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REVIEW ARTICLE

Unveiling thermosensitive genetic male sterility in rice - molecular insights and approaches

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Abstract

The application of male sterility has facilitated the commercialization of heterosis in rice, resulting in an enhanced yield and output of this staple food crop. The application of three-line hybrid rice technology is decreasing due to limited heterosis, lack of good combiner in cytoplasmic male sterile lines, poor reproducibility of hybrid seeds and limited commercial acceptance. Two-line heterosis breeding can overcome these issues. Two-line breeding allows for more diverse germplasm as parents, including any line as female and 97% of germplasm as male. Moreover, two-line breeding can lead to lower production costs. Systematic research can enable the widespread deployment of two-line hybrid rice technology. Identifying the novel genetic, molecular and cellular factors and understanding the regulatory networks regulating male sterility in rice is vital for maximizing heterosis and ensuring global food security. In this review, we have briefly discussed the types of environment-sensitive genetic male sterility systems for two-line hybrid seed production. Furthermore, we have discussed the genetic, molecular and cellular basis for the thermosensitive genetic male sterility (TGMS) mechanism. We have provided insights on molecular and biotechnological interventions such as CRISPR and omics techniques in the TGMS system to address the existing concerns and to overcome the problems related to two-line rice breeding.

Keywords

cellular mechanism; EGMS; molecular mechanism; TGMS; two-line hybrids;

Introduction

Rice is the backbone of sustenance serves as a primary calorie source for both rural and urban populations and constitutes the main dietary staple for over half of the global population (1). Over the 20th century, significant advancements had been made in the scientific foundation of plant breeding. Jones reported heterosis in rice for the first time. In India, K. Ramaiah and Kadam were the first to report heterosis in rice. The genetic basis of heterosis in rice was laid by the father of hybrid rice, Yuan Long Ping in 1960 (2).

The introduction of semi-dwarf varieties in the 1960s enabled India to achieve food self-sufficiency. Historically, the 1970s and 1980s saw significant improvements in grain crop yields around the world. The first is due to the “Green Revolutions”, which saw significant enhancements in wheat and rice yields, largely due to the attribution of semi-dwarf genes. “The Second Green Revolution” is leveraging heterosis through the male sterility technique, contributing to a notable increase of over 20% in grain yield (3). The first high-yielding semi-dwarf rice variety IR8 also called “miracle rice” was developed by Peter Jennings from the cross between DGWG and the vigorous and tall Peta variety (4). The global rice production accounts for 522.18 million metric tonnes during 2023-2024 and India contributes 26% of the global rice production which is about 137.83 million metric tonnes (5). India has witnessed a notable increase in rice production a rise from 34.5 million tonnes in 1960-61 to 135.75 million tonnes in 2022-23. A continuous rise in rice yield has been observed since the fiscal year 1991. Upon reviewing the historical data, it becomes evident that despite efforts spanning the past two decades, annual rice production only saw a marginal 1% increase (1). Environmental changes and biotic stress have hindered the achievement of the targeted high yield. Consequently, crop adaptation necessitates a range of genomic adjustments and scientists are continuously striving to enhance crop production in major crops to fulfill the requirements of the expanding population.

Heterosis in rice was first time studied by Professor Yuan Longping. In 1961, he observed natural male sterile mutants, which gave him the idea to use them for hybridization (4). In 1974, the first three-line commercial rice hybrid Nanyou 2 was released from the cross between n Erjiunan 1 A and IR24 (4). The success of hybrid rice has substantially accelerated the research and investigation of male sterility in rice. Hybrid rice technology consists of two -line and three-line hybrid rice which is developed *via* photo/thermo-sensitive male sterility and cytoplasmic male sterility, respectively (6). Cytoplasmic male sterility is widely used among all the male sterility systems but it is difficult to maintain the A-line, lack of diversity in R lines and the negative effect of sterile cytoplasm limits the use of the CMS system. The two-line system or Environment-sensitive genetic male sterility system (EGMS) is controlled by environmental factors such as Photoperiod and temperature. In India, the photoperiodic differences are negligible or marginal, the ideal EGMS system would be the TGMS system. Several recent articles have examined the crucial genes and networks involved in the TGMS system. Some of them are *tms5* in the AnnongS-1 line and tms_{10} gene in *japonica* cv. 9522 TGMS line (7). Creating and utilizing the TGMS lines encounters challenges from modifier gene complexes that impact the full expression of the TGMS trait (8). Mapping and tagging various TGMS genes with closely associated markers will facilitate reliable identification and selection using Marker-Assisted Selection (MAS). Temperature variations influence male sterility in TGMS lines through genetic and epigenetic modifications. This includes regulation of non-coding RNAs, RNA metabolism and involvement of transcriptional factors (TFs) (9).

Grain yield in rice is mostly determined by the rice inflorescence (10). The Asian cultivated rice *Oryza sativa* is a diploid species having 12 chromosomes (2n = 24) and the inflorescence is called panicle which has a main rachis, spikelets, primary and secondary branches. Rice spikelet has six stamens, the pistil contains two styles, each with a plumose and laterally extended stigma ovary and a single ovary and the fruit is called a caryopsis. Rice is typically referred to be a self-pollinating crop. Anther and pollen development is a complex genetic process that demands a deep knowledge of molecular and cellular regulation of male sterility to enhance the potential for hybrid development, as disruptions can easily impede progress (11). Numerous genomics studies have discovered several genes linked to the TGMS trait and investigated the genetic, molecular, biochemical and epigenetic pathways that control the development of male gamete. In this review, we've explored the latest advancements concerning the genetic components of TGMS and various molecular regulations affecting the TGMS traits. Various types of EGMS systems have been briefly introduced. Moreover, we introduced the male fertility recovery mechanism in TGMS lines and the molecular mechanism of critical sterility-inducing temperature (CSIT) to better understand the adaption of TGMS in two-line hybrid development.

Male sterility

In 1763, Joseph Gottlieb Kolreuter observed the occurrence of male sterility for the first time, he noted that some species and particular hybrids had anther abortion. The inability of a plant to generate viable pollen, anthers or male gametes is known as male sterility. Significant gains in food productivity have been noted since the agricultural exploitation of hybrid crop varieties due to their greater uniformity and hybrid vigour (12). Male sterility is simple to identify as a lot of pollen grains are generated and are amenable to study. Staining methods (carmine, lactophenol or iodine) are used to assay male sterility. Based on the male sterile lines, the first single cross-grain hybrid "HB_1" in Bajra was introduced in India in 1965 which outyielded local open-pollinated varieties (OPVs) with up to a 100% yield advantage (13).

Why is male sterility more prevalent than female sterility : Compared to the ovule and embryo sac, the male saprophyte and gametophyte have less environmental protection.

Male sterility is simple to identify because there are many pollen samples accessible for research.

Male sterility is easily established using staining techniques (carmine, lactophenol or iodine); female sterility necessitates crossing.

While female sterility is not significant for crop breeding, male sterility has the ability to propagate in nature (it may still set seed).

It occurs naturally as a result of cytoplasmic and/or nuclear gene mutations.

Classification of male sterility

A. Phenotypic

1. Structural male sterility (peculiarities in male sex)

2. Sporogenous male sterility (pollen absent but stamens present)

3. Functional male sterility (viable pollen but a barrier stops fertilization)

B. Genotypic

1. Genetic male sterility

2. Cytoplasmic male sterility

3. Cytoplasmic-genetic male sterility

EGMS (Environment-sensitive genetic male sterility)

It is a genetic male sterility (GMS) mechanism whose expression is controlled by fluctuations in environmental factors. In two-line hybrid rice, the fertility transition is regulated by the EGMS (PGMS, TGMS, PTGMS, MGMS and HGMS) systems in response to changes in the environment (14-15). In Arabidopsis and rice, EGMS genes, in conjunction with MYB transcription factors, noncoding RNA such as RNase ZS^1, E_3 ubiquitin ligase, leucine-rich-repeat receptor-like kinase (LRR-RLK) and UDP glucose pyrophosphorylase, have been elucidated (14). EGMS may be crossed with most of the traditional rice types (two-line restorer lines) to create heterotic hybrids. Classification of environmentally sensitive genetic male sterility (Fig 1).

PGMS (Photoperiod-sensitive genetic male sterility)

Professor Shi Ming in the Hubei region of China, identified this particular kind of male sterility in rice in 1973. When the photoperiod was less than 13 hours and 45 minutes, numerous male sterile plants were observed in the late japonica cultivar Nongken 58S which regained fertility when daylight was more (16). PGMS may be classified into two types *viz* short photoperiod sensitive genetic male sterile type (S-PGMS) and long photoperiod sensitive genetic male sterile type (L-PGMS), based on the fertility of rice under various photoperiod circumstances. In L-PGMS, MS occurs in long-day conditions and fertility during the short day, whereas, in S-PGMS MS type occurs during the short day and fertility during the long days (17). Other researchers have found that temperature also affects PGMS in a certain way, a high temperature will make PGMS more sterile (18).

As of right now *pms1, pms2, pms3* and *pms4* have been found as long photoperiod sensitive genetic male sterile genes (Table 1.). These genes are mostly obtained from the *indica* rice Guangzhan 63S and *japonica* rice Nongken 58S. Zhang used RAPD and RFLP markers to transfer the NK58S MS gene to the *indica* PGMS line 32001S. This facilitated the discovery of two male sterile genes *pms1* located on chromosome 7 and *pms2* situated on chromosome 3 (19).

TGMS (Thermo-sensitive genetic male sterility)

TGMS trait is predominantly influenced by temperature, with a critical phase of fertility transition occurring between the production of pollen mother cells (PMC) and meiosis. It was reported first in China (20) then in Japan followed by Philippines, India and Vietnam (21-23). Through introgressive backcrossing of the PGMS line Nongken 58S (NK58S) with an indica background, the successful production of Peiai 64S (PA64S) was achieved, which exhibited TGMS trait (24). The majority of TGMS mutants that have been reported to date, including Annong S-1 from China, Norin PL-12 from Japan, IR 32364 TGMS from the Philippines and several mutants reported from India and Vietnam exhibited sterility at the sensitive stage when temperatures are higher (above 30°C) while fertility was observed at lower temperatures (below 24°C) (12).

The TGMS genes tms_5, tms_{10}, $tms_{9\text{-}1}$, $ostms_{18}$, ugp_1 and $t/pms_{12\text{-}1}$, have now been cloned (Table 4). TMS_5 gene codes for RNase ZS^1 a conserved RNA enzyme, which cleaves and degrades the Ub_{L40} mRNA (three-ubiquitin ribosomal L40 fusion proteins). In the wild type, elevated temperatures trigger the accumulation of excess Ub_{L40} mRNA, which is typically cleaved by RNase ZS^1, encoded by TMS5. This cleavage ensures normal anther development. However, in *tms5* mutants lacking $RNaseZS^1$ function, the absence of cleavage results in the buildup of Ub_{L40} mRNA at high temperatures. This accumulation disrupts pollen development, causing pollen abortion (25).

rTGMS (Reverse thermo-sensitive genetic male sterility)

A rare kind of sterility known as the reverse TGMS type manifests sterility at lower temperatures whereas fertility at higher temperatures, contrasting typical TGMS. Mutant Diaxin 1A and 4A; 26 Zhaizao Chinese mutant cultivars and Indian mutant variety JP-38S have been described as reverse TGMS types (26). JP38S discovered by Ali and Siddiq was a spontaneous mutant that exhibited a reverse TGMS characteristic, acting as fertile at temperatures over 30.5 °C and sterile at temperatures below 24 °C (27). Three loci of rTGMS were identified as *TMS* from 8987 line, *rtms1* from G207S line and *tms6(t)* from G20S line (26–28). These genetic loci were assigned to chromosomes 6, 10 and 10, correspondingly (29).

PTGMS (Photo-thermo sensitive genetic male sterility)

Numerous male sterile lines of *indica* and *japonica* strains

Table 1. Genes for PGMS and its types

PGMS	gene	Chromosome	Source/ parent	Function	Reference
Long-photoperiod sensitive sterility	*pms1*	7	NK58S (Nongken 58S)	Long-chain non-coding RNA	(31)
	pms2	3	NK58S	Unknown	(106)
	pms3	12	32001S	Long-chain non-coding RNA	(107)
	pms4	4	Mian9S	Unknown	(108)
	ptgms2-1	2	Guangzhan 63S	Unknown	(54)
Short-photoperiod sensitive sterility	*rpms1*	8	Yi D1S	Unknown	(109)
	rpms2	9	Yi D1S	Unknown	(109)
	csa	1	csa mutant	MYB transcription factor	(55)

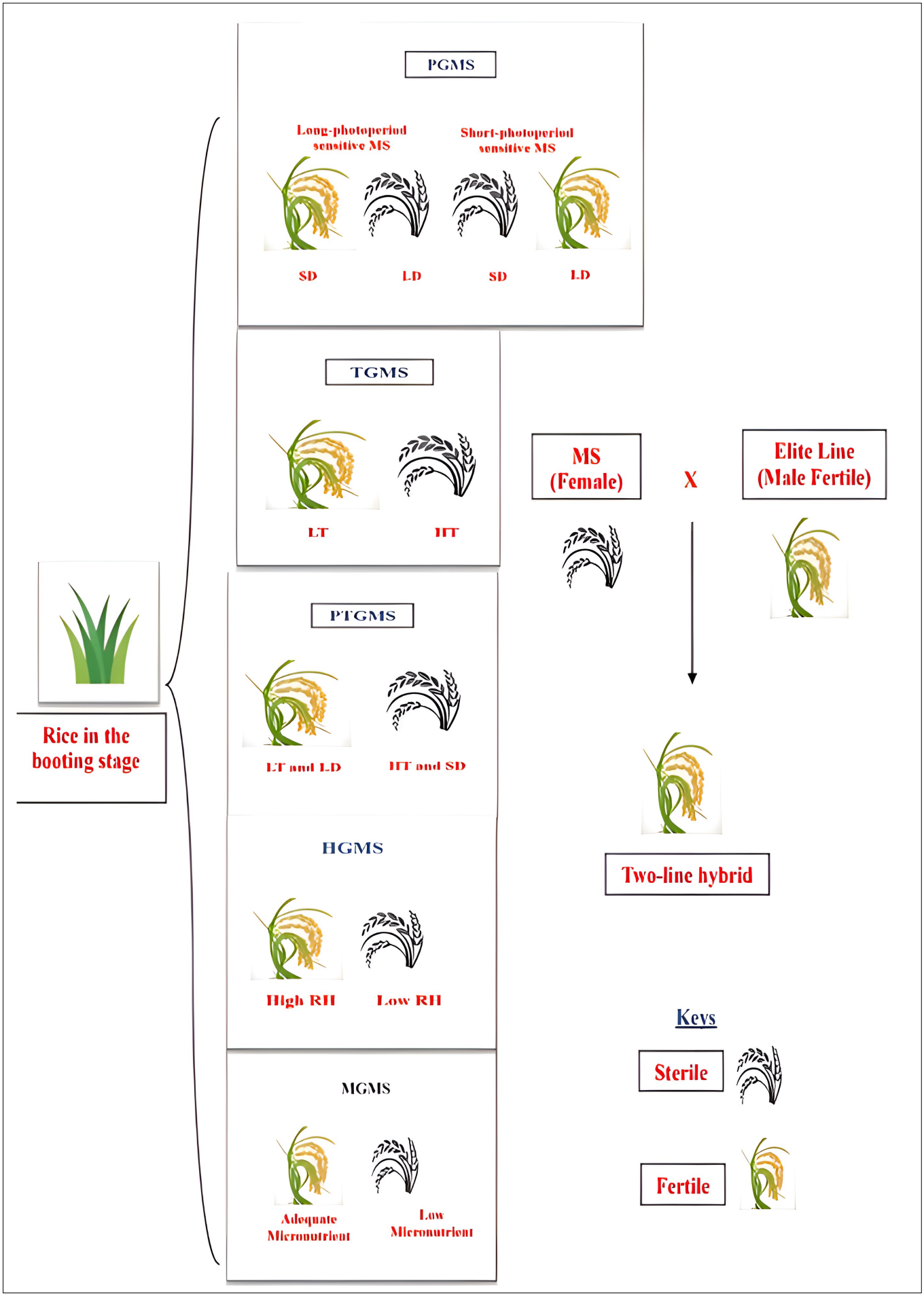

Fig. 1. Two-line rice breeding via different EGMS lines.

Note: SD - Short day, LD - long day, LT - low temperature, HT- high temperature, RH- relative humidity.

have been created using the Nongken 58S mutant. In PTGMS system, temperatures, as well as photoperiod have an interaction effect, leading to a male sterility type. When Photoperiod impact occurs within the temperature range it is termed as "Temperature Range of Photosensitivity" (30). Male gametes were found to be sterile under conditions of high temperatures (HT) and short-day lengths (SD) in contrast to a critical threshold, but fertile under low temperatures (LT) and long-day lengths (LD). A comprehensive study of NK58S a PGMS mutant revealed that male sterility or fertility phenotype was influenced by a significant interaction between temperature and photoperiod, rather than solely by changes in either photoperiod or temperature (25). In China, it is estimated that over 95% of the EGMS lines utilized in hybrid rice development originated from three distinct genetic sources: PGMS lines derived from Nongken 58S and TGMS lines derived from Zhu1S and Annong S-1 (31, 32). In China, there were 427 registered two-line hybrid rice combinations utilizing P/TGMS, which accounted for approximately 20% of the total cultivated area of hybrid rice (33).

MGMS (Micronutrient-sensitive genetic male sterility)

Copper deficiency, boron deficiency and insufficiency of various other micronutrients have been documented as a cause of male sterility in many crops such as wheat (34, 35). Male sterility induced by boron deficiency has also been documented in rice (36) and barley (35). There have been reports of high genetic diversity in response to sensitivity to various micronutrient deficiencies. In circumstances when micronutrients are insufficient, very sensitive plant types are male-sterile. Some researchers proposed that these sensitive genotypes can be employed as females with tolerant genotypes as males to produce F_1 hybrid seed. Grow females in settings with adequate micronutrients to multiply the micronutrient-sensitive varieties.

HGMS (Humidity sensitive genetic male sterility)

Recent studies have demonstrated that certain varieties of rice possess male sterile traits that are susceptible to changes in humidity levels. Related genes including *OsHMS1, OsCER1 (OsGL1-4)* and *OsOSC12* have been documented. *OsCER1* is a rice gene which is homologous to Arabidopsis' *CER1* gene (wax synthesis) and is expressed exclusively in rice tapetum during anther developmental stage S_{10}–S_{11}. Its suppression prolongs tapetum survival, leading to pollen failure and altering lipid metabolism-related genes (37). Xue reported that the *OsOSC12* gene encodes a conserved triterpene synthase crucial for producing "cereal tapetum alcohol," which is a key component of pollen coating in herbaceous plants (38). Mutant O*sOSC12* leads to defective pollen coating, causing rapid dehydration and male sterility under low humidity (<60%), mitigated by linolenic acid, palmitic acid or stearic acid application. Yun found that *OSHMS1*, encoding a β-ketoacyl-CoA synthetase in rice is pivotal in VLCFAs (very-long-chain fatty acids) synthesis. It initiates and controls the production of C28 and C26 VLCFAs, crucial for forming rod-shaped structures and tyrosine in the wall of pollen protects pollen from drying out (39).

TGMS system for hybrid seed production in rice

Hybrid crops can be commercialized successfully only if affordable technical methods for hybrid seed production are accessible. The first attempt was made by using chemical hybridizing agents in the 1970s however, as MS systems became available, this strategy was no longer used. To develop two-line hybrid rice, EGMS lines such as TGMS and PGMS lines are mostly used, which unlike CMS lines impose no constraints on the restorer-maintainer relationship. Two-line hybrid breeding originated in China (9). Seed production through TGMS is best suitable for the Indian scenario since photoperiodic differences are negligible or marginal. Temperature plays a key role in controlling fertility in TGMS lines. In TGMS, the plants are male sterile at higher mean temperatures (more than 30 ºC day and 24 ºC night) and convert into fertile plant at lower mean temperatures (less than 24ºC day and more than 16 ºC night) (40). The TGMS lines can be identified by critically monitoring when exposed to natural conditions post-panicle initiation, particularly at temperatures exceeding 30-35°C. Male sterility is identifiable by partly filled drooping panicles or sterile upright green panicles on the same plant (30, 40). For TGMS lines to be properly exploited in the target locations, the lines need to be characterized for important physiological and agronomical traits, such as the critical sterility-inducing temperature (CSIT) and critical fertility-inducing temperatures (CFIT) (41). According to the findings of Rekha et al, in a majority of TGMS lines, the critical stage for fertility-sterility transition occurs during panicle growth, roughly 5 to 26 days prior to heading (41). The low-CSTP TGMS lines that show complete sterility are preferred. In IRRI, TGMS lines developed using DH technology such as A07, have been confirmed to be highly stable and exhibit a CSTP of 24 °C. TNAU 95S was reported for stable pollen sterility. Newly developed TGMS lines must undergo evaluation for their outcrossing potential, before being utilized in hybrid breeding (42).

TGMS lines can be employed in mountains or during a cold season for seed multiplication (Fig 2). In two-line hybrid rice seed production, TGMS plants are grown in six rows, flanked by two rows of non-TGMS lines on each side (30).

In TGMS/two-line hybrid breeding, unlike in three-line HR technology, a specific restorer-rice line is not required. The sterile line serves a double function as both the maintainer and sterile line during the crossing process (9). Due to its versatility nearly any rice variety can function as a restorer line, facilitating flexible crossing and harnessing heterosis between subspecies, ultimately enhancing the yield, grain quality and resistance of HR combinations.

Genetics of TGMS trait in rice

Understanding the genetic basis of the TGMS trait is crucial for leveraging this technology. The recent identification of novel TGMS lines with low CSTP and the discovery of complete sterility occurring at an average temperature of 24°C, has reignited interest in two-line hybrid rice breeding methods.

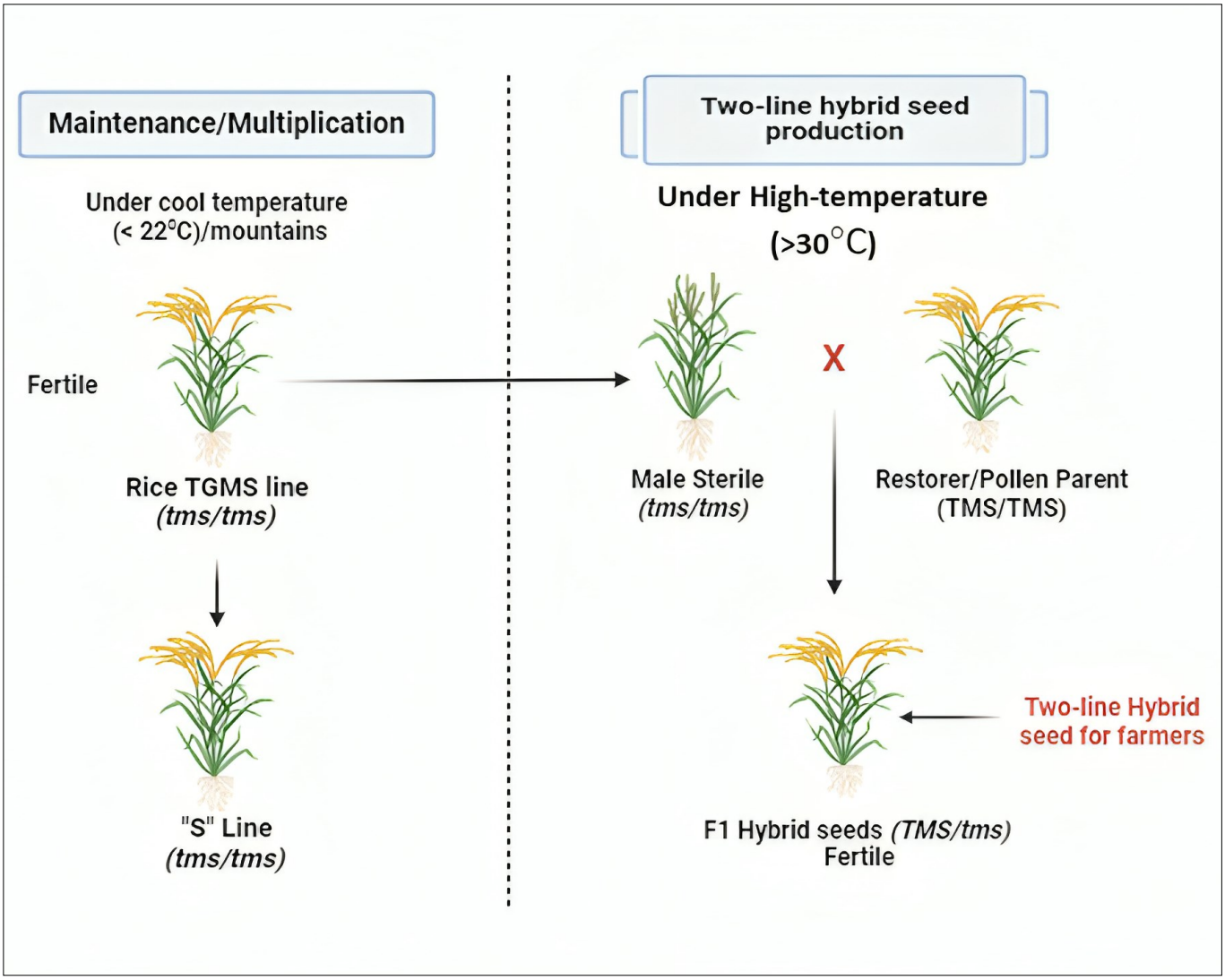

Fig. 2. TGMS system for Two-line hybrid seed production in rice.

Chromosomal location of TGMS genes and their sources

So far, thirteen different TGMS genes and their allelic variations have been found such as *tms1* was mapped on chromosome 8 of the 5460S line on (43), *tms2* on chromosome 7 of Norin PL 12 line (44), *tms3* on chromosome 3 of IR32364 (45), *tms4* on chromosome 2 of SA 2 (46), *tms5* on chromosome 2 of Annong S-1 (47), tms*6* on chromosome 5 of the SoKcho-MS line (48), *tms*6(t) on chromosome 3 of 0A15-1 (48), *tms7*(t) in was mapped in chromosome 3 of UPRI-95-140 TGMS line (49), *tms8* was mapped in chromosome number 11 of the F61 line on (50), *tms9* on chromosome 2 of Zhu1S line on (51), *tms9-1* in Hengnong S-1on chromosome 9 (52), *tms*10 on chromosome 2 of japonica cv. 9522 (7) and *tms*X in Xian S was also found to be present on chromosome 2 (53) (Table 2). Several independent TGMS mutants have been found in breeding programmes and further offspring selected from the NK58S line were used for TGMS line development (31). TGMS genes from Zhu 1S (*tms9*), Annong S-1 *(tms5*) and Guangzhan 63S (*ptgms2-1*) have been precisely located on chromosome 2 through fine mapping. While *tms5* and *ptgms2-1* were largely overlapped, *tms9* was fine-mapped to a section similar to *ptgms2-1*/ *tms5.* Several candidate genes were postulated for *ptgms2-1* (a ribonuclease Z homolog, *RNZ*) (53) and *tms5* (*OsNAC6*)(24) none for *tms9*. Recently it's found that Annong S-1, Zhu 1S and Guangzhan 63S had allelic TGMS genes, specifically *tms5*, *tms9* and *ptgms2-1.* Analysing over 300 non-EGMS and EGMS lines revealed a nonsense mutation in the *RNZ* gene, known as *RNZm* caused TGMS in Annong S-1, Zhu 1S, Guangzhan 63S and other lines (54).

Inheritance of TGMS trait

According to genetic research conducted at IRRI, in Japan (21) and India (23, 56), the TGMS character was governed by the expression of the single recessive gene as in Norin PL 12, IR32364S and SA 2. Many TGMS sources of either induced or spontaneous origin have been identified, including Anxiang S and Annong S-1 in China (57, 58). SA 2, F61 and SM 5 (23, 49, 59) and IR32364 at IRRI (22) in India. Norin PL 12 in Japan (21). Studies on allelic relationships revealed that the TGMS genes of the two mutants were distinct (Table 3). Because of the combined influence of the trigenetic inheritance pattern, genetic background and environmental temperature, hybrids of the TGMS line UPRI 95-140TGMS and 44 normal male fertile lines exhibited a more complex TGMS inheritance pattern and gene expression (60).

Molecular regulation of TGMS genes

The development of two-line HR systems has also thrived, making them a perfect CMS substitute. Moreover, the two-line-based hybrid yields approximately 5-10% higher than

Table 2. TGMS genes and their chromosomal location in rice

Genes	Sources	Chromosome	Nearest flanking markers	Distance (cM)	Reference
tms$_1$	5460S	8	RZ562-RG978	6.7	(43)
	Norin PL12	7	R643A-R1440 (D24156)	0.3	(110)
tms$_2$	Norin PL12	7	RM11-RM2	5.0, 16.0	(111)
	KDML105	7	0s7g2690	15.4, 16.9	(44)
tms$_3$	IR32364S	6	OPAC3640-OPAA7550	7.7, 10.0	(112)
	IR32364S	6	F18F, F18RM, F18FM/F18M	2.7	(45)
tms$_4$	TGMS-VN1	2	E5/M12600	3.3	(46)
	SA2	9	RM257, EAA/MCAG	6.2, 5.3	(59)
tms$_5$	Annong S-1	2	RM174, R394	0, 2.5	(26)
	Annong S-1	2	C365-1, G227-1	1.04, 2.08	(113)
	M105S	2	RM174	0	(114)
	Annong S-1 and Y58S	2	4039-1 and4039-2	-	(24)
	103S	2	RM71, RM3294 and RM6378	-	(115)
	IR68301S	2	RM12676, 2gAP0050058	-	(116)
tms$_6$	Sokcho-MS	5	E60663, RM3351	1.9, 0.1	(48)
tms$_{6(t)}$	140TGMS -UPRI 95	3	-	-	(117)
	G20S	10	RM4455, RM3152	1.10, 3.0	(28)
tms$_{7(t)}$	140TGMS -UPRI 95	7	-	-	(117)
tms$_8$	F61	11	RM224, RM21	3.0, 4.3	(50)
tms$_9$	Zhu1S	2	Indel 57, Indel 37	0.31, 0.12	(51)
tms$_{9-1}$	HengnongS-1	9	QY-9-27, QY-9-19	0.07, 0.22	(52)
tms$_{10}$	*japonica* cv. 9522	2	Os02g18320	-	(7)
tmsX	XianS	2	RMX21, RMAN81	-	(53)

Table 3. TGMS gene source and their inheritance pattern in rice

Norin PL 12, IR32364S	*tms3* and *tms 1*	Single recessive gene (for all)	(56)
SA 2 and ID24	*tms4* and *tms2*		(59)
Annong S-1	*tms5*		(24)
Anxiang S	-		(57)
R59TS	*tmsX*		(118)
H89-1	-	Single recessive gene (for all)	(21)
Sokcho-MS	tms6		(48)
Xians	*tms5*		(53)
F61	*tms8*		(50)
HengnongS-1	tms9-l		(52)

that of three-line-based (CMS) HR systems. It may be possible to add more TGMS system(s) for increased genetic diversity across various crop species by uncovering molecular mechanisms and identifying the underlying variables. This would give a solid basis for novel hybrids.

Genetic influence on TGMS trait

Genetic background plays a crucial role when attempting to comprehend the mechanisms that may give rise to new HR (Hybrid rice). Previous research has demonstrated that single/two or even more genes may be responsible for the expression of male sterility depending on environments as well as genetic resources. As demonstrated in an experiment by Virmani (40), crossing the *indica* and *japonica* with Nongken 58S, all of the F_1 siblings were fertile. The results of reciprocal crossings between F_2 and Nongken 58S revealed that male sterility is implied by a single recessive gene. Some research found that crossing an *indica* variety to Nongken-58S resulted in the segregation of two recessive genes (61). Many male sterile lines with an Indica background showed comparable segregation genetic ratios, such as Peiai64S (46).

Regulation by RNA metabolism and noncoding RNAs

The widely used temperature-inducible gene *tms5, is* used as a genetic modification to produce TGMS lines for HR. RNase Z an endoribonuclease, has been discovered across all kingdoms of life. Eukaryotes possess two distinct variants of RNase Z, termed RNase Z^{S-} theshort form and RNase Z^{L-} the long form, while prokaryotes exclusively harbour RNase Z^S. In addition to tRNA processing, RNase Z has vital function in mRNA maturation (62-63).

In eukaryotes, a single conserved mutation was found in RNase Z, also known as RNase Z^{S1}, it is thought to function independently of temperature changes and to be detectable in numerous tissues at both permissive temperature (PT) and RT (9). Additionally, gene *tms5* encodes a conserved protein RNase Z^{S1}, which controls the abundance of the mRNA for Ub_{L40} (ubiquitin-60S ribosomal protein L40) (64). Indirectly responding to temperature changes, the *tms5* may also degrade the Ub_{L40} mRNA rather than directly altering the amounts of mRNA or protein (Fig 3). TMS5 protein is present in the cytoplasm, whereas TMS5 mRNA is more abundant in PMC (64). RNase Z^{S1} can slice mRNA that translates three Ub_{L40} proteins, which are mostly produced in pollen mother cells and can be stimulated by warm temperatures. In mutant *tms5,* at position 71nt a base transition of Cytosine to Adenine (C to A) results in an early stop codon in *TMS5*. Ub_{L40} mRNA levels were low at low temperatures and did not cause any abnormalities in the anther, allowing *tms5* lines to produce normal pollen grains (65). Under high temperatures, Ub_{L40} mRNA levels were elevated which could not undergo splicing in *tms5* lines, leading to defective pollen growth and male sterility.

Table 4. List of Cloned TGMS-related genes in rice

Gene ID	Gene Name	Transcript Product	Biological Product	References
Os02g0214300	*tms5*	RNase Z^{S1}	RNA metabolism	(64)
Os02g0283800	*tms10*	LRR-RLK	Signal Transduction	(7)
Os09g0449000	*tms9-1*	PHD Transcription factor	Transcriptional regulation	(52)
Os10g0524500	*ostms18*	Glucose methanol choline (GMC) oxidoreductase	Pollen wall synthesis	(92)
Os09g0553200	*ugp1*	UDP glucose pyrophosphorylase	RNA Metabolism	(66)

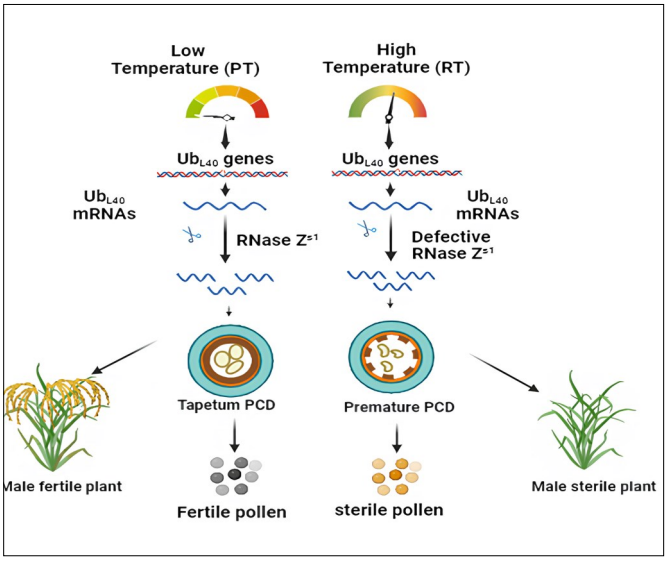

Fig. 3. In rice anthers, Ub_{L40} genes are expressed in microspore mother cells (MMCs), particularly under high temperatures. Wild-type plants utilize RNase Z^{S1} to regulate Ub_{L40} mRNA levels effectively. However, RNase Z^{S1}-deficient mutants experience mRNA accumulation, causing anther abnormalities and resulting in male infertility due to Ub_{L40} overexpression.

The mRNA splicing and fertility transition in TGMS lines

In an investigation of Ugp1 (UDP-glucose pyrophosphorylase1) rice it was observed that temperature changes affect mRNA splicing and the aggregation of un-spliced or spliced mRNA may be the underlying molecular reason for the change in fertility status of Upg1 TGMS line (66). In rice, the *Upg1* is essential for PMC meiosis and microspore formation. When *Upg1* was co-suppressed or silenced by RNA interference (RNAi), callose deposition was disrupted, leading to abnormalities in the development of the pollen wall. Thus, MS in HR resulted from PMC degeneration during the meiosis start stage. The transformants from the Ugp1-OX lines were segregated into two subgroups. One subgroup exhibited full inhibition of the native Ugp1 expression, indicating co-suppression. This subgroup was labeled as the co-suppressing plants. At the vegetative-growth stage, co-suppressing plants did not differ phenotypically from one another. However, in the reproductive growth stage, co-suppressing plants showed complete male sterility during the natural season but displayed fertility during the autumn season.

Additionally, Chen proposed that the buildup of UGPase-protein within florets of cosuppressive plants at low temperatures is the reason for the fertility change. When co-suppressing plant florets were grown at a low temperature, mRNA splicing of the Ugp1 was strongly controlled and spliced mRNA of the Ugp1 accumulated more in the florets cultured at a high temperature (66). The fertility phenotype is caused by the correctly spliced Ugp1 mRNA, which allows for the aggregation of a high amount of UGPase in the florets of plants undergoing co-suppression, leading to the expression of fertility phenotype (Fig 4A). These findings revealed that temperature rather than photoperiod, controls the fertility transition under SD in co-suppressing plants, which are classified as TGMS.

Metabolism of structural substances and miRNAs regulate TGMS

MicroRNAs (miRNAs) aid in the development of plants and respond to changes in their surroundings. Many studies have shown that specific metabolic and biochemical pathways are associated with anther development. Wu et al., carried out an extensive investigation to demonstrate that miRNAs with varying expression patterns concerning the TGMS gene take part in the biochemical pathways and synthesis of secondary metabolites (67). The metabolism of starch and sucrose are the two notable routes. Sphingolipid metabolism is another route that is controlled by miRNA target genes. Sphingolipids also control male fertility and programmed cell death (PCD). Proline and arginine metabolism are two proteome pathways that are critical for plant fertility, they involve the transformation of aspartic acid into proline and the slowing down of glutamic acids which is thought to be the cause of male sterility in rice sterile lines. Chen investigated the effects of overexpressing Ugp1 under the control of the ubiquitin promoter (66). Surprisingly, the results led to the development of a rice line with thermosensitive genetic male sterility, attributed to the suppression of Ugp1 rather than its overexpression (Fig. 4A).

Argonaute protein and phased secondary siRNAs (phasiRNAs) regulate TGMS

Research on the phasiRNA biochemical pathways linked to reproduction in rice has shown the importance of the generation and function of 21-nt phasiRNAs in anther development (68). It is commonly documented across flowering plants that 22-nt miR2118 and miR2275 trigger the generation of 21-nucleotide and 24-nucleotide phasiRNAs, respectively (69, 70). Disruption of miR2118, which initiates the production of 21-nt phasiRNAs, leads to abnormalities in anther wall formation, accompanied by a reduction in the abundance of 21-nt phasiRNAs (71). Mutant *dcl5* in maize was unable to produce 24-nucleotide phasiRNAs and exhibited temperature-dependent fertility (72). Additionally, reported that mutant ago1d exhibited pollen sterility when relocated to a glasshouse environment with a mean temperature of 22° C during the phase of panicle initiation. Microscopic examination revealed that exposing *ago1d* mutants to 22°C primarily resulted in abnormalities in PCD and pollen starch accumulation. Decreasing the levels of *AGO1d* leads to a decrease in the level of both phasiRNAs *viz.,* 21- and 24-nt and leads to the expression of TGMS trait (Fig 4B). In cooler temperatures, the ago1d mutant's anthers primarily exhibit an abundance of tapetal cells and reduced starch accumulation during pollen formation, potentially due to disrupted cellular metabolism (68).

LRR-RLK regulates TGMS expression

Leucine-rich repeats receptor-like kinases (LRR-RLK) are crucial in controlling the defining motions of the meiocytes and tapetum cells during anther formation (73). According to Yu et al., mutant *tms10* showed the transition from male sterility to fertility occurring from high to low temperatures and *tms10L*, its homolog, encodes LRR-RLK (7). Due to expanded and vacuolated tapetum at high temperatures, the *tms10* mutant exhibited a sterility phenotype, which caused

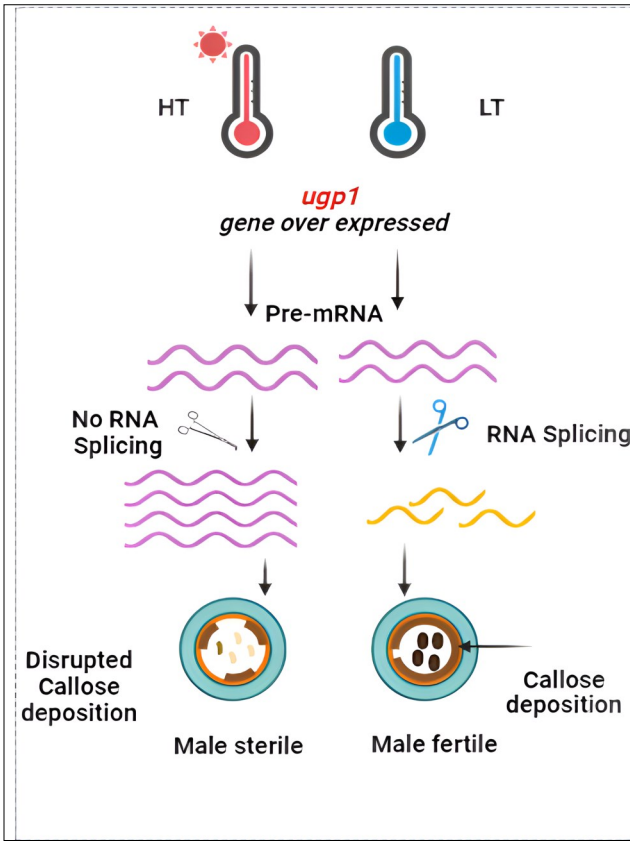

Fig. 4A. Overexpressing UPD-glucose pyrophosphorylase1 (Ugp1) in plants results in excessive unprocessed mRNA, leading to male sterility at high temperature. During low temperature mRNA undergoes proper splicing resulting in fertility.

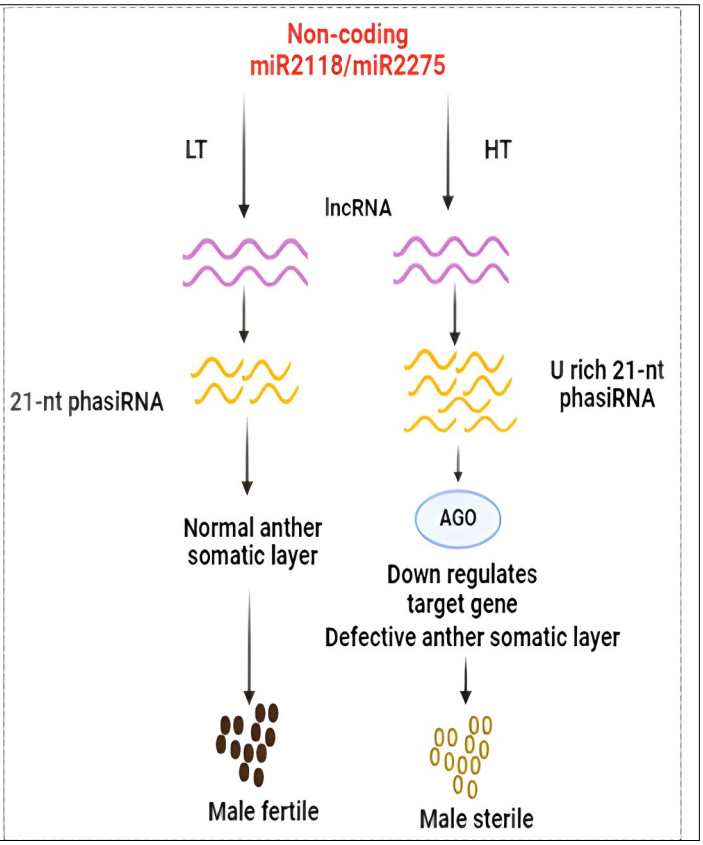

Fig. 4B. miR2118 generates an abundance of U-rich 21-nt phasiRNAs. These, along with AGO proteins, downregulate target genes, inducing male sterility in high temperature conditions but restoring fertility in low temperature conditions.

the pollen grains to abort at the S_9 stage. The results showed that the plants having double allelic mutations with the *tms10* and *tms10L* alleles resulted in the expression of male sterility at both high and low temperatures and the single mutation-containing *tms10* or *tms10L* plants demonstrated male fertility only at low temperatures indicating redundancy in function. According to the current findings, *tms10* and *tms10L* both have monitoring switches for temperature changes that might potentially buffer anther development form during the last stages of meiosis (7).

Yang discovered an additional LRR-RLK protein, previously identified as MSP1, which is now referred to as OsTMS15 (74). This protein has a pivotal role in the initiation of tapetum and pollen development, reacting to changes in temperature.

Han suggested that *ostms15* sterility under high temperatures is more consistent than that of the *tms5* gene, which will be crucial for the breeding of rice (75). The TIR motif found in the LRR region of *MSP1/OsTMS15* engages with its ligands through interaction, they generated several TGMS lines with targeted modifications in this region, enabling both mechanistic studies and the breeding of robust rice varieties.

Plant homeodomain finger proteins effect on TGMS trait

Plant homeodomain (PHD) finger proteins, classified as transcription factors (TFs), hold considerable importance in the male reproductive functions within plant biology (76). These transcription factors possess a PHD domain whereas others contain additional feature LXXLL motifs (where L represents leucine and X denotes amino acid). These motifs are associated with protein interaction control the activation or suppression of transcription and determine their subcellular distribution (77). In a recent investigation, it was revealed that a genetic mutation in rice occurring in the PHD finger protein's LXXLL motif MS1wenmin1 and MS1 (Male Sterile 1), is responsible for conferring TGMS. The abundance of both MS1 and MS1wenmin1 is regulated by ambient temperature, with higher temperatures (above 27° C) causing a greater decrease in MS1wenmin1 compared to MS1 within the nuclei. This imbalance ultimately leads to male sterility (78).

Transcription factors regulate TGMS

Transcription factors (TF) are crucial in regulating anther and pollen development in response to environmental signals (79). OsBHLH138 is a basic helix-loop-helix (bHLH) transcription factor primarily localized in the nucleus. It possesses the ability to create the fundamental helix-loop-helix configuration and attach its bHLH domain to the core promoter region of *tms5-2*. Additionally, it activates the expression level of *tms5-2* through its acidic amino acid-rich domain. Manipulating the expression of OsbHLH138 allows us to control the levels of *TMS5* expression and the aggregation of Ub_{L40} mRNAs, thereby governing male fertility across varying temperatures (80). The transcription factor GATA10 directly regulates the expression of Ub_{L40}. Knockout mutations in *Osgata10* leading to reduced Ub_{L40} expression, were associated with a trend towards increased male fertility. This confirms that GATA10 controls fertility conversion by influencing Ub_{L40} levels (81).

Cellular pathways controlling pollen development and fertility

Rice undergoes anther development through a series of 14 stages (82). The specification, growth and breakdown of the anther are intricately controlled by numerous genes and pathways. Any malfunction in these genes could lead to MS (83). Prior to meiosis, the microspore mother cell (MMC) wall is mostly composed of pectin after the cellulose degrades (84). Following meiosis, four individual haploid microspores are formed, which are surrounded by a tetrad wall comprising two layers: the primary outer pectin wall and the inner callose wall (85). The tetrad wall during the later tetrad stage dissolves, facilitating the tapetal deposition of pollen wall components onto microspores for exine synthesis (86). After meiosis, the anther tapetal layer will undergo PCD. This process is controlled by two transcription factors: TDR (Tapetum Degradation Retardation) and PTC1 (Persistent Tapetal Cell1) (87). Timing is crucial for tapetal PCD, since premature or delayed PCD might lead to male sterility. AnS -1, Guangzhan 63S and Xian 1S TGMS lines have empty anthers (53). Early tapetal PCD starts in the MMC stage and lasts until the tapetal cells are destroyed in Annong S-1 cultivated at high temperature (88). Premature tapetal PCD leads to early deterioration of the tapetum, reducing the availability of nutrients and other components like sporopollenin to microspores. The timing of premature tapetal PCD in TGMS and PGMS lines affects pollen development differently *i.e.* in TGMS lines, no pollen is produced in the pollen sac while PGMS lines experience pollen abortion. The PGMS and TGMS genes are neither directly nor indirectly linked in any known PCD pathway, making it unclear whether MS induces premature tapetal PCD (89). The TGMS trait observed in HengnongS-1 is governed by the native variant of *OsMS1/PTC1*, known as *OsMS1wenmin1*. This variant codes for a transcription factor responsible for regulating the development of the final stages anther (78, 90) (Table 5). AnnongS-1's TGMS trait arises from cytosine to adenine base substitution in the locus of *TMS5*, leading to early termination. The deficiency arises in the MMC prior to meiosis in the TGMS lines carrying the *tms5* locus (64). Microspore growth was aberrant at high temperatures and tapetal cells were expanded at another TGMS gene, *tms10* (7).

Fertility restoration by slow growth in TGMS lines

Fertility restoration in various TGMS lines primarily occurs via delayed development induced by low temperatures (83). According to this hypothesis, anther development can be slowed down by cold temperatures, reduced light intensity and photoperiod with shorter daylight duration, thereby enabling the production of functional pollen in P/TGMS lines (91, 92). However, it is unclear how slow growth allows faulty pollen to survive in TGMS lines.

The first evidence comes from the discovery of the reversible male sterile *(rvms)* Arabidopsis TGMS line, which is male fertile at permissive temperatures (17 °C) and sterile at HT 24 °C (91). *rvms* mutant has lipase activity by GDSL lipase protein. *rvms* mutant exhibits irregular exine formation and leakage of cytoplasm during pollen growth. A mutant for two genes *res1* and *rvms* exhibited male fertility under high temperature. Gene *RES1* codes for A-type Cyclin-Dependent Kinase-1 (CDKA-1) essential for cell multiplication during male gametogenesis (Fig 5). At 24 °C the *res1* mutant represents a mild allele that delays meiosis in male cells and micro gametogenesis. It showed that low temperatures slow pollen growth, as shown by cytological and statistical research. They suggested that slowing growth overcomes flaws in rvms microspores, resulting in functioning pollen. The mechanisms identified in Arabidopsis are likely conserved in other plant species. New findings from other restorers of *rvms* offer new

Table 5. Genes involved in the environmental adaption of male reproduction controlled by temperature

Crop	Gene name	Gene product	Pathway	Type of EGMS	Reference
Rice	*UGP1*	UDP-glucose pyrophosphorylase1	Processing of RNA	TGMS	(66)
	TMS5	RNase ZS[1]	Processing of RNA	TGMS	(64)
	TMS10 -TMS10L	LRR-RLK	Signaling transduction	TGMS	(7)
	TMS9-1/OSMS1	PHD finger protein	Protein location and transcriptional regulation	TGMS	(78)
	AGO1d	Argonaute protein	Phasi- RNAs production	TGMS	(68)
	OsNP1/ OsTMS18	Glucose–methanol–choline oxido- reductase	Pollen exine formation	TGMS	(92)
	ORMDL/ tms2	Orosomucoid	Sphingolipid homeostasis, PCD	TGMS	(119)
	OsTMS15	LRR-RLK	Tapetum development	TGMS	(75)
Arabid-opsis	*ABCG26*	ATP-binding cassette transporter G26	Pollen exine formation	TGMS	(90)
	IRE1A IRE1B	IRE	Pollen coat formation	TGMS	(120)
	AtSec62	Translocation protein	Unfolded Protein translocation	TGMS	(121)
	PEAMT	S-adenosyl-l-methionine: phosphoethanolamine N- methyltransferase	Signal transduction	TGMS	(122)
	COI1	E3 ligase	Protein degeneration	TGMS	(88)
Maize	*TMS5*	RNase ZS1	mRNA decay	TGMS	(123)
	DCL5	Dicer-like 5	PhasiRNA production	TGMS	(72)
	MAGO1, MAGO2	MALE-ASSOCIATED ARGONAUTE	Pre-meiotic phasiRNA pathways	TGMS	(124)
	INVAN6	Alkaline/neutral invertase	Sugar accumulation, metabolism and signaling	TGMS	(125)

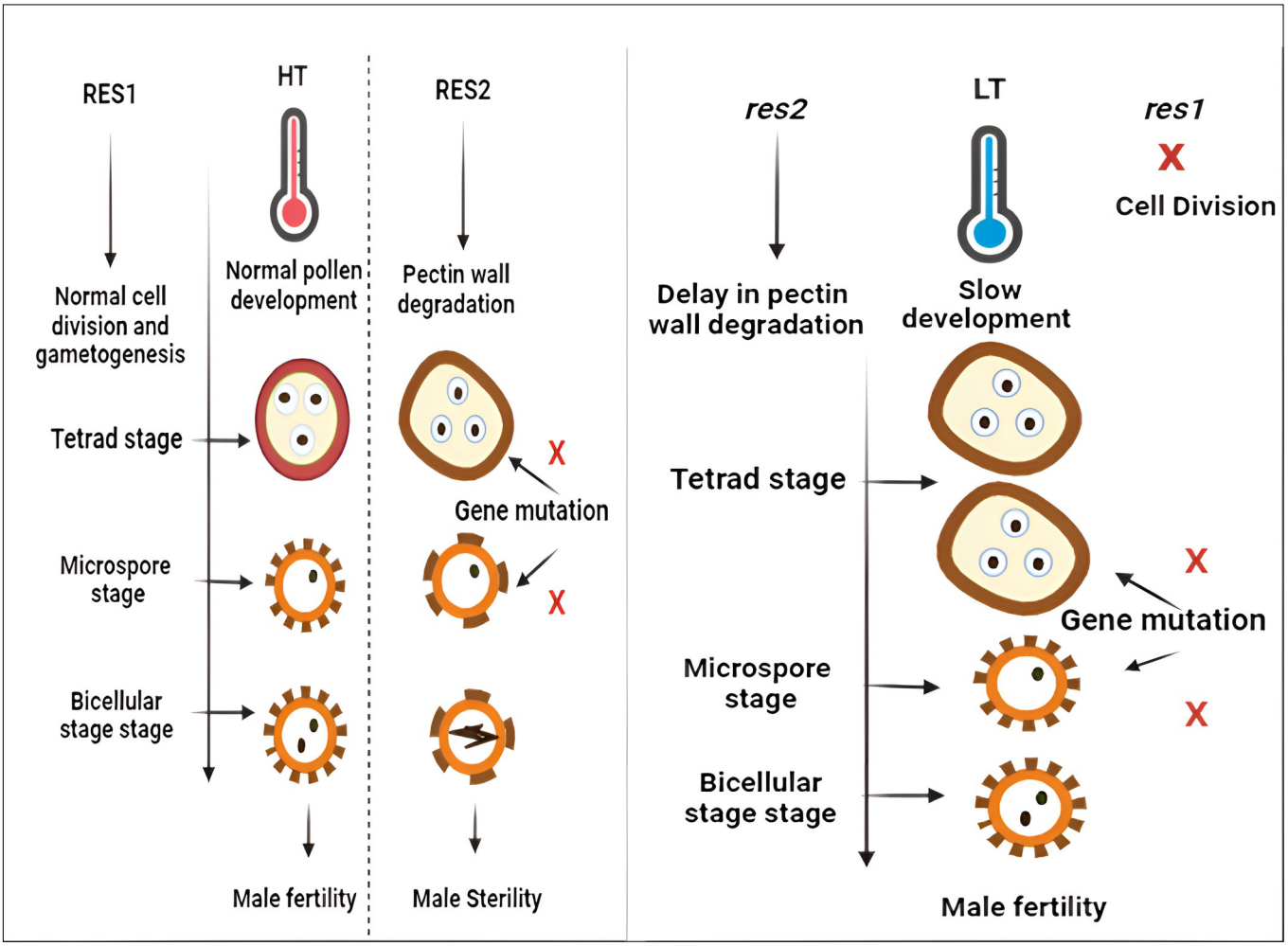

Fig. 5. Low-temperature leads to slow pollen development in Arabidopsis mutants. The *res1* and *res2* mutations can restore fertility by slowing pollen development and delaying pectin wall degradation respectively. (- Pectin) .

perspectives on the transition from sterility to fertility in PGMS/TMGS lines. Shi discovered the restorer of *rvms-2 (res2*) corresponds to an allele of QUARTET 3 (*QRT3*), which codes for a polygalacturonase enzyme delaying tetrad pectin wall degradation and can revert to fertility in *rvms*/ rvms-2 and some additional P/TGMS lines (92). *MS188,* a transcription factor unique to tapetum, plays a role in pollen wall construction by activating *QRT3* expression, which promotes pectin breakdown. Suggesting an external pathway's involvement in regulating the transition of the cell wall (92). Restoration of fertility in *rvms* and other photoperiod/thermosensitive genic male sterile lines are caused by a delay in tetrad pectin wall disintegration. However, *QRT3*'s early expression abolishes the restoration of *rvms-2* fertility under low temperatures. *res3*, carrying a *UPEX1* (UNEVEN PATTERN OF EXINE 1) point mutation, exhibited delayed breakdown of the callose wall in tetrads, it reverted male fertility in TGMS having defects in the pollen (rvms–2) (76). It suggests that fertility transition in TGMS is due to the slow development of microspores during the cell wall transition which may reduce the need for wall protection, resulting in functional pollens and restored fertility.

Molecular regulation of critical sterility-inducing temperature (CSIT)

One of the barriers preventing the development of two-line hybrid rice is high or inconsistent critical sterility-inducing temperatures (CSIT) in TGMS lines. These limitations constrain the production of TGMS hybrid seeds. The '*tms5*' gene, which was initially discovered in the AnnongS-1, has a stable inheritance and is presently utilized in over 95% of hybrid rice produced through two-line breeding (19). CSIT vary greatly in *tms5*-based TGMS lines across genetic backgrounds. Cloning and uncovering the molecular mechanism of the genes controlling CSIT offers fundamental solutions (93). The first gene found to be associated with CSIT, *CSIT1*, was successfully cloned (94). Later they cloned the *CSIT2* gene that regulates CSIT in TGMS lines based on *tms5*. A mutation in *CSIT2*, which encodes a RING-type E_3 ubiquitin ligase, causes AnS-1's CSIT to rise from 26°C to 32°C. According to Chen *indica* TGMS lines with *tms5* usually possess low critical sterility-inducing temperatures, whereas the *japonica* TGMS lines carrying the *tms5* gene typically show higher CSIT around 28°C to 32°C. Sequence variation for the haplotype of *CSIT2* between indica and japonica rice related to the CSIT differentiation (95).

A protein quality control (PQC) mechanism identifies and eliminates the irregular proteins to avert the harmful buildup of such proteins via ubiquitin-proteasome system or autophagy, safeguarding cells from cytotoxic accumulation (96). Ribosome-linked quality control (RQC) monitors protein synthesis, which also eliminates defective proteins/polypeptides (97). When ribosomes encounter problems during translation, certain RQC

cofactors recognize interrupted ribosomes and bind to the 80S ribosome engaging an E_2-binding enzyme responsible for ubiquitinating the 40S ribosomal protein to promote the separation of the 60S and 40S subunits (91). After the 40S subunits separate, the 60S ribosomal subunit along with the nascent polypeptide chain (RNC), attaches to a protein called NEMF (nuclear export mediator factor - Rqc2). NEMF then brings in an enzyme called E_3 ubiquitin ligase CSIT1 that adds ubiquitin to the newly formed polypeptide chain for deterioration (94).

Peng reported that elevated temperatures (RT) can lead to improperly folded peptide chains, prompting the RQC system to eliminate these defective proteins (93). According to Zhou the accumulation of surplus ubiquitin proteins occurs in the anthers when *TMS5* function is lost in Annong S-1 under elevated-temperature conditions (64). The translation of protein in the anther becomes more vulnerable to high temperature, while CSIT2 separates 80S ribosomes, decreasing protein translation and resulting in male sterility. In mammals, the *TMS5* homologue ELAC1 is involved in repairing the 2'-3' cyclic phosphate of ΔCCA tRNA, allowing tRNA recycling and the re-addition of 3'CCA during RQC. Mutations in AnS-1's TMS5 might cause ΔCCA tRNA accumulation, potentially impacting male development via protein translation disruption in high temperatures (Fig 6).

In *tms5 csit2* mutants, disrupted the RQC process, resulting in the production of both functional and abnormal anther-related proteins (ARPs). This mutation also decreases ΔCCA tRNA accumulation, impacting male fertility and elevating the critical sterility-inducing temperature (CSIT).

Exploiting CRISPR/Cas9 technology in TGMS System

The demand for rice is expected to surge, there is a need to create ways to effectively feed this greatly increased population. A breakthrough technique the CRISPR/Cas system identified as an excellent technique for creating genetically inheritable alterations in crops. The genome editing assembly consists of a single guide RNA (sgRNA) and Cas proteins. Upstream of the CRISPR array, there is an A-T base pair-rich leader sequence. The end of the CRISPR loci includes conserved genes that encode Cas proteins (98).

CRISPR/Cas9 system for the precise modification of the *OsTMS5* gene. To knock down the OsGS3 gene, a guide RNA (gRNA) expression cassette targeting the *OsTMS5* gene's 20 nucleotide sequence was produced (99). They reported that *the OsTMS5* promoter harbors cis-elements which were responsive to light and hormones, which were active in a variety of rice tissues and across developmental stages. To achieve scientific goals through the introduction of precise DNA targets, in vivo genome editing methods are essential tools in gene engineering. Zhou et al, by utilizing the CRISPR/Cas9 technology, knocked out the expression of the OsGS3 gene to produce transgene-free TGMS lines within a year (100). Using this technology *tms10* gene of a few other rice varieties that were edited also displayed TGMS traits, offering a genetic source for hybridization (7).

TGMS lines with free T-DNA background can be created using the CRISPR/Cas9 system, eliminating deleterious mutations that might increase the quality of two-line hybrid rice. GXU 47, a super grain quality Basmati rice was used in the study to create a novel TGMS line with *tms5* mutation. The mutants showed good thermo-sensitive male fertility transition features, displaying complete male sterility at temperatures above or at 24 ℃ and desirable fertility at 21 ℃.

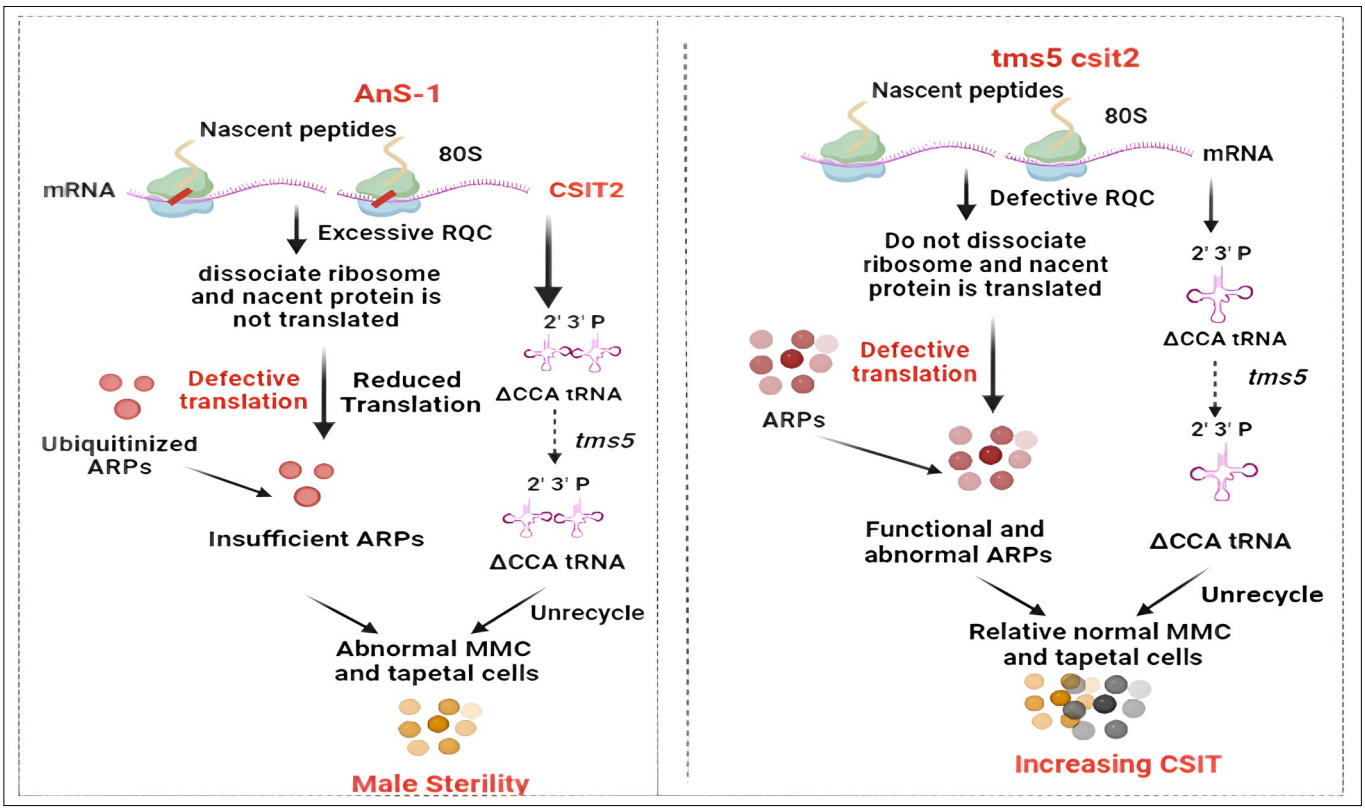

Fig. 6. Molecular control of critical sterility-inducing temperature (CSIT) of rice TGMS (*csit2 tms5*) lineNote: RQC: ribosome-linked protein quality control, MMC: microspore mother cell; ARPs: anther-related proteins; PCD: programmed cell death and CSIT: critical sterility-inducing temperature.

They conducted iTRAQ-based proteomics investigations on CRISPR/Cas9-mediated *TMS5* mutations, helping to explain how a gene mutation affects the entire plant proteome (101). Furthermore, the CRISPR/Cas9 multiplex gene editing technique has facilitated the incorporation of disease-resistant genes into the *TMS5* gene to produce disease-resistant TGMS lines. For instance, the *Xa13* gene and the recessive *pi21* gene which confer protection from bacterial blight and rice blast, respectively. For target genome editing, Ma demonstrated CRISPR/Cas9 technology which showed an average mutation frequency of 85.4% in the rice genome and attained 82% of the targeted editing efficiency for the desired trait (102).

Further research will help in identifying the molecular basis of *tms5,* which causes the expression of male sterility in rice, making it easier to develop high-quality TGMS lines as *tms*5 is the principal TGMS gene, which was discovered in over 70% of the Chinese-produced commercial TGMS lines (101). Plant breeding by utilizing genetic engineering techniques would provide advantages over traditional breeding, including reduced time and labour requirements and increased efficacy. The CRISPR/Cas9 system, a well-developed genetic engineering tool, has been widely used for crop quality improvement and/or increased yield.

Omics approach in TGMS system

RNA-Seq analysis of rice TGMS line identified 1070 DEGs enriched in transcriptional regulation, TF activity, protein folding and metabolism. Kinases, Ub_{L40}s, RNA polymerase subunit and HSPs regulate fertility changes in rice TGMS lines (103). In P_{23} rice line, male fertility was governed by 391 regulated and 315 upregulated genes in response to temperature fluctuations. Electron microscopy revealed temperature-induced sterility due to deficient starch accumulation, abnormal exine growth and absent inner pollen grains. qPCR confirmed defective sporopollenin synthesis, transport and pollen wall construction (104).

Proteomic assessment of rice AnnongS-1 anthers under varied temperatures revealed 89 differentially accumulated proteins, with 46 upregulated and 43 downregulated. The majority of these proteins were enzymes that play a role in influencing processes such as photosynthesis, metabolic regulation, protein and carbohydrate metabolism and antioxidative defense mechanisms (105). Sun performed a metabolomic analysis in anthers of the P23 line in rice. They found that fertile anthers displayed upregulation of lipids and flavonoids, crucial for the formation of pollen walls and male reproductive success. Additionally, C-type lignin and lignin G units were pivotal for pollen wall formation (104).

Understanding the transcriptome, proteome and metabolome will provide a deep understanding of molecular processes governing male sterility in response to environmental changes.

Conclusion and future directions

TGMS system is gaining significant attention in research due to its numerous advantages compared to the three-line system, making it increasingly popular for hybrid breeding worldwide. In the TGMS system sterility to fertility transition regulation is affected by environmental factors like temperature. Yet a small number of TGMS genes have been cloned and characterized for their mechanisms. In this paper, we have reviewed the MS systems employed to develop rice EGMS lines and highlighted potential areas for improvement. The high critical sterility-inducing temperature (CSIT) of TGMS lines (in a *japonica* background) largely limits the adoption of the two-line HR system. We have briefly provided insight into the molecular control of CSIT of TGMS lines harbouring the *tms5* gene. Finding the molecular mechanism of CSIT for various TGMS genes will greatly influence the two-line HR application. We emphasized the importance of functional characterization of target genes in future studies.

The application of sophisticated biotechnology and functional genomics is expected to yield significant progress in male sterility and hybrid rice breeding. Genome editing technologies now allow for more accurate and stable expression of desired genes, including the TGMS genes. In many countries, including the Philippines, genome editing is still considered a genetically modified (GM) domain. Multiplex gene editing by identifying transgene-free homozygous triple *tms5/pi21/xa13* mutants developed in the T_1 generation with TGMS characteristics enhanced resistance against rice blast and bacterial leaf blight (65). Over the past two decades, numerous TGMS genes have been discovered and their regulatory pathways have been extensively examined. However, further research is required to delve deeper into the genetic and molecular mechanisms to prevent the genetic susceptibility of hybrids. Moreover, the current advancements in rice functional genomics research and gene engineering represent a crucial milestone towards achieving future objectives, including the development of HR germplasm and promoting environmentally sustainable agriculture.

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Authors' contributions

TK helped in conceptualization, supervision and editing. BB helped in writing the original draft. SM worked on the conceptualization and did supervision. BA, NS and GSK supervised the study. All authors were involved in the manuscript preparation and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical issues: None

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