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Molecular Mechanisms and Genetic Insights into Thermo-Sensitive Genic Male Sterility (TGMS) in Rice: A Mini Review

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Among hybrid seed production, rice is among most successful crops. The two-line hybrid rice system has several advantages as compared with conventional three-line system one of the most important is eradicating the need of restorer gene and maintainer line. Thermosensitive genic male sterility (TGMS), in rice is regulated by temperature i.e. sterile at high temperatures (>29 °C) but becomes fertile at lower temperatures (<24 °C). The TGMS plants can serve as both male-sterile and maintainer lines in this hybrid rice production. For better understanding TGMS system in rice breeding, here we summarized the current information related to identification and mapping of TGMS genes in rice, including linked markers, chromosomes and involved molecular mechanisms. Further, research gaps and possible future directions for applying TGMS genes in rice hybrid breeding are discussed

Keywords: Thermo-sensitive genic male sterility (TGMS), Hybrid Rice, TGMS Gene regulation, Gene expression profiling

INTRODUCTION

Rice has been cultivated for more than ten years and it is substantial in carbohydrates, low in fat, and rich in nutrients. It is chief food in more than 110 countries. Rising rice consumption is correlated with growing populations [1]. Pakistan is ranked 10th in the world for rice production and accounting for 10% of the cultivated land, 3.1% of agricultural value added, and 0.6% of GDP [2].

Hybrid rice production is obligatory to increase crop productivity and improve food security [3]. The first filial generation of a cross between two genetically diverse parents is referred to as a "hybrid" [4]. It is easy to gain a production increase of 10% by cultivating hybrid rice instead of the common rice varieties [5]**.**

The two-line hybrid rice system reveals several benefits upon comparing with the conventional three-line system. It utilizes male sterility that responds to temperature and photoperiod among other environmental variables This technique improves the breeding process and increases the economy and productivity of seed production by eradicating the need for restorer gene and maintainer line. Hybrid rice production by use of thermosensitive genic male sterility in two line breeding system is simpler and cost effective as compared to the traditional three-line system [5].

There are several types of male sterility, including cytoplasmic male sterility (CMS) and genic male sterility as: photoperiod-sensitive genic male sterility (PGMS), and thermosensitive genic male sterility (TGMS), along with other forms of genic male sterility [6].

The sterility of TGMS rice is regulated by temperature. It remains sterile at high temperatures (>29 °C) but becomes fertile at lower temperatures (<24 °C). This characteristics allows TGMS plants to serve as both male-sterile and maintainer lines in hybrid rice production [5]. TGMS lines have been developed from various indica varieties, showing temperature-regulated male sterility [7]. The first TGMS mutant was reported in 1986 from a CMS restorer line 5460. Growth chamber testing revealed that 5460S was fertile at low temperatures but became sterile at high ones [8].

To advance the understanding and TGMS applications in rice breeding, this review purposes to: 1) Assess the present knowledge on the identification and mapping of TGMS genes in rice, including linked markers and chromosomes; 2) Investigate molecular mechanisms regulating TGMS in rice; and 3) Identify research gaps and suggest future directions for studying TGMS genes and their applications in crop breeding.

Genetic analysis and mapping of TGMS genes:

The study on thermo-sensitive genic male sterility (TGMS) in rice has resulted in the identification and mapping of various vital TGMS genes across various chromosomes [9]. Many scientists have investigated the genetic basis of TGMS lines and discovered a number of genes accountable for male sterility, including TMS1, TMS-2, TMS3, TMS4, TMS4(t), TMS5, TMS6, TMS6-1, TMS6-2, TMS6(t), TMS8, TMS9-1, and RTMS-10 [10]. For instance, the tms1 gene is linked to Chromosome 8 through RAPD markers, while tms2 is associated with Chromosome 7, identified by microsatellite markers. The tms4 gene is located on Chromosomes 2 and 8, with several AFLP and microsatellite markers contributing to its mapping. tms3(t), mapped to Chromosome 6, was studied using RAPD markers in a specific TGMS mutant line. tms5 is situated on Chromosome 2, between STS and CAPS markers. The tms6 gene, found on Chromosome 5, is associated with markers RM3351 and E60663. Other genes like tms6-1 and tms6-2 have been mapped to Chromosomes 3 and 5, respectively, with specific RAPD and microsatellite markers. The tms8 gene on Chromosome 11 is linked with a SCAR marker, while tms9-1 on Chromosome 9 is flanked by several SSR markers. Finally, RTMS10 on Chromosome 10 has been characterized with SSR markers, revealing its linkage between specific markers. These mappings are crucial for enhancing hybrid rice breeding and understanding the genetic foundation of TGMS. If TGMS genes are cloned and transported into other crops, it will significantly advance both theoretical research and practical applications in hybrid rice production. However, chromosomal mapping is essential before cloning these genes. The following table highlights TGMS genes and their linked markers and chromosome [5].

Table 1: **Chromosomal locations, linked markers, TGMS genes, and parent lines for rice thermo-sensitive genic male sterility (TGMS)**

Parental line	TGMS gene	Chromosome no.	Linked marker	Reference
5460S	tms1	Chromosome 8	RAPD marker: 1.2-Kb product of OPB 19	[11] [12]
Norin PL12 and KDML 105	tms2	Chromosome 7	Microsatellite markers: RM2, RM10, RM11, and RM214)	[13]
TGMS indica mutant: TGMS–VN1 and a fertile indica line: CH1	tms4(t)	Chromosome 2	Microsatellite marker: RM27 AFLP Markers: E2/M5–600, E3/M16–400, E5/M12–600, and E5/M12–200	[13]
Mutant SA2	tms4	Chromosome 8	AFLP marker: EAA/MCAG SSR marker RM257	[12]
IR32364TGMS and IR68	tms3(t)	Chromosome 6	RAPD markers: OPF18$_{2600}$, OPB19$_{750}$, OPAA7$_{550}$, and OPAC3$_{640}$	[14]
AnnongS-1 and Nanjing11	tms5	Chromosome 2	STS marker C365-1 and CAPs marker G227-1	[15]
Sokcho-MS and an *indica* variety Neda	tms6	Chromosome 5	RM3351 and E60663	[16]
Somatic mutant 0A15-1	tms6-1	Chromosome 3	RAPD marker S187-770	[12]
Mutant Sokcho-MS	tms6-2	Chromosome 5	RM335 and E60663	[12]
G20S and Jing226 UPRI 95 and 140TGMS	tms6(t)	Chromosome 10 Chromosome 7	SSR markers: RM3152 and RM4455	[17] [12]
F61 and IR 58025B	Tms8	Chromosome 11	SCAR marker (0.8 cM)	[12]
Hengnong S-1 Minghui63, T92, T95, R96, R98) TGMS Lines: C815S, Y58S, Zhu1S	tms9-1 gene	Chromosome 9	RM24413 and RM24537	[18]
YannongS (YnS) and L422	RTMS10 locus	Chromosome 10	ID11087 and RM25292.	[19]

Molecular mechanisms of TGMS genes regulation:

Temperature-Sensitive Splicing

Temperature-sensitive splicing is significant post-transcriptional molecular regulation mechanism of thermosensitive genic male sterility (TGMS) in rice. It modifies gene expression and effects development and differentiation. Cis splice sites, which are critical for the precise recognition of intron-exon boundaries; small nuclear RNAs (snRNAs), which are vital components of the spliceosome; trans pre-mRNA splicing proteins, which enable the splicing process; and SR proteins, which are involved in both the recognition of splice sites and the regulation of splicing efficiency are important components of temperature-sensitive splicing. Temperature fluctuations may have impact on these splicing factors by impacting the stability and functionality of the spliceosome and associated proteins. Understanding these interactions is essential for elucidating the molecular mechanisms behind TGMS [6].

Regulation of TGMS by CSIT2

In TGMS rice, CSIT2, an E3 ubiquitin ligase, is critical for governing the crucial sterility-inducing temperature (CSIT). This gene is essential for ribosome-associated protein quality control (RQC), which involves the degradation of misfolded and ribosomal proteins by ubiquitinating them. CSIT2 prevents the accumulation of harmful reactive oxygen species (ROS) by regulating translational efficiency and protein quality. The partial restoration of male fertility and increase in levels of protective proteins caused by mutation in CSIT2. The molecular mechanisms underlying TGMS in rice can be better understood by analyzing CSAIT2 role in regulating protein stability and maintain CSIT [20].

Regulation of TGMS by RNase ZS1

The TMS5 gene, which encodes the RNase ZS1 protein, plays a critical role in temperature-sensitive male sterility (TGMS) in rice. A mutation in TMS5 results in a nonfunctional RNase ZS1, which affects the processing of specific mRNAs from the ubiquitin-60S ribosomal protein L40 family. While RNase ZS1 itself is not affected by temperature, its inability to process these mRNAs leads to their accumulation at high temperatures, causing male sterility. This insight is valuable for developing male-sterile lines in hybrid rice breeding and understanding plant male fertility [10].

Epigenetic Regulation:

Epigenetic modifications, particularly DNA methylation, is essential in plant development. The study of DNA methylome of P/TGMS rice under varying day lengths and temperatures show the involvement of mechanisms such as oxidative methylation, photosynthesis, and circadian rhythm in the fertility transition [21].

Future Prospects:

Despite tremendous progress in understanding the molecular mechanisms of TGMS, many gaps remain in our knowledge. Ku et al. (2003) reported that the male sterility of TGMS is related with the early programmed cell death of the tapetum in rice. UDP-glucose pyrophosphorylase1 is vital for callose deposition during pollen wall development, and its suppression in plants led to a TGMS phenotype. Furthermore, temperature-sensitive splicing has been proposed as an important regulatory mechanism of TGMS in rice. These studies suggest that TGMS is controlled by various molecular mechanisms. However, little is known about the exact molecular pathways involved [18]. To date, none of the TGMS genes have been cloned [11]. There is need to identify and characterize novel TGMS genes across different rice verities and wild relatives to advance hybrid rice breeding. Despite the advances made in the study of DNA methylation in P/TGMS in rice, there is a need to learn more about the role of epigenetic regulations [21]. Addressing these gaps presents an auspicious avenue for future research.

CONCLUSION

The exploration of thermo-sensitive genic male sterility (TGMS) in rice has provided significant insights into its genetic and molecular mechanisms. Studies have identified and mapped various TGMS genes, revealing their critical roles in male sterility and fertility transitions. Key molecular mechanisms include temperature-sensitive splicing, regulation by CSIT2 and RNase ZS1, and epigenetic modifications like DNA methylation. Despite these advancements, there are still substantial gaps in our understanding of the exact molecular pathways and the full range of regulatory mechanisms involved in TGMS. Notably, the cloning of TGMS genes remains a significant challenge. Future research should focus on identifying and characterizing novel TGMS genes across diverse rice varieties and wild relatives, and further investigating the role of epigenetic regulations. Addressing these research gaps will enhance our knowledge of TGMS and support the development of more effective hybrid rice breeding strategies, ultimately contributing to improved crop productivity and food security.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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