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REVIEW ARTICLE

Molecular regulation and breeding prospects of second-generation hybrid rice

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Abstract

Second-generation hybrid rice breeding, using two-line systems also called Environment-sensitive Genic Male Sterility (EGMS), represents a significant advancement in hybrid rice. By eliminating the need for a maintainer line, this method simplifies hybrid seed production and reduces associated costs, thereby offering promising solutions for yield improvement and food security. EGMS encompasses various systems, including Temperature-sensitive (TGMS), Reverse TGMS (RTGMS), Photoperiod-sensitive (PGMS), Photoperiod and Temperature-sensitive (PTGMS) and Humidity-sensitive (HGMS), each of which is regulated by specific environmental cues. This review highlights the evolution, genes associated with and classification of EGMS systems, as well as their roles in hybrid rice production. The different methods of production and selection of location for the EGMS lines are also discussed. Advances in biotechnology, such as CRISPR/Cas9 gene editing, mutation breeding and speed breeding, have significantly improved the stability, adaptability and faster development of EGMS lines. Furthermore, gene pyramiding and marker-assisted selection (MAS) enhance trait integration, facilitating the creation of hybrids with superior agronomic traits, disease resistance and environmental stress tolerance. India's contributions in two-line breeding, especially the development of several TGMS lines suitable for regional climatic conditions, demonstrate the potential of TGMS in hybrid rice technology. This will help meet the demands of a growing population while enhancing climate resilience.

Keywords: breeding methods; classification; CRISPR/Cas9; evolution; TGMS in India; two-line breeding

Introduction

The population of the world is predicted to increase at a rate of 2.3 billion people from 2009 to 2050, reaching 9.1 billion. More than half of this population, especially in South-Eastern Asia, relies on rice for their staple consumption. Rice is cultivated on more than 160 million hectares worldwide, with annual global production exceeding 750 million metric tons of paddy rice, as reported by the Food and Agriculture Organization in 2023. Asia dominates global rice production, accounting for over 90 % of both area and output, with China and India being the top producers. Despite changes in people's eating habits over time, there is an increasing demand for rice and its byproducts. One of the most effective strategies to enhance yield potential has been the development and adoption of hybrid rice, which exploits heterosis or hybrid vigour to produce superior-performing varieties. Thus, hybrid rice has a significant role in increasing yield potential and ensuring food security. Research on hybrid rice began in China during the early 1970s, led by Yuan Long Ping and later spread to India (1), Philippines and other tropical countries (2). Hybrid rice technology is crucial, particularly in tropical areas with limited land and water resources, to fulfil the rising demands of food. According to FAO, rice hybrids yield 20 % more than cultivated varieties. It also offers a faster growth rate, better agronomic and floral traits, multiple resistance and adaptability, which have expanded hybrid rice to cover over half of the total rice-growing areas (3).

Rice is a self-fertilizing, short-day plant that requires male -sterile lines (MS) to produce hybrid seeds in the breeding program. Rice hybrids are broadly developed using either the three-line or the two-line breeding systems. The most used male sterility system, followed by the three-line breeding (cytoplasmic male sterility - CMS) system, is two-line breeding/environmental sensitive genetic male sterility (EGMS) (4). Recently, genetic engineering (5) and apomictic methods (6) have also gained attention to produce MS lines. The three-line system involves a cytoplasmic male sterile (CMS) line, a maintainer line and a restorer line. MS lines from CMS result from the interaction of the nuclear and cytoplasmic genes, making them sterile (S) lines, which are stable. While effective, this method is limited by the complex process of maintaining sterility and restoring fertility. In contrast, EGMS is a cause of mutated nuclear genes that are sensitive to environmental changes. These lines require specific environmental conditions to produce MS lines. When the environment is favorable, EGMS lines have several advantages over CMS lines; it does not require a maintainer line and can cross with a wide range of genotypes as restorers. The two-line hybrid system marks a new generation of hybrid rice called the second-generation hybrid rice. Sterility in EGMS lines occurs during the early reproductive stage/booting stage of plant due to the loss of viability of pollen/pollen abortion (7), even with a normal pistil. They can revert to normal fertile plants under a fertility-favoring environment due to the suppression of sterility-inducing genes. This flexibility simplifies hybrid seed production, enhances the exploitation of heterosis and broadens the genetic base for hybrid combinations (8). These MS plants can be effectively used as 'S' lines in the development of two-line hybrids by crossing with a desirable pollen parent. Fig. 1 illustrates the development of a two-line hybrid using the EGMS system.

For the successful development of two-line hybrids and multiplication of seeds, it is essential to understand environmental cues like temperature, light hours, humidity, etc., which have a direct effect on sterility expression. Several genes responsible for sterility, induced by the environment, have been discovered, characterized and successfully exploited in EGMS hybrid production. However, the actual molecular and physiological mechanisms have not been achieved. Advances in molecular biology, genomics and gene editing technologies will greatly accelerate the identification and manipulation of such genes, allowing for the precise development of stable and efficient EGMS lines. Techniques like CRISPR/Cas9 have been successfully applied to edit the genes responsible for sterility (9) to create new TGMS lines. Additionally, molecular marker-assisted selection (MAS), transcriptomic profiling and genome-wide association studies (GWAS) will enhance the understanding of the genetic networks underlying male sterility. Integration of these biotechnological tools with conventional breeding can significantly lead to a new era of two-line hybrid rice systems. This review highlights the genetic control, advances in EGMS breeding and its breeding prospects to understand its scope for future research.

The classification of the EGMS system in rice

The classification of EGMS lines in rice is primarily based on the environmental factors that trigger male sterility. EGMS lines are crucial for two-line hybrid rice breeding systems, as they eliminate the need for a maintainer line. The types of EGMS are photoperiod-sensitive genic male sterile (PGMS) lines, reverse PGMS lines, thermo-sensitive genic male sterile (TGMS) lines, Reverse TGMS lines, humidity-sensitive genic male sterility (HGMS) and photo-thermo-sensitive genic male sterile (PTGMS) lines. Selection and usage of a particular type depend on the environmental conditions of the region. This classification aids breeders in selecting suitable EGMS lines according to the specific environmental conditions of their target cultivation regions, ultimately enhancing the efficiency and adaptability of hybrid rice production. Fig. 2 gives the classification of the EGMS system in rice.

Temperature-sensitive genic male sterility (TGMS)

Temperature changes influence the sterility/fertility phase in this type. At low temperatures, the pollen is fertile and shows sterility at higher temperatures. This makes seed production in TGMS lines possible only in low-temperature regions. Generally, the TGMS lines remain sterile at a day/night temperature of >30 °C/ >24 °C and they can revert to fertility at a day/night temperature of <24 °C/>18 °C (10). The first stable TGMS line, AnnongS-1, was a spontaneous mutant (11) derived from the F_3 population of Chao -40 and shows complete pollen sterility at 33-37 °C (12). The genes for the TGMS trait are expressed differently at various temperatures.

The anthers that produce pollen develop normally up to a certain temperature threshold. When the temperature exceeds this critical threshold, the TGMS genes are activated. The temperature above which the pollen becomes sterile is known as the critical sterility point (CSP) (13) and it varies for each TGMS

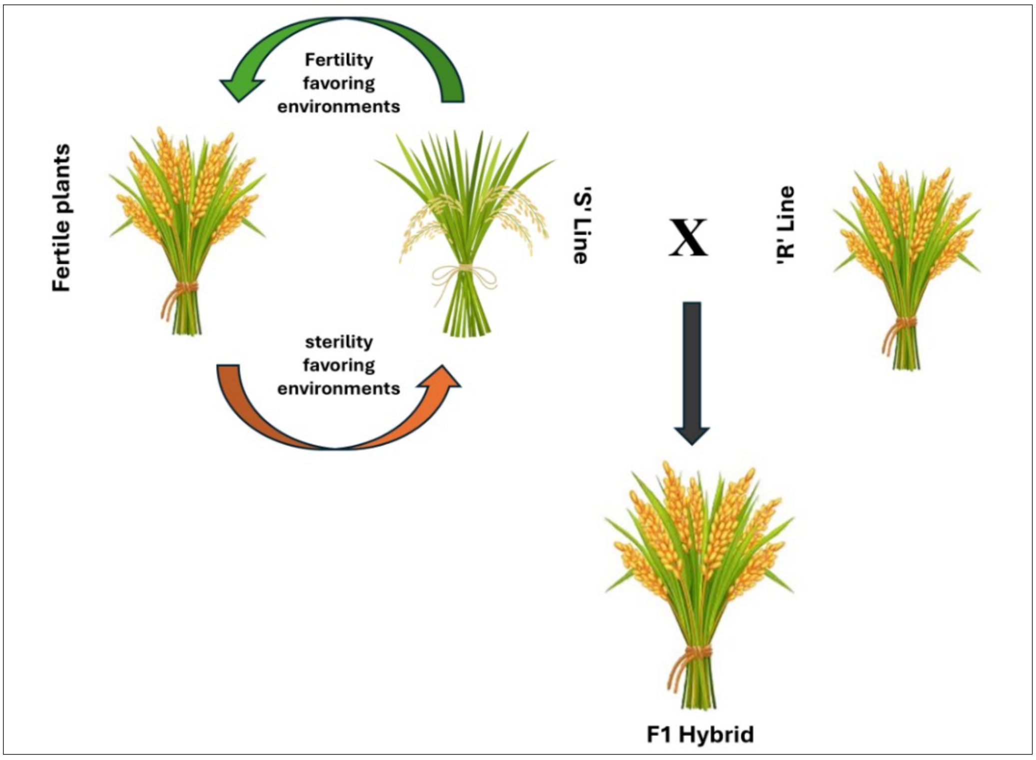

Fig. 1. The development of a two-line hybrid using the EGMS system.

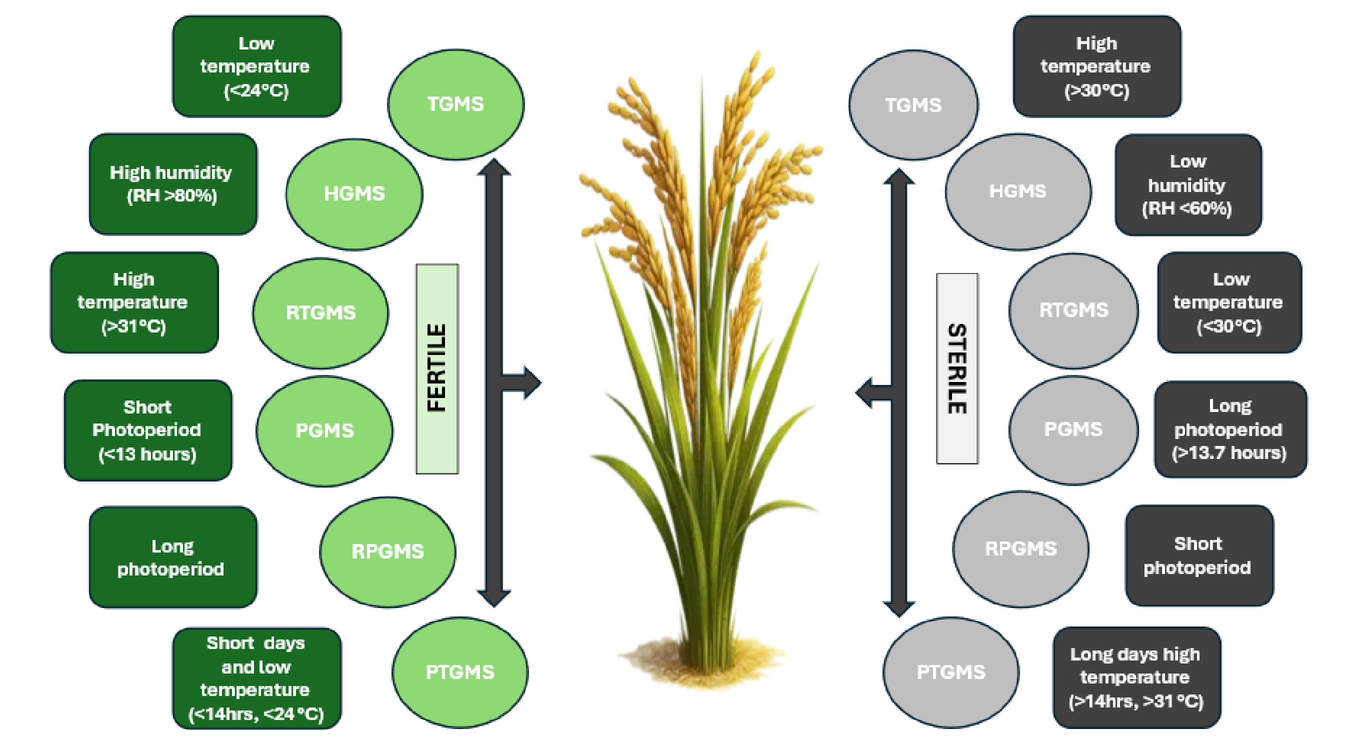

Fig. 2. Classification of the EGMS system in rice.

line. The gene responsible for sterility was found to be recessive and sterility occurs due to the abnormal chromosome segregation disrupting normal meiosis. This leads to non-functional pollen grains or pollen abortion. Increased temperatures also harm functional tapetal cells (14, 15). These cells are generally involved in pollen adherence and providing nutrients for germination. In India, TGMS lines were developed at IRRI by transferring the sterility gene from *japonica* TGMS mutant Norin PL 12 into *indica* lines (16). The varied agro-climatic conditions of India make it a prominent region for the development of TGMS hybrids.

Reverse temperature sensitive genic male sterility (RTGMS)

Few TGMS lines showed sterility at lower temperatures, which paved the way to discover the RTGMS lines of rice. RTGMS is the reverse of the TGMS lines, wherein the plants are sterile under low temperatures and fertile in high temperatures (17). The first RTGMS line J207S (18) developed in China showed sterility when the temperature was less than 31 °C and fertility when above 31 °C. This type of MS line can be technically used for hybrid production in low-temperature regions and the multiplication of seeds can be done at higher temperature regimes. But in tropical crops like rice, hybrid production using this type of MS line becomes quite difficult, since it requires a threshold temperature for flowering.

The sterility in the reverse TGMS *japonica* line, DiannongS -1 Xuan, was also found to be controlled by a recessive gene, *rtms1-D*, located on chromosome 10 (19). Another gene, *rtms10*, was mapped on chromosome 10 in YannongS (YnS) (20), which produced abnormal pollens at low temperatures. These genes, in contrast to TGMS in rice, cause pollen abortion at lower temperatures, resulting in sterility of RTGMS lines.

Photoperiod-sensitive genic male sterility (PGMS)

PGMS in rice was discovered in 1973 by Professor Shi Ming Song in Hubei, China. It is the first type of second-generation hybrid discovered where the length of the day has an important impact. It was found that when the day length is greater than 13.75 hr, the lines are sterile and when less than 13 hr, the lines are fertile. PGMS is a condition where the male gametes/pollen become sterile during the long-day conditions and become fertile when under short-day conditions. The first PGMS line identified in rice was from a *japonica* cultivar, Nongken58 and was named Nongken58S in China (21). It shows sterility when the day length is 13.75 to 14.00 hours per day with light intensity of 5 to 50 lux (22) and vice versa. This type of PGMS is referred to as the long-photoperiod sensitive type.

PGMS can also be classified as short-photoperiod sensitive type (reverse PGMS) (23), which is the reverse of the PGMS of rice, where the plant is sterile when under short-day conditions. Nongken58 and Peiai64S are the commercially exploited PGMS lines for hybrid rice breeding. The most critical stage for PGMS lines in rice is the two to three-leaf stage, before heading, the right stage for microsporogenesis. By altering the photoperiod to longer day length conditions, the heading stage (critical fertility point), the PGMS lines would become sterile (24). Defective exine and intine pollen layers were found in the sterile PGMS line ZAU11S106, along with an abnormal and sometimes missing nucleus (25). It is because the functional nucleus carrying the fertility genes is damaged due to the light hour changes. The major loci controlling PGMS in the *japonica* variety are *pms1* (26), *pms2* and *pms4* (27). These genes are widely exploited to produce successful PGMS lines in hybrid rice breeding.

Photo-thermo sensitive genic male sterility (PTGMS)

Both photoperiod and temperature influence this type of EGMS system. The alteration in fertility/sterility is due to the interaction between temperature and photoperiod (22). Temperature is one of the important factors as these lines are completely sterile or fertile in a particular range of temperature, i.e., greater than 30 °C/ less than 24 °C, respectively, even though there is no influence by the photoperiod. However, photoperiod affects the lines between 24 °C and 32 °C, i.e., the longer the photoperiod, the greater the male sterility at a lower temperature and the shorter the photoperiod, the greater the male sterility at high temperatures (12).

In the PGMS mutant NK58S, it was found that the male sterile or fertile conditions were due to the combined influence of temperature and photoperiod (28) and rather than the influence of altered photoperiod. The most sensitive period for PTGMS lines is during the development of the panicle, i.e., from the differentiation of the secondary branch to the PMC formation. In this period, a day length greater than 14 hr and a light intensity of at least 50 lux can cause sterility in NK58S, while normal fertility happens with a day length shorter than 13.75 hr (29). Like the gene *pms3* controlling PGMS in rice (30), a gene *ptgms2-1* was found to control PTGMS in rice line in Guangzhan63S (31). Huhan74S, a new PTGMS line with a critical fertility alteration at 23 °C was developed, which is found to have resistance to drought and rice blast, by integrating blast-resistant genes into Huhan1S (32), hence can be effectively utilized for developing climate-resilient hybrids.

Humidity-sensitive genic male sterility (HGMS)

HGMS is a condition wherein male gamete development is affected by variable humidity conditions, where pollen aborts at a low humidity and remains fertile at a higher humidity. Here, relative humidity (RH) is the prime factor that influences the production of fertile or sterile male gametes (32). This type of MS system was first observed in *Arabidopsis thaliana* in 2003 (34). It is the only method of producing a two-line rice hybrid that is not influenced by the temperature and photoperiodic system (12). Mutant lines show full sterility when RH lies below 60 % and are fertile when RH exceeds 80 % (33). This type of sterility system can be used in regions with RH of less than 60 % (34), where the lines remain MS that can be crossed with a desirable male parent.

The sterility was due to abnormal pollen germination and adherence caused by abnormal trypsin structure. This issue occurs in two HGMS OsCER1Cas lines that result in male sterility (35). The gene *hms1* helped in the synthesis of C26 and C28 very long chain fatty acids that produce bacula and tryptamine in pollen walls. This reduces the pollen adhesion and germination on stigmas, contributing to HGMS conditions (37). Research findings show that mutant with OsGL1-4 affects pollen adhesion and the water retention potential. This will be helpful in the development of the HGMS lines (35) suitable for humidity-favoring regions. The rice lines Zhonghua11 (ZH11) and Nipponbare also exhibit HGMS (35, 37) and OsCER1, OsOSC12 and OsHMS1 are some of the genes that contribute to HGMS conditions (34-36).

Evolution of two-line hybrids

The emergence of EGMS hybrids began in 1973 when a spontaneous mutant NK58S was identified in segregating generations of a *japonica* rice variety Nongken58. Further studies showed that NK58S was thermo-sensitive in addition to photosensitivity, which led to the discovery of TGMS lines in rice. The *japonica* two-line rice hybrids, 7001S/Xiushui 04 and 7001S/Wanhui, significantly increased rice production in China during the 1980s (38). Eventually, collaborative research to develop the two-line hybrid exploitation of PGMS/TGMS lines was initiated in China in 1987. The TGMS traits in AnnongS-1, a spontaneous mutant of *indica* rice Annong, were later reported.

Extensive research was undertaken in the TGMS line Pei'ai64S (PA64S), which was discovered after introgression and backcrossing of NK64S in an *indica* background (20), which led to the development of hybrids with superior performance. China's contribution to hybrid rice over the past 60 years, advanced two-line hybrid breeding technology and broadened its research prospects through technical extension and commercialization of hybrid rice globally (39). The intensive research on two-line breeding in rice not only improved China's self-sufficiency in food grain production but also helped global food security and future research (37). India, next to China, contributed significantly to the field of research on hybrid rice in the world, using both three-line and two-line hybrids (10). Using modern breeding and molecular techniques has led to the development of many new EGMS sources with great potential. Table 1 shows the timeline of the evolution of EGMS systems in hybrid rice breeding.

Advantages and disadvantages of EGMS

The two-line hybrids show superior performance qualitatively and quantitatively in terms of yield, grain quality, etc. It has been found that it gives 10-20 % higher yield than the inbred varieties (8). Moreover, the hybrid seed multiplication is simple and saves labour costs and breeding cycles (12). In the CMS system, the sterility-inducing cytoplasm may have adverse effects on the hybrids, which leads to the development of undesirable characters. But a second-generation hybrid rice system has no such effects (8) and it does not require a specific restorer line, as it can be easily crossed with almost all rice genotypes. These features lead to increased breeding efficiency, especially for heterosis at the subspecies level of *indica* and *japonica* rice (12). It is an ideal method for the development of *indica/japonica* hybrids where identification of the restorer is difficult.

Any genetic background can inherit the EGMS genes, which broadens the diversity of the female parental lines. Its characters can be explored and exploited under varying climatic conditions (54), especially in favorable environments. Some TGMS lines are also resistant to biotic and abiotic stressors like drought, blast and bacterial blight (48, 49). Similarly, other desirable traits for quality and yield can also be integrated with TGMS traits through introgression or gene pyramiding.

However, long-term maintenance of EGMS lines without controlled conditions may result in instability, potentially leading to reduced sterility expression. These lines are highly sensitive to environmental factors like temperature, photoperiod and humidity; even slight deviations can trigger partial fertility restoration, thereby compromising seed purity (55). With increasing climate variability, unpredictable weather poses a significant risk to EGMS-based systems due to their dependence on precise environmental cues for sterility expression. Despite their utility in simplifying hybrid breeding by eliminating the need for a maintainer line, these limitations are to be considered for improved stability and adaptability in future EGMS lines.

Selection of location

The breeding of EGMS lines and the production of hybrid seeds depend on the location and the weather/climate of the region. For hybrid seed production of a TGMS line, a region/location with a critical fertility temperature (CFT) point is critical. The CFT is the critical temperature during the sensitive stage of a TGMS line, which results in complete/partial fertility. It allows the TGMS line to produce fertile and partially fertile pollen grains. This temperature range ensures optimal flowering and seed development. It allows the TGMS line to produce fertile and partially fertile pollen grains. Identification of such locations plays a major role in successful hybrid seed production in TGMS breeding.

Similarly, the ideal location for self-pollinated TGMS lines/S lines in rice is determined by critical sterility temperature (CST).

Table 1. Timeline of the evolution of two-line breeding in rice

Year	Event	Details	Type of EGMS	Reference
1973	Identification of Nongken58S	Nongken58S was identified in China by Professor Shi Ming Song as a spontaneous mutant derived from the *japonica* rice variety Nongken58	PGMS	(40)
1987	Development AnnongS-1	AnnongS-1 was identified as a spontaneous mutant from the *indica* rice cultivar 'Annong 1'	PTGMS	(41)
1987	Exploitation of two-line system of breeding in rice	A collaborative research project was initiated in China involving the MS lines to develop rice hybrids using two-line hybrid breeding	PGMS/TGMS	(42)
1991	Development of TGMS in Pei'ai64S (PA64S)	PA64S was developed by introgressing the PGMS trait from NK58S into an *indica* rice background, resulting in a line whose sterility is predominantly governed by temperature	TGMS	(38)
1999	Identification of reverse thermo-sensitive genic male sterility (RTGMS) in rice	A spontaneous mutant exhibiting reverse behaviour of TGMS was discovered in a farmer's field which exhibited fertility at temperature >30 °C and sterility at temperature <24 °C	RTGMS	(43)
2001	Recognition of two-line hybrids	Pei'ai64S, won China's National Science and Technology Progress Award in 2001	TGMS	(44)
2003	Fine mapping of the gene *tms5*	The gene responsible for sterility in the TGMS line AnnongS-1, *tms5* was mapped on chromosome 2. STS marker C365-1 and CAPs marker G227-1 were used to map the gene	TGMS	(45)
2004	New TGMS line identification	A new TGMS line, TS6 was identified at Tamil Nadu Agricultural University, Coimbatore and was found to be sterile for 78 consecutive days at 32-35 °C maximum and 22-23 °C minimum temperatures	TGMS	(46)
2005	First gene pyramiding	Using microsatellite markers, three genes, *tms2*, *tms5* and *tgms* were inserted in SA2 by gene pyramiding	TGMS	(47)
2012	Mapping of PTGMS gene (*pms3*)	*PMS3*, a key gene controlling PTGMS, was fine mapped to chromosome 12 in rice	PTGMS	(28)
2014	Establishing *tms5* as the key gene controlling thermo-sensitive genic male sterility (TGMS) in rice	Researchers identified *tms5* codes for a encoding a ribonuclease critical for processing UbL40 mRNAs during pollen development. The mutation in *tms5* results in the accumulation of unprocessed mRNA under high-temperature conditions, leading to male sterility	TGMS	(11)
2014-2022	Integration of EGMS systems with climate-resilient traits	Development of EGMS lines resistant to diseases like blast and false smut and drought thus breeding for climate resilient rice	All	(32, 48-52)
2023	Gene pyramiding to improve rice blast resistance with higher yields	Using NIL-C5S and Chuang5S (C5S) as recurrent parents, three genes (*Pigm* + *Pi48* + *Pi49*) were inserted to develop lines, which showed resistance to rice blast without reduction in yield	P/TGMS	(53)

The CST is the temperature below which these lines changes into fertile from sterile conditions (56). For seed multiplication of TGMS lines, a region with a temperature consistently below the CST of the specific TGMS line during the flowering period is required. Unlike CGMS, seed multiplication in TGMS lines does not require maintainer lines, relying instead on the CST of the location, which is the main advantage. Similarly, locations with suitable day lengths should be selected for the hybrid development using a PGMS line.

Many rice varieties show different temperatures for sterility regulation, especially for *japonica* rice, which has a wider range of sterility temperatures (57). All these differences have led to the exploitation of climate-resilient male sterile lines. The identification of these critical locations helps in characterizing TGMS lines for their morphological, physiological and yield-related traits in both sterility and fertility-favouring environments. For instance, TGMS red rice better suitable to Kerala's climatic conditions was developed through introgression and backcrossing, using a stable TGMS line EC720903, which was imported from the International Rice Research Station (IRRI), Philippines (58). Similarly, some identified TGMS lines were found to have superior and stable performance in Coimbatore and Kerala's environmental conditions (59-61). Genetic diversity and characterization of the TGMS lines under a sterile conducive environment at Coimbatore were reported (62, 63). In Tamil Nadu, which is pioneering in TGMS, Coimbatore, Trichy, Sathyamangalam and Madurai are identified as the best locations for TGMS seed multiplication (10, 63), as these regions have a mean temperature high enough to cause sterility. For now, the hybrid seed multiplication is convenient only in the low temperature zone at Gudalur in Tamil Nadu (64).

The identification of the critical locations also helps in characterizing the EGMS lines for their morphological, physiological and yield-related traits in that location. Like TGMS, PGMS and HGMS in rice also requires regions with a particular critical day length and RH respectively, for its sterility expression. Rice breeding using PGMS is well-suited for temperate countries with significant day length variation and distinct seasons, such as China, India and Japan (64).

Genetic control of EGMS lines

Most of the TGMS lines are influenced by a monogenic recessive allele. The TGMS traits in Norin PL12 and IR32364S were found to be controlled by a recessive allele (65). A single recessive gene (*tms5*) was responsible for the sterility in the first TGMS line, AnnongS and its derivatives, AnnongS-1 and Y58S. The gene was genetically mapped to chromosome 2 (66). Later, using SSR markers, the complete mapping of the *tms5* gene was established (66, 67). Reciprocal crosses to Nongken58S also showed that male sterility may be caused by a single recessive gene (12, 68). However, studies on allelic relationships showed that the TGMS genes of Norin PL12 and the mutants of IR32364S were not the same. Wild rice and landraces serve as a hub for several genes inducing sterility, which can be exploited using modern biotechnological methods. The introgressed lines were used to identify the traits for yield, low-temperature tolerance and drought stress to detect favorable genes from common wild rice (69). Later, the TGMS trait, governed by several genes, was reported.

The identified *tms5* gene is responsible for the production of Ub_{L40}mRNAs (11) expressed in the pollen mother cell (70) and the increased production causes male sterility by producing defective pollen. It was also found that RNA polymerase subunit is directed by DNA, along with HSPs and kinases (71) are also involved in the alteration of fertility in rice. The pollen was not produced when the temperature was greater than 30 °C and at 28 °C abortive pollen was produced in Zheda13S. At this point, there was degeneration of the tapetum and the microspore mother cells, which led to the production of small whitish anthers. In the TGMS-CO27 male sterile line, the TGMS conditions were caused by co-suppression of the ogUgp1 gene (72), which is responsible for UDP glucose pyrophosphorylation. In UPRI 95-140, two non-allelic TGMS genes *tms*6(t) and *tms7*(t) were found to be responsible for sterility, which were mapped on chromosomes 3 and 7, respectively.

TGMS line 5460S, developed in China, has the *tms1* allele on the sixth chromosome, whereas in Norin PL 12, it is designated as *tms2* (73). On studying the allelic relationships of other TGMS lines, a new non-allelic TGMS trait, *tms4*, in SA2, a sodium azide-induced TGMS mutant (74, 75) was discovered. Varying alterations of sterility and fertility were observed on the transfer of the Norin PL 12 gene (*tms2*) into IRRI cultivars such as IR6949S, IR68945S and IR68294S, which indicated that these altered conditions are responsible for changes in the expression of the TGMS trait in various genetic backgrounds (8). Among them, *tms*5 is a key gene regulating thermosensitive sterility in many TGMS lines.

The key genes regulating PGMS and PTGMS in rice include *pms1* and its allelic variant *pms1(t)* on chromosome 7, *ptgms2-1* on chromosome 2 and *pms3* on chromosome 12 (31, 36, 76, 77); these genes are associated with the induction of male sterility under longer day lengths. Another genetic locus conferring the PGMS trait in Mian 9S was mapped on chromosome 4 and is called *pms4* (78). Likewise, genes like *rpms1* and *rpms2* were found to be responsible for the RPGMS trait in rice (79). These genes regulate the expression of certain factors that are specific to the tapetal cells and influence pollen development. Under long photoperiods, epigenetic modifications and inactivation of fertility-restoring genes lead to abnormal microspore development, leading to pollen abortion, ensuring complete male sterility. However, under short photoperiods, the expression of fertility-restoring factors is upregulated, enabling normal anther dehiscence and viable pollen formation in PGMS lines.

Few novel genes controlling RTGMS in rice, namely *rtms1*, *rtms1-D* and *rtms10*, induce sterility at lower temperatures (19, 20, 80). Among them *rtms10* gene in YannongS was found to be semi-dominant, shifting its sterility behaviour with increasing ambient temperature. The region occupied by *rtms1-D* is more specifically defined than *rtms1* and gives a better precision of the gene. However, interestingly, all the genes governing RTGMS in rice were mapped on chromosome 10, which indicates that the trait is localized on that chromosome. Though it was found that the genetic control of HGMS in rice was due to *hms1* (37), the literature available regarding its mechanism and mapping is less. Table 2 gives the important genes associated with EGMS in rice.

Breeding of two-line hybrids

Pedigree selection

Pedigree selection is the most used breeding method for developing EGMS lines. It helps in the identification of a EGMS mutant, which can be utilised for breeding programmes. For example, the temperate *japonica* TGMS mutant Norin PL 12 was one such mutant identified (16) and was utilized for crossing with other rice genotypes (*indica* or tropical *japonica*). In this method, the selection of plants in the segregating population that show EGMS traits under a specific environmental condition is followed by continuous crossing and selection until stable EGMS lines are identified. Further, the identified EGMS lines are characterized in sterility-favoring environments for agro-morphological, molecular and floral traits. The seed multiplication of EGMS lines is taken up under fertility-favoring environments. The stable EGMS lines with good floral traits are used for two-line hybrid seed production. A summary of the steps involved in the process is given in Fig. 3. This method is simple and conventionally used to produce EGMS lines.

Introgression

The introduction of sterility genes can be an alternative method for the integration of the EGMS trait in two-line breeding. A07 is one such TGMS line, which was used as a donor for introgressive hybridization of the *tms5* gene (64) into environmentally adapted materials by backcrossing twice and selection of progenies for the gene *tms5* at

Table 2. Some of the important genes associated with EGMS in rice

Gene	Source	Chromosome	Markers	Trait	Reference
pms1	32001S	7	RG477 and R1807	PGMS	(26)
pms1(t)	Pei'ai64S	7	RM21242 and YF11	PGMS	(76)
pms3	Nongken58S	12	R2708 and C751	PGMS	(77)
pms4	Mian 9S	4	RM6659 and RM1305	PGMS	(78)
ptgms2-1	Guangzhan63S	2	RM12521 and RM12823	P/TGMS	(31)
rpms1	YiD1S	8	RM22980 and RM23017	RPGMS	(79)
rpms2	YiD1S	9	RM23898 and YDS926	RPGMS	(79)
tms1	5460S	8	RM12713 and RM12722	TGMS	(81)
tms2	Norin PL12	7	RM12713 and RM 12722	TGMS	(73, 82)
tms3	IR32364S	6	OFP18 and OPAC3	TGMS	(83, 84)
tms4	TGMS-VN1	2	E2/M5-600, E3/M16-400, E5/M12-600 and E5/M12-200	TGMS	(74)
tms5	AnnongS-1	2	4039-1 and 4039-2		(45, 66, 85)
	103S	2	RM22 and RG257	TGMS	(86)
	TNAU60S	2	RM12713 and RM12722		(81)
tms8	F61	11	RM21 and RM224	TGMS	(87)
tms9	Zhu1S	2	Indel 37 and Indel 57	TGMS	(88)
tms9-1	HengnongS-1	9	dCAPS marker	TGMS	(89)
rtms1	J207S	10	RM239 and RG257	RTGMS	(19)
rtms10	YannongS	10	ID13116 and ID1318	RTGMS	(20)
rtms1-D	Diannong S-1 xuan	10	RM25271 and RM25289	RTGMS	(80)

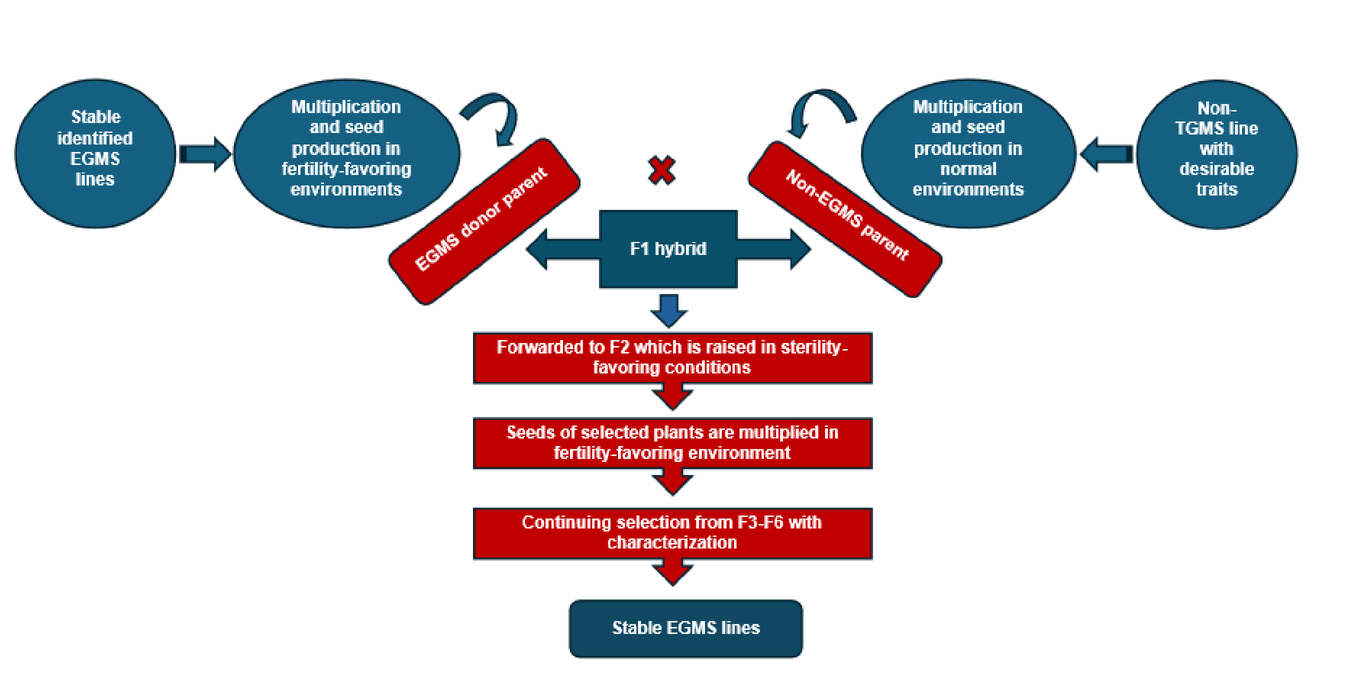

Fig. 3. Steps involved in the pedigree method of breeding in EGMS.

IRRI, Philippines. The transfer of the *tms5* gene into a well-developed genetic background is found to be possible by using SNP markers that help to identify target genes and select resembling individuals. Leaf blast-resistant TGMS lines were developed by incorporating the durable and broad-spectrum gene *Pi2* in China. As a result, four lines carrying the resistance to blast gene *Pi2* were developed from C815S and VE6219. These new lines showed a blast-resistant frequency equivalent to that of the donor parent VE6219, making them valuable for blast control in hybrid rice production (48). This method along with MAS will serve as a precise method of breeding EGMS lines with the sterility inducing genes.

Mutation

Mutation breeding is a process where mutations are induced in the genetic material of a plant to create new genetic variations using physical, chemical, or biological mutagens. Mutation breeding helps to broaden the diversity and introduction of new alleles into the gene pool, along with the rapid development of lines compared to conventional breeding methods. For example, the TGMS lines TS6 and CBTS0282 were subjected to gamma radiation of 300 and 350 Gy and an EMS concentration of 0.5 % and 0.6 % to induce mutation, which can be used to develop rice hybrids (90). Though it allows precise targeting of specific EGMS traits, screening of large populations and unpredictable outcomes are also expected, which can lead to unstable mutants.

CRISPR/Cas9 editing system

CRISPR-Cas9 is a tool that has transformed the field of genetic engineering by enabling precise DNA modifications, which have opened new possibilities in research, especially in agriculture. The CRISPR/Cas9 editing system allows for targeted genome editing through a complex of Cas9 endonuclease and guide RNA, providing a simple and efficient technique compared to other genome engineering technologies. Simultaneous mutagenesis of three genes (*OsPi21*, *OsXa5* and *OsBADH2*) into an elite TGMS variety, Zhi5012S, was done using CRISPR/Cas9 (91). The resulting sterile lines showed popcorn-like fragrance and improved resistance to blast and bacterial blight. Using CRISPR/Cas9, mutation was also produced in the Zhongjiazao17 background, which gave rise to two TGMS mutants, tms5-1 and tms5-2. These mutants lack any residual T-DNA (92) and exhibit TGMS, which is important for two-line hybrid rice breeding.

Using CRISPR/Cas9, mutations were induced in Pinzhan material for the genes *tms5*, *Pi21* and *Xa13*. A multiplex gene editing method was used to find the triple mutants for the genes that had TGMS characteristics, along with blast and blight resistance in rice. This method can be a promising method in producing stress-resilient EGMS hybrids that gives a better yield.

Rapid generation advancement of TGMS lines

For the development of population in rapid generation advance (RGA), the selection is delayed until the F_6 generation, unlike the pedigree method. This population is grown in structures like greenhouses or on seedling trays with less spacing and limited inputs, where higher temperatures limit root growth, leading to early flowering and maturity, which shortens crop duration. This method is advantageous for developing EGMS lines due to its controlled and stable conditions. It also allows for higher genetic gain by handling many populations with wide variation (92).

This method can be used to conserve time and limit the costs incurred. Under controlled conditions, it also hastens the fixation of new lines by single-seed descent from a mixed (93). Doubled haploids are used to speed up the fixation and have been successful in TGMS lines A36, A07, A37 and A32 (94) from the mutant segregants. This will further help the researchers in exploiting the EGMS traits for better hybrid production.

Gene pyramiding method

Gene pyramiding in plant breeding involves incorporating multiple desired genes into a single genetic background to enhance its resistance or tolerance to various diseases/stresses. This process requires successive rounds of crossing and selection to accumulate the desired genes in a single genetic background. Using this method, EGMS lines with additional superior traits, which will have cumulative effects such as resistance to biotic stress and abiotic stress, good floral traits, along with increased yield, can be developed. Using backcross breeding and gene pyramiding along with molecular MAS, Hua1228S (PTGMS line) was developed in China, carrying *Pi2*, *Xa7* and *tms5* genes, which showed leaf and neck blast resistance at higher levels (49). Two genes, *Pi2* from VE6219 (rice blast resistant) and *Xa23* from HBQ810 (bacterial blight genes), were introgressed into a TGMS line Guangzhan63-4S by marker-assisted pyramiding, which led to the development of a blast and bight resistant Hua1015S, a pyramided line (95). The newly developed TGMS line Hua1015S and its derived hybrids showed resistance to both rice blast and bacterial blight (94). Pyramiding of three thermosensitive genetic male sterility (TGMS) genes, *tgms*, *tms2* and *tms5*, was also done using linked microsatellite markers (47). Gene pyramiding is considered a promising method in the development of climate-resilient hybrids, which will help in coping with the environmental stressors.

Growth of two-line breeding in India

Among the different types, TGMS system is widely exploited in India. Diverse agro climatic conditions, altitude variations, large rice cultivation areas, high demand for rice and the economic, market and practical advantages offered by TGMS technology make it highly conducive for the Indian subcontinent. TGMS hybrids in India are mainly cultivated in the northern states like Chhattisgarh, Punjab and Haryana (10). ICAR has done intensive research in two-line breeding and characterized several TGMS lines over the past decade. It has done molecular characterization and evaluation of six TGMS lines viz., DRR-5S, DRR-1S, IR73834-21S IR73827-23S, UPRI-95-140S and UPRI-95-167S (96), which showed significant genetic diversity and potential for high heterosis in hybrids, which can be selected as potential parents. The development of these lines has contributed to the advancement of rice breeding programs, particularly in regions with variable temperatures that are conducive to the expression of TGMS traits.

In Tamil Nadu, the Paddy Breeding Station has made significant contributions in the field of two-line breeding and released TGMS lines TNAU19S, TNAU45S, TNAU60S, TNAU95S and TNAU39S which were stable, showing complete pollen sterility with good floral traits (10). These identified TGMS lines were further characterized (96, 97) for floral and morphological traits, which are used as promising 'S' lines in the production of two-line hybrids. TNAU 60S was identified from PKM3 (98) as a spontaneous mutant with desirable sterility and floral characters, it was found to be a promising TGMS for the development of rice hybrids. Another spontaneous mutant of RP 2161-106-1-1 called TS6 was identified, which is found to be successful in breeding programmes. The TGMS lines, TNAU 45S, TNAU 95S and TNAU 39S showed desirable floral and morphological traits like stable sterility in pollens, wide-opening glumes and exerted stigmas (61). A total of 119 TGMS rice lines were characterized for yield and floral attributes in both sterility and fertility-favoring environments of Coimbatore and Gudalur, respectively (62, 64). The hybrid CORH5 was the first two-line rice hybrid, released by TNAU, Coimbatore in 2024. The parentage of this hybrid CORH5 is TNAU 60S and CBSN 405. A private seed company, Savannah Private Limited, has also released two-line rice hybrids, namely SAVA-124 and SAVA-134, for commercial cultivation (10).

Many TGMS lines with desirable traits suitable for several regions of India have been developed. The variability of the genes governing and the floral traits of the identified TGMS lines implies the broad genetic background of the TGMS trait. Despite the stable pollen sterility, the performance of these lines in different conditions varies. This might be due to varying environmental conditions, the influence of the other genes and the combining ability of the line. Table 3 shows some of the important TGMS lines developed in India (64).

Conclusion

The development of two-line breeding for rice has improved yield and stress resistance because of the discovery of several TGMS sources. Farmers now have access to superior hybrid rice varieties, which not only meet the growing food demands but also provide better economic returns due to high yield and adaptability. The two-line hybrids show multiple stress resistance and adapt well to the local conditions, enhancing hybrid rice production, offering significant advantages in terms of yield potential and adaptability. It helps to overcome the bottlenecks of the three-line rice breeding systems and shows successful results in breeding programmes. Modern methods like mutation breeding, gene pyramiding, RGA and CRISPR-Cas9 have helped in achieving the stability of TGMS lines. These advancements will pave the way for the development of a new generation rice hybrids with superior performance, not only in India but also globally. Further morphological and molecular characterization of identified TGMS lines will help researchers for a better understanding of TGMS traits and further exploitation. As the demand for food security continues to rise globally, the integration of molecular tools into two-line breeding systems holds great promise for creating high-yielding, resilient rice varieties, thereby contributing to sustainable agriculture and meeting future food challenges.

Future Research Priorities

Discovering and utilizing new genes for male sterility that respond to temperature and photoperiod changes can help to create better hybrids with desirable characteristics. The success of two-line hybrids depends on stable pollen sterility, which is a major problem of concern due to environmental fluctuations. Understanding the genetic control of the TGMS trait will aid in the exploitation of the TGMS trait to develop stable sterile lines. The PGMS gene from NK58 in *japonica* rice does not produce stable offspring. However, when it is transferred to PA64S in *indica* rice, it shows a TGMS trait (12). Various gene editing tools and modern techniques can be utilized for this purpose, which can lead to a new hybrid rice breeding.

Table 3. Some of the important hybrid rice sources developed in India

Name of the line	Ecospecies	Gene origin	Place of development	References
JP38, JP 2	*indica*	Spontaneous mutant	IARI	(64)
TS6, TNAU 60S, TNAU 45S, TS29	*indica*	Spontaneous mutant	TNAU	(10, 13, 81, 97)
TNAU 27S, TNAU 19S, TGMS 94S, TGMS 93S, TGMS 92S, TGMS 91S, TGMS 82S, TGMS 81S, TGMS 74S, TNAU 39S, TNAU 45S, TNAU 95S, TS0912, TS0925, TNAU 39S, TNAU 60S	*indica*	Selection	TNAU	(13, 81, 99, 100)
SA 2, F 61	*indica*	Induced mutation (C) India	IARI	(64)
JP 8-1A-12	*indica*	Breeding population, India	IARI	(64)
JP 24A	*indica*	India	IARI	(64)

Plants can be tested in different locations with different temperatures and day lengths to discover the novel lines. No specific restorers are required, so inter-subspecies crosses can be done to widen the genetic diversity of the parents for two-line breeding. This method can also produce hybrids with higher yields than the current commercial varieties. This hybrid may also possess resistance to biotic and abiotic stress, like diseases, pests, salinity, etc. MAS can speed up the process of breeding by allowing early selection of desirable traits in plant development. Gene pyramiding aids in introgressing multiple desirable traits into an elite cultivar for the development of stable climate-resilient hybrids.

Advancing two-line hybrid breeding in India requires an integrated approach of traditional breeding techniques with modern biotechnological and genomic tools like CRISPR-Cas9 gene editing. Speed breeding can be effectively utilised to reduce the breeding duration for the rapid development of such hybrids. Research should focus on genetic, environmental and socio-economic factors that will be critical to developing high-yielding, resilient and sustainable two-line hybrids. These hybrids should be suited to the diverse conditions of Indian as well as global agriculture.

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Authors' contributions

All authors contributed equally to the preparation of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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References

1. Singh SK, Bhati PK, Sharma A, Sahu V. Super hybrid rice in China and India: Current status and future prospects. Int J Agric Biol. 2015;17:221232.
2. Virmani SS. Advances in hybrid rice research and development in the tropics. Hybrid rice for food security, poverty alleviation and environmental protection. Los Baňos, Philippines: International Rice Research Institute; 2003. p. 7-20.
3. Spielman DJ, Kolady DE, Ward PS. The prospects for hybrid rice in India. Food Secur. 2013;5(5):651-65. https://doi.org/10.1007/s12571-013-0291-7
4. Huang JZ, E ZG, Zhang HL, Shu QY. Workable male sterility systems for hybrid rice: Genetics, biochemistry, molecular biology and utilization. Rice. 2014;7:13. https://doi.org/10.1186/s12284-014-0013-6
5. Rao GS, Deveshwar P, Sharma M, Kapoor S, Rao KV. Evolvement of transgenic male-sterility and fertility-restoration system in rice for production of hybrid varieties. Plant Mol Biol. 2018;96(1-2):35-51. https://doi.org/10.1007/s11103-017-0678-5
6. Moon S, Lee YS, Gutierrez-Marcos J, Jung KH. Advancements in hybrid rice production: improvements in male sterility and synthetic apomixis for sustainable agriculture. Plant Biotechnol. 2025;23(6):2330-45. https://doi.org/10.1111/pbi.70057
7. Sheng Z, Tang L, Shao G, Xie L, Jiao G, Tang S, et al. The rice thermo-sensitive genic male sterility gene tms9: pollen abortion and gene isolation. Euphytica. 2015;203(1):145-52. https://doi.org/10.1007/s10681-014-1285-z
8. Virmani SS. Two-line hybrid rice breeding manual. IRRI; 2003. 88 p.
9. Fang Y, Yang J, Guo X, Qin Y, Zhou H, Liao S, et al. CRISPR/Cas9-induced mutagenesis of TMS5 confers thermosensitive genic male sterility by influencing protein expression in rice (*Oryza sativa* L.). Int J Mol Sci. 2022;23(15):8354. https://doi.org/10.3390/ijms23158354
10. Swaminathan M. Progress and prospects of two-line rice breeding in India. Intech Open; 2022. https://doi.org/10.5772/intechopen.99545
11. Zhou H, Zhou M, Yang Y, Li J, Zhu L, Jiang D, et al. RNase Z^{S1} processes Ub_{L40} mRNAs and controls thermosensitive genic male sterility in rice. Nat Commun. 2014;5:4884. https://doi.org/10.1038/ncomms5884
12. Ashraf MF, Peng G, Liu Z, Noman A, Alamri S, Hashem M, et al. Molecular control and application of male fertility for two-line hybrid rice breeding. Int J Mol Sci. 2020;21(21):7868. https://doi.org/10.3390/ijms21217868
13. Rajesh T, Radhakrishnan VV, Kumari KT, Francies RM, Sreenivasan E, Ibrahim KK, et al. Critical stages of thermo-sensitive genic male sterile lines of rice in Kerala. ORYZA - Int J Rice. 2017;54(4):401-6. https://doi.org/10.5958/2249-5266.2017.00054.6
14. Ku S, Yoon H, Suh HS, Chung YY. Male-sterility of thermosensitive genic male-sterile rice is associated with premature programmed cell death of the tapetum. Planta. 2003;217(4):559-65. https://doi.org/0.1007/s00425-003-1030-7
15. Zhang C, Zhu Q, Li J, Zhang H, Lee D, Chen L. Regulatory mechanisms of temperature, light and water on the expression of male sterility genes in rice. Mol Plant Breed. 2024;15(6):429-41. https://doi.org/10.5376/mpb.2024.15.0040
16. Lopez MT, Virmani SS. Development of TGMS lines for developing two-line rice hybrids for the tropics. Euphytica. 2000;114:211-5. https://doi.org/10.1023/A:1003947219699
17. Wang N, Li X, Zhu J, Yang ZN. Molecular and cellular mechanisms of photoperiod- and thermo-sensitive genic male sterility in plants. Mol Plant. 2025;18(1):26-41. https://doi.org/10.1016/j.molp.2024.12.012
18. Dora SA, El-Dinary ME, Yousef MA, Selim ME. Genetic behavior for reverse thermo-sensitive genic male sterility (RTGMS) through segregating generations of some rice genotypes in Egypt. J Sus Agric Sci. 2017;43(1):1-8.
19. Jia JH, Zhang DS, Li CY, Qu XP, Wang SW, Chamarerk V, et al. Molecular mapping of the reverse thermo-sensitive genic male-sterile gene (rtms1) in rice. Theor Appl Genet. 2001;103(4):607-12. https://doi.org/10.1007/PL00002916
20. Jin-Long NI, De-zheng W, Da-Hu NI, Feng-shun S, Jian-bo Y, Da-nian Y. Characterization and fine mapping of RTMS10, a semi-dominant reverse thermo-sensitive genic male sterile locus in rice. J Integr Agric. 2022;21(2):316-25. https://doi.org/10.1016/S2095-3119(20)63563-8
21. Zhang Q, Shen BZ, Dai XK, Mei MH, Saghai Maroofo MA, Li ZB, et al. Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. Genetics. 1994;91(18):8675-9. https://doi.org/10.1073/pnas.91.18.8675
22. Fan Y, Zhang Q. Genetic and molecular characterization of photoperiod and thermo-sensitive male sterility in rice. Plant Reprod. 2018;31:3-14. https://doi.org/10.1007/s00497-017-0310-5
23. Yuan SC, Zhang ZG, He HH, Zen HL, Lu KY, Lian JH, et al. Two photoperiodic reactions in photoperiod-sensitive genic male-

sterile rice. Crop Sci. 1993;33(4):651-60. https://doi.org/10.2135/cropsci1993.0011183X003300040002x

24. Nthakanio PN, Qingzhong X. Defining the effects of day-length and temperature in PGMS rice trait. African Crop Science Conference Proceedings; 2013. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20163288722

25. Nthakanio NP, Qingzhong X. Programmed cell death-like behavior in photoperiodsensitive genic male sterile (PGMS) rice. Afr J Biotechnol. 2011;10(16):3027-34. https://doi.org/10.5897/AJB08.1220

26. Liu N, Shan Y, Wang FP, Xu CG, Peng KM, Li XH, et al. Identification of an 85-kb DNA fragment containing pms1, a locus for photoperiod sensitive genic male sterility in rice. Mol Gene Genomics. 2001;266(2):271-5. https://doi.org/10.1007/s004380100553

27. Huang T-y, Wang Z, Hu Y-g, Shi S-p, Peng T, Chu X-d, et al. Genetic analysis and primary mapping of pms4, a photoperiod-sensitive genic male sterility gene in rice (*Oryza sativa*). Rice Sci. 2008;15(2):153-6. https://doi.org/10.1016/S1672-6308(08)60035-9

28. Zhou H, Liu Q, Li J, Jiang D, Zhou L, Wu P, et al. Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. Cell Res. 2012;22(4):649-60. https://doi.org/10.1038/cr.2012.28

29. Ziguo Z, Hanlai Z, Jing Y, Shengchao Y, Duanpin Z, et al. Conditions inducing fertility alteration and ecological adaptation of photoperiod-sensitive genic male-sterile rice. Field Crops Research. 1994;38(2):111-120. https://doi.org/10.1016/0378-4290(94)90005-1

30. El-Mowafi HF, AlKahtani MDF, El-Hity MA, Reda AM, Al Husnain L, El-Degwy ES, et al. Characterization of fertility alteration and marker validation for male sterility genes in novel PTGMS lines hybrid rice. Saudi J Biol Sci. 2021;28(8):4109-16. https://doi.org/10.1016/j.sjbs.2021.04.058

31. Xu J, Wang B, Wu Y, Du P, Wang J, Wang M, et al. Fine mapping and candidate gene analysis of ptgms2-1, the photoperiod-thermo-sensitive genic male sterile gene in rice (*Oryza sativa* L.). Theor Appl Genet. 2011;122(2):365-72. https://doi.org/10.1007/s00122-010-1452-0

32. Liu Y, Zhang F, Luo X, Kong D, Zhang A, Wang F, et al. Molecular breeding of a novel PTGMS line of WDR for broad-spectrum resistance to blast using Pi9, Pi5 and Pi54 genes. Rice. 2021;14(1):96. https://doi.org/10.1186/s12284-021-00537-1

33. Xue Z, Xu X, Zhou Y, Wang X, Zhang Y, Liu D, et al. Deficiency of a triterpene pathway results in humidity-sensitive genic male sterility in rice. Nat Commun. 2018;9(1):604. https://doi.org/10.1038/s41467-018-03048-8

34. Xu Y, Yu D, Chen J, Duan M. A review of rice male sterility types and their sterility mechanisms. Heliyon. 2023;9(7):e18204. https://doi.org/10.1016/j.heliyon.2023.e18204

35. Yu B, Liu L, Wang T. Deficiency of very long chain alkanes biosynthesis causes humidity-sensitive male sterility via affecting pollen adhesion and hydration in rice. Plant Cell Environ. 2019;42(12):3340-54. https://doi.org/10.1111/pce.13637

36. Ni E, Deng L, Chen H, Lin J, Ruan J, Liu Z, et al. OsCER1 regulates humidity-sensitive genic male sterility through very-long-chain (VLC) alkane metabolism of tryphine in rice. Funct Plant Biol. 2021;48(5):461-8. https://doi.org/10.1071/FP20168

37. Chen H, Zhang Z, Ni E, Lin J, Peng G, Huang J, et al. HMS1 interacts with HMS1I to regulate very-long-chain fatty acid biosynthesis and the humidity-sensitive genic male sterility in rice (*Oryza sativa*). New Phytol. 2020;225(5):2077-93. https://doi.org/10.1111/nph.16288

38. Li J, Luo X, Zhou K. Research and development of hybrid rice in China. Plant Breed. 2024;143:96-104. https://doi.org/10.1111/pbr.13134

39. Qian Q, Zhang F, Xin Y. Yuan Longping and hybrid rice research. Rice. 2021;14(1):101. https://doi.org/10.1186/s12284-021-00542-4

40. Mei MH, Dai XK, Xu CG, Zhang Q. Mapping and genetic analysis of the genes for photoperiod-sensitive genic male sterility in rice using the original mutant Nongken 58S. Crop Sci. 1999;39(6):1711-5. https://doi.org/10.2135/cropsci1999.3961711x

41. Zhang X, He Q, Zhang W, Shu F, Wang W, He Z, et al. Genetic relationships and identification of core germplasm among rice photoperiod- and thermo-sensitive genic male sterile lines. BMC Plant Biol. 2021;21(1):313. https://doi.org/10.1186/s12870-021-03062-x

42. Pardeep K. Two-line hybrid production system and their applications in rice. Int J Agric Sci. 2016;8:3502-4.

43. Ali AJ, Siddiq EA. Isolation and characterization of a reverse temperature sensitive genic male sterile mutant in rice. Indian J Genet Plant Breed. 1999;59(04):423-8.

44. He Q, Deng H, Sun P, Zhang W, Shu F, Xing J, et al. Hybrid Rice. Engineering. 2020;6(9):967-73. https://doi.org/10.1016/j.eng.2020.08.005

45. Wang YG, Xing QH, Deng QY, Liang FS, Yuan LP, Weng ML, et al. Fine mapping of the rice thermo-sensitive genic male-sterile gene tms5. Theoret Appl Genet. 2003;107:917-21. https://doi.org/10.1007/s00122-003-1327-8

46. Latha R, Thiyagarajan K, Senthilvel S. Genetics, fertility behaviour and molecular marker analysis of a new TGMS line, TS6, in rice. Plant Breed. 2008;123(3):235-40. https://doi.org/10.1111/j.1439-0523.2004.00975.x

47. Nas TMS, Sanchez DL, Diaz GQ, Mendioro MS, Virmani SS. Pyramiding of thermosensitive genetic male sterility (TGMS) genes and identification of a candidate tms5 gene in rice. Euphytica. 2005;145:67-75. https://doi.org/10.1007/s10681-005-0206-6

48. Jiang J, Mou T, Yu H, Zhou F. Molecular breeding of thermo-sensitive genic male sterile (TGMS) lines of rice for blast resistance using Pi2 gene. Rice. 2015;8(1):11. https://doi.org/10.1186/s12284-015-0048-3

49. Mi J, Yang D, Chen Y, Jiang J, Mou H, Huang J, et al. Accelerated molecular breeding of a novel P/TGMS line with broad-spectrum resistance to rice blast and bacterial blight in two-line hybrid rice. Rice. 2018;11(1):11. https://doi.org/10.1186/s12284-018-0203-8

50. Wang WM, Fan J, Jeyakumar JMJ. Rice false smut: an increasing threat to grain yield and quality. In: Protecting rice grains in the post-genomic era. Intech; 2019. p. 89-108. https://doi.org/10.5772/intechopen.84862

51. Lore JS, Jain J, Kumar S, Kamboj I, Sidhu N, Khanna R, et al. Identification of potential donors for false smut resistance in elite breeding lines of rice (*Oryza sativa L.*) under field conditions. Plant Genet Resour. 2022;20(1):7-14. https://doi.org/10.1017/S1479262122000041

52. Tang S, Gao D, Chen Y, Li Y, Zhang S, Zhao Z. Inheritance pattern of two-line hybrid rice resistance to false smut. Agric Sci Technol. 2014;15(4):557.

53. Peng P, Jiang H, Luo L, Ye C, Xiao Y. Pyramiding of multiple genes to improve rice blast resistance of photo-thermo sensitive male sterile line, without yield penalty in hybrid rice production. Plants. 2023;12(6):1389. https://doi.org/10.3390/plants12061389

54. Yu J, Han J, Kim YJ, Song M, Yang Z, He Y, et al. Two rice receptor-like kinases maintain male fertility under changing temperatures. Proc Natl Acad Sci USA. 2017;114(46):12327-32. https://doi.org/10.1073/pnas.1705189114

55. Virmani SS. Hybrid rice. In: Sparks DL, editor. Advances in agronomy. Vol. 57. Academic Press; 1996. p. 377-462.

56. Gayathri R, Stephen R. Differential expression of transport and signalling genes in leaves and panicle regulates the development of pollen-free anthers in TGMS red rice. Cereal Res Commun. 2021;49(3):465-73. https://doi.org/10.1007/s42976-020-00124-y

57. Zhou H, He M, Li J, Chen L, Huang Z, Zheng S, et al. Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. Sci Rep. 2016;6:37395. https://doi.org/10.1038/srep37395

58. Gayathri R, Stephen R. Characterization of thermo sensitive red rice line for its suitability in the State of Kerala, India. Madras Agric J. 2018;105(Special Issue).

59. Chandra N. Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa L.*). M.Sc. Kerala Agricultural University; 2014. https://krishikosh.egranth.ac.in/items/e3eb51c3-d59d-4ecc-ac37-e47b639edc07/full

60. Kalaiyarasi R, Binodh AK, Thiyagarajan K. Evaluation of promising TGMS lines for exploitation of two-line heterosis in rice (*Oryza sativa L.*). Indian J Genet Plant Breed. 2006;66(03):225-6.

61. Srimathi K, Arumugam Pillai M, Aananthi N, Rajababu C. Genetic studies on TGMS lines for development of superior two-line rice hybrids. EJPB. 2019;10(2):620-6. http://doi.org/10.5958/0975-928X.2019.00078.4

62. Roy RR, Kumaresan D. Genetic variability and association studies for yield and floral traits in temperature sensitive male sterile lines (TGMS) of rice (*Oryza sativa L*). EJPB. 2019;10(3):1200-9. https://doi.org/10.5958/0975-928X.2019.00152.2

63. Vishvapriya M, Binodh AK, Manonmani S, Kumaresan D, Senthil A, Kumar GS. Genetics of sterility behaviour in thermo sensitive genic male sterility system in rice (*Oryza sativa L.*). EJPB. 2023;14(3):1105-10. https://doi.org/10.37992/2023.1403.126

64. Ali J, Dela Paz M, Robiso CJ. Advances in two-line heterosis breeding in rice via the temperature-sensitive genetic male sterility system. In: Rice improvement: physiological, molecular breeding and genetic perspectives. Springer International Publishing; 2021. p. 99-145. https://doi.org/10.1007/978-3-030-66530-2_4

65. Borkakati RP, Virmani SS. Genetics of thermosensitive genic male sterility in rice. Euphytica. 1996;88:1-7. https://doi.org/10.1007/BF00029259

66. Yang Q, Liang C, Zhuang W, Li J, Deng Q, Wang B, et al. Characterization and identification of the candidate gene of rice thermo-sensitive genic male sterile gene tms5 by mapping. 2007;225:321-30. https://doi.org/10.1007/s00425-006-0353-6

67. Jiang D, Lu S, Zhou H, Wu X, Zhuang C, Liu Y, et al. Mapping of the rice (*Oryza sativa* L.) thermo-sensitive genic male sterile gene tms5 with EST and SSR markers. Chin Sci Bull. 2006;51:417-20. https://doi.org/10.1007/s11434-006-0417-9

68. Minghua M, Liang C, Zhang Z, Li Z, Caiguo XU, Zhang Q. pms 3 is the locus causing the original photoperiod-sensitive male sterility mutation of "Nongken 588". Science in China (Series C). 1999;42.

69. Tian F, Li DJ, Fu Q, Zhu ZF, Fu YC, Wang XK, et al. Construction of introgression lines carrying wild rice (*Oryza rufipogon Griff.*) segments in cultivated rice (*Oryza sativa L.*) background and characterization of introgressed segments associated with yield-related traits. Theoret Appl Genet. 2006;112(3):570-80. https://doi.org/10.1007/s00122-005-0165-2

70. Jiang D, Shahid MQ, Wu J, Deng R, Chen Z, Wang L, et al. Thermo-sensitive genic male sterile lines of neo-tetraploid rice developed through gene editing technology revealed high levels of hybrid vigor. Plants. 2022;11(11):1390. https://doi.org/10.3390/plants11111390

71. Li C, Tao RF, Li Y, Duan MH, Xu JH. Transcriptome analysis of the thermosensitive genic male-sterile line provides new insights into fertility alteration in rice (*Oryza sativa*). Genomics. 2020;112(3):2119-29. https://doi.org/10.1016/j.ygeno.2019.12.006

72. Pan Y, Li Q, Wang Z, Wang Y, Ma R, Zhu L, et al. Genes associated with thermosensitive genic male sterility in rice identified by comparative expression profiling. BMC Genomics. 2014;15(1):1114. https://doi.org/10.1186/1471-2164-15-1114

73. Xinggui L, Virmani SS, Yang R. Advances in two-lines hybrids rice breeding. In: Advances in hybrid rice breeding. Phillippines: IARI; 1998. p. 89-98.

74. Dong NV, Subudhi PK, Luong PN, Quang VD, Quy TD, Zheng HG, et al. Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. Theoret Appl Genet. 2000;100:727-34. https://doi.org/10.1007/s001220051345

75. Reddy OUK, Siddiq EA, Sarma NP, Ali J, Hussain AJ, Nimmakayala P, et al. Genetic analysis of temperature-sensitive male sterilty in rice. Theoret Appl Genet. 2000;100:794-801. https://doi.org/10.1007/s001220051345

76. Zhou YF, Zhang XY, Xue QZ. Fine mapping and candidate gene prediction of the photoperiod and thermo-sensitive genic male sterile gene pms1(t) in rice. J Zhejiang Univ Sci B. 2011;12(6):436-47. https://doi.org/10.1631/jzus.B1000306

77. Li X, Lu Q, Wang F, Xu C, Zhang Q. Separation of the two-locus inheritance of photoperiod sensitive genic male sterility in rice and precise mapping the pms3 locus. Euphytica. 2001;119:343-8. https://doi.org/10.1023/A:1017570025805

78. Huang T-y, Wang Z, Hu Y-g, Shi S-p, Peng T, Chu X-d, et al. Genetic analysis and primary mapping of pms4, a photoperiod-sensitive genic male sterility gene in rice (*Oryza sativa*). Rice Sci. 2008;15(2):153-6. https://doi.org/10.1016/S1672-6308(08)60035-9

79. Peng HF, Zhang ZF, Wu B, Chen XH, Zhang GQ, Zhang ZM, et al. Molecular mapping of two reverse photoperiod-sensitive genic male sterility genes (rpms1 and rpms2) in rice (*Oryza sativa L.*). Theoret Appl Genet. 2008;118(1):77-83. https://doi.org/10.1007/s00122-008-0877-1

80. Zhang X, Chang G, Wu Z, Wan J, Yang J, Wang F, et al. Identification and fine mapping of rtms1-D, a gene responsible for reverse thermosensitive genic male sterility from Diannong S-1X. Plant Divers. 2022;44(2):213-21. https://doi.org/10.1016/j.pld.2021.05.002

81. Kadirimangalam SR, Hifzur R, Saraswathi R, Kumar M, Raveendran M, Robin S. Fine mapping and expression analysis of thermosensitive genic male sterility gene (tms) in rice (*Oryza sativa* L.). Plant Gene. 2019;19:100186. https://doi.org/10.1016/j.plgene.2019.100186

82. Lopez MT, Toojinda T, Vanavichit A, Tragoonrung S. Microsatellite markers flanking the tms2 gene facilitated tropical TGMS rice line development. Crop Sci. 2003;43(6):2267-71. https://doi.org/10.2135/cropsci2003.2267

83. Subudhi PK, Borkakati RP, Virmani SS, Huang N. Identification of RAPD markers linked to rice thermosensitive genetic male sterility gene by bulk segregant analysis. Rice Genet Newsl. 1995;12:228-31.

84. Lang NT, Subudhi PK, Virmani SS, Brar DS, Khush GS, Li Z, et al. Development of PCR-based markers for thermosensitive genetic male sterility gene tms3 (t) in rice (*Oryza sativa L.*). Hereditas. 1999;131(2):121-7. https://doi.org/10.1111/j.1601-5223.1999.00121.x

85. Song FS, Ni JL, Qian YL, Li L, Ni DH, Yang JB. Development of SNP-based dCAPS markers for identifying male sterile gene tms5 in two-line hybrid rice. Genet Mol Res. 2016;15(3). https://doi.org/10.4238/gmr.15038512

86. Hien V, Yoshimura A. Identifying map location and markers linked to thermosensitive genic male sterility gene in 103S line. J Sci Dev. 2015;13(3):331-6.

87. Hussain AJ, Ali J, Siddiq EA, Gupta VS, Reddy UK, Ranjekar PK. Mapping of tms8 gene for temperature-sensitive genic male sterility (TGMS) in rice (*Oryza sativa L.*). Plant Breed. 2012;131(1):42-7. https://doi.org/10.1111/j.1439-0523.2011.01897.x

88. Sheng Z, Wei X, Shao G, Chen M, Song J, Tang S, et al. Genetic analysis and fine mapping of tms9, a novel thermosensitive genic

male-sterile gene in rice (*Oryza sativa L*.). Plant Breed. 2013;132 (2):159-64. https://doi.org/10.1111/pbr.12024

89. Qi Y, Liu Q, Zhang L, Mao B, Yan D, Jin Q, et al. Fine mapping and candidate gene analysis of the novel thermo-sensitive genic male sterility tms9-1 gene in rice. Theoret Appl Genet. 2014;127(5):1173-82. https://doi.org/10.1007/s00122-014-2289-8
90. Thiyagarajan K, Abirami S, Robin S, Manonmani S, Jambhulkar SJ. Development of TGMS lines with improved floral traits through mutation breeding in rice. Dept. of Atomic Energy; 2006. https://inis.iaea.org/records/r6h5h-vh846
91. Zhang H, Liang M, Chen J, Wang H, Ma L. Rapid generation of fragrant thermo-sensitive genic male sterile rice with enhanced disease resistance via CRISPR/Cas9. Planta. 2024;259(5):112. https://doi.org/10.1007/s00425-024-04392-4
92. Barman HN, Sheng Z, Fiaz S, Zhong M, Wu Y, Cai Y, et al. Generation of a new thermo-sensitive genic male sterile rice line by targeted mutagenesis of TMS5 gene through CRISPR/Cas9 system. BMC Plant Biol. 2019;19(1):109. https://doi.org/10.1186/s12870-019-1715-0
93. Collard BCY, Beredo JC, Lenaerts B, Mendoza R, Santelices R, Lopena V, et al. Revisiting rice breeding methods-evaluating the use of rapid generation advance (RGA) for routine rice breeding. Plant Prod Sci. 2017;20(4):337-52. https://doi.org/10.1080/1343943X.2017.1391705
94. Ali J, Dela Paz M, Marfori CM, Nicolas KL. Environment sensitive genic male sterility (EGMS) based hybrid breeding in rice. Molecular breeding strategies for crop improve. One-day Dialogue; 2017. p. 120-42.
95. Jiang J, Yang D, Ali J, Mou T. Molecular marker-assisted pyramiding of broad-spectrum disease resistance genes, Pi2 and Xa23, into GZ63-4S, an elite thermo-sensitive genic male-sterile line in rice. Mol Breed. 2015;35(3):83. https://doi.org/10.1007/s11032-015-0282-9
96. Singh VK, Upadhyay P, Sinha P, Mall AK, Ellur RK, Singh A, et al. Prediction of hybrid performance based on the genetic distance of parental lines in two-line rice (*Oryza sativa L*.) hybrids. J Crop Sci Biotechnol. 2011;14(1):1-10. https://doi.org/10.1007/s12892-010-0111-y
97. Kanimozhi P, Pushpam R, Binodh AK, Kannan R, Pillai MA. Evaluation of TGMS lines for good floral and out crossing related traits in rice. EJPB. 2018;9(4):1497-502. https://doi.org/10.5958/0975-928X.2018.00185.0
98. Robin S, Manomani S, Pushpam R, Arasakesary SJ, Thiyagarajan K. TNAU 60S (IC0622805; INGR17028), a TGMS line of rice (*Oryza sativa*). Indian J Plant Genet Resour. 2019;32(2):239-40.
99. Manonmani S, Pushpam R, Robin S. Stability of TGMS lines under different temperature regimes for pollen sterility. J Rice Res. 2016;9(1):17-9.
100. Sasikala R, Kalaiyarasi R, Paramathama M. Exploitation of twoline heterosis utilizing thermosensitive genic male sterility system in rice (*Oryza sativa L*.). Int J Trop Agric. 2015;33(2):1111-7.

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