sensitive genic male sterility and fertility restoration. *Seed Biology* **2**.

Liu, Y., Xu, Z., Du, Y., Huang, B., Li, M., Zhang, L., Zhao, X. *et al*. (2023b) Mapping of Rf20(t), a minor fertility restorer gene for rice wild abortive cytoplasmic male sterility in the maintainer line 'Zhenshan97B'. *Theor. Appl. Genet.* **136**, 248.

Liu, Z., Lin, S., Shi, J., Yu, J., Zhu, L., Yang, X., Zhang, D. *et al*. (2017b) Rice No Pollen 1 (NP1) is required for anther cuticle formation and pollen exine patterning. *Plant J.* **91**, 263–277.

Luan, J., Liu, T., Luo, W., Liu, W., Peng, M., Li, W., Dai, X. *et al*. (2013) Mitochondrial DNA genetic polymorphism in thirteen rice cytoplasmic male sterile lines. *Plant Cell Rep.* **32**, 545–554.

Luo, D., Xu, H., Liu, Z., Guo, J., Li, H., Chen, L., Fang, C. *et al*. (2013) A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. *Nat. Genet.* **45**(5), 573–577.

Lv, J., Yu, K., Wei, J., Gui, H., Liu, C., Liang, D., Wang, Y. *et al*. (2020) Generation of paternal haploids in wheat by genome editing of the centromeric histone CENH3. *Nat. Biotechnol.* **38**, 1397–1401.

Mamun, E.A., Alfred, S., Cantrill, L.C., Overall, R.L. and Sutton, B.G. (2006) Effects of chilling on male gametophyte development in rice. *Cell Biol. Int.* **30**, 583–591.

Margis, R., Fusaro, A.F., Smith, N.A., Curtin, S.J., Watson, J.M., Finnegan, E.J. and Waterhouse, P.M. (2006) The evolution and diversification of Dicers in plants. *FEBS Lett.* **580**, 2442–2450.

Marimuthu, M.P., Jolivet, S., Ravi, M., Pereira, L., Davda, J.N., Cromer, L., Wang, L. *et al*. (2011) Synthetic clonal reproduction through seeds. *Science* **331**(6019), 876.

Matsuda, T., Sugita, M. and Ichinose, M. (2020) The L motifs of two moss pentatricopeptide repeat proteins are involved in RNA editing but predominantly not in RNA recognition. *PLoS One* **15**, e0232366.

Melonek, J., Stone, J.D. and Small, I. (2016) Evolutionary plasticity of restorer-of-fertility-like proteins in rice. *Sci. Rep.* **6**, 35152.

Mieulet, D., Jolivet, S., Rivard, M., Cromer, L., Vernet, A., Mayonove, P., Pereira, L. *et al*. (2016) Turning rice meiosis into mitosis. *Cell Res.* **26**(11), 1242–1254.

Moon, S. and Jung, K.H. (2020) First Steps in the Successful Fertilization of Rice and Arabidopsis: Pollen Longevity, Adhesion and Hydration. *Plants* **9**(8), 956.

Ni, E., Deng, L., Chen, H., Lin, J., Ruan, J., Liu, Z., Zhuang, C. *et al*. (2021) OsCER1 regulates humidity-sensitive genic male sterility through very-long-chain (VLC) alkane metabolism of tryphine in rice. *Funct. Plant Biol.* **48**, 461–468.

Nonomura, K., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H. and Kurata, N. (2007) A germ cell specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. *Plant Cell* **19**, 2583–2594.

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

Notsu, Y., Masood, S., Nishikawa, T., Kubo, N., Akiduki, G., Nakazono, M., Hirai, A. *et al*. (2002) The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: frequent DNA sequence acquisition and loss during the evolution of flowering plants. *Mol. Genet. Genomics* **268**, 434–445.

Okazaki, M., Kazama, T., Murata, H., Motomura, K. and Toriyama, K. (2013) Whole mitochondrial genome sequencing and transcriptional analysis to uncover an RT102-type cytoplasmic male sterility-associated candidate Gene Derived from *Oryza rufipogon*. *Plant Cell Physiol.* **54**, 1560–1568.

Omukai, S., Arimura, S.I., Toriyama, K. and Kazama, T. (2021) Disruption of mitochondrial open reading frame 352 partially restores pollen development in cytoplasmic male sterile rice. *Plant Physiol.* **187**, 236–246.

Peng, G., Liu, M., Luo, Z., Deng, S., Wang, Q., Wang, M., Chen, H. *et al*. (2024) An E3 ubiquitin ligase CSIT2 controls critical sterility-inducing temperature of thermo-sensitive genic male sterile rice. *New Phytol.* **241**, 2059–2074.

Peng, G., Liu, M., Zhu, L., Luo, W., Wang, Q., Wang, M., Chen, H. *et al*. (2023a) The E3 ubiquitin ligase CSIT1 regulates critical sterility-inducing temperature by ribosome-associated quality control to safeguard two-line hybrid breeding in rice. *Mol. Plant* **16**, 1695–1709.

Peng, G., Liu, Z., Zhuang, C. and Zhou, H. (2023b) Environment-sensitive genic male sterility in rice and other plants. *Plant Cell Environ.* **46**, 1120–1142.

Preuten, T., Cincu, E., Fuchs, J., Zoschke, R., Liere, K. and Borner, T. (2010) Fewer genes than organelles: extremely low and variable gene copy numbers in mitochondria of somatic plant cells. *Plant J.* **64**, 948–959.

Purwestri, Y.A., Lee, Y.S., Meehan, C., Mose, W., Susanto, F.A., Wijayanti, P., Fauzia, A.N. *et al*. (2023) RWP-RK Domain 3 (OsRKD3) induces somatic embryogenesis in black rice. *BMC Plant Biol.* **23**, 202.

Qi, X., Zhang, C., Zhu, J., Liu, C., Huang, C., Li, X. and Xie, C. (2020) Genome editing enables next-generation hybrid seed production technology. *Mol. Plant* **13**, 1262–1269.

Rovekamp, M., Bowman, J.L. and Grossniklaus, U. (2016) Marchantia MpRKD regulates the gametophyte-sporophyte transition by keeping egg cells quiescent in the absence of fertilization. *Curr. Biol.* **26**, 1782–1789.

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

Shi, C., Zhang, J., Wu, B., Jouni, R., Yu, C., Meyers, B.C., Liang, W. *et al*. (2022) Temperature-sensitive male sterility in rice determined by the roles of AGO1d in reproductive phasiRNA biogenesis and function. *New Phytol.* **236**, 1529–1544.

Shi, C., Zou, W., Zhu, Y., Zhang, J., Teng, C., Wei, H., He, H. *et al*. (2024) mRNA cleavage by 21-nucleotide phasiRNAs determines temperature-sensitive male sterility in rice. *Plant Physiol.* **194**, 2354–2371.

Shimada, A., Nagai, T. and Kimura, Y. (1992) Sterility in rice induced by chemical treatment. *Biosci. Biotechnol. Biochem.* **56**, 1619–1622.

Shimada, T., Endo, T., Fujii, H., Nakano, M., Sugiyama, A., Daido, G., Ohta, S. *et al*. (2018) MITE insertion-dependent expression of CitRKD1 with a RWP-RK domain regulates somatic embryogenesis in citrus nucellar tissues. *BMC Plant Biol.* **18**, 166.

Singh, A., Baranwal, V., Shankar, A., Kanwar, P., Ranjan, R., Yadav, S., Pandey, A. *et al*. (2012) Rice phospholipase A superfamily: organization, phylogenetic and expression analysis during abiotic stresses and development. *PLoS One* **7**, e30947.

Song, M., Wang, W., Ji, C., Li, S., Liu, W., Hu, X., Feng, A. *et al*. (2024a) Simultaneous production of high-frequency synthetic apomixis with high fertility and improved agronomic traits in hybrid rice. *Mol. Plant* **17**, 4–7.

Song, S., Li, Y., Qiu, M., Xu, N., Li, B., Zhang, L., Li, L. *et al*. (2024b) Structural variations of a new fertility restorer gene, Rf20, underlie the restoration of wild abortive-type cytoplasmic male sterility in rice. *Mol. Plant* **17**, 1272–1288.

Song, S., Wang, T., Li, Y., Hu, J., Kan, R., Qiu, M., Deng, Y. *et al*. (2021) A novel strategy for creating a new system of third-generation hybrid rice technology using a cytoplasmic sterility gene and a genic male-sterile gene. *Plant Biotechnol. J.* **19**, 251–260.

Song, X., Li, P., Zhai, J., Zhou, M., Ma, L., Liu, B., Jeong, D.H. *et al*. (2012a) Roles of DCL4 and DCL3b in rice phased small RNA biogenesis. *Plant J.* **69**, 462–474.

Song, X., Wang, D., Ma, L., Chen, Z., Li, P., Cui, X., Liu, C. *et al*. (2012b) Rice RNA-dependent RNA polymerase 6 acts in small RNA biogenesis and spikelet development. *Plant J.* **71**, 378–389.

Sun, G., Geng, S., Zhang, H., Jia, M., Wang, Z., Deng, Z., Tao, S. *et al*. (2022) Matrilineal empowers wheat pollen with haploid induction potency by triggering postmitosis reactive oxygen species activity. *New Phytol.* **233**(6), 2405–2414.

Takatsuka, A., Kazama, T., Arimura, S.I. and Toriyama, K. (2022) TALEN-mediated depletion of the mitochondrial gene orf312 proves that it is a Tadukan-type cytoplasmic male sterility-causative gene in rice. *Plant J.* **110**, 994–1004.

Takatsuka, A., Kazama, T. and Toriyama, K. (2021) Cytoplasmic male sterility-associated mitochondrial gene orf312 derived from rice (*Oryza sativa* L.) Cultivar Tadukan. *Rice* **14**, 46.

Tamotsu, H., Koizumi, K., Briones, A.V. and Komiya, R. (2023) Spatial distribution of three ARGONAUTEs regulates the anther phasiRNA pathway. *Nat. Commun.* **14**, 3333.

Tanaka, Y., Tsuda, M., Yasumoto, K., Yamagishi, H. and Terachi, T. (2012) A complete mitochondrial genome sequence of Ogura-type male-sterile cytoplasm and its comparative analysis with that of normal cytoplasm in radish (*Raphanus sativus* L.). *BMC Genomics* **13**, 352.

Tang, H., Luo, D., Zhou, D., Zhang, Q., Tian, D., Zheng, X., Chen, L. *et al*. (2014) The rice restorer Rf4 for wild-abortive cytoplasmic male sterility encodes a mitochondrial-localized PPR protein that functions in reduction of WA352 transcripts. *Mol. Plant* **7**, 1497–1500.

Tang, H., Zheng, X., Li, C., Xie, X., Chen, Y., Chen, L., Zhao, X. *et al*. (2017) Multi-step formation, evolution, and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. *Cell Res.* **27**, 130–146.

Teng, C., Zhang, H., Hammond, R., Huang, K., Meyers, B.C. and Walbot, V. (2020) Dicer-like 5 deficiency confers temperature-sensitive male sterility in maize. *Nat. Commun.* **11**, 2912.

Toriyama, K. (2021) Molecular basis of cytoplasmic male sterility and fertility restoration in rice. *Plant Biotechnol.* **38**, 285–295.

Tucker, M.R. and Koltunow, A.M.G. (2009) Sexual and asexual (apomictic) seed development in flowering plants: molecular, morphological and evolutionary relationships. *Funct. Plant Biol.* **36**, 490–504.

Underwood, C.J. and Mercier, R. (2022) Engineering Apomixis: Clonal Seeds Approaching the Fields. *Annu. Rev. Plant Biol.* **73**, 201–225.

Vernet, A., Meynard, D., Lian, Q., Mieulet, D., Gibert, O., Bissah, M., Rivallan, R. *et al*. (2022) High-frequency synthetic apomixis in hybrid rice. *Nat. Commun.* **13**, 7963.

Virmani, S.S. and Ilyas-Ahmed, M. (2001) Environment-sensitive genic male sterility (EGMS) in crops. *Adv. Agron*. Vol. **72**, 139–195.

Waki, T., Hiki, T., Watanabe, R., Hashimoto, T. and Nakajima, K. (2011) The Arabidopsis RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Curr. Biol.* **21**, 1277–1281.

Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., Wu, M. *et al*. (2019) Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nat. Biotechnol.* **37**, 283–286.

Wang, D., Li, J., Sun, L., Hu, Y., Yu, J., Wang, C., Zhang, F. *et al*. (2021a) Two rice MYB transcription factors maintain male fertility in response to photoperiod by modulating sugar partitioning. *New Phytol.* **231**, 1612–1629.

Wang, J., Ying, S., Long, W., Luo, L., Qian, M., Chen, W., Luo, L. *et al*. (2024) Integrated transcriptomic and metabolomic analysis provides insight into the pollen development of CMS-D1 rice. *BMC Plant Biol.* **24**, 535.

Wang, K., Gao, F., Ji, Y., Liu, Y., Dan, Z., Yang, P., Zhu, Y. *et al*. (2013) ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice. *New Phytol.* **198**, 408–418.

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

Wang, Q., Bin, B. and Wang, H. (2023) Dynamic diffusion of hybrid rice varieties and the effect on rice production: evidence from China. *Front. Sustain. Food Syst.* **7**, 1071234.

Wang, X., Xu, Y., Zhang, S., Cao, L., Huang, Y., Cheng, J., Wu, G. *et al*. (2017) Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. *Nat. Genet.* **49**(5), 765–772.

Wang, Z., Zou, Y., Li, X., Zhang, Q., Chen, L., Wu, H., Su, D. *et al*. (2006) Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. *Plant Cell* **18**, 676–687.

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

Wu, J., Qiu, S., Wang, M., Xu, C., Deng, X.W. and Tang, X. (2021) Construction of a weight-based seed sorting system for the third-generation hybrid rice. *Rice* **14**, 66.

Wu, L., Jing, X., Zhang, B., Chen, S., Xu, R., Duan, P., Zou, D. *et al*. (2022) A natural allele of OsMS1 responds to temperature changes and confers thermosensitive genic male sterility. *Nat. Commun.* **13**, 2055.

Wu, Y., Fox, T.W., Trimnell, M.R., Wang, L., Xu, R.J., Cigan, A.M., Huffman, G.A. *et al*. (2016) Development of a novel recessive genetic male sterility system for hybrid seed production in maize and other cross-pollinating crops. *Plant Biotechnol. J.* **14**, 1046–1054.

Xia, R., Chen, C., Pokhrel, S., Ma, W., Huang, K., Patel, P., Wang, F. *et al*. (2019) 24-nt reproductive phasiRNAs are broadly present in angiosperms. *Nat. Commun.* **10**, 627.

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

Xie, H., Peng, X., Qian, M., Cai, Y., Ding, X., Chen, Q., Cai, Q. *et al*. (2018) The chimeric mitochondrial gene orf182 causes non-pollen-type abortion in Dongxiang cytoplasmic male-sterile rice. *Plant J.* **95**, 715–726.

Xu, Y., Yu, D., Chen, J. and Duan, M. (2023a) A review of rice male sterility types and their sterility mechanisms. *Heliyon* **9**, e18204.

Xu, Z., Du, Y., Li, X., Wang, R., Chen, Z.a., Zhao, X., Liu, Q. *et al*. (2023b) Identification and fine mapping of a fertility restorer gene for wild abortive cytoplasmic male sterility in the elite *indica* rice non-restorer line 9311. *Crop J.* **11**, 887–894.

Xue, Z., Xu, X., Zhou, Y., Wang, X., Zhang, Y., Liu, D., Zhao, B. *et al*. (2018) Deficiency of a triterpene pathway results in humidity-sensitive genic male sterility in rice. *Nat. Commun.* **9**, 604.

Yan, Z., Chen, F., Mishra, A.K. and Sha, W. (2022) An Economic Assessment of Adoption of Hybrid Rice: Micro-Level Evidence from Southern China. *Front. Sustain. Food Syst.* **6**, 1066657.

Yang, Z., Liu, L., Sun, L., Yu, P., Zhang, P., Abbas, A., Xiang, X. *et al*. (2019) OsMS1 functions as a transcriptional activator to regulate programmed tapetum development and pollen exine formation in rice. *Plant Mol. Biol.* **99**, 175–191.

Yao, L., Zhang, Y., Liu, C., Liu, Y., Wang, Y., Liang, D., Liu, J. *et al*. (2018) OsMATL mutation induces haploid seed formation in *indica* rice. *Nat. Plants.* **4**(8), 530–533.

Yin, P.P., Tang, L.P., Zhang, X.S. and Su, Y.H. (2022a) Options for engineering apomixis in plants. *Front. Plant Sci.* **13**, 864987.

Yin, T., Luo, J., Huang, D. and Li, H. (2022b) Current progress of mitochondrial genome editing by CRISPR. *Front. Physiol.* **13**, 883459.

Yu, B., Liu, L. and Wang, T. (2019) Deficiency of very long chain alkanes biosynthesis causes humidity-sensitive male sterility via affecting pollen adhesion and hydration in rice. *Plant Cell Environ.* **42**, 3340–3354.

Yu, J., Han, J., Kim, Y.J., Song, M., Yang, Z., He, Y., Fu, R. *et al*. (2017) Two rice receptor-like kinases maintain male fertility under changing temperatures. *Proc. Natl. Acad. Sci. USA* **114**, 12327–12332.

Yuan, L. (2019) Strategic vision for hybrid rice. *Crop Breed. Genet. Genomics.* **1**, e190020.

Zhai, J., Zhang, H., Arikit, S., Huang, K., Nan, G.L., Walbot, V. and Meyers, B.C. (2015) Spatiotemporally dynamic, cell-type-dependent premeiotic and meiotic phasiRNAs in maize anthers. *Proc. Natl. Acad. Sci. USA* **112**, 3146–3151.

Zhai, L., Sun, W., Zhang, K., Jia, H., Liu, L., Liu, Z., Teng, F. *et al*. (2014) Identification and characterization of Argonaute gene family and meiosis-enriched Argonaute during sporogenesis in maize. *J. Integr. Plant Biol.* **56**, 1042–1052.

Zhang, H., Li, X., Xu, Z., Wan, Z., Wang, R., Zhao, X., Tu, G. *et al*. (2022a) Precise genetic mapping of Rf18(t), a new fertility restorer gene from 'Nipponbare' for wild abortive cytoplasmic male sterility in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **135**, 2687–2698.

Zhang, H., Liang, W., Yang, X., Luo, X., Jiang, N., Ma, H. and Zhang, D. (2010) Carbon starved anther encodes a MYB domain protein that regulates sugar partitioning required for rice pollen development. *Plant Cell* **22**, 672–689.

Zhang, H., Xu, C., He, Y., Zong, J., Yang, X., Si, H., Sun, Z. *et al*. (2013) Mutation in CSA creates a new photoperiod-sensitive genic male sterile line applicable for hybrid rice seed production. *Proc. Natl. Acad. Sci. USA* **110**, 76–81.

Zhang, Y.F., Li, Y.L., Zhong, X., Wang, J.J., Zhou, L., Han, Y., Li, D.D. *et al*. (2022b) Mutation of glucose-methanol-choline oxidoreductase leads to thermosensitive genic male sterility in rice and Arabidopsis. *Plant Biotechnol. J.* **20**, 2023–2035.

Zhao, Z., Ding, Z., Huang, J., Meng, H., Zhang, Z., Gou, X., Tang, H. *et al*. (2023) Copy number variation of the restorer Rf4 underlies human selection of three-line hybrid rice breeding. *Nat. Commun.* **14**, 7333.

Zhou, H., Liu, Q., Li, J., Jiang, D., Zhou, L., Wu, P., Lu, S. *et al*. (2012) Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. *Cell Res.* **22**, 649–660.

Zhou, H., Zhou, M., Yang, Y., Li, J., Zhu, L., Jiang, D., Dong, J. *et al*. (2014) RNase Z(S1) processes UbL40 mRNAs and controls thermosensitive genic male sterility in rice. *Nat. Commun.* **5**, 4884.

Zhou, L., Mao, Y.C., Yang, Y.M., Wang, J.J., Zhong, X., Han, Y., Zhang, Y.F. *et al*. (2024) Temperature and light reverse the fertility of rice P/TGMS line ostms19 via reactive oxygen species homeostasis. *Plant Biotechnol. J.* **22**(7), 2020–2032.