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Research Article

Genetics of sterility behaviour in Thermo Sensitive Genic Male Sterility system in rice (*Oryza sativa L.*)

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Abstract
A total of 1493 F_2 populations of five crosses were evaluated to study the TGMS nature under high temperature regime at Coimbatore. The present study revealed that the observed variation among 1493 F_2 populations showed 25 per cent of population were completely sterile. The chi-square analysis did not yield significant outcomes for the crosses TNAU 45 S x CB15127, TNAU 60 S x CBSN 17, TNSAU 60 S x CBSN 20, and TNAU 95 S x CBSN 366. The expected segregation ratio of 75:25 (fertile: sterile) was not significant ($P>0.05$) in crosses *viz.*, TNAU 45 S x CB15127, TNAU 60 S x CBSN 17, TNAU 60 S x CBSN 20 and TNAU 95 S x CBSN 366 indicating that the observed ratio was a good-fit. Stigma length, stigma breadth, anther length, and glume opening angle showed positive skewness and platykurtic distributions, suggesting that the genes were under the influence of dominant x dominant-based complementary gene behaviour and controlled by a larger number of genes. However, stigma exsertion, anther breadth, sterile lemma length, panicle length, and panicle exsertion exhibited negative skewness and leptokurtosis distribution patterns, suggesting gene regulation by a smaller number of genes.

Keywords: Frequency-density curve, Rice TGMS, Chi-square, Skewness and Kurtosis

INTRODUCTION

Rice (*Oryza sativa* L.) is an important and essential food source for more than half of the world's population. Two significant advances in rice breeding history are the Green Revolution in the 1960s and the introduction of hybrid technologies in the 1970s resulted in significant gains in rice breeding. Rice provides 20% of the calories consumed by more than half of the world's population, making it essential to global nutrition. Over 90% of the world's production and consumption of rice is concentrated in Asia (Ali *et al.,* 2021). To adequately nourish the projected population of nine billion people by 2050, it is predicted that rice yields must grow by at least 50% (Feng *et al.,* 2017). "Hybrid Rice Technology" is an efficient tool to increase rice yield many folds. Rice hybrids have demonstrated an excellent production increase of 20–30% when compared to conventional methods under the same watering conditions (Viramani *et al.,* 2003). A key turning point in the history of rice breeding was reached in China in the late 1970s when hybrid rice technology was first successfully developed and adopted for commercial use (Li and Yuan, 2009). This discovery not only changed rice yield and production, but it also rekindled international interest in hybrid rice breeding. However, India faced a number of difficulties in the practical production of hybrid seeds, which discouraged the commercialization of heterosis. Fortunately, a significant advance was made in 1976 when it was realized that the use of male sterility (MS) systems could be used to produce hybrid rice on a large scale (Lin and Yuan*,* 1980). This discovery opened the door for more improvements in hybrid rice cultivation and gave rise to fresh optimism for sustainably raising rice yields.

In China, hybrid rice production was first established in 1964, while in India, it was introduced in 1989. Currently, commercial hybrid rice production is active in over 40 nations (Changxiang Mao *et al.,* 2012). The overwhelming reliance on WA (Wild abortive) as the sole source of cytoplasmic male sterility (CMS) and the difficulties in seed production and parental line development, however, make the investigation of alternate strategies to harness hybrid vigour in rice is necessary. The two-line method, which combines thermosensitive genic male sterility (TGMS) lines with fertile lines or varieties to produce commercial F1 hybrids, is one an efficient strategy in rice. The two-line system of hybrid breeding gets its name from the fact that it only needs two lines to maintain and propagate male sterile lines and produce hybrid seeds. This breeding program has had a significant influence and has marked a revolutionary development in agricultural technology.

The identification of thermosensitive genic male sterility (TGMS) was a substantial advance in this field. The genic male sterile lines that are temperature sensitive shows sterility at high temperatures (>25°C) and fertility at low temperatures (<23°C) (Arasakesary 2015). This discovery has created new opportunities for producing hybrid rice that is both efficient and effective. Two-line hybrids exhibit a high level of heterosis that is between 5 and 10% higher than three-line hybrids (Swaminathan, 2021). The traditional three-line hybrid rice technology is expected to be gradually replaced by TGMS based two-line hybrid rice breeding over the course of the next decade due to recent scientific advancements. This change is anticipated to significantly increase rice yield and further exploit the benefits of heterosis. Hence the proposed study is aimed at identifying new TGMS lines with better outcrossing traits.

MATERIALS AND METHODS

The genetic material consisted of 1493 F_2 population derived from five different crosses namely, TNTRH 128 (TNAU 45 S x CB 15127), TNTRH 167 (TNAU 60 S x CBSN 17), TNTRH 168 (TNAU 60 S x CBSN 20), TNTRH 173 (TNAU 95 S x CBSN 366), and TNTRH 175 (TNAU 95 S x CBSN 383). The whole set of F_2 population was planted at the Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore located at 11° N latitude and 77° E longitude with an elevation of 426.7 m above mean sea level during *Rabi* 2022 – 2023. The temperature regime in 2023 from 15 th March 2023 to 15 th May 2023 is presented in (**Fig. 1**).

The study concentrated on a number of traits, all studied under sterility-favouring conditions, including anther length, anther breadth, stigma length, stigma breadth, glume opening angle, pollen sterility, stigma exsertion, sterile lemma length, anther colour, stigma colour, sterile lemma colour, sterile lemma shape, panicle length, panicle exsertion, and spikelet fertility. Pollen fertility was assessed under a light microscope, using 1% Iodine-Potassium iodide) solution as stain. A stereomicroscope was used to measure the anther length, anther breadth, stigma length, stigma breadth, stigma colour, and sterile lemma length (ElShamey *et al.,* 2022). To ascertain the number of genes governing sterility across various F_2 population crosses, the anticipated values aligned with observed values for each cross were computed based on the projected ratios for F_2 generation crosses. The projected ratio for a singular gene within F_2 crosses is 3:1. The disparities between observed and projected outcomes were evaluated using the chi-square test, employing the formula provided by Little and Hills (1978). R software was used to do statistical analysis on the collected data (Fathima *et al.,* 2021). Descriptive statistics included skewness and kurtosis to explain the distribution pattern of fertility and sterility.

RESULTS AND DISCUSSION

A total of 1493 F_2 populations of 5 crosses were evaluated to study the TGMS nature under high temperature regime at Coimbatore during *Rabi* 2022-23. Visual studies of pollen in the unopened spikelets revealed that F_2 populations produced fertile pollen grains as fertile parents. The F_2 population was further tested under a microscope with a 1% Iodine Potassium Iodide (IKI) solution using 15 spikelets from newly developed panicles of all the plants. The observed variation among the F_2 group for the attributes revealed considerable differences among the population. Twenty five per cent of the population (373 lines) were observed as completely sterile and seventy-five per cent as fertile population (1120 lines) were fertile under high temperature regime at Coimbatore (**Fig. 2**). All the F_2 plants of five crosses judged by pollen fertility data indicated a dominant nature of the fertility restoration gene(s). The frequency of sterile and fertile plants indicated significant genetic variability, implying that genetic improvement for the characters analysed is possible.

In the F_2 population of five crosses, there was considerable diversity in spikelet fertility ranging from completely sterile to highly fertile spikelets. Specifically, about 25% of the genotypes had completely sterile spikelets, 5% had highly sterile, 13% had partially sterile, 22% had fertile, and 35% had highly fertile spikelets. According to the findings of this study, F_2 in all crosses were fertile, indicating dominance of fertility over sterility. The number of sterile plants observed suggests the possibility of using the 25% sterile lines for heterosis in two line rice breeding. Similar result was obtained by Vinodhini *et al.*(2019). The information regarding the fit of observed data to expected genetic ratios for various

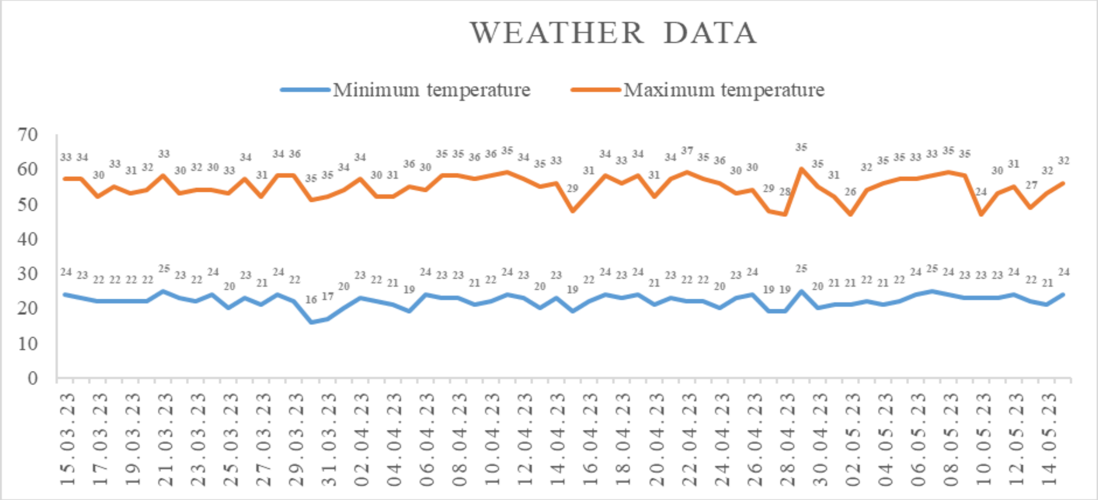


Fig. 1. The recorded temperatures in experimental field, from 15 th March 2023 to 15 th May 2023

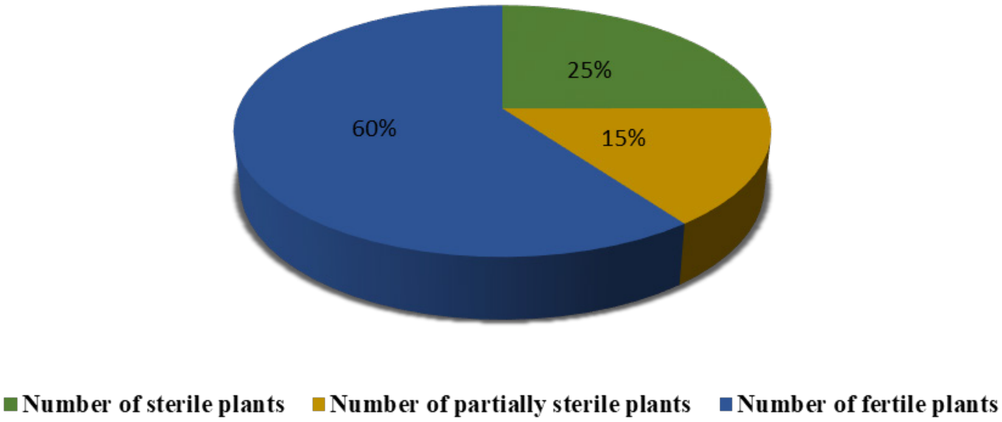


Fig. 2 Fertility/Sterility behaviour of F_2 population

F_2 generation crosses is presented in **Table 1**. The chi-square analysis did not yield significant outcomes for the crosses TNAU 45 S x CB15127, TNAU 60 S x CBSN 17, TNSAU 60 S x CBSN 20, and TNAU 95 S x CBSN 366. However, a significant result from the chi-square test was obtained for the F_2 generation of the cross TNAU 95 S x CBSN 383 cross. This suggests that the observed ratio aligns well with expectations, and any deviations from it are likely due to random chance. Thus, the null hypothesis remains valid in this context. The conformity of the 75:25 segregation ratio in the mentioned crosses indicates a prevalence of sterile genes over fertile ones in these crosses, supporting the assumption that sterility is controlled by a single recessive gene in these breeding combinations. This finding was similar to the observations made by Lawan *et al.*(2018) in their study on sorghum, the anticipated 75:25 ratio of segregation between fertile and sterile individuals in the BC_1F_1 generation.

The range of sterile plants was 7 to 105, with crosses TNAU 95 S x CBSN 383 and TNAU 45 S x CB15127 having the lowest and greatest numbers, respectively. The range for fertile plants was 96 to 287, with cross TNAU 95 S x CBSN 383 having the account on higher side (93%). The cross TNAU 45 S x CB15127 yielded a total of 392 plants, comprising of 287 fertile and 105 sterile ones. This resulted in an observed ratio of 73:27 (fertile: sterile). Similarly, the cross TNAU 60 S x CBSN 17 produced 377 plants, with 280 being fertile and 97 sterile, presenting an observed ratio of 74:26 (fertile: sterile). In the case of the cross TNAU 60 S x CBSN 20, a total of 327 plants emerged, including 240 fertile and 87 sterile plants, giving an observed ratio of 73:27 (fertile: sterile). Moreover, the cross TNAU 95 S x CBSN 366 led to 294 plants, out of which 217 were fertile and 77 were sterile, demonstrating an observed ratio of 74:26 (fertile: sterile). Furthermore, the cross TNAU 95 S x CBSN 383 displayed 103 plants in total, with 96 being fertile and 7 being sterile, resulting

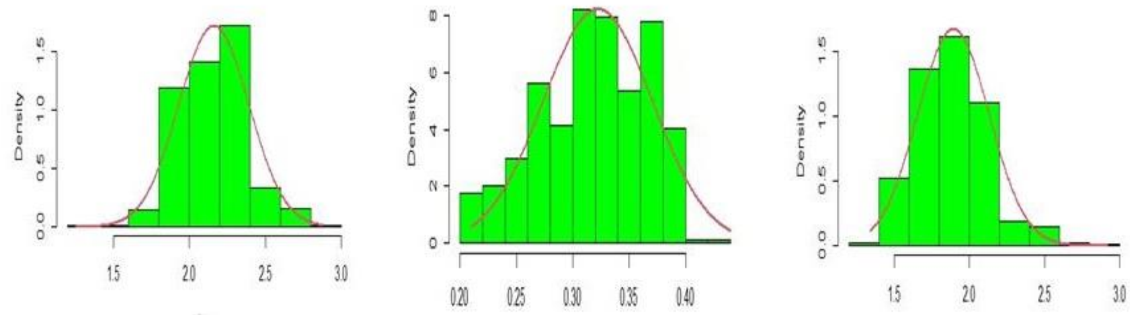
Table 1. Test of goodness of fit to genetic ratios for different F_2 crosses

Crosses	Observed frequency			Expected frequency			χ^2 value			Ratio
	Fertile plants	Sterile plants	Total	Fertile Plants	Sterile plants	Total	Fertile Plants	Sterile plants	Total	
TNAU 45 S x CB15127	287	105	392	294	98	392	0.17	0.50	0.67^{ns}	3:1
TNAU 60 S x CBSN 17	280	97	377	282.75	94.25	377	0.03	0.08	0.11^{ns}	3:1
TNAU 60 S x CBSN 20	240	87	327	245.25	81.75	327	0.11	0.34	0.45^{ns}	3:1
TNAU 95 S x CBSN 366	217	77	294	220.5	73.5	294	0.06	0.17	0.22^{ns}	3:1
TNAU 95 S x CBSN 383	96	7	103	77.25	25.75	103	4.55	13.65	18.20^{*}	15:1

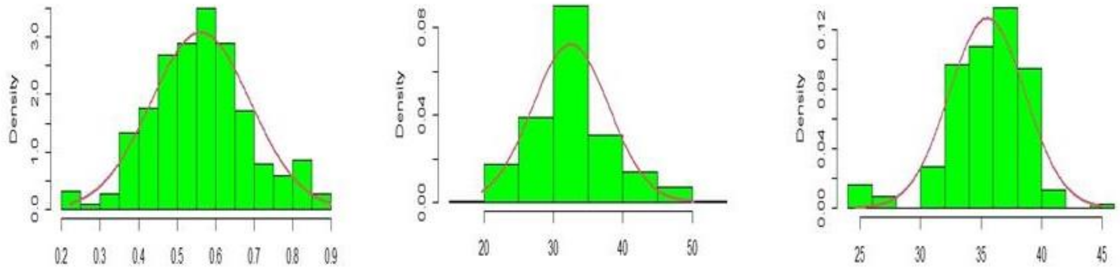
in an observed ratio of 93:7 (fertile: sterile) (**Table 1**). The expected segregation ratio of 75:25 (fertile: sterile) was not significant (P>0.05) in crosses *viz.*, TNAU 45 S x CB15127, TNAU 60 S x CBSN 17, TNAU 60 S x CBSN 20 and TNAU 95 S x CBSN 366 indicating that the observed ratio was a good-fit. Whatever deviation observed from the expected, was due to chance and the null hypothesis holds true. The 75:25 segregation ratio in the above crosses suggests that sterile genes outnumbered fertile genes in crossings and that the hypothesis that sterility is mediated by a single recessive gene is still valid in the crosses made. The expected segregation ratio of 75:25 (fertile: sterile) was significant (P<0.05) in cross TNAU 95 S x CBSN 383 indicating that the observed ratio was not a good fit. The influence of the female parent and/or a potential variation in the expression of the weaker gene in various genetic backgrounds could also be responsible for these changes in the type of gene interaction. Similar results were also reported by Reddy *et al.* (2010) indicating a goodness of fit between restorer parents and A-lines. According to research by Raj and Virmani (1988) and Mann (1994), specific modifier genes have been identified as being responsible for modifying the segregation ratio over generations of rice and wheat. The F_2 population of the crosses TNAU 95 S x CBSN 383 had chi-square values that were significant at the projected segregation ratio of 93:7 (fertile: sterile). Because chance alone was not the origin of the observed inequalities, the null hypothesis was rejected, demonstrating a poor fit of the observed ratios. Elkonin *et al*. (2009) and Nikolova *et al*. (2012) obtained similar results. Reddy *et al*. (2010) also observed that the 93.75:6.25 ratio in BC_3F_1 for crosses 85 x 86 and 477 x 478 was not significant, showing that sterility is mediated by a single recessive gene.

Skewness and kurtosis are advanced statistical measures to examine the characteristics of gene behaviour and the number of genes influencing traits. Skewness offers insights into the concentration of deviations above or below the central measurement, indicating the degree of positivity or negativity in the population. Positive skewness is linked to complementary gene behaviour, while negative skewness is associated with duplicated gene behaviour. Kurtosis assesses the sharpness of the distribution in the population. The dispersion of the different parameters measured was examined graphically, by plotting the graphing in frequency distribution as per Anupriya *et al.* (2020) (**Fig. 3**). A probability density curve was employed to assess the distribution patterns of the recorded parameters within the 1493 TGMS genotypes. Stigma length displayed a positive skewness (0.78) and a leptokurtic (1.27) distribution. This positive skewness and leptokurtic distribution indicated the presence of complementary gene behaviour influenced by a smaller number of genes. Stigma breadth exhibited a positive skewness (0.19) and a platykurtic (-0.62) distribution. Anther length demonstrated a positive skewness (0.09) and a platykurtic (0.28) distribution. Glume opening angle showcased a positive skewness (0.61) and a platykurtic (0.60) distribution (**Table 2**). Notably, a positive skewness and platykurtic distribution were observed in terms of single plant yield, suggesting that the genes were under the influence of dominant x dominant-based complementary gene behaviour and controlled by a larger number of genes. The outcomes align with the earlier research on maize by Sarankumar *et al.* (2019), wherein it was established that a positive kurtosis indicates a genetic activity governed by a limited number of genes, while a negative kurtosis suggests a lack of genetic activity controlled by a greater number of genes.

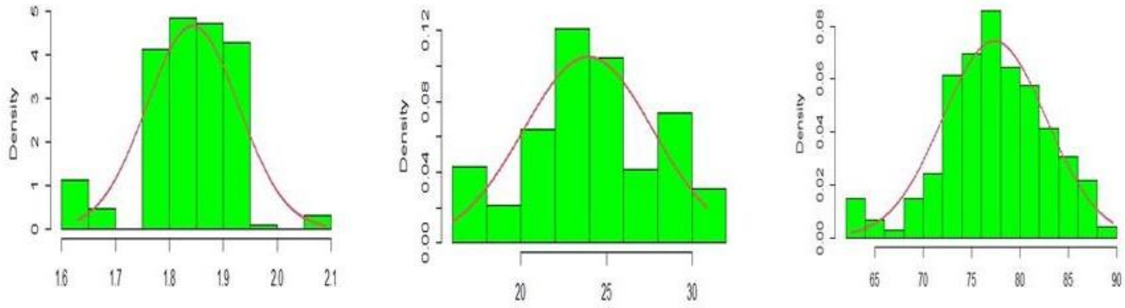
Nevertheless, stigma exsertion displayed negative skewness (-1.01) and leptokurtosis distribution (2.33). This specific distribution pattern, characterized by negative skewness and leptokurtosis, signifies the presence of complementary gene action and suggests that the trait is regulated by a smaller number of genes. On the contrary, anther breadth exhibited a distribution featuring negative skewness (-0.39) and platykurtosis (-0.62) (**Table 2**). Similarly, sterile lemma length, panicle length trait and panicle exsertion demonstrated a distribution with negative skewness and platykurtosis. The distribution pattern, marked by negative skewness and platykurtosis, is an indicative of duplicate epistasis gene action. The results suggested that the traits such as sterile lemma length, panicle length trait and panicle exsertion were controlled by a larger number of genes. The outcomes correspond with the findings of Neelima *et al.* (2020). Furthermore, the negatively skewed platykurtic distribution implies that these traits are governed by a larger number of genes, which encompass dominant and dominance-based duplicate epistasis.



a) Anther Length b) Anther Breadth c) Stigma Length



d) Stigma Breadth e) Glume Angle f) Stigma Exsertion



g) Sterile lemma Length h) Panicle Length i) Panicle Exsertion

Fig. 3. Frequency distribution of nine parameters among the TGMS lines under sterility favouring environment

Table 2 Skewness and Kurtosis of floral and out crossing related traits of TGMS lines

Characters	Skewness	Kurtosis
Stigma length	0.78	1.27
Stigma breadth	0.19	-0.62
Anther length	0.09	0.28
Anther breadth	-0.39	-0.62
Glume angle	0.61	0.60
Stigma exsertion	-1.01	2.33
Sterile lemma length	-0.36	0.62
Panicle length	-0.01	-0.69
Panicle exsertion	-0.35	0.27

The findings of this study, indicated that the nature of gene action and the number of genes controlling the trait favours the type of selection to be carried out for improving the crop.

The calculated χ2 value was lower than the χ2 value found in the table suggesting that a recessive nuclear gene is responsible for governing thermo sensitive genic male sterility in the TGMS lines.

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