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Feature Review **Open Access**

Regulatory Mechanisms of Temperature, Light, and Water on the Expression of Male Sterility Genes in Rice

Cui Zhang [1,3], Qian Zhu [1,2,3], Juan Li [1,2,3], Hui Zhang [1,3], Dongsun Lee [1,2,3], Lijuan Chen [1,2,3]
1 Rice Research Institute, Yunnan Agricultural University, Kunming, 650201, Yunnan, China
2 The Key Laboratory for Crop Production and Smart Agriculture of Yunnan Province, Yunnan Agricultural University, Kunming, 650201, Yunnan, China
3 College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming, 650201, Yunnan, China
Corresponding email: chenlijuan@hotmail.com

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Abstract Temperature, light, and humidity significantly influence the expression of male sterility (MS) genes in rice. High temperatures were found to induce MS by disrupting pollen development and gene expression in the anther. Genes involved in sugar, lipid, phytohormone, and phenylpropanoid various metabolisms were differentially expressed under varying temperature, light, and humidity conditions, affecting pollen wall development and MS. For instance, cooling water temperature prior to panicle initiation was shown to affect chilling-induced MS, with stress-responsive genes being down-regulated under low water temperature conditions. The findings of valuable reports provide a comprehensive understanding of how temperature, light, and water or humidity regulate male sterility genes in rice. This review aims to elucidate the regulatory mechanisms of temperature, light, water or humidity on the expression of MS genes in rice, focusing on the interaction between environmental factors and genetic responses that govern MS, thereby enhancing two-line hybrid rice production and food security.

Keywords Male sterility (MS); Rice; Temperature regulation; Light sensitivity; Water stress

1 Introduction

Male sterility (MS) in plants, particularly in rice (*Oryza sativa* L.), plays a crucial role in the development of hybrid crops, which significantly contribute to global crop productivity. The concept of MS, which includes both cytoplasmic male sterility (CMS) and genic male sterility (GMS) (Budar and Pelletier, 2001; Chen and Liu, 2014), has been extensively utilized in rice breeding programs to enhance yield potential and grain quality. The discovery of photoperiod-sensitive genic male sterility (PGMS), temperature-sensitive genic male sterility (TGMS), and humidity-sensitive genic male sterility (HGMS) has provided valuable germplasms for the breeding of "two-line" hybrids, which have been instrumental in increasing rice production (Li et al., 2007; Fan and Zhang, 2017; Abbas et al., 2021; Xue et al., 2018; Chen et al., 2020). The identification and characterization of various MS genes have deepened researchers' understanding of the molecular mechanisms controlling anther and pollen development, thereby facilitating the efficient use of biotechnology-based MS systems in hybrid breeding (Wan et al., 2019; Sun et al., 2021b).

Environmental factors such as temperature, light (photoperiod), and water (humidity) availability play a significant role in regulating the expression of male sterility genes in rice. Photoperiod and temperature-sensitive genic male sterility (P/TGMS) and HGMS lines are particularly sensitive to changes in these environmental conditions, which can trigger transitions between male fertility and sterility. For instance, the PGMS, TGMS, and HGMS lines exhibit sterility under specific photoperiod, temperature, and humidity conditions, respectively, and revert to fertility when these conditions change (Zhou et al., 2012; Fan and Zhang, 2017; Wang et al., 2021; Xue et al., 2018; Chen et al., 2020). The molecular mechanisms underlying these transitions involve complex regulatory networks, including noncoding RNAs, transcription factors, and various metabolic pathways (Chen et al., 2010; Sun et al., 2021b; Kan and Lin, 2021; Xue et al., 2018; Chen et al., 2020). Understanding these regulatory mechanisms is essential for developing strategies to breed rice varieties that can maintain high yields and quality under varying environmental conditions.

This review systematically investigates the regulatory mechanisms of temperature, light, and water (humidity) on MS gene expression in rice, including identifying key genetic factors and understanding their interaction with environmental factors that control MS. This review highlights the molecular and genetic basis of MS regulation, with a focus on PGMS, TGMS, and HGMS lines, and to reveal new insights into the environmental regulation of MS genes by integrating data from various genomic, transcriptomic, and molecular analyses. This review aims to develop new strategies for breeding disaster-resistant rice varieties that can adapt to changing environmental conditions, thereby ensuring food security and sustainable agricultural practices.

2 Temperature Regulation of MS Genes

2.1 TGMS in rice

TGMS in rice is a crucial mechanism that facilitates the development of hybrid rice varieties (Hussain et al., 2012). TGMS lines exhibit MS under specific temperature conditions, which can be reversed when the temperature changes. This characteristic is essential for the production of two-line hybrid rice systems, as it allows for controlled pollination and hybrid seed production. The TGMS trait has been extensively studied, and various TGMS lines have been developed to enhance rice breeding programs (Viraktamath and Virmani, 2001; Lee et al., 2005; Fan and Zhang, 2017; Sun et al., 2021b; Yan et al., 2024).

2.2 Molecular mechanisms underlying temperature regulation

The molecular mechanisms underlying photoperiod-and-temperature regulation of P/TGMS in rice involve complex interactions between genes, *microRNAs* (*miRNAs*), and environmental factors (Figure 1) (Fan and Zhang, 2017). For instance, the TGMS locus *TMS5*, identified in the TGMS line Annong S-1, encodes an RNase Z, which plays a significant role in the regulation of MS. The TGMS trait of AnS-1 was found to be caused by C-to-A mutation in *TMS5*, resulting in a premature stop codon in the RNase Z^{S1} protein (Zhou et al., 2014). Remarkably, male fertility of *tms5* mutant was found to be completely restored at high temperatures by knocking out *OsVms1* which encodes the enzyme for cP-ΔCCA-tRNA generation (Yan et al., 2024). Additionally, miRNAs such as miR156, miR5488, and miR399 have been found to regulate male fertility by influencing various metabolic pathways, including fatty acid metabolism and lignin synthesis in anther walls (Sun et al., 2021b). These findings highlight the intricate molecular networks that govern TGMS in rice.

2.3 Case study: *OsTMS6* and its role in TGMS

OsTms6 is another critical gene associated with TGMS in rice. Studies have shown that the expression of *OsTms6* is highly sensitive to temperature variations, and its regulation is crucial for maintaining male sterility under specific temperature conditions. For example, exposure to temperatures above 32°C for more than 8 hours can induce complete male sterility in certain *indica* TGMS lines, while a shorter exposure is sufficient for *japonica* lines (Viraktamath and Virmani, 2001). The genetic background of the rice lines also influences the expression of *OsTms6*, indicating the need for developing diverse TGMS lines to ensure stable sterility under varying environmental conditions (Lee et al., 2005; Liu et al., 2010).

2.4 Impact of global warming on TGMS

Global warming poses a significant challenge to the stability of TGMS in rice. As global temperatures rise, the critical temperature thresholds for inducing MS may be frequently exceeded, leading to unintended fertility and reduced effectiveness of TGMS lines. This necessitates the development of TGMS lines with higher critical sterility-inducing temperatures and greater resilience to temperature fluctuations (Viraktamath and Virmani, 2001; Liu et al., 2010; Zhang et al., 2022; Lin et al., 2023). Furthermore, understanding the molecular mechanisms and genetic factors involved in temperature regulation can aid in breeding more robust TGMS lines that can withstand the impacts of climate change (Viraktamath and Virmani, 2001; Fan and Zhang, 2017; Sun et al., 2021b; Zhang et al., 2022; Lin et al., 2023). By integrating genetic, molecular, and environmental insights, researchers can develop innovative strategies to enhance the stability and effectiveness of TGMS lines, ensuring sustainable rice production in the face of global warming.

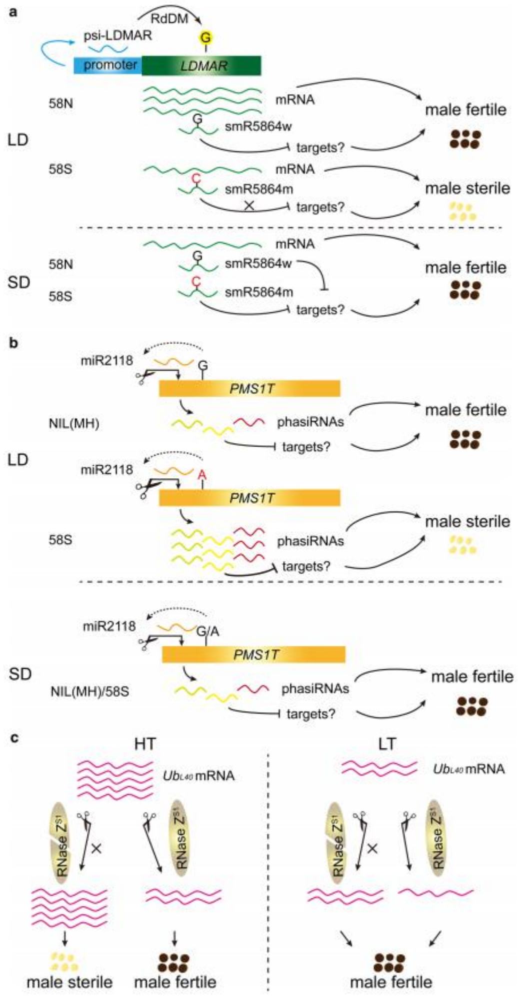

Figure 1 Models of the current understanding for the three cloned EGMS genes (Adopted from Fan and Zhang, 2017)

Image caption: a. An lncRNA *LDMAR* controls the PGMS trait at the *PMS3* locus. A small RNA psi-LDMAR generated from the promoter mediated the DNA methylation of LDMAR regulating the level of transcript for pollen development under long-day conditions. Or alternatively, a G-to-C mutation leads to a loss of-function of small RNA osa-smR5864m in targeting different genes, resulting in PGMS. b. An lncRNA *PMS1T* targeted by the miR2118 confers the PGMS at the *PMS1* locus. The SNP nearby the cleavage site may influence the cleavage efficiency for higher accumulation of phasiRNAs, causing male sterility in 58S under long-day conditions. c. The TGMS gene *TMS5* encodes RNase Z^{S1} processing Ub_{L40} mRNA whose expression is temperature-sensitive. The loss-of-function mutation of RNase Z^{S1} results in the overaccumulation of Ub_{L40} mRNA, leading to male sterility at high temperature. LD, long-day conditions; SD, short-day conditions; HT, high temperature; LT, low temperature (Adopted from Fan and Zhang, 2017)

3 Light Regulation of MS Genes

3.1 PGMS in rice

PGMS in rice is a critical trait for hybrid rice breeding, as it allows for the production of MS lines under specific daylength conditions. The PGMS trait is controlled by various genetic loci, such as *PMS1* and *PMS3,* which encode long noncoding RNAs essential for male fertility under long-day conditions (Fan and Zhang, 2017). The *carbon starved anther* (*csa*) mutant is another example of a PGMS line (Zhang et al., 2013), where the *CSA* gene plays a pivotal role in regulating sugar metabolism and cell wall synthesis in anthers under short-day conditions (Figure 2) (Li et al., 2022). Additionally, the *pms3* locus, which encodes the long non-coding RNA LDMAR, is crucial for normal male fertility under long-day conditions, with increased methylation in its promoter leading to MS (Ding et al., 2012).

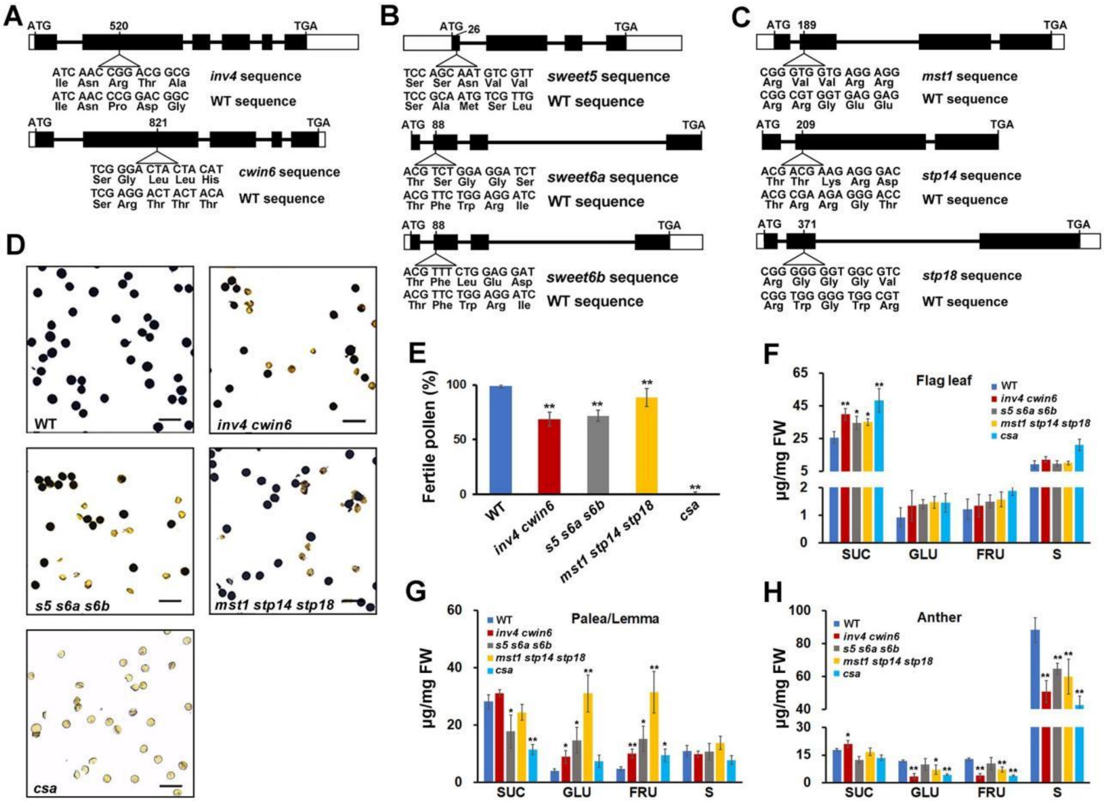

Figure 2 Phenotyping mutants of *CSA* target genes (Adopted from Li et al., 2022)

Image caption: A, Mutation sites in *INV4* and *CWIN6* genomic sequences. B, Mutation sites in *SWEET5*, *SWEET6a*, and *SWEET6b* genomic sequences. C, Mutation sites in *MST1*, *STP14*, and *STP18* genomic sequences. D, Iodine staining of pollen grains from WT and mutant anthers. Viable pollen grains stain darkly. Scale bar, 100 μm. E, Percentage of fertile pollen grains in WT and mutant anthers. Data indicate mean ± STD of three pollen grains from each of three plants. Significant differences were assessed using Student's *t* test compared with WT (***P*<0.01). F-H, Soluble sugar and starch content in flag leaf (F), palea/lemma (G), and anthers (H) of WT and mutant plants. Data indicate mean ±STD of three biological replicates. Significant differences were assessed using Student's *t* test compared with WT (**P* < 0.05, ***P* < 0.01). s5 s6a s6b, sweet5 sweet6a sweet6b; SUC, sucrose; GLU, glucose; FRU, fructose; S, starch (Adopted from Li et al., 2022)

3.2 Mechanisms of light perception and signal transduction

The perception of light and its subsequent signal transduction are fundamental processes in regulating PGMS in rice. Key components of the circadian clock, such as *Circadian Clock-Associated 1* and *Pseudo-Response Regulator* (*PRR95*), are involved in sensing photoperiod signals in leaves, which are then weakly transduced to anthers (Li et al., 2022). The *CSA* gene, a MYB TF, is specifically expressed in anthers and is indispensable for sugar partitioning under long-day conditions, highlighting its role in PGMS (Wang et al., 2021). Furthermore, the small RNA osa-smR5864w, produced by the noncoding RNA P/TMS12-1, is involved in the regulation of male fertility in response to photoperiod and temperature changes (Zhou et al., 2012).

3.3 Gene networks involved in light-responsive MS

Gene networks play a crucial role in mediating the effects of light on male sterility in rice. Transcriptome analyses have identified numerous differentially expressed genes (DEGs) in response to varying day lengths, with significant enrichment in transport, carbohydrate, and lipid metabolic processes, as well as phytohormone signaling pathways (Figure 3) (Sun et al., 2021a). Co-expression network analysis has revealed several hub genes, including *Carbon Starved Anther* and *UDP-glucose pyrophosphorylase*, which are strongly correlated with photoperiod sensitivity and male fertility (Sun et al., 2021a). Additionally, miRNAs such as miR156, miR5488, and miR399 have been implicated in regulating male fertility by influencing SPLs, lignin synthesis, and flavonoid metabolism pathways (Sun et al., 2021b).

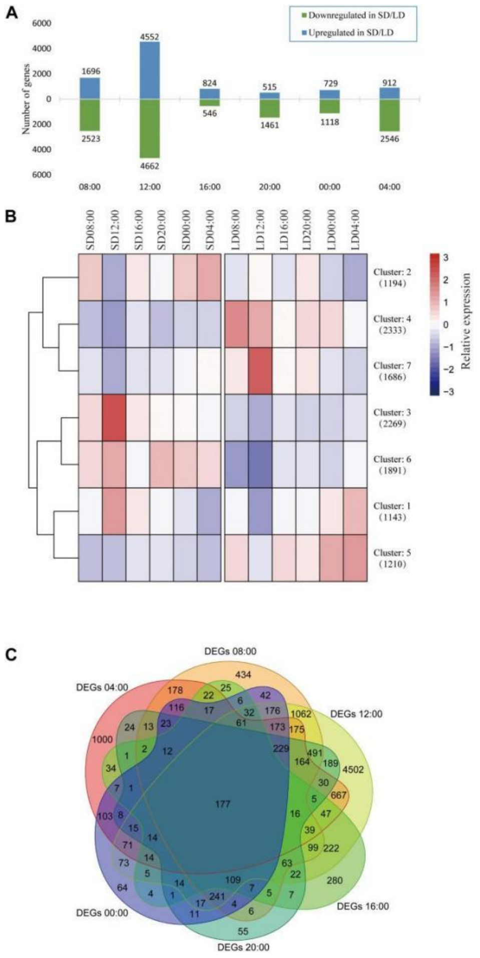

Figure 3 Expression of 11 726 DEGs in response to photoperiod conditions (Adopted from Sun et al., 2021a)

Image caption: SD, short day; LD, long day. (A) Number of up- and down-regulated DEGs at each time point. (B) Heatmap and dendrogram of all DEGs in all samples ($k = 7$). Gene numbers in each cluster given in brackets. DEGs assembled to seven clusters: three clusters up-regulated in SD (2, 3, and 6); three clusters up-regulated in LD (4, 5, and 7); and Cluster 1, with higher daytime expression under SD conditions, and nighttime expression under LD conditions. The value of center point of seven clusters represents relative expression. (C) Venn diagram showing all DEGs at six time points. A total of 177 genes showed differential expression between SD and LD at all time points (Adopted from Sun et al., 2021a)

3.4 Interaction between light and other environmental factors

The interaction between light and other environmental factors, such as temperature, further complicates the regulation of MS in rice. For instance, the reverse PGMS line YiD1S exhibits MS primarily regulated by day-length but also influenced by temperature, with two major genes, *rpms1* and *rpms2*, controlling this trait (Peng et al., 2008). The fertility transition in P/TGMS lines is a result of the cooperative regulation of major-effect sterile genes with photoperiod and/or temperature-sensitive genes (Chen et al., 2010). This interaction underscores the complexity of environmental regulation of MS and the need for a comprehensive understanding of the underlying molecular mechanisms. In summary, the regulation of MS genes in rice by light involves intricate mechanisms of photoperiod perception, signal transduction, and gene network interactions, with significant interplay between light and other environmental factors such as temperature. Understanding these processes is essential for optimizing hybrid rice breeding strategies and improving crop yields.

4 Water and Humidity Regulation of MS Genes

4.1 Water stress and its effects on plant physiology

Water stress, encompassing both drought and flooding, significantly impacts plant physiology, particularly in rice. Drought stress leads to a reduction in water availability, causing physiological changes such as reduced cell turgor, impaired photosynthesis, and altered nutrient uptake (Wu et al., 2022). These changes can severely affect reproductive development, including male fertility. For instance, drought stress during the reproductive stage can lead to aberrant anther development and reduced pollen viability, as observed in rice plants subjected to water stress (Jin et al., 2013). Additionally, water stress can disrupt carbohydrate metabolism and phytohormone signaling, further complicating the plant's physiological responses (Jin et al., 2013).

4.2 Water-responsive gene expression in rice

The expression of genes in rice is highly responsive to water stress. Under drought conditions, a multitude of genes are differentially expressed to mediate stress responses. For example, a meta-analysis of microarray data identified numerous drought-responsive genes, including those involved in abiotic stimulus response, water deprivation, and abscisic acid (ABA) signaling (Soltanpour et al., 2022). These genes play crucial roles in modulating physiological processes to enhance drought tolerance. Similarly, specific TFs such as *OsAL5* have been shown to regulate both drought-related genes and TGMS genes, linking drought response to MS (Wen et al., 2021).

4.3 Influence of drought and flooding on MS genes

Drought and flooding have distinct yet profound effects on the expression of MS genes in rice. Drought stress can lead to the down-regulation of genes involved in pollen development, resulting in MS. For instance, genes associated with tapetum and microspore development are often down-regulated under drought conditions, leading to defective pollen development and reduced fertility (Jin et al., 2013). Conversely, flooding can also impact MS by altering the expression of stress-responsive genes. For example, low water temperatures before panicle initiation can increase chilling-induced MS and down-regulate stress-responsive genes such as *OsFKBP65* and heat shock proteins, which are crucial for protecting proteins from oxidative damage (Suzuki et al., 2015).

4.4 Adaptive mechanisms in water-responsive MS

Rice plants have evolved various adaptive mechanisms to cope with water stress and maintain MS. One such mechanism involves the regulation of reactive oxygen species (ROS) scavenging. The gene *OsPP18*, regulated by the TF *SNAC1*, enhances drought and oxidative stress tolerance by modulating ROS homeostasis through ABA-independent pathways (You et al., 2014). Additionally, the overexpression of *OsAL5* has been shown to improve drought tolerance by regulating both drought-related genes and MS genes, suggesting a potential strategy for breeding drought-tolerant rice varieties with stable MS (Wen et al., 2021). These adaptive responses highlight the complex interplay between water stress and male sterility gene regulation in rice.

4.5 Impact of humidity on HGMS lines

HGMS is a new type of EGMS discovered in rice recent year, which normally shows fertility under high humidity and sterility under low humidity. For instance, the spontaneous sterile mutant from the *japonica* rice cultivar ZH11, has defects in the structure of the pollen wall, and the pollen is prone to dehydration and inactivation in low humidity environments, but remains viable in high humidity environments (Xue et al., 2018; Chen et al., 2020). In the natural field conditions of Guangzhou, China (24°C~35°C, 50%~90% RH), this mutant showed a significantly reduced seed-setting rate (c. 10%) compared with that of ZH11 (Chen et al., 2020). Xue et al. (2018) demonstrate that deficiency of a triterpene pathway results in HGMS in rice, *OsOSC12/OsPTS1* encodes a triterpene synthase, which affects the biosynthesis of C16 and C18 fatty acids in tryphine and regulates HGMS in rice. Another study reveals the molecular mechanism by which the rice *HMS1* and *HMS1I* genes interact to regulate the synthesis of very-long-chain fatty acids and the formation of the oil layer in the pollen wall, thereby controlling HGMS (Figure 4) (Chen et al., 2020).

5 Cross-Talk Between Environmental Factors

5.1 Interaction between temperature and light in gene regulation

The interaction between temperature and light plays a crucial role in the regulation of MS genes in rice. P/TGMS rice lines, such as *Peiai64S* (*PA64S*), exhibit fertility changes in response to varying temperature and light conditions. Studies have shown that the expression of microRNAs (miRNAs) and their target genes, which are involved in fatty acid metabolism and phenylalanine metabolism, are significantly influenced by temperature changes under long light conditions. Specifically, miR156, miR5488, and miR399 have been identified as key regulators affecting male fertility by influencing SPLs, lignin synthesis of anther walls, and the flavonoid metabolism pathway (Sun et al., 2021b). Additionally, high temperatures have been found to disrupt the function of certain tapetal genes, which are essential for pollen adhesion and germination on the stigma, further highlighting the complex interplay between temperature and light in gene regulation (Endo et al., 2009).

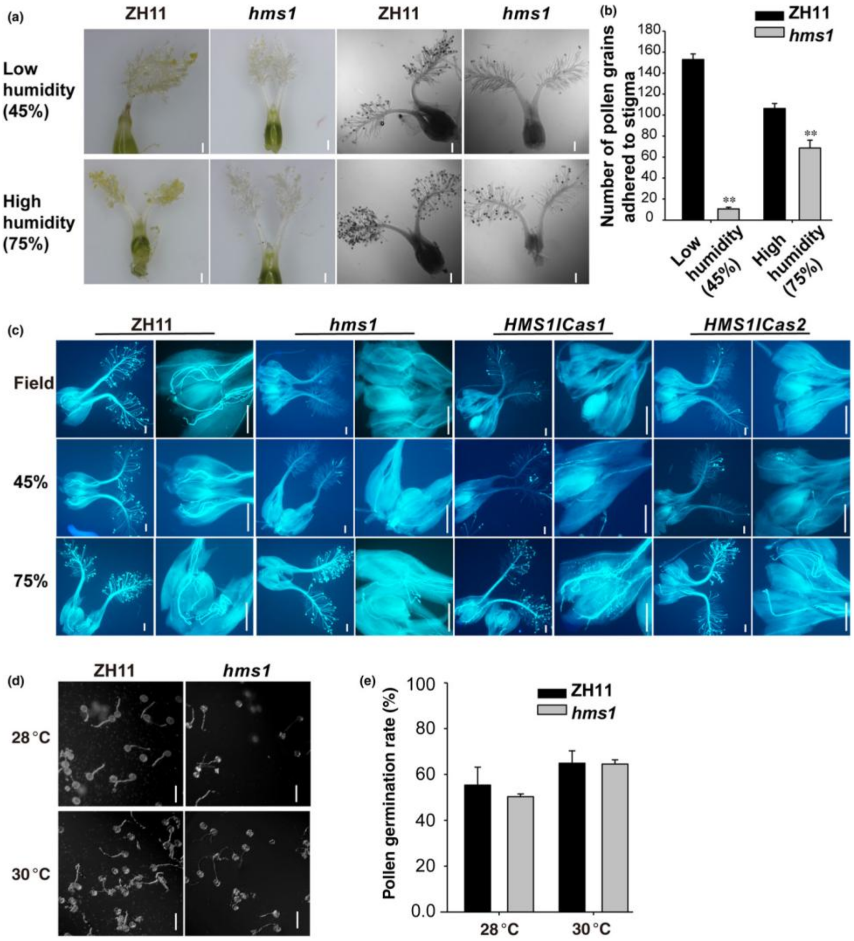

Figure 4 The adhesion and germination of rice (*Oryza sativa*) Zhonghua11 (ZH11), *humidity-sensitive genic male sterility 1* (*hms1*) and *HMS1- INTERACTING PROTEIN (HMS1I)*Cas pollen grains under different humidity conditions (Adopted from Chen et al., 2020).

Image caption: (a) The adhesion of ZH11 and hms1 pollen grains on their own stigmas under different humidity conditions. (b) Number of pollen grains adhered to their own stigmas of the ZH11 and *hms1* plants under different humidity conditions. (c) The germination of ZH11, *hms1* and *HMS1I*Cas pollen grains on their own stigmas under different humidity conditions. (d) *In vitro* germination of ZH11 and *hms1* pollen grains under different temperatures. (e) In vitro germination rates of ZH11 and *hms1* pollen grains under different temperatures. Bars: (a, c) 0.5 mm; (d) 100 lm. Error bars indicate SD (n = 5) (two-tailed Student's t-test; **, P < 0.01) (Adopted from Chen et al., 2020)

5.2 Combined effects of water and temperature on MS

The combined effects of water and temperature on MS in rice are significant, particularly in the context of drought stress and TGMS. The TF *OsAlfin like 5* (*OsAL5*) has been identified as a key regulator linking drought stress response and TGMS. Overexpression of *OsAL5* in rice plants results in increased sensitivity to temperature changes, with plants becoming sterile under high temperatures (28°C) and fertile under low temperatures (23°C). Moreover, these *OsAL5* overexpression plants exhibit higher survival rates under drought stress compared to wild-type plants, indicating that *OsAL5* plays a dual role in regulating both drought tolerance and MS (Wen et al., 2021). Additionally, cooling water before panicle initiation has been shown to increase chilling-induced MS and disable the expression of stress-responsive genes, such as *OsFKBP65* and heat shock proteins, in rice spikelets, further demonstrating the impact of water and temperature interactions on MS (Suzuki et al., 2015).

5.3 Integrative model of environmental regulation of MS genes

An integrative model of environmental regulation of MS genes in rice involves the interaction of temperature, light, and water stress. High temperatures have been shown to cause MS by altering gene expression during pollen development, with specific genes being repressed in the anther under high-temperature conditions (Endo et al., 2009). Additionally, the expression of critical genes and the accumulation of metabolites in the metabolism of sugar, lipid, and phenylpropanoid are essential for MS formation, as demonstrated by transcriptomic and metabolomic analyses (Sun et al., 2022). The regulatory mechanisms of MS also involve miRNAs and their target genes, which respond to temperature changes and influence various metabolic pathways (Sun et al., 2021b). Furthermore, the TF *OsAL5* links drought stress response and TGMS, highlighting the complex interactions between environmental factors in regulating MS genes (Wen et al., 2021).

5.4 Implications for breeding and crop management

Understanding the regulatory mechanisms of temperature, light, and water or humidity on the expression of MS genes in rice has significant implications for breeding and crop management. Breeding programs can focus on developing genetically diverse TGMS lines with critical sterility-inducing temperatures that are not affected by sudden interruptions with lower temperatures, ensuring stable MS under varying environmental conditions (Viraktamath and Virmani, 2001). Additionally, manipulating the expression of key regulatory genes, such as *OsAL5*, can enhance drought tolerance and improve the resilience of TGMS lines to temperature fluctuations (Wen et al., 2021). Integrating knowledge of miRNA-mediated regulation and metabolic pathways involved in MS can further aid in the development of rice varieties with optimized fertility and stress tolerance (Sun et al., 2021b). Overall, a comprehensive understanding of the cross-talk between environmental factors and MS genes will enable the development of more robust and high-yielding rice cultivars, contributing to food security in the face of climate change.

6 Research Methods for the Regulation of MS Gene Expression

6.1 Techniques for studying gene expression in response to environmental factors

To understand the regulatory mechanisms of temperature, light, and water or humidity on the expression of MS genes in rice, various techniques are employed to study gene expression in response to these environmental factors. One such approach is the use of Environmental Gene Regulatory Influence Networks (EGRINs), which integrate multiple genome-scale measurements to uncover how gene expression is coordinated in response to environmental signals. EGRINs have been used to study the response of rice cultivars to high temperatures, water deficit, and agricultural field conditions by integrating time-series transcriptome data, patterns of nucleosome-free chromatin, and the occurrence of known cis-regulatory elements (Wilkins et al., 2016).

6.2 High-throughput sequencing and transcriptomics

High-throughput sequencing and transcriptomics are pivotal in identifying and quantifying gene expression changes under different environmental conditions. For instance, transcriptome analysis of anther development stages in maize has identified numerous TF genes that are differentially expressed, providing insights into the regulatory pathways underlying MS (Jiang et al., 2021). Similarly, proteomic analysis using isobaric tags for relative and absolute quantification (iTRAQ) has been employed to study the proteomic changes in TGMS rice lines, revealing differentially expressed proteins involved in various biosynthetic and metabolic processes (Fang et al., 2022).

6.3 CRISPR-Cas9 and other gene editing tools

CRISPR-Cas9 and other gene editing tools have revolutionized the study of gene function by enabling precise modifications in the genome. In rice, CRISPR/Cas9 has been used to create novel mutants of the TGMS gene *tms5*, resulting in lines with specific mutations that exhibit complete MS at high temperatures and restored fertility at lower temperatures (Fang et al., 2022). Additionally, CRISPR interference (CRISPRi) provides a method for sequence-specific control of gene expression, allowing for targeted silencing of transcription and facilitating the study of gene functions and regulatory systems (Larson et al., 2013).

6.4 Field trials and phenotypic analyses

Field trials and phenotypic analyses are essential for validating the effects of genetic modifications and understanding the practical implications of gene expression changes under natural environmental conditions. For example, CRISPR/Cas9-mediated gene mutagenesis in maize and rice has been followed by phenotypic and cytological analyses to identify new *GMS* genes and understand their roles in anther and pollen development (Jiang et al., 2021; Chen et al., 2020; Yan et al., 2024). These field trials help in assessing the stability and effectiveness of the genetic modifications in real-world agricultural settings. By integrating these experimental approaches and methodologies, researchers can gain a comprehensive understanding of how environmental factors regulate the expression of male sterility genes in rice, ultimately aiding in the development of improved crop varieties.

7 Future Directions and Perspectives

7.1 Potential for developing climate-resilient MS lines

The development of climate-resilient MS lines in rice is crucial for ensuring stable two-line hybrid seed production under varying environmental conditions. Research has shown that the regulation of MS in rice is highly sensitive to temperature, photoperiod, and humidity changes. For instance, the identification of miRNAs such as miR156, miR5488, and miR399, which influence male fertility by affecting SPLs, lignin synthesis, and flavonoid metabolism, provides a new understanding of the regulatory mechanisms involved (Sun et al., 2021b). Additionally, the discovery of TGMS loci and their associated regulatory mechanisms offers potential targets for breeding thermo-tolerant rice species without yield penalties (Kan and Lin, 2021; Lee et al., 2005; Liu et al., 2010; Zhang et al., 2022; Lin et al., 2023). Future research should focus on identifying and characterizing more such loci and understanding their interactions with environmental factors to develop robust MS lines.

7.2 Advances in precision breeding and genetic engineering

Advances in precision breeding and genetic engineering hold significant promise for the development of MS lines in rice. Techniques such as CRISPR/Cas9 can be employed to edit specific genes involved in MS regulation, such as the *p/tms12-1* locus, which has been shown to control P/TGMS through a point mutation in a noncoding RNA (Zhou et al., 2012; Ding et al., 2012). Moreover, transcriptome and metabolome analyses have revealed that genes involved in sugar, lipid, and phenylpropanoid metabolism are essential for male fertility, providing additional targets for genetic manipulation (Sun et al., 2022). By leveraging these advanced techniques, it is possible to create rice lines with precise and stable MS traits, enhancing the efficiency of hybrid seed production.

7.3 Importance of multi-disciplinary approaches

The complexity of MS regulation in rice necessitates a multi-disciplinary approach that integrates genetics, molecular biology, environmental science, and breeding strategies. For example, the cooperative regulation of major-effect sterile genes with photoperiod-,,temperature-, and humidity-sensitive genes, as well as the role of minor-effect genes in determining the critical temperature of sterility transition, highlights the need for a comprehensive understanding of the genetic and environmental interactions (Chen et al., 2010; Ding et al., 2012; Yan et al., 2024; Chen et al., 2020). Additionally, the integration of transcriptomic, metabolomic, and electron microscopy analyses has provided valuable insights into the molecular and physiological processes underlying MS (Sun et al., 2022). Collaborative efforts across these disciplines will be essential for developing innovative solutions to the challenges posed by environmental variability.

7.4 Anticipated challenges and solutions

Several challenges are anticipated in the development and application of MS lines in rice. One major challenge is the variability in environmental conditions, which can affect the expression of MS genes and compromise hybrid seed production. For instance, the response of TGMS lines to varying temperature situations has been shown to be influenced by the genetic background of the recipient lines and the duration of temperature exposure (Viraktamath and Virmani, 2001; Lin et al., 2023; Yan et al., 2024). To address this, hybrid rice breeders need to develop genetically diverse TGMS lines with critical sterility-inducing temperatures that are resilient to sudden temperature fluctuations. Another challenge is the potential yield penalties associated with breeding thermo-tolerant rice species. Future research should focus on identifying natural alleles and employing transgenic editing to develop thermo-tolerant lines without compromising yield (Kan and Lin, 2021). By addressing these challenges through targeted research and breeding strategies, it will be possible to enhance the stability and productivity of two-line hybrid rice systems.

8 Concluding Remarks

This review has elucidated the intricate regulatory mechanisms by which temperature, light, and water or humidity influence the expression of MS genes in rice. High temperatures have been shown to significantly impact MS by altering gene expression and metabolic pathways essential for pollen development. For instance, heat stress affects membrane fluidity, heat shock proteins, and reactive oxygen species (ROS) scavenging, which are crucial for maintaining cellular homeostasis during stress conditions. Additionally, EGRINs have been identified as key players in coordinating gene expression in response to environmental signals, including high temperatures and water deficit. PGMS lines have been found to rely heavily on circadian clock components and sugar metabolism for fertility regulation under varying day lengths. Similarly, P/TGMS lines exhibit differential gene expression in response to environmental changes, with significant roles played by miRNAs and various metabolic pathways involving lipids and phenylpropanoids etc.

Understanding the environmental regulation of MS genes in rice is paramount for sustainable agriculture. The ability to manipulate these regulatory mechanisms can lead to the development of more resilient rice varieties that can withstand the adverse effects of climate change, such as increased temperatures and water scarcity. For example, breeding thermo-tolerant rice species without yield penalties through natural allele mining and transgenic editing holds promise for future agricultural practices. Moreover, the insights gained from EGRINs and the role of circadian clocks in PGMS lines can be leveraged to optimize hybrid seed production, ensuring food security.

In conclusion, the regulatory mechanisms of temperature, light, and water or humidity on the expression of MS genes in rice are complex and multifaceted. Future research should focus on further elucidating these mechanisms at the molecular level, particularly the roles of noncoding RNAs, TFs, and metabolic pathways. Additionally, the development of more stable hybrid rice varieties through advanced breeding techniques, such as apomixis, could revolutionize seed production and enhance crop yields. Continued exploration of these regulatory networks will not only contribute to our fundamental understanding of plant biology but also pave the way for innovative solutions to the challenges posed by global climate change.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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