Chapter

Progress and Prospects of Two Line Rice Breeding in India

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Abstract

Increasing the yield potential through hybrid rice technology was very well proved in nearby countries. Three line hybrid rice technology is encounted with some of the difficulties in seed production. Identification of Environmentally influenced male sterility overcomes the problem encountered in seed production since it is regulated by the temperature it is called temperature regulated male sterility and hybrids produced using this line is called two line rice hybrids. Types of male sterility and methods to identify the new TGMS lines and breeding methods employed for producing the tgms lines were described. Characterization of tgms lines by both conventional method and molecular tools has been enumerated. Seed multiplication of tgms under fertility inducing environment and seed production of two line hybrids has been explained. Seed production location was exclusively identified for seed multiplication of tgms lines. Heterotic potential of two line rice hybrids has been studied. Future prospectes in tgms research and two line heterotic potential was explained for increasing the yield potential in rice.

Keywords: rice, TGMS lines, characterization, new line development, two line rice hybrids, yield

1. Introduction

Rice is the staple food for about half of the world's population. The global population is expected to reach 9 billion people by 2050 [1]. This demands for significant efforts to increase grain production, and it is expected to add 44 million tons staple crops per year for ensuring sufficient food production for such huge population [2]. Although rice production has significantly increased from 34.5 million tonnes in 1960–1961 to 117.5 million tonnes in 2020–2021, this significant increase over years was achieved by introduction of semi dwarf varieties, through improved crop management, adoption of hybrid varieties and improved plant protection practices. Rice production needed to be increased 42% by 2050 to feed the demands of an ever-increasing human population globally. Due to the potential of hybrid rice in increasing both rice production and productivity, many countries are focusing on exploiting the benefits of this technology. Success of hybrid rice technology and commercial exploitation was proved in China by the late 1970s marked the second major landmark in the history of rice breeding. It showed commendable breakthroughs in rice production and productivity, made other countries to revive its interest in hybrid rice breeding.

The use of hybrid rice has proved to be an effective and economical way to increase rice production. In rice, the phenomenon of heterosis was reported earlier by Jones [3] and Ramaiah [4]. However, several problems experienced in the production of hybrid seeds discouraged the commercial exploitation of heterosis in India. Later in the year 1976, it was accepted that large scale production of hybrid rice could be achieved through utilization of male sterility (MS) systems [5]. China initiated hybrid rice production in the year 1964 followed by India in the year 1989. Presently around 40 countries are actively involved in commercial hybrid rice production [6].

The over dependence on a single source of cytoplasmic male sterility (CMS) via WA (Wild abortive) and the difficulties in seed production and parental line development warrant the identification of alternate approaches to exploit hybrid vigor in rice. Two line breeding is one such possibility that emerged following the chance discovery of a photoperiod-sensitive genic male sterile plant called Nongken 58S, in the japonica variety Nongken 58 by Prof. Shi Ming Song of China [7–9] which was found to be sterile under longer photoperiods (> 14 hr) and fertile under shorter photo periods (13 hr) subsequently. Temperature sensitive genic male sterility (TGMS) line was identified by Chinese and Japanese scientists which was completely sterile under high temperature (> 32°C) and under low temperature (24°C) it was fertile [10, 11]. Using the PGMS system Yuan [12] put forth a new strategy of hybrid rice breeding which did not involve a maintainer, as the maintenance is taken care off by the shorter photoperiod (13 hr), hence it was called as two line method. During the sterile phase, EGMS plants can be used as a female parent to produce hybrid seed through self-fertilization without the use of a maintainer line as required in the CMS system. Since only two lines are required for the maintenance and multiplication of male sterile lines and production of hybrid seed, the system using this type of male sterility is known as the two-line system of hybrid breeding.

2. Advantages of two-line over three-line system of heterosis breeding

- More number of heterotic hybrids can be developed because of wide choice of parental lines.

- Simpler and more efficient seed Production system.

- Large scale use of single source of cytoplasm and the risk of outbreak of epidemics as well as the negative effects of sterility inducing cytoplasm are avoided.

- In Rice, two-line system is specifically useful for developing aromatic and inter racial hybrids.

- Two-line hybrids are having magnitude of heterosis 5 to 10% higher than in three line hybrids.

3. Types of environmental sensitive genic male sterility (EGMS)

Comprises mainly of four types namely*:*

3.1 Photoperiod-sensitive genic male sterility (PGMS)

This type of male sterility was discovered in rice by Professor Shi Ming Song in Hubei Province of China in 1973. Several male sterile plants were noticed in a late japonica cultivar Nongken 58, when exposed to photoperiod of more than 14 hours. The same sterile plants when grown in photoperiod of less than 13 hours and 45 minutes, turned to fertility. Subsequently detailed investigations were carried out and the findings have been reported by Shi [7, 8]. The male sterile mutant was originally designated as Hubie Photoperiod-Sensitive Genic Male Sterile Rice (HPGMSR). Subsequently the mutant was named as Nongken 58 S. Pioneering and extensive work has been done on this mutant at Wuhan in Hubei Province and at other centers in China. PGMS trait from Nongken 58 S has been transferred to several elite japonica and indica cultivars through backcrossing in China.

PGMS system is useful and can be deployed in temperate countries where the day length differs considerably during different seasons.

3.2 Thermo-sensitive genic male sterility (TGMS)

This type of male sterility which is controlled by the temperature prevailing at sensitive stage of the crop, was discovered in China [13, 14].

In most of the TGMS mutants reported so far, such as Annong – 1S from China, Norin PL-12 from Japan, IR 32364 TGMS from IRRI, Philippines and several mutants reported from India and Vietnam, the sterility is caused by higher temperatures (generally above 30°C) at the sensitive stage whereas at lower temperatures (generally below 24°C) fertility is observed. However, in few cases, sterility is observed at lower temperatures and fertility is observed at higher temperatures. Such type of male sterility is referred to as 'Reverse TGMS type'. Examples of reverse TGMS type reported are mutant Diaxin 1A and IV A and a mutant in variety 26 Zhaizao from China [15–17], JP-38S from India.

In tropical and sub-tropical countries, where there are large temperature differences across locations, regions, seasons and at different attitudes TGMS system can be utilized. India is one of the country with various regions and seasons and with attitude ranging from sea level to several thousand meters in hilly areas, is highly suitable for exploiting TGMS system for deployment and development of two-line hybrids.

3.3 Photo-thermo-sensitive genic male sterility (PTGMS)

Several japonica and indica male sterile lines have been developed, utilizing the Nongken 58S mutant. All such lines developed utilizing the PTGM mutant, were found to interact both with photoperiod as well as temperature. Such type of male sterility is called photo thermosensitive genic male sterility (PTGMS). In such a system, photoperiod is effective between critical fertility point (CFT) and critical sterility point (CSP). This range of temperature is called as Temperature Range of Photo sensitivity.

3.4 Micronutrient-deficiency induced male sterility

Deficiencies of copper, Boron and some other micronutrients are reported to cause male sterility in wheat and some other crops. High genetic variability has been reported in sensitivity to deficiency of these micronutrients. Very sensitive types are completely male sterile under micronutrient deficient conditions. It has been suggested that these sensitive genotypes can be used under deficient conditions as females and tolerant genotypes as males for producing F_1 hybrid seed. The sensitive types can be multiplied by growing them under micro nutrient sufficient conditions.

The PGMS, TGMS and PTGMS lines are governed by 2–3 recessive genes, hence they can be easily transferred through backcrossing to known elite, good combining varieties. TGMS system can be utilized for tropical countries like India where low and high temperature prevails in high altitudes and in plains respectively, whereas PGMS system for the temperate countries like China and Japan where the daylength variation is significant. PTGMS can be utilized in both the tropical and temperate conditions. The sensitive stage to temperature, photoperiod or both is generally stage IV (stamen and pistil primordia), to stage VI (Meiosis) of the developmental stages of the rice plant.

On the basis of the critical sterility point (CSP) the temperature at which complete sterility is induced and critical fertility point (CFP) the temperature at which maximum fertility is achieved, it can be classified into four types.

3.4.1 Type 1: High CSP (>32°C) Low CFP (<24°C)

This type is recognized by Chinese as ideal, as it is safe for both hybrid seed production and multiplication of PTGMS. According to Yuan [18] such an ideal type still, remains to be identified. Although no one is certain as to where to draw the lines for high CSP and Low CFP, based on the prevalent temperature and photoperiod regimes in a region, a narrow range can be determined. Spontaneous Mutant lines SM3 and SM5 fall under this category. SM5 with a CSP of 32.3°C is just on the border line [19].

3.4.2 Type 2: High CSP (>32°C) High CFP (>24°C)

Chinese have reported several of the EGMS lines identified by them to fall under this category. Zhang *et al.,* [20] classified this category as 7001 S type. Under this category TGMS like 8902S and W7415S have been listed. It is not suited to Chinese condition, as it would introduce risk in hybrid seed production. Zhang *et al.,* [20] suggested their usefulness in tropics. JP 2 falls under this category [19].

3.4.3 Type 3: Low CSP (<32°C) – Low CFP (<24°C)

By virtue of its stable sterility duration over a large region in China, it can be used in hybrid seed production without any problem. However in this type of EGMS lines, their seed multiplication becomes difficult and hence limits their wide utilization in China [20]. Nevertheless this type (Pei ai 64S type) was preferred until ideal lines were bred [18, 20]. In subtropical countries like India however this type would be most suited, as only sterile phase is required to be more stable in such situation. Most of the TGMS Lines viz., TNAU 45S, TNAU 60S, TNAU 95S etc., developed from Department of Rice, TNAU, India fall under this category.

3.4.4 Type 4: Low CSP (< 32°C) - High CFP (>24°C)

Some of the lines *viz*., GDR 38S, GDR 39S and GDR40S developed from Hybrid Rice Evaluation Centre recorded this type of category.

4. Detection and identification

A detailed procedure for identifying Thermo-sensitive genic male sterile (TGMS) lines under field conditions, from germplasm and mutagenised populations in rice, has been given by Virmani *et al* [21]. These populations are critically observed for occurrence of male sterility when the crop gets exposed under natural conditions after panicle initiation stage to temperatures of above 30–35^0 Male sterility can be easily identified in the field, by the presence of partially filled hanging panicles and completely sterile erect green panicles in the same plant. Those showing complete lack of seed formation in self pollinated crop or partial seed set in cross pollinated crops under sterility inducing phase and partial seed set both in self and cross pollinated crops under fertility inducing phase are suspected to be EGMS. Such suspected EGMS plants are them studied critically in phytotrons or growth chambers under appropriate environmental conditions to confirm the presence of EGMS. If such facilities are not available, then periodical sowings over a period of time can be resorted to with change in temperatures under natural conditions, to observe the transformation from fertility to sterility vice-versa.

5. Characterization of EGMS

Characterization of EGMS lines, essentially involves precise determination of sensitive stage and in case of TGMS lines, determination of critical sterility point (CSP) and critical fertility point (CFP). In case of PGMS lines, critical light length is determined.

5.1 Characterizing TGMS lines under field conditions

- Meteorological Data of 10–15 years can be collected on minimum and maximum temperature, day length, humidity, etc., of the location where the lines are to be characterized.

- During the year, Identify 3–4 distinct periods of high and low temperatures.

- Select the sowing season with the period of 15–25 days before heading (5–15 after PI) coincides with the high temperature. Such plants which remain sterile at high temperature will be selected.

- Note the temperature data pertaining to 15–25 days before heading, this is the critical sterility point of a given line.

- Allow and multiply the plants (selected in #3) by ratooning and subject them to lower temperature regimes at the same growth stage. Plants showing partial fertility or become fully fertile will be identified.

- Record the temperatures which prevailed during the period 15–25 days prior to heading. This is the critical fertility point of a TGMS line.

6. Inheritance of EGMS

In most of the studies inheritance of EGMS has been reported as monogenic recessive. However, there are few studies, where it has been reported as digenic and in one case as dominant, depending upon the crosses in which inheritance has been studied. In PGMS mutant Nongken 58S, Shi [8] reported the inheritance of the character as monogenic recessive, whereas reported it as digenic. Oard and Hu [22] in PGMS mutant M-201S, reported this trait to be controlled by one to three recessive genes. Huang and Zhang [17] in the mutant CIS-25-10 S, reported a single dominant gene. However, Xue and Deng [23] reported that the PGMS trait was quantitatively inherited.

TGMS trait is reported to be monogenic recessive in 5460 S [14], R 59 TS [24] H89–1 [25, 26] and IR 32364 TGMS [25]. However, this trait was reported to be controlled by two recessive genes in Annong S-1 [27] and UPRI 95–140.

TGMS genes in 5460S and H 89–1 (later renamed as Norin PL-12) were designaed as tms_1, and tms_2 respectively Virmani and Borkakati [25] found TGMS gene in IR 32364 TGMS to best non-allelic with the gene in Norin PL-12 and tentatively designated it as tms_3. For lack of accessibility to Chinese TGMS mutant 5460S, allelic test with tms_1, could not be carried out. Subsequently, Reddy *et al.* [28] and Dong *et al.* [29] reported tms_4(t) gene in their studies. Recently Wang *et al.* [30, 31] reported tms_5 gene in Annong S-1 mutant.

Ashraf et al., [32] reported that pollen-mother-cell (PMC) formation, as well as meiosis stages, are induction detection sites for TGMS because at high-temperature wrinkled or abortive pollen grains were produced due to abnormal meiosis in microspore-mother-cells (MMC). Zhou et al. [33] quoted that, other TGMS-lines were also reported from Japan, The Philippines, India, and Vietnam [26, 34–36]. Mostly, reported TGMS-lines or mutants induce male sterility at high temperatures and male fertility at low temperatures [37–39]. The stated TGMS genes/lines are tms1, tms2, tms3, tms4, tms5, tms6, tms7(t), tms8, tms9, tms9–1, and tms10 [30, 31, 40–47] and Zao25S, Lu18S, N28S, 95,850 ms, XianS, Zhu1S, Meixiang851S, and HD9802S [48–53], that provide useful material for two-line HR production. Intriguingly, the reverse phenomena were also observed such as male sterility induced at low temperature and fertility restored at high temperature. Such kinds of TGMS rice-lines are termed as reverse TGMS (rTGMS) lines. Herein, the reported rTGMS genes/lines are rtms1, Diaxin-1A, and IVA and the mutant of Indica-rice variety 26-Zhaizao from China and JP-38S from India [16, 54–57]. The tms5 is an important factor that regulates thermosensitive sterility among many tgms lines.

6.1 Linkage with molecular markers

Linkage with morphological or molecular markers facilitates transfer of EGMS genes to desirable agronomical backgrounds, since mutants are rarely suitable for direct utilization in plant breeding programs. Linkage with morphological markers are rare. Secondly, this character of sensitivity to environmental factors is expressed only under certain specific ranges or conditions of these factors. Under such situation molecular markers are very handy and useful.

A summary of the molecular markers linked to the EGMS genes and the chromosomes on which they are located is given in **Table 1**.

The molecular mechanism underlying the TGMS Lines are studied by Pan *et al*., [61]. They reported the usefulness of thermosensitive genic male sterile (TGMS) lines and photoperiod-sensitive genic male sterile (PGMS) lines to improve rice yields. The male sterility in recently developed TGMS CO 27 is based on co-suppression of a UDP-glucose pyrophosphorylase gene (Ugp1). They studied Microarray-based transcriptome profiling by growing the TGMS-Co27 line and wild-type Hejiang 19 (H1493) line at high and low temperatures. A total of 8303 genes were differentially expressed in the two lines, under the two conditions, or both. Global gene expression was strongly affected by environmental factors. Some genes were strongly repressed in TGMS-Co27 at high temperature were important for pollen development. Notably, series-cluster analysis of differentially expressed genes (DEGs) between TGMS-Co27 plants grown under the two conditions showed that low temperature induced the expression of a gene cluster. This cluster was found to be essential for sterility transition. Many meiosis stage-related genes were included that are probably important for thermosensitive male sterility in TGMS-Co27. Temperature plays a major role in global gene expression and may be the common regulator of fertility in PGMS/TGMS rice lines.

EGMS gene	Linked molecular markers	Chromosomal location	Reference
PGMS Pms_1	RG 477 and RG 511	Chro-7	Zhang et al. [58]
PGMS Pms_2	RG 191 and RG 348	Chro-3	Zhang *et al* [58]
TGMS tms_1	RAP marker 1.2 TGMS	Chro-8	Wang *et al.* [45]
TGMS tms_2	RFLP Marker R 643 A and R 1440	Chro-7	Yamaguchi *et al.* [46]
TGMS tms_3	RAPD Markers OPF 18_{2600} $OPAC3_{640}$ $OPAA7_{550}$ $OPM19_{750}$	Chro-6	Subudhi *et al. [59]*
TGMS tms_4	RM-27 RM-257	Chro-2 and Chro-9	Dong *et al.* [29] Reddy *et al.* [28]
TGMS tms_5	STS Marker C 365–1	Chro-2	Wang *et al.* [30, 31]
TGMS tms_6	RM 3476	Chro-5	Robin *et al.[60]*

Table 1.
Molecular markers for EGMS genes.

7. Breeding of TGMS Lines

Procedures for breeding TGMS lines are similar to conventional breeding procedures, with one major difference. The trait to be selected is male sterility, which requires a particular set of conditions for it's expression and another set of conditions for multiplication of selected segregants. Sterile plants are selected under appropriate conditions in F_2 generation. Such plants are ratooned and grown under fertility inducing conditions to obtain seed of the selected segregants. Since TGMS is a recessive character, and if it is controlled by a single gene in selected segregants, there will be no further segregation for sterility/fertility in F_3 and subsequent generations, though there may be segregation for other plant characters. Thus F_3 generation onwards, the selected segregants can be grown in fertility inducing conditions for selection and forwarding the generations. By F_6 generation, stabilized elite TMGS lines can be developed.

8. Breeding procedures for TGMS Lines

8.1 Germplasm search

Make a detailed and systematic study on germplasm or any stabilized breeding material and look out for spontaneous sterile mutants which may revert to fertility under low temperature. Robin *et al*., [62] developed the new TGMS line (TNAU 60S) and was identified as spontaneous mutant from the rice variety PMK 3 with desirable floral characteristics and stable sterility. This TGMS line has the duration of 125 days with semi dwarf plant type. The panicle exertion percentage is 76.9% with wide angle of glume opening which makes the line with higher out crossing potential highly amenable for commercial exploitation. The grain quality of the TGMS line is highly preferable. TNAU 60 S has been used in hybridization and many heterotic hybrids were developed. This line was registered with NPBGR for its unique TGMS trait as IC 0622805 and INGRI 17028.

8.2 Pedigree breeding

Agronomically adopted line or variety will be crossed with TGMS donor and F1 will be studied under normal Environments and selfed seeds of F1 will be raised under sterility inducing Environment. From this F2 population Sterile plants will be are selected under high temperature *ie* sterility inducing Environment. Such plants are ratooned and grown under fertility inducing conditions to obtain seed of the selected segregants. Since EGMS is a recessive character, and if it is controlled by a single gene in selected segregants, there will be no further segregation for sterility/fertility in F_3 and subsequent generations, though there may be segregation for other plant characters. Thus F_3 generation onwards, the selected segregants can be grown in fertility inducing conditions for selection and forwarding the generations. By F_6 generation, stabilized elite TMGS lines can be developed. Kavithamani et al. [63] developed the many new tgms lines by crossing CO 49 an agronomically adopted variety with TS 29 (promising TGMS line) as a donor line. The lines shows superiority over their parents in sterility with good floral traits and grain yield. The details were given in **Figure 1**.

One of the line were characterized by [64]. TNAU 135S (TS 29/CO 49) with a long duration line showed 100% sterility during January month sowing at Department of Rice, Coimbatore and its complete sterility is under maximum temperature of 31.2°C, and minimum temperature of 21. ^{0}C and critical stage for expression of pollen sterility is 85–104 days. Other lines TNAU 137S developed from TNAU TNAU4S-1-2 /CB 06–564 with CSP of 20.3 also showed complete sterility whereas another line with same female parent TNAU 4S with BPT 5204 showed partial sterility indicated that it requires different month of sowing.

8.3 Molecular tagging of TGMS gene and development of new lines

The TGMS parental line, TS 29 has a stable sterile and fertile phases with substantially low critical temperature. Inheritance studies of the F_2 population revealed that the thermo-sensitive genic male sterility in TS 29 was under the control of single recessive gene. Molecular tagging of TGMS genes in the F_2 mapping population was done by using SSR markers. Out of 50 primer pairs (putatively linked to the six reported TGMS genes in rice) assayed for studying polymorphism, 19 primer pairs produced polymorphic alleles between the parents. The SSR markers revealed 38 percentage of polymorphism between TS 29 and CO(R) 49, the recipient fine grain parent. The identified 19 primer pairs were used for bulked segregant analysis A total of 400 F_3 progenies were raised during summer (fertility

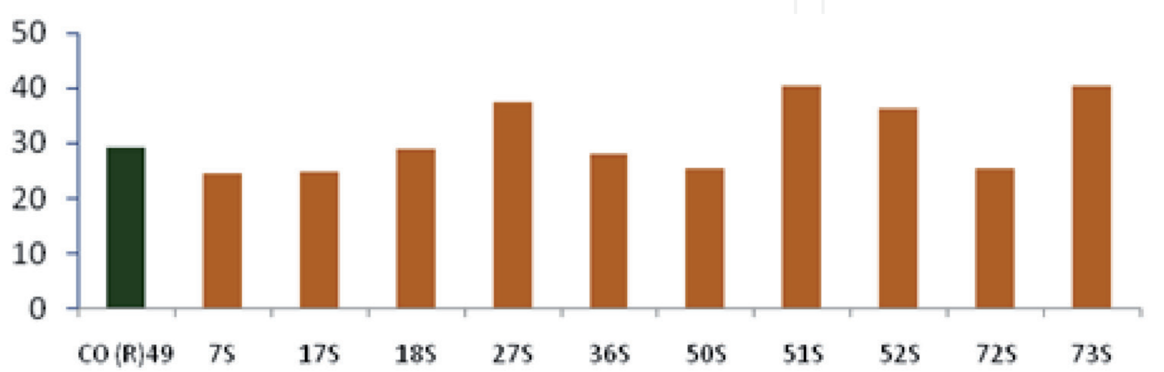

Figure 1.
Promising TGMS lines in comparison with its parents.

limiting season). The DNA of F2 plant which contributed the sterile/ fertile F3 plants were identified and bulked. The study identified one SSR marker, RM 3476 which co-segregated with the phenotypic observations recorded under the field condition. The marker, RM 3476 has already been identified as located adjoining *tms* 6 gene in chromosome 5 of rice. Population advancement for fixing stable segregants with TGMS trait and diversifying the resistance through other crosses and MAS are in progress. However the stability and heterotic potential of TGMS segregant lines were assessed in F4 generation by crossing with tester parents. The F_1s were evaluated along with check hybrids few promising hybrids *viz.*, TNAU 61 S/DE 2, TNAU 31S/ JGL 385 were identified as superior in grain yield and grain quality. Robin *et al*., [60].

8.4 Mutation breeding

Any breeding materials can be mutated and the progenies will be screened for the presence of TGMS gene. Strict observations are to be made in M2 generation planted under high temperature region, as the trait is governed by a recessive gene. To improve the floral traits favoring out crossing two stable TGMS lines *viz.*, TS 6 and CBTS 0282 were subjected to gamma rays (300 and 350 Gy). In the M_2 generation, a total of 469 progeny rows with 1,28, 975 plants of CBTS 0282 and 854 progeny rows with 1,28,100 of TS 6 were raised. 361 sterile plants with good stigma exertion and wide angle of glume opening were selected and stubble planted at Hybrid Rice Evaluation Centre, Gudalur, a low temperature region for inducing pollen fertility and further seed increase. The M_3 and M_4 generations of the selected plants with desirable floral traits were evaluated at high temperature conditions at Coimbatore and seed increase was done at Gudalur. Finally a total of 11 TGMS lines were developed with good floral traits *viz.*, better stigma exertion percentage, wider angle of glume opening and better panicle exertion than the control were identified and are being utilized for two line hybrid rice development [65]. Out of these 11 lines two lines TNAU 84S (TS 29 150Gy) and TNAU 139S(TS 29 100 Gy-3) were characterized by [64] and found that TNAU 139S was completely sterile in all five staggering during January sowing but TNAU 84S did not showed complete pollen fertility but it showed complete spikelet sterility. TNAU 139S recorded CSP of 20.3. Apart from using already available TGMS lines released varieties were also used for developing new TGMS lines.

Mutation studies were further followed with released rice varieties by [66]. Two rice varieties viz., ADT 39 and CR 1009 were utilized to generate genetic variability by exposing them to gamma rays at 50, 100, 150, 200, 250 and 300 Gy. The main focus of this study is to identify TGMS mutants which could help in hybrid breeding programme. Chlorophyll mutants were observed in both the varieties in M_2 generation. The male sterile plants were identified in M_2 generation under high temperature condition (Coimbatore) and the reverted lines in the low temperature region (Gudalur) were planted again in the high temperature condition to confirm their TGMS nature. All the plants expressed complete sterility. Seven plants (comprising five plants from ADT 39 and two plants from CR 1009) isolated from M_3 generation recorded 100 percent pollen and spikelet sterility under high temperature condition and more than 60 percent spikelet fertility under low temperature condition. These lines were further advanced for attaining homozygosity and out of seven lines one of the promising line TNAU 100S was Isolated from the ADT 39 100 Gy. The line was characterized and found that 72–91 days were critical stages for expression of pollen sterility and CSP was 20.5°C [64]. This line also showed complete sterility with wider sterility period. This line is having good grain quality *ie* medium slender grain type which is highly preferred by south Indian people can be exploited for commercial two line hybrid development.

8.5 Backcross programme

Transferring the genes from already available sources to elite genotypes or lines with high combining ability. Tanee et al., [67] utilized this metod for developing new TGMS lines. To transfer *tgms* gene(s) controlling TGMS to Thai rice cultivars by backcross breeding method, Thai rice cultivars ChaiNat 1, PathumThani 1, and Suphan Buri 1 were used as recurrent parents and a male sterile line was used as a donor parent. The BC_2F_2 lines were developed from backcrossing and selfing. An individual plants were evaluated for tmsX gene by conventional breeding and 28 plants were selected from the total plants 78. A total of 18 SSR markers covering the 12 rice chromosomes were employed to select the outstanding genetic background for backcrossing in order to maintain genetic background of the recurrent parent. Selected 60 plants were screened for tmsX gene by phenotyping, subsequently 32 selected plants were screened by added more 18 SSR markers for genetic background in BC2F1 generation. In order to increase the recurrent parent genetic background, MAS was applied at BC2F1 generation by using more number of loci for providing opportunity to find individual plants with the highest genetic background of the recurrent parent. Two plants were isolated from BC2F1 generation and were found to carry 97.22% genetic background. These plants had genetic background higher than BC2F1 generation. Selfed seeds from selected BC2F1 plants were planted at a temperature higher than 30°C and phenotypic selection was employed at flowering stage for selection of the sterile plants. In this way they successfully introgressed the *tgms* gene into Thai rice cultivars.

9. Characterization of the TGMS lines

To study the critical fertility (CFT) and critical sterility (CST) point for TS 29, staggered sowing of seeds in weekly interval was taken up in large cement pots. Pollen fertility was observed. Fertility variation in comparison with maximum temperature prevailed during that month was compared. Complete sterility was observed at a temperature of more than 32/19°C and it was fertile below this temperature. But occasionally sterility was observed above and below this temperature that may be due to combined influence of the other weather parameters. The results of correlation analysis between pollen sterility and weather factors revealed that maximum and mean temperature were the primary factors influencing fertility transition. In this result the negative association was observed between pollen sterility and relative humidity. During the sterile phase relative humidity was low (< 85 per cent) and during fertile phase relative humidity was high (> 90 per cent). Minimum temperature was also observed a significant association with pollen sterility in the latter phase of panicle developments. Sunshine hours had lower level of influence over pollen sterility. Negative significant association was observed between relative humidity and pollen sterility percentage (**Figures 2–4**) [60].

A study was carried out an experiment at the Paddy Breeding Station, TNAU, Coimbatore. The materials comprised, 60 suspected TGMS lines from different populations viz., for screening the sterility /fertility expression. All the population of TGMS lines initiated panicle development during April when the maximum/

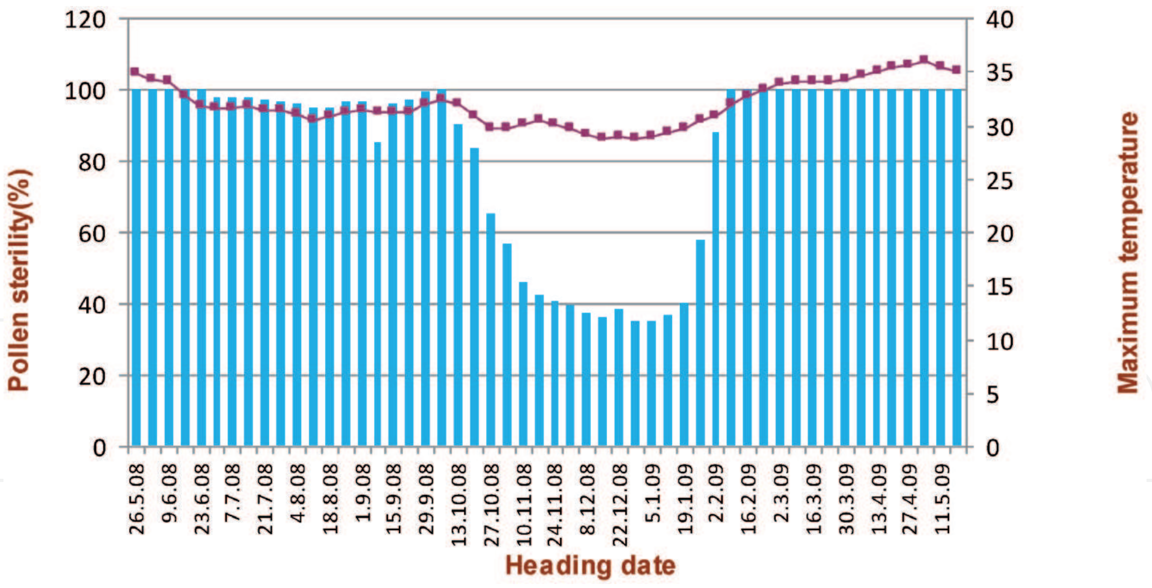

Figure 2.
Fertility behavior of TS 29 for maximum temperature.

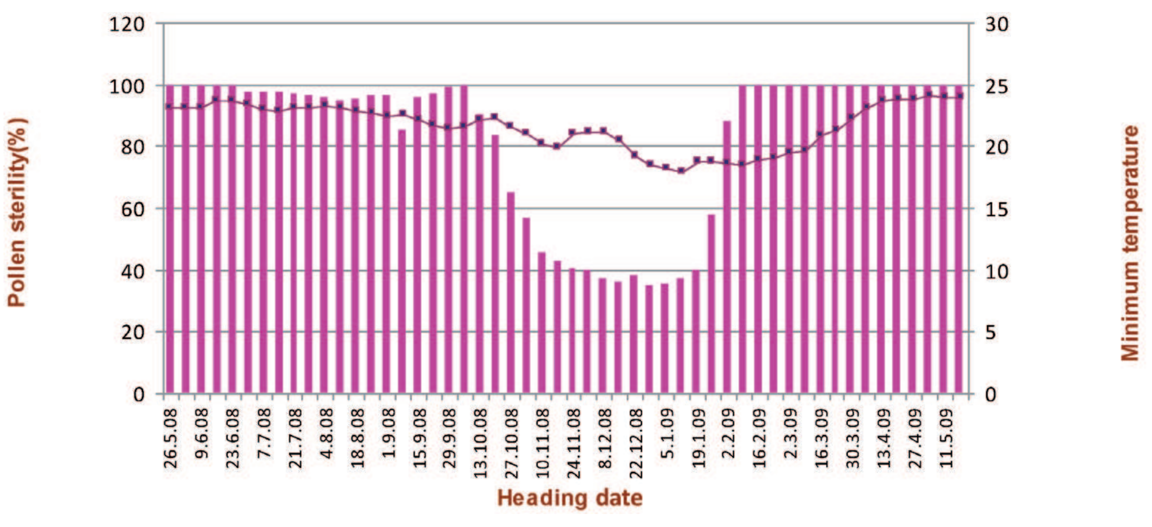

Figure 3.
Fertility behavior of TS 29 for minimum temperature.

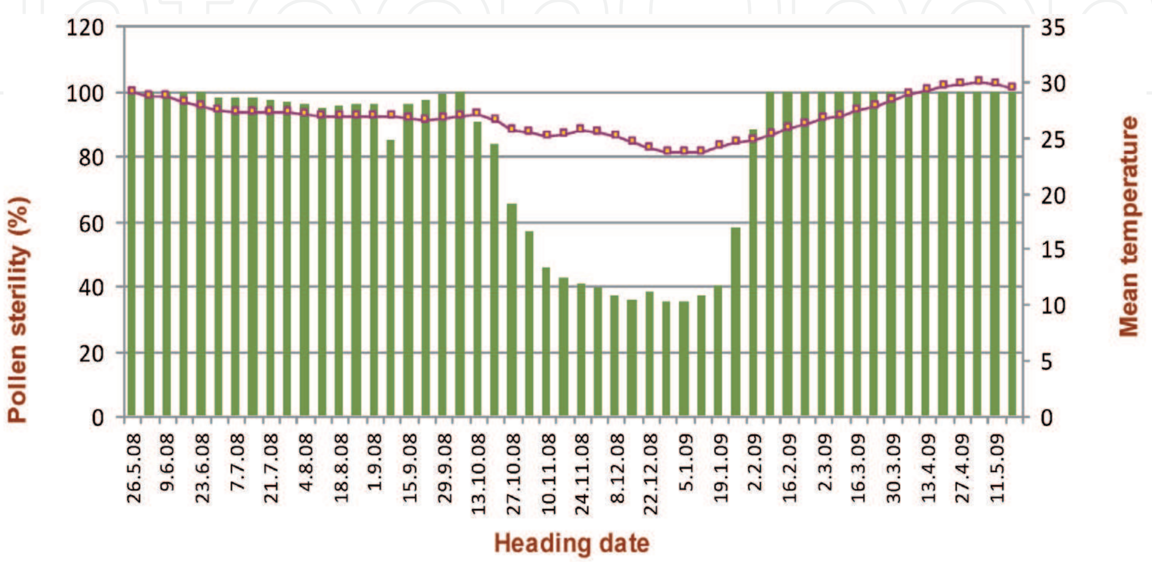

Figure 4.
Fertility behavior of TS 29 for mean temperature.

minimum temperature (day/night) was 30.5–37.8 0 C/22.0–26.4°C. The suspected TGMS lines were evaluated for pollen fertility by using 1% Iodine Potassium Iodide (I-KI) solution. Pollen grains from three randomly chosen fields were evaluated and pollen fertility was expressed in percentage. Five panicles per plant were evaluated for spikelet fertility. Sterile plants identified from promising TGMS lines were ratooned for self multiplication of seeds to confirming fertility transformation during kharif. Pollen fertility/spikelet fertility observation were recorded for ratooned plants. Seeds were collected from each ratooned promising TGMS lines and raised for evaluation during rabi. Pollen/spikelet fertility was assessed for all TGMS lines. Promising TGMS lines identified during summer. Out of 60 population evaluated for TGMS expression, 175 sterile plants were identified based on pollen/spikelet sterility. The sterile plants consisted of 27 F4'S, 40 Fs's, 20 DH's and 88 GD No's. The results showed that 25 lines found promising for stable TGMS expression along with good floral traits. Among the TGMS lines CBTS 0280, CBTS 0283, CBDHTS 025, GD 98014, GD 98028 had early flowering. All the twenty five promising TGMS lines exhibited 100 per cent pollen/spikelet sterility during summer season when the maximum, minimum temperature was 30.5–37.8/22.0–26.4 (day/night). The TGMS lines viz., CBTS 0268 and CBTS 0272 are found to be possessing long slender grain, purple tip, well exerted purple stigma. The TGMS line viz., CBTS 0252 and CBTS 0254 were developed from Indica/Japonica crosses which showed 100% pollen sterility, medium slander grain with purple stigma. The results revealed that the TGMS line viz., CBTS 0252 and CBTS 0254 could be useful to produce two line hybrids with high heterosis for yield with good plant type [68].

Newly developed 66 tgms lines were screened under sterility favoring environment for tgms gene expression during summer and sterility limiting environment during winter at Paddy Breeding Station, Coimbatore for the past five years. Out of 66 TGMS lines, 15 lines showed stable performance and seven TGMS lines *viz*., COTGMS 02, COTGMS 07, COTGMS 10, COTGMS 11, COTGMS 12, COTGMS 13 and COTGMS 15 were completely pollen sterile through out the summer period. It shows that, these lines are having wider sterility expression period. These lines also recorded very good floral traits *viz.,* higher pollen sterility per cent, panicle and stigma exsertion per cent, wider glume opening favorable for enhancing out-crossing rate and seed set percentage during seed production [69].

A total of 21 TGMS lines which showed complete sterility were raised during kharif for seed multiplication in the fertile phase [70]. Among the 21 TGMS lines, 12 TGMS lines viz., TNAU 9S, TNAU 14S, TNAU 15S, TNAU 28S, TNAU 30S, TNAU 32S, TNAU 63S, TNAU 64S, TNAU 67S, TNAU 69S, CBTS 0282-27-1 and TS 6–182-1 were found to be highly fertile and uniform in plant and grain type. Utilizing these lines Crossing block with 7 TGMS lines and 120 male parents were raised for developing new two line hybrid combinations and 23 hybrids were synthesized. All these hybrids were evaluated with already existing hybrids and promising hybrids were forwarded. A total of 9 two line rice hybrids were raised in the advanced yield trial to assess the yield performance along with the check varieties / hybrids viz., CO 48, CORH 3, CORH 2 and ADTRH 1. Six hybrids viz., TNTRH 1, TNTRH 2, TNTRH 5, TNTRH 8, TNTRH 10 and TNTRH 12 performed well over the checks for which mini seed production has been taken up for further evaluation. First time in India these two line hybrids were developed and evaluated under yield trial. The hybrid combination viz., TNTRH 5 has been nominated for Initial hybrid rice trial (IHRT-Medium) during Kharif 2007. This hybrid recorded 6893 kg/ha of grain yield. The promising medium duration hybrid TNTRH 19 recorded the grain yield of 8210 kg/ha which is 28.12% increased yield over CO (R) 49 and was tested under MLT 2010 hybrid Rice – Medium Trial.

Salgotra et al. [71] characterized eight TGMS lines, DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29, showed complete sterility at low altitude and satisfactory seed-set percentage at high altitude. Characterization of floral traits and sterility-sensitive stage were determined by the tracking method. At low altitude, with an average air temperature of 35.4°C, TGMS lines DRR 19S, DRR 20S and DRR 29S displayed a sterility-sensitive stage at 21 days prior to normal heading. For complete sterility the TGMS line DRR 1S requires a temperature of 36.6°C at 17 days prior to normal heading. The temperature for complete sterility ranged from 33.9°C to 35.8°C at low altitude in the remaining seven lines. A significant positive correlation with opening duration of lemma and palea and with size of stigma and angle of opened lemma and palea.

9.1 Out crossing potential of TGMS lines

The newly developed TGMS Lines are to be assessed for its outcrossing potential then only it can be successfully exploited for hybrid breeding programme. [72] studied the outcrossing potential of TGMS lines. In this study average style length of the TGMS lines was 1.87 mm. Maximum style length was recorded in TNAU 18 S with 2.01 mm while TS-29–150 GY and TNAU 60 S had the styles with 1.85 mm and 1.75 mm, respectively. TNAU 18 S had the maximum spikelet opening angle of 23.01^0 and the TS-29–150 GY and TNAU 60 S had the angles of 20.23^0 and $18.54^{0,}$ respectively. TNAU 60 S involved in five cross combination and the minimum of 60.8 cm and the maximum of 74.4 cm plant height was obtained. Similarly TS-29–150 GY had the minimum height of 62.2 cm and the maximum plant height of 66.80 cm involving in two cross combinations. It was noted that the tillering and flowering of TGMS lines were prolonged even after the completion of flowering in male parents. TNAU 18 S showed the 92.8 per cent with highest panicle exertion rate, TS 29 150 GY had the medium value of 66.6 per cent while TNAU 60 S had the lowest of 51.8 per cent. This parameter did not show significant difference among the lines tested. The degree of spikelet opening angle and the duration of panicle opening usually bear significant influence on seed setting percentage. The height difference also played a major role in seed setting percentage. The height difference between the male and the female plants was the maximum of 54 cm in TNAU 18 S x IET 27044. The medium height difference was observed with the cross involved in the crosses of TNAU 18 S as female and the minimum height difference of 22.0 cm was observed in the hybrid generated form TNAU 60 S and CB-09–106. The height differences between the parents of ten hybrids during the flowering period showed notable influence in out-crossing percentage. The medium differences between two parents (22.8 cm to 51.20 cm) had great influences in the seed productions. This is in accordance with the statement elaborated by Virmani *et al*, [73] as this may be attributed to the appropriate height differences which might ensure the contacts of female line's stigma with the maximum pollens at peak anthesis period of the parents concerned.

The seeding interval was determined by the growth duration between the two parental lines. The one with longer duration was sown earlier according to the number of days of difference between the two parents in terms of days to 50 percent flowering [73]. Synchronizing period, though it did not have much variation in this study, had a little effect on the out-crossing potentials of these TGMS by exhibiting varying seed yield in different cross combinations. Three of four cross combination involving TNAU 18 S had the highest out-crossing. Cross combinations with TS-29–150 GY revealed the second highest out-crossing rate.

Manonmani [74] studied Tgms gene introgressed 200 lines forpollen fertility in plains (lowaltitude300 MSL) at Coimbatore during the summer. During the critical period of the crop growth the average temperature was 25–29°C. Then the selected lines were stubbleplanted in high altitude (1500MSL) at Hybrid Rice Evaluation Centre, Gudalur during Khariff. Average temperature of less than 20°C was recorded. During flowering stage, on microscopic observation with potassium iodide stain, some of the sterile lines recorded pollen free anthers (GDR 33S, TNAU 84S & TNAU 86S) and also differences in size of the pollens (GDR 29S). Pollen sterility level observed was 0–98%.All the seventy sterile stubbles from coimbatore were planted at Gudalur, fertility reversion rate was studied and selfed seeds were collected. Based on the pollen fertility observation the70 lines were grouped into four categories. Thirty eight lines showed >90% reversion, 13 lines showed 50–90% and < 11 lines showed <50% reversion and 8 lines showed no reversion. The Selfed seeds from the revesed lines were collected. These lines will be further exploited for their stability and will be used for the development of the two line hybrid in Tamil Nadu.

TGMS lines were also characterized with molecular markers. [75] investigated to study the genetic relationship of thermosensitive genic male sterile lines developed at Tamil Nadu Agricultural University, Coimbatore using morphological traits and SSR markers. Wide genetic variation among TGMS lines were observed for morphological and floral traits. SSR markers survey using 100 SSR markers revealed that 27 were polymorphic, amplifying a total of 71 alleles with an average of 2.67 alleles. TNAU 18S exhibited better performance based on the morphological characters, for a number of tillers per plant, angle of glume opening and panicle length and TNAU 45S expressed good floral characters. Cluster analysis differentiated six TGMS lines into four clusters.

Two TGMS lines (TNAU 60S and TNAU 95S) showed 100 per cent pollen and spikelet sterility and the remaining lines are in the range of 97–98 per cent pollen and spikelet sterility. The stable pollen sterility showed by TNAU 95S was also reported by Srimathi et al. [76] and Kanimozhi et al. [77]. There is a narrow variation in the angle of glume opening which ranged from 20 to 23° among the lines. Panicle length was observed to be more in TNAU 18S (20.25 cm) and while less in TNAU 39S (11 cm). There is a narrow variation in 100 seed weight ranged from 1.97 to 2.44 g. TNAU 45S had the highest stigma length (0.27 mm), stigma breadth (0.08 mm), anther length (0.31 mm) and anther breadth (0.06 mm) among all TGMS lines and TNAU 95S (0.13) had the highest pollen volume compared to all TGMS lines. Euclidean distance values ranged from 4.464 to 6.558 indicating the presence of a wide range of genetic diversity among the six TGMS lines. The ED value was maximum (6.558) between the genotypes TNAU 39S and TNAU 95S, indicating that these genotypes are diversely related to each other. Meanwhile, the minimum ED value was observed between genotypes TNAU 95S and TNAU 60S (4.464) and followed by genotypes TNAU 14S and TNAU 39S (4.507) indicating that these genotypes were closely related to each other.

Identification of the new polyploid rice photoperiod - and thermo-sensitive genic male sterile lines will provide material for further research into polyploidy and hybrid vigor in rice and promote the exploitation of polyploid hybrid rice [78].

Pardeep et al., [79] investigated the eighteen TGMS lines and being used for molecular characterization by sixteen SSR markers and correlated with critical sterility temperature. Based on the data generated on 18 TGMS lines, the UPGMA dendrogram was constructed using Jaccard's similarity coefficients. A total of 47 alleles were amplified using 16 SSR primer pairs. All the lines except for marker RM499 were found to be polymorphic. The range of alleles was 2–5, while the average number of alleles per primer was 2.93. All the three clusters contained one or two fertile lines in each namely, cluster I (TGMS-6), cluster II (TGMS-9) and (TGMS-18) and cluster III (TGMS-1). These fertile lines separated to other sterile lines by three markers with unique bands. TGMS-6 and TGMS-9 showed 200 bp specific band by RM 324 marker, TGMS-1 and TGMS-18 showed 180 bp and 200 bp specific bands and in TGMS-1 also showed 180 bp specific band with RM 254 marker, it means that the specific bands 180 bp and 200 bp generated by different markers in different lines responsible for fertility.

Grouping of TGMA lines based on molecular markers were studied by Mengchen *et al.,* [80]. They studied 48 simple sequence repeat (SSR) markers, and genotyped a panel of 208 *indica* P/TGMS lines and confirmed three subgroups, named *indica*-I, *indica*-II and *indica*-III, in *indica* P/TGMS lines. Further diversity analysis indicated *indica*-II had the highest genetic diversity. The genetic differentiation between *indica*-II and *indica*-III was demonstrated as the largest among the three subgroups. Moreover, *indica/japonica* component identification was detected that five P/TGMS lines possess *indica* components less than 0.900. These results improve our knowledge on the genetic background for P/TGMS lines in China and will be beneficial for hybrid rice breeding programs.

TGMS Lines also introgressed with broad spectrum resistance for many diseases. Wang et al., [81] successfully bred the broad-spectrum resistance gene Xa23 through marker-assisted selection (MAS) combined with phenotypic selection in two novel inbred rice varieties and two photoperiod - and thermosensitive genic male sterility (P/TGMS) lines. All of the developed lines and derived hybrids exhibited enhanced resistance to BB with excellent yield performance.

9.2 Stability of TGMS lines under fertility inducing and sterility inducing environments

In Tamil Nadu, there is an exclusive centre for two line hybrid rice research under Tamil Nadu Agricultural university, Coimbatore. The centre was established during 1996 at Gudalur, Nilgiris District. The seed production procedure mentioned above is followed for evaluating tgms line at Gudalur and Coimbatore. For exploiting the two line breeding system a stable TGMS Lines are needed and seed production to be standartised based on the line to be used and prevailing weather parameters in the particular location.

Manonmani *et al.,* [82] studied the stability of new TGMS lines for sterility and standardized the seed multiplication of tgms lines at sterility and fertility favoring Environments. The experiments were conducted at Paddy Breeding Station, Coimbatore, Farmers field at Sathiyamangalam and Hybrid Rice Evaluation Centre, Gudalur during the *rabi and khariff* seasons in 2013 & 2014 to assess the pollen fertility expression under different temperature regimes in new generation temperature sensitive genic male sterile lines of TNAU. Weather parameters at Coimbatore and Gudalur during for the past fifteen years was analyzed for fixing the sowing season (**Figure 5** and **6**).

Tgms lines were evaluated under sterility inducing Environments *viz.*, Coimbatore and Sathiyamangalam during the month of December 2013 & 2014 (Rabi 2013 & 2014). The same lines were stubble planted and evaluated for pollen sterility under pollen fertility inducing Environment during the month of July 2014 at high altitude (1500 MSL) with cool climate at Hybrid Rice Evaluation Centre, Gudalur. The new TGMS lines developed at TNAU *viz.*, TNAU 45S, TNAU 60S, TNAU 95 S, TNAU 19S and TNAU 39S were evaluated for their stability of pollen sterility under different temperature regimes were given in the **Table 2**.

At sterility inducing Environments the lines showed 100% pollen sterility. These lines were seeded during December at Coimbatore and Sathiyamangalam to expose them to a sterility inducing temperature (>29°C /< 23°C day night) during panicle initiation to flowering stage to test their sterility behavior so that their critical stage

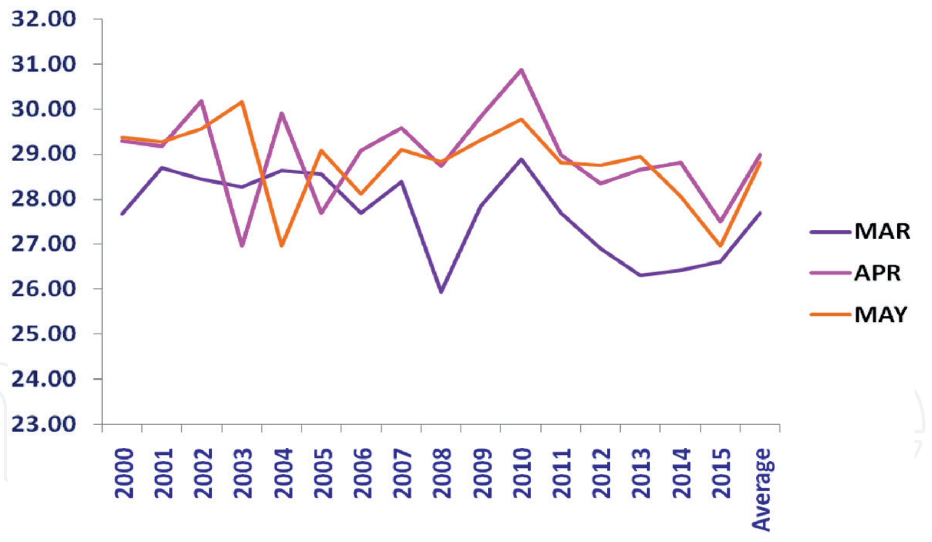

Figure 5.
Mean Weather data at Coimbatore Location.

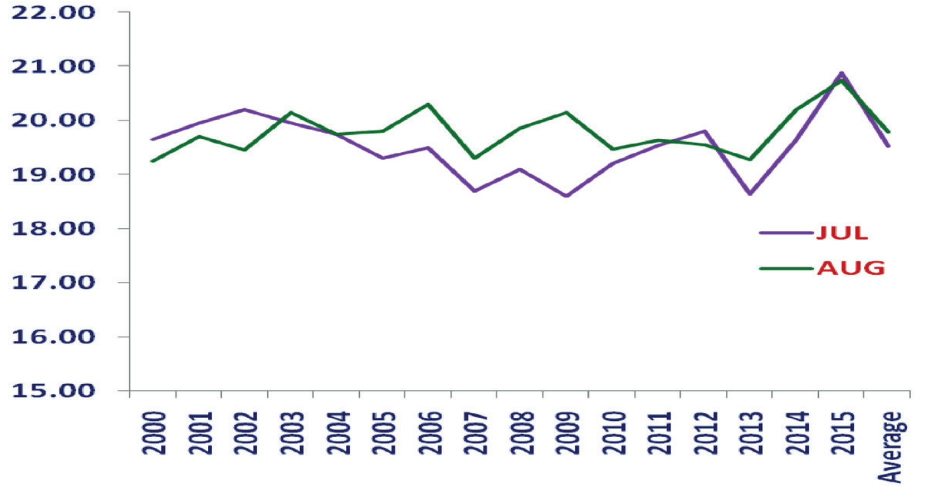

Figure 6.
Mean Weather data at Gudalur Location.

TGMS lines	Rabi 2013		Rabi 2014		Kharif 2013	Khariff 2014
	Coimbatore	Sathy	CBE	Sathy	Gudalur	Gudalur
TNAU 45S	100	100	100	100	5	4
TNAU60S	100	100	100	100	3	5
TNAU95S	100	100	100	100	6	5
TNAU 19S	100	100	100	100	7	9
TNAU39S	100	100	100	100	4	6

Table 2.
Pollen sterility of different TGMS lines in Rice.

of flowering coincides with more than 29°C. Mean weather data for both locations was provided in the **Figures 6** and **7** and it showed that the temperature recorded in both the places exceeded >25°C during the month of March, April and May. During the flowering stage all these lines showed 100% pollen sterility at both the locations for more than 60 days and was test verified for next year also.

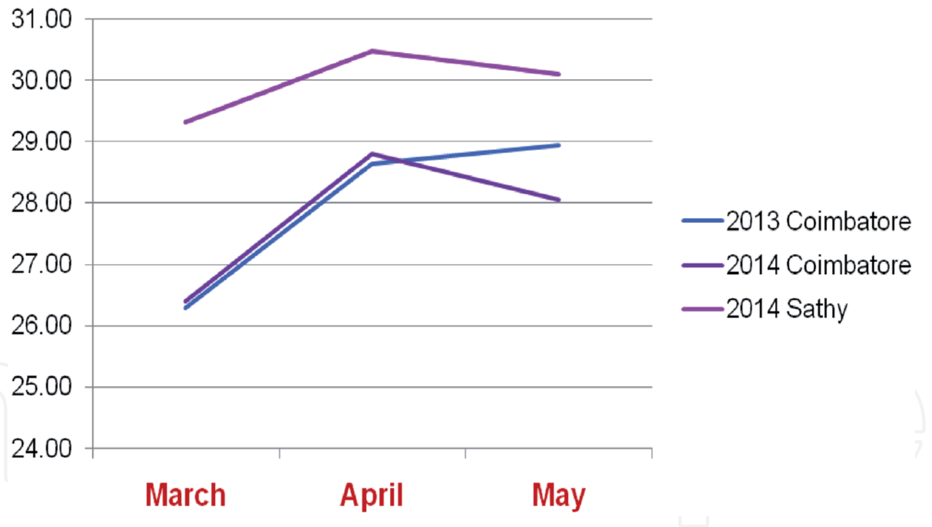

Figure 7.
Mean Weather data at Coimbatore and Sathyamangalam.

The daily mean temperature of 24 to 26°C was found to be the critical temperature for fertility alteration. The sterile stubbles of these lines were planted at HREC, Gudalur during May to induce fertility for their seed multiplication and were exposed at critical stages to fertility inducing temperature (24°C / 18°C day / night). Maximum, minimum, and mean temperature significantly influenced the pollen and spikelet fertility in all five TGMS lines at high altitude. At Gudalur the temperature range during the month of July and August was less than 20°C. The appropriate sowing date of TGMS lines was fixed during June–July in such a way that the critical stages of panicle development would be exposed to the required temperature. The individual lines were maintained under isolation and genetically pure seeds were produced at Gudalur.

The above TGMS lines with wider pollen sterility period under plains can be very well exploited for developing two line rice hybrids during the period of December to April at Coimbatore. The same lines can be easily seed multiplied at Gudalur during July to November.

10. Two line hybrid rice seed production

The TGMS seed produced from high altitude can be brought to locations like Coimbatore as proved above, where stable high temperature prevails during its sensitive stage for nearly 30 days. TGMS plants are planted in 6 rows and sandwiched with two rows of non TGMS good combiner lines on either sides. TGMS line must be randomly checked for complete pollen sterility during its sterile phase at high temperature. Supplementary pollination techniques as applied to three line system must be adopted such as GA_3 spray, rope pulling, flag leaf clipping etc. to increase hybrid seed production. At the time of harvest care must be taken to harvest separately the non TGMS lines first. Seeds harvested from the TGMS line must be cleaned, packed, labeled, and sold to farmers as two line hybrid seed.

Hence Coimbatore and Gudalur locations were identified for the TGMS Seed multiplication and hybrid seed production in Tamil Nadu.

Based on the studied conducted at Department of Rice, Coimbatore and Hybrid Rice Evaluation Centre, Gudalur with Stability of TGMS lines compared with weather parameters over the ten years study resulted in the identification of season for seed multiplication and hybrid seed production as follows in the **Table 3**.

S. No.	Location	Sowing season	Suitable for
1.	Gudalur	June–July	Seed increase of TGMS lines
2.	Coimbatore	December–January	Hybrid seed production
3.	Coimbatore	September–October	Seed increase of TGMS lines
4.	Sathyamangalam	December–January	Hybrid seed production

Table 3.
Standardization of sowing season for the TGMS lines.

10.1 Multiplication of EGMS lines

EGMS lines, if multiplied continuously for several generations without any selection, may segregate for Critical Sterility Point, thereby causing major problems in maintaining purity of the hybrid seeds. Therefore, nucleus and breeder seed production must be taken up on a continual basis.

10.1.1 Method I

- Seeding of TGMS or PGMS lines is arranged in such a way that the sensitive stage occurs when the temperature or photoperiod is favorable for a higher seed set. Nucleus seed production of an EGMS (TGMS or PGMS) line begins in the fertility inducing environment.

- About 100 plants will be selected at the time of flowering, from the population of an EGMS (TGMS or PGMS) line and their panicles are bagged. Within a week selection process should be completed.

- After the harvest, 50 plants with higher spikelet fertility (above 30%) are selected.

- About 30 seeds are taken from each of the selected plants to grow single-row progenies and the remaining seeds are stored carefully. Progenies of the selected plants are grown in the sterility-inducing environment. The balance of the seeds of the progenies that are uniform and completely male sterile must be marked and bulked to form the nucleus seed.

- Nucleus seed of the EGMS line is used for producing breeder seed under strict isolation. Breeder seed for the EGMS line is produced in the fertility-inducing environment.

- The breeder seed produced under the direct supervision of the plant breeder has high genetic purity and is used for producing foundation seed of parental lines, which in turn will be used for producing hybrid seed.

10.1.2 Method II

- Under a sterility-inducing environment select a completely male sterile plant with typical characteristics of the original EGMS line.

- Ratoon the selected plant. Multiply the ratooned stubbles under a fertility-inducing environment. The nucleus seed will be harvested from the ratooned stubbles.

- The nucleus seed is used for producing breeder seed and the latter for producing foundation seed.
- Preserve the selected stubbles under favorable temperature conditions with good management. The new nucleus seed will be produced continuously.

10.2 Seed multiplication of TGMS Lines

Seed production potential in the TGMS lines were studied at Tamil Nadu. During fertility reversion phase can be enhanced by growing them under medium hill regions of Gudalur (1500 m MSL) in Nilgiris district, vTamil Nadu [83]. At Gudalur, the temperature range during the month of July and August was less than 20°C. The appropriate sowing date of TGMS lines was fixed during June–July in such a way that the critical stages of panicle development would be exposed to the required temperature. The individual lines were maintained under isolation and genetically pure seeds were produced at Gudalur. The TGMS line TNAU 60S was evaluated at different locations for their stability in sterility and it was proved that under high temperature (Coimbatore) it expressed 100% sterility and at low temperature it produced more than 90% seed set at Gudalur [74]. This line with wider pollen sterility period under plains can be very well exploited for developing two line rice hybrids during the period of December to April. The same lines can be easily seed multiplied at Gudalur during July to November.

Alternate to hilly areas fertility reversion was studied at plains also in the cooler months by [84]. They evaluated 255 TGMS derivatives generated at IARI-RBGRC, Aduthurai, for fertility phase changes during kharif season. The lines were grown during Apr – Aug at Aduthurai (elevation 19.5 m) conditions when ambient temperature was above 27°c (Season 1). Fully sterile plants identified were stubble planted during Aug – Dec at Gudalur (elevation 416 m) for testing the reversion phase (Season 2). Concurrently, a subset of 43 random fully sterile stubbles were retained at Aduthurai during the same period (Aug-Dec) and their fertility behavior was also observed. There was 69% of the lines showing SF of >45%, since 45% seed set or more is desirable for commercial seed production. In Season 2, the lines stubble planted at Aduthurai also showed fertility reversion between 12 and 65% with 35.6% of the population showing >45% seed set. Temperature of Gudalur ranged between 19 and 33°c during flowering (Nov), while at Aduthurai, it was between 21 and 34°c in the same season. Results indicate that minimum temperature is more crucial than the maximum temperature in fertility reversion behavior.

11. Heterotic potential of two line hybrids

The magnitude of heterosis in two line hybrid is also 5–10% higher than in three line hybrids as it does not have a cytoplasmic penalty. Reported that for most of the characters, the mean heterosis percent was in the order of indica/japonica F1 > Tropica japonica/indica F1 > indica/indica F1 > Tropical japonica/japonica F1.

A comparative studies on two - line, three - line and conventional hybrids of rice (l.) was made at TNAU by [85]. To compare the efficiency of the available systems in hybrid rice technology, a study was conducted to evaluate 120 hybrids belonging to four different group of combinations using cytoplasmic genic male sterile lines (CMS), temperature sensitive genic male sterile lines (TGMS), temperature sensitive genic male fertile lines and well adapted varieties as female parents for their genetic potential related to yield and yield components.

The two line hybrids, TNAU (TGMS)4 x BPT5204, TNAU (TGMS)4 x JGL1798 and TNAU (TGMS)4 x Karnataka Deluxe Ponni were the high yielders and in CMS based hybrids, the hybrid IR 79156 A x Karnataka Deluxe Ponni possessed high yield and good restoration capacity. Of the conventional hybrids, IR 79156B x BPT5204, IR80151B x PSBRC82, IR80151B x WGL32100 and IR80151B x Karnataka Deluxe Ponni have exhibited significant *sca* and standard heterosis for yield [86]. Among the three groups of hybrids the two line hybrids were superior for yield compared to the CMS based and conventional hybrids and its application has great potential to revolutionize rice production in breaking the yield plateau.

Highly heteotic two line hybrids were identified by [87]. They studied three TGMS lines and 20 testers were used to generate 60 two line rice hybrids in a LxT mating design. All the three TGMS lines *viz.,* TNAU 18 S, TNAU 60 S and TS-29–150 GY and nine testers, *viz.*, CB 493, CB 55, CB 508, CB 513, CB 004, CB 921, CB-09–104, CB-09–106, CB 306 showed desirable general combining ability values for two or more characters of which three testers *viz.*, CB 044, CB 009 and CB 306 had positive and significant general combining ability values for single plant yield. Thirty eight hybrids had desirable *sca* effects for at least one of the eight characters. Top hybrids selected with high standard heterosis were TNAU18 S X CB 55, TNAU 18 S X CB 508, TNAU 18 S X CB 044, TNAU18 S X CB 921, TNAU 60 S X CB-09–106, TNAU 60S X CB 493, TNAU 60S/CB 55, TNAU60 S X CB 513 and TS-29–150 GY X CB 306 (**Table 4**).

Considering both physical and cooking quality traits primarily with head rice recovery, the four hybrids namely TNAU 60 S X CB 009, TNAU 60 S X CB-09–106, TNAU 18 S X CB 921 and TNAU 18 S X CB 044 had acceptable grain quality traits with maximum phenotypic scores. These hybrids were studued for their adoptability under three environments. Comparing both Eberhart and Russell and AMMI models to all hybrids and checks, four two - line hybrid*s viz.,* TNAU 60 S X IET 21009, TNAU 60 S X CB-09–106, TNAU18 S X CB 921 and TNAU 60 S X CB 513 gave above average values and outperformed the checks thus showing considerable stability in the tested locations. On comparison of the two line rice hybrids with their test entries of similar maturity groups belonging to CGMS system and HYVs, obviously the two line rice hybrids gave additional per day productivity ranging from 3.23 to 44.53 percent increase. The hybrid TNAU 60 S

SN	Hybrid combination	Single plant Yield (Kg/plant)		
		d_i	d_{ii}	d_{iii}
1	TNAU18SX CB 55	122.36**	113.3**	108.88 **
2	TNAU18S X CB 508	124.81**	122.5**	122.49 **
3	TNAU18SX CB 044	203.18**	202.7**	196.45 **
4	TNAU18SX CB 921	118.54**	77.26**	178.99 **
5	TNAU 60SXCB-09–106	80.48**	70.39*	66.86 **
6	TNAU60SX CB 493	70.34**	27.76	98.82 **
7	TNAU60SX CB 55	141.98**	125.7**	102.96 **
8	TNAU60SX CB 513	224.32**	151.00**	95.27 **
9	TS-29–150GYX CB 306	118.2**	100.5**	151.48 **

,Significant at 5% and 1%, respectively.*

Table 4.
Heterotic potential of Two line rice hybrids.

X CB 55 significantly differed from other entries for six traits in initial hybrid evaluation and notably recorded 34.30 g of single plant yield. This hybrid also had medium slender grain type, high head rice recovery with intermediate amylose content and desirable pasting properties leading to a score of 40 in IRRI quality scale. This cross combination yielded fairly good amount of hybrid seed in mini seed production plot and also the female parent TNAU 60 S had acceptable out-crossing percentage. Multi location trials revealed that this particular hybrid fell under medium maturity group with 131 days of maturity, highest panicle length and also the highest yielded grain yield of 13082 $kgha^{-1}$ with 99.6 $kgha^{-1}$ day^{-1} productivity.

The hybrid combination was given with name TNTRH 55. The new two line rice hybrid TNTRH 55 with a duration of 125 days was synthesized using tgms line TNAU 60S with CB 55. The hybrid seeds were produced with minimum staggering between the parental lines. The hybrid was tested with station trials at Coimbatore and also at HREC, Gudalur the exclusive station for tgms line multiplication situated at 1500 MSL in Tamil Nadu for three seasons (Khariff 2014, 2015 & 20 (**Tables 5** and **6**) [88]. All the biometrical traits along with blast reaction were also studied with check varieties. Per day productivity of this hybrid was 39.7 kg/day.

This hybrid showed 13–25% yield increase over the check variety. It showed resistance to blast with the score of 1. The hybrid produces medium slender grain type with Intermediate amylose content and Gel consistency. In Multilocation Evaluation Trial it recorded a grain yield of 6562 kg/ha which was 17.4% over ADT 39 and on par with medium duration check TNAU rice hybrid CO 4 (6578 kg/ha). At present the hybrid is under advanced stage of evaluation in Tamil Nadu. If this hybrid qualifies the criteria for release it will be the first two line rice hybrid ever released for cultivation in India. By the release of two line rice hybrid we can reduce the hybrid cost drastically as it involves only two parental lines for seed production [82].

Chandrasekhar [89] studied 1000 hybrids by crossing 500 germplasm lines (male) with one CGMS female line (IR79156A) and one TGMS female line (IR75589) and were evaluated in test cross nursery. Among the lines tested in test cross nursery on CGMS female, 60% lines are either partial restorers or partial maintainers. The maintainers proportion was 9% and restorer was 33%. TGMS female on an average across 161 combinations yielded 6.25 F_1 seed yield per plant in comparison to 4.95 g F_1 seed in CMS female. It was observed that TGMS females in general have higher seed production potential than the CMS female and TGMS female yielded 26% higher seed yield that CGMS female on an average across male lines in the study. For grain yield 187 hybrids recordedsignificant

Entries	DFF	Plant Ht (Cm)	Panicle length	Spikelet fertility	Duration	Grain yield (kg/ha)	Per day productivity (kg/day)
CO R 51	95	82.33	20.8	70.32	115	4373	**38.028**
CORH 3	95	70.66	17.46	76.28	115	4276	37.178
TNTRH 58	105	72.66	21.66	46.38	130	2478	19.063
TNTRH 55	**105**	**75.00**	**22.53**	**80.10**	**125**	**4956**	**39.650**
CORH4	109	96.00	25	66.95	139	3644	26.218
CO (R) 50	112	90.16	22.13	64.32	142	5102	35.930

Table 5.
Evaluation of two line rice hybrids at HREC, Gudalur.

Genotypes	Yield (kg/ha)			
	Kharif 2014	Kharif 2015	Kharif 2016	Average
TNTRH 55	4956	4194	6410	5186
ADT 39	3644	3065	5128	3945

Table 6.
Performance of two line rice hybrid over seasons.

Hybrid code	Combination	Days to 50% flowering	Grain Yield (t/a)	Standard heterosis
H137	IR75589TGMS x PLASD20	82	9.80	34.4**
H203	IR75589TGMS x PLIR547452231983	86	9.62	31.9**
H323	IR75589TGMS x PLSANTOSH	88	9.24	26.7**

, Significant at 5% and 1%, respectively.*

Table 7.
Heterotic potential of the hybrids.

positive heterosis over better parent and 128 hybrids over standard check. The top two line hybrids H137 (34.4%), H203 (31.9%) and H323 (26.7%) recorded the highest significant positive heterosis for grain yield (**Table 7**). TGMS hybrids exhibited higher average grain yield heterosis than the CGMS hybrids.

12. Status of two line rice hybrids in India

TGMS or two line system based rice hybrids are predominantly cultivated in Northern India (Punjab, Haryana) and some parts of Chhattisgarh. Commercially in India, Savannah Seeds Private Limited is the major player which supplies their TGMS hybrids in the brand name of “SMART Rice”. Currently based on estimates the two line hybrids occupy area of around 400000–450000 acres in India. Some of the prominent hybrids in the market are Sava smart rice −127, Sava smart rice −134, Sava smart rice −200, Sava smart rice −300 etc. The two line system hybrid seed production requires stable weather parameters and currently in India major hybrid seed production in the northern states of Haryana, parts of Rajasthan and Punjab during Kharif season. This is quite opposite to 3 line system in which the major seed production area in southern states and in rabi season. Some of the other companies actively involved in the parental line and hybrids development in 2 line system are Syngenta, Corteva Agriscience, Monsanto-Bayer etc.

13. Future prospects

- Long lasting research in TNAU resulted in the development of short duration, medium duration and long duration TGMS lines with better out crossing percentage, good grain quality with better agronomic traits can be exploited for developing good grain quality two line rice hybrids. It can be achieved through the selection of good grain quality similar duration tgms lines subsequently by heterosis breeding approach.

- Development of TGMS lines with herbicide tolerance for making the seed production ease and maintaining the genetic purity enables to reduce the seed production cost. Markers are developed for this herbicide tolerant line. By adopting marker assisted back cross breeding method one can convert tgms line with herbicide tolerant trait.

- Application of Marker Assisted Breeding for introgression of biotic and abiotic stress tolerant gene in to the TGMS line and male parents. Promising donors are available with multiple stress tolerance that can be utilized.

- Exploitation of *japonica* germplasm with wide compatible gene for developing new TGMS lines.

- Using the conventional and molecular approach it can be achieved.

- Developing seed production packages for enhancing the row ratio and seed produce ability of two line hybrids by formulating the experiments with more number of female rows it can be standardized.

- Exploiting the ideal locations already identified places for seed production and seed multiplication. As it was indicated in the text one can exploit their own locality for seed production utilizing the past ten to fifteen years weather data.

- Employing Genome editing techniques for quick development of TGMS lines with proven or mega varieties. The success report was already published by employing the Crisper CAS 9 technique they developed the tgms line. Similar approach can be adopted.

- Molecular characterization of available TGMS lines and identification of mechanism involved in regulation of genes through omic technologies.

- Development and testing of large number of two line rice hybrids across the locations. The hybrids developed will be tested across the locations and stability models can be exploited for identifying the adoptable hybrids with higher yield potential.

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