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RESEARCH ARTICLE

Comprehensive analysis of morphological and genetic traits in advanced thermo-sensitive genic male sterile (TGMS) lines of rice (*Oryza sativa* L.)

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Abstract

The main aim of this study was the characterization of genetic, morphological and floral traits of TGMS lines to evaluate their potential for large-scale hybrid seed production. Ten TGMS lines were evaluated for fertility-sterility transformations under changing temperatures and the critical sterility and fertility temperatures were ranged from 24 °C to 28.9 °C. Variations in floral biology, out-crossing rates, glume opening angles and flower opening duration were observed among the lines. The genetic advance in the study ranged from 0.19 % to 125.29 %, with the highest recorded for number of spikelets per panicle (125.29 %) and seed yield per plant (27.64 %). Traits such as time of anthesis showed a low genetic advance (0.91 %), while critical sterility temperature and panicle length exhibited moderate advances of 3.77 % and 7.83 %, respectively. Phenotypic coefficients of variation were high for out-crossing rate (34.82 %), angle of glume opening (24.91 %) and number of spikelets per panicle (23.42 %). Genetic gains ranged from 1.29 % to 70.61 %, with notable gains in out-crossing rate (70.61 %) and seed yield per plant (41.81 %). Path coefficient analysis revealed significant positive correlations between number of tillers per plant and angle of glume opening (0.512), spikelets per panicle (0.513), out-crossing rate (0.519), plant height (0.643) and seed yield per plant (0.515), while negatively correlated with critical temperature (-0.626). The highest heritability values were recorded for out-crossing rate (98.43 %), followed by ratoon yield per plant (95.98 %), number of tillers per plant (95.06 %), angle of bloom opening (96.70 %) and others.

Keywords: floral trait; out-crossing; TGMS; temperature sensitive genic male sterile line; two-line hybrids

Introduction

A genetic system known as thermo-sensitive genic male sterility (TGMS) causes plants' male fertility to change in response to temperature. This technique has proven to be a useful tool for creating hybrid rice, particularly in tropical areas where temperature fluctuations are constant throughout the year and at various elevations. A maintenance line is not necessary for the male sterile line's seed replication in TGMS, in contrast to the cytoplasmic-genetic male sterility (CMS) system. Instead, TGMS hybrid creation dramatically simplifies the process by utilizing only the TGMS line and a pollen parent.

Three paternal lines; male sterile (A), maintainer (B) and restorer (R)-are necessary in the CMS system, which adds complexity and raises expenses. Furthermore, some difficulties that CMS-based hybrid systems occasionally encounter are partial sterility, problems restoring fertility and genetic limitations related to cytoplasmic inheritance. By avoiding these restrictions, TGMS systems provide a productive and affordable substitute for hybrid breeding. The TGMS system controls male fertility by taking advantage of its sensitivity to temperature variations. The TGMS line demonstrates total male sterility in specific temperature ranges necessary for producing hybrid seeds. These lines become fertile again under various temperature settings, producing male sterile lines without needing an additional maintenance line. In hybrid breeding operations, this dynamic makes the TGMS method especially resource-efficient and flexible. When compared to hybrids based on CMS, rice hybrids created with TGMS lines have shown more significant heterosis. The improved performance of hybrid offspring in characteristics like yield, disease resistance and stress tolerance is known as heterosis or hybrid vigour (1). Due to the larger genetic pool made available by the two-line breeding method, TGMS-based hybrids exhibit higher levels of heterosis expression. Breeders can now use a greater variety of germplasm thanks to this approach, which eliminates the requirement for compatibility with particular cytoplasmic backgrounds.

Besides its biological benefits, the TGMS approach lessens the logistical and operational difficulties of producing hybrid seeds. The lack of a maintenance line lowers expenses and facilitates breeding processes, increasing the accessibility and scalability of hybrid seed production for commercial uses. Additionally, because of their adaptability, TGMS lines can be grown in various ecological settings, increasing the possibility of hybrid rice production across a range of agro-climatic zones. The main goal of this study is to find and isolate stable TGMS lines in order to take advantage of their potential in two-line hybrid rice breeding. A revolutionary development in hybrid rice breeding, TGMS utilizes the CMS system's simplicity and effectiveness while eliminating its drawbacks (2). Its implementation can improve rice farming's sustainability and production, especially in areas where temperature-sensitive breeding techniques can be used successfully (3).

Hybrid rice breeding was transformed by identifying rice genotypes that may undergo reversible changes in male fertility depending on environmental conditions, like temperature or photoperiod, throughout crucial growth phases. As a result of this innovation, two-line breeding has been developed, providing a more efficient substitute for the conventional three-line system extensively used in China (4). Temperature-sensitive genic male sterile (TGMS) lines in two-line heterosis breeding have several benefits, including a wider variety of male parents, the lack of cytoplasm that causes sterility and eliminating the need for maintenance lines. TGMS lines show fertility at lower temperatures (<23 °C) and heritable sterility at higher temperatures (>30 °C). This versatility increases agricultural output by improving hybrid seed production, grain yield and seed efficiency (5). Two-line hybrids produced from thermo-sensitive genic male sterile (TGMS) lines have been shown by the IRRI, Philippines, to have a greater frequency of heterotic combinations than three-line hybrids produced from cytoplasmic male sterile (CMS) lines (6). Furthermore, the inherent benefit of TGMS-based seed manufacturing is that it does not require a maintainer line, making it more efficient. It is well known that fewer efforts are needed to develop two-line hybrids. Still, the fundamental problems of temperature fluctuations are faced during the reproductive phase because the self-seed in TGMS lines deteriorates the genetic purity of the hybrid, which is observed with a high average sterility temperature, i.e. 29 °C. Hence, the need for low average sterility temperature, i.e. 24 °C TGMS lines, can serve the purpose of developing the genetically pure two-line rice hybrids. Here, the TGMS lines like PTG-101, PTG-102, PTG-103 and PTG-106 under study showed 24 °C average sterility temperature with no self-seed at Crop Research Centre Pantnagar during *Kharif* 2021 and 2022.

Finding and using TGMS lines to produce two-line hybrids was the ultimate objective of this investigation. The study's primary objectives were to screen TGMS lines for fertility and sterility temperatures, characterize their morphology and perform genetic analysis on recently created TGMS lines. By effectively utilizing TGMS lines in crop development initiatives, these initiatives help to advance hybrid breeding techniques.

Materials and Methods

The present experiment was carried out by using ten Thermo-sensitive Genic Male Sterile (TGMS) lines-PTG 5-1, PTG-7, PTG-12, PTG-28, PTG-101, PTG-102, PTG-103, PTG-104, PTG-105 and PTG-106-developed through recombination and mutation breeding at the Crop Research Centre, GBPUA&T, Pantnagar. Favourable weather conditions during the crop-growing periods of 2021 and 2022 supported optimal rice growth (Fig.1). The experiment followed a Randomized Block Design (RBD), with each TGMS line treated as a separate entry and replicated thrice. The experimental setup comprised six-row plots for each TGMS line, with two rows of male parents flanking four central rows of female (TGMS) plants. The planting schedule synchronized flowering by sowing the female lines on 15 May and male parents between May 10 and 20. Seeds were produced as a ratoon crop in the cooler season, November–December during both years. To examine seed set under varying temperature conditions, 1-2 tillers of individual plants were alternately covered with butter paper bags during flowering from 15 September to 15 October. This approach enabled the assessment of critical sterility and fertility phases. The morphological and floral characters were assessed using the standard evaluation system of the IRRI, Philippines (3). The study aimed to provide insights into the performance of TGMS lines under a controlled environment.

A comprehensive study was conducted on five tagged TGMS (Thermosensitive Genic Male Sterile) plants to analyze seven morphological traits-flag leaf orientation, leaf sheath, panicle type, stigma exsertion, apiculus pigmentation, stigma colour and grain type-and twelve flowering traits, including

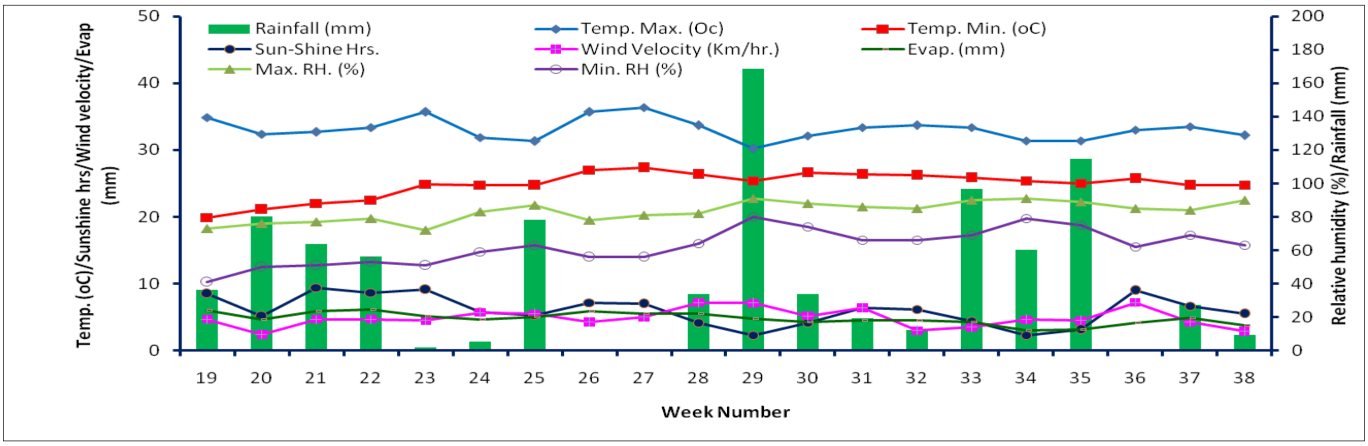

Fig. 1. Graph depicting the average weather for each month of the typical meteorological week in 2022 at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

days to 50 % flowering, tiller number, panicle exertion percentage, time of anthesis, angle of bloom opening, panicle length, number of spikelets per panicle, out-crossing rate (%), critical sterility temperature, plant height, seed yield per plant (g) and ratoon yield per plant (g). Data were recorded during the flowering to maturity stages under sufficient pollen availability (Table 1-2). Seed-setting rates of out-pollinated panicles were also observed. Statistical evaluation was performed using ANOVA, while genotypic (σ^2g) and phenotypic (σ^2p) variances, heritability, genetic advance, genetic gain, correlation and path coefficients were computed according to standard methodologies (7-9). Correlation and path coefficient analyses were performed using (10) methodology, with significance evaluated using table "r" values at 5 % and 1 % levels (n-2 degrees of freedom). Path coefficients were categorized as negligible (0.00-0.09), low (0.10–0.19), moderate (0.20-0.29), high (0.30-0.99), or very high (>1.00) (11). Genetic gain, expressed as a percentage of the population mean (8), provided insights into the expected genetic improvements. These analyses collectively informed the heritability and genetic potential of TGMS lines, emphasizing their implications for breeding programs.

Results

Coefficients of variability

The phenotypic difference in the trait from one genotype to another arises from the interaction of a particular genotype with environmental conditions. Although the environment is not changeable, the analysis (Table 3) showed that for all these traits, the phenotypic coefficient of variation (PCV) was found to be higher than that of the genotypic coefficient of variation (GCV), with an indication of a highly interacting factor of the environment also during the expression of different traits.

Table3. illustrated that the high phenotypic coefficient of variation for out-crossing rate (34.82 %), angle of glume opening (24.91 %), number of spikelets per panicle (23.42 %) and number of tillers per plant (22.97 %), which signify that these traits have the opportunity for significant genetic improvement. Whereas traits like days to 50 % flowering (1.69 %), time of anthesis (2.16 %), panicle exsertion percentage (6.01 %) and critical sterility temperature (9.10 %) had low PCV, thus indicating a narrow potential for improvement through genetics because the trait expressions were stable across the genotypes.

The GCV was high for out-crossing rate (34.55 %), angle of glume opening (24.50 %), number of spikelets per panicle (23.42 %) and number of tillers per plant (22.50 %). In contrast, low GCV values were obtained for days to 50 % flowering (1.03 %), time of anthesis (1.40 %), panicle exsertion percentage (4.26 %) and critical sterility temperature (8.01 %). A moderate GCV value of 11.51 % was found for plant height in Fig. 2.

Heritability

Heritability in the broad sense, which indicates the proportion of variability due to genetic factors, was high for most traits in this study. The highest heritability was observed for out-crossing rate (98.43 %), followed by number of tillers per plant (95.06 %), angle of glume opening (96.70 %), number of spikelets per panicle (95.82 %), seed yield per plant (95.76 %), ratoon yield per plant (95.98 %) and panicle length (91.94 %) (Table 3). Moderate heritability values were observed for days to 50 % flowering (37.04 %), time of anthesis (41.63 %) and panicle exsertion percentage (50.29 %) in the Fig. 3.

Table 1. Morphological characters for ten thermo-sensitive genic male sterile lines of rice

S. No.	Characters	PTG 5-1	PTG-7	PTG-12	PTG-28	PTG-101	PTG-102	PTG-103	PTG-104	PTG-105	PTG-106
1	**Flag leaf orientation**	Erect short	Erect short	Erect short	Medium erect	Long erect	Long erect	Erect short	Erect short	Medium erect	Medium erect
2	**Leaf sheath colour**	Green	Green	Green	Green	Dark green	Green	Dark	Dark	Dark	Dark
3	**Panicle type**	Straight	Semi erect	Semi erect	Bushy	Bunchy	Busy	Bushy	Droopy	Semi erect	Droopy
4	**Panicle exsertion**	Complete	Complete	Complete	incomplete	Complete	Incomplete	Complete	incomplete	incomplete	incomplete
5	**Apiculus pigmentation**	Purple	Purple	Purple	Green	Green	Green	Green	Purple	Purple	Purple
6	**Stigma colour**	Purple	Purple	Purple	Green	Green	Green	Green	Purple	Purple	Purple
7	**Grain type**	Medium bold, awnless	Long slender tip awned	Long slender, awnless	Medium bold, awnless	Long bold tip awned	Long slender, awnless	Long slender awnless	Long slender awnless	Long slender awnless	Medium long awnless

Table 2. Flowering traits of thermo-sensitive genic male sterile (TGMS) of rice

S. No.	Characters	PTG 5-1	PTG-7	PTG-12	PTG-28	PTG-101	PTG-102	PTG-103	PTG-104	PTG-105	PTG-106
1	**Number of tillers per plant**	13	14	15	12	16	13	15	20	15	20
2	**Days to 50 % flowering**	100	98	98	96	100	99	98	100	98	98
3	**Panicle exsertion percentage**	100	100	85	100	100	95	100	95	95	95
4	**Time of anthesis (hr: min) A.M.**	10:30	10:00	10:00	10:00	10:00	10:30	10:30	10:30	10:00	10:20
5	**Angle of glume opening (°)**	15	20	15	15	25	25	30	25	20	25
6	**Panicle length(cm)**	25	28	22	28	32	30	33	32	34	34
7	**Number of spikelets per panicle**	220	200	180	200	258	300	300	310	365	320
8	**Out crossing percentage**	20	20	15	20	32	38	39	40	43	38
9	**Critical sterility temperature (°C)**	28	28.5	28.9	28	24	24	24	24	26	24
10	**Plant height (cm)**	100	84	92	90	105	100	110	120	84	105
11	**Seed yield per plant(gm)**	50	65	38	68	68	60	72	78	82	80
12	**Ratoon yield per plant (gm)**	20	30	12	20	22	22	21	25	27	28

Table 3. Assessment of range, genotypic and phenotypic coefficient of variation, heritability, genetic advance and genetic gain of newly developed TGMS lines

Genotypes	Range	Coefficient of variability		Heritability (%)	Genetic advance	Genetic gain
		GCV (%)	PCV (%)			
Number of tillers per plant	20.0-12.0	17.82	18.28	95.06	5.48	35.80
Days to 50 % flowering	100.0 -96.0	1.03	1.69	37.04	1.27	1.29
Panicle exsertion	100.0- 85.0	4.26	6.01	50.29	6.01	6.23
Time of anthesis	10.4- 10.0	1.40	2.16	41.63	0.19	1.86
Angle of glume opening (°)	30.0 - 15.0	24.50	24.91	96.70	10.67	49.62
Panicle length(cm)	34.0- 22.0	13.30	13.88	91.94	7.83	26.28
Number of spikelets per panicle	365.0- 180.0	23.42	23.92	95.82	125.29	47.22
Out crossing rate (%)	43.0-15.0	34.55	34.82	98.43	21.53	70.61
Critical sterility temperature(°C)	28.4- 24.0	8.01	9.10	77.47	3.77	14.52
Plant height (cm)	120.0-84.0	11.51	12.20	88.94	22.13	22.35
Seed yield per plant (g)	82.0-38.0	20.74	21.20	95.76	27.64	41.81
Ratoon yield per plant (g)	30.0-12.0	22.50	22.97	95.98	10.31	45.41

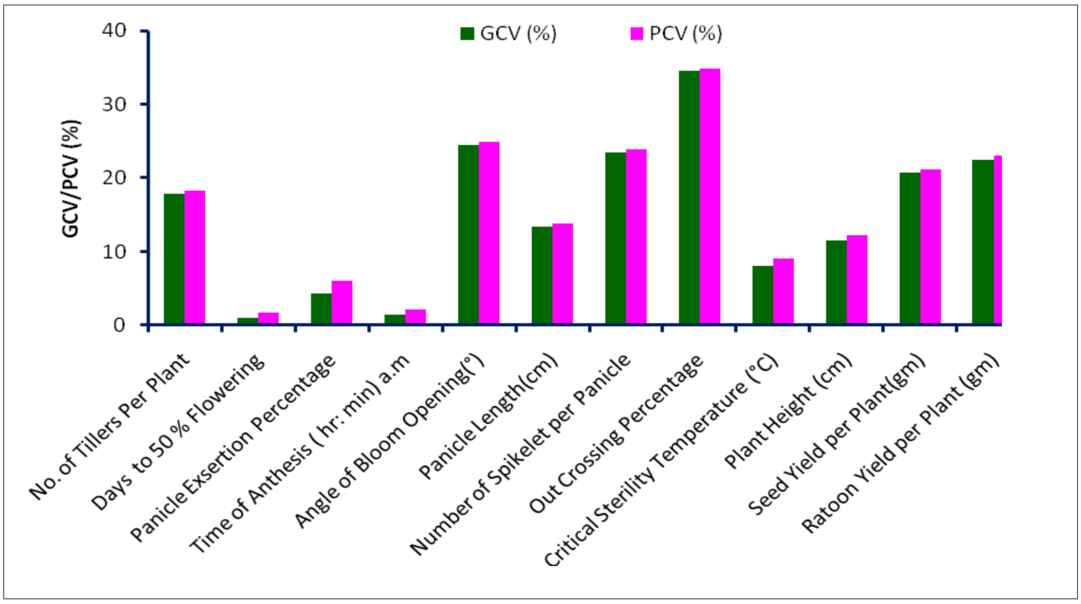

Fig. 2. Histogram displaying GCV/PCV values for different traits.

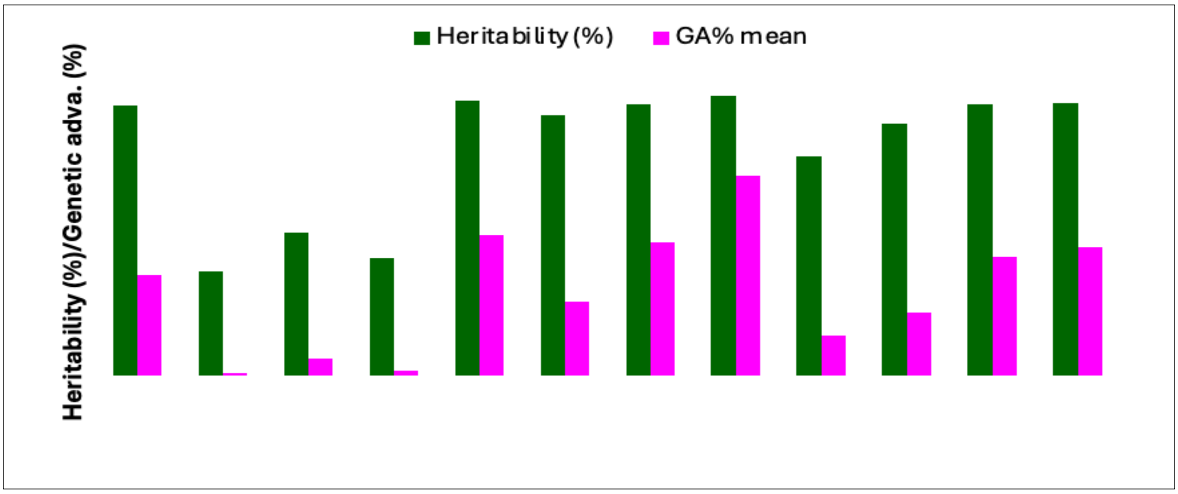

Fig. 3. Histogram depicting heritability and genetic advance for different characters.

Genetic advance

The genetic advance in the study varied between 0.19 % and 125.29 %, with the highest number of spikelets per panicle at 125.29 % and seed yield per plant at 27.64 %. The time of anthesis showed a low genetic advance of 0.91 %, while critical sterility temperature and panicle length averaged 3.77 % and 7.83 %, respectively. Heritability is less informative without high genetic advance as a percent of the mean. Table 3 showed high heritability and genetic advance for the number of spikelets per panicle (125.29 %) and seed yield per plant (27.64 %), indicating additive gene action and thus selection for these traits would be very effective for improvement (7). On the other hand, high heritability coupled with low genetic advance was observed for characteristics like the number of tillers per plant (5.48 %) and panicle length (7.83 %) (Table 3). This represents that the traits could be improved through selection due to presence of the action of additive genes (8).

In the current study, traits like the number of tillers per plant (5.48 %) and panicle length (7.83 %) indicate high heritability and low genetic advance. These findings illustrated that choosing these traits would be futile in improving them. These characters' heritability was wholly influenced by non-additive gene action, which means the presence of non-additive (environmental factors), suggesting that heterosis breeding could be fruitful (8, 9).

Genetic gain

The data (Table 3) showcased that the genetic gain is from 1.29 % to 70.61 %, with high genetic gain found for traits like out-crossing rate (70.61 %), angle of glume opening (49.62 %), number of spikelets per panicle (47.22 %), ratoon yield per plant (45.41 %) and seed yield per plant (41.81 %). The critical sterility temperature had a moderate genetic gain of 14.52 %. Low genetic gain was noted at the time of anthesis with 1.86 % and days to 50 % flowering at 1.29 %. The out-crossing rate, which was at 70.61 % and the angle of glume opening, which was at 49.62 %, had high heritability with a potential for a high genetic gain, which may be exploited strongly**.** Conversely, the traits have high heritability but low genetic gain and are unlikely to be fruitful from selection.

Correlation studies

Correlation coefficient analysis for determining component traits for improvement in yield through genetics was applied in this research. The traits' phenotypic and genotypic correlation coefficients were investigated, but the genotypic showed higher magnitudes than those of the phenotypic. As highlighted above, an analysis of grain yield's association with its component traits is quite essential in rice breeding. The various characteristics' phenotypic and genotypic correlation coefficients are summarized (Table 4). The number of tillers per plant significantly positively correlated with the angle of glume opening (0.512), number of spikelets per panicle (0.513), out-crossing rate (0.519), plant height (0.643) and seed yield per plant (0.515) but showed a significant negative correlation with

Table 4. Analysis of genotypic correlation (above diagonal) and phenotypic correlation (below diagonal) for 12 characters among different newly developed TGMS lines of rice

Characters		NTPP	DF50	PE	TA	ABO	PL	NSPP	OC	CST	PH	SYPP	RYPP
NTPP	G	1.000	0.443*	-0.306	0.288	0.512**	0.498**	0.513**	0.519**	-0.626**	0.643**	0.515**	0.353
	p	1.000	0.225	-0.183	0.117	0.475**	0.528**	0.472**	0.506**	-0.456*	0.582**	0.470**	0.352
DF50	G			0.190	0.424*	0.307	0.121	0.219	0.326	-0.414*	0.648**	-0.153	0.066
	P			-0.078	0.348	0.286	0.004	0.211	0.162	-0.378*	0.499**	-0.024	0.018
PE	G				0.442*	0.287	0.414*	0.091	0.082	-0.269	0.076	0.522**	0.503**
	P				-0.051	0.163	0.313	-0.026	0.142	-0.044	0.089	0.274	0.453*
TA	G					0.637**	0.351	0.442*	0.567**	-0.619**	0.815**	0.113	0.039
	P						0.485**	0.126	0.317	0.306	-0.449*	0.649**	0.113
ABO	G						0.797**	0.664**	0.798**	-0.945**	0.641**	0.580**	0.398*
	P						0.725**	0.644**	0.772**	-0.832**	0.640**	0.565**	0.362*
PL	G							0.894**	0.919**	-0.906**	0.377*	0.966**	0.687**
	P							0.819**	0.879**	-0.666**	0.319	0.881**	0.665**
NSPP	G								0.989**	-0.797**	0.359	0.759**	0.520**
	P								0.944**	-0.750**	0.322	0.768**	0.491**
OC	G									-0.934**	0.478**	0.789**	0.492**
	P									-0.791**	0.449*	0.751**	0.492**
CST	G										-0.776**	-0.632**	-0.343
	P										-0.627**	-0.600**	-0.290
PH	G											0.248	-0.038
	P											0.221	-0.041
SYPP	G												0.788**
	P												0.741**
RYPP	G												1.000
	P												1.000

*, ** significant at 5 % and 1 % level, respectively

NTPP: No. of Tillers Per Plant, DF50: Days to 50 % Flowering, PE: Panicle Exsertion, TA: Time of Anthesis, ABO: Angle of Bloom / glume Opening (°), PL: Panicle Length (cm), NSPP: Number of Spikelets per Panicle, OC: Out Crossing, CST: Critical Sterility Temperature (°C), PH: Plant Height (cm), SYPP: Seed Yield per Plant (g), RYPP: Ratoon Yield per Plant (g) critical sterility temperature (-0.626). The time from days to 50 % flowering was positively correlated with anthesis time (0.424) and plant height (0.648) but negatively correlated with the critical sterility temperature (-0.414). Panicle exsertion percentage showed high significant positive correlations with the rate of out-crossing (0.919), height (0.377), seed yield per plant (0.966) and ratoon yield per plant (0.687). Panicle length was positively correlated with the number of spikelets per panicle (0.894), out-crossing rate (0.984), seed yield per plant (0.759) and ratoon yield per plant (0.520). The number of spikelets per panicle exhibited significant positive correlations with the out-crossing rate (0.984), seed yield per plant (0.759) and ratoon yield per plant (0.520). The out-crossing rate was positively correlated with plant height (0.478), seed yield per plant (0.789) and ratoon yield per plant (0.492). Seed yield per plant showed a significant positive correlation with ratoon yield per plant (0.788).

The ultimate objective is to implement continuous selection, improve maintenance and enhance other related grain yield components to develop new rice cultivars with more significant genetic potential to increase grain yield. Because these are polygenically controlled and expression depends on the performance of numerous component characters, direct selection is inefficient for increasing the number of spikelets per panicle. To create successful crop improvement projects, it is crucial to understand the extent and type of the relationship between component characters and their effects on yield, such as traits like the number of spikelets per panicle and its associated qualities. Although most of the examined features showed high intrinsic associations with one another, phenotypic correlations for most were lower than genotypic correlations. However, the phenotypic value is diminished by the significant interaction of the environment. The results suggest that the numbers of spikelets per panicle and seed yield per plant are important yield-related traits and could be considered as selection criteria to increase rice grain yield.

Path coefficient analysis

Path coefficient analysis was done among ten TGMS lines in this study to assess the direct and indirect effects of various traits, with results illustrated (Table 5). The diagonal values in the table depicted that seed yield per plant (1.201) had the maximum positive effect on critical sterility temperature (1.17), angle of glume opening (0.972) and days to 50 % flowering (0.469). The number of tillers per plant directly positively affected days to 50 % flowering (0.098 phenotypic, 0.1686 genotypic), out-crossing rate (0.43 phenotypic, 0.516 genotypic), seed yield per plant (0.618 phenotypic, 0.29 genotypic) and ratoon yield per plant (0.353 phenotypic). Panicle exsertion had indirect adverse effects (-0.1035 phenotypic, -0.1038 genotypic), panicle length (-0.46 phenotypic, -0.14 genotypic), number of spikelets per panicle (-0.101 phenotypic, -0.502 genotypic) and plant height (-0.341 phenotypic, -0.878 genotypic).

It positively contributed to the out-crossing rate (0.27 phenotypic, 0.16 genotypic) and ratoon yield per plant (0.066 phenotypic, 0.018 genotypic). The factors were negatively contributing indirectly to panicle length (-0.11 phenotypic, -0.00 genotypic), number of spikelets per panicle (-0.04 phenotypic, -0.22 genotypic), plant height (-0.34 phenotypic, -0.75 genotypic) and seed yield per plant (-0.18 phenotypic, -0.01 genotypic). This displayed a direct positive effect in the following traits: number of spikelets per panicle on ratoon

Table 5. Assessment of direct and indirect effects of different traits on the number of tillers per plant among newly developed TGMS lines

Characters		NTPP	DF50	PE	TA	ABO	PL	NSPP	OC	CST	PH	SYPP	RYPP
NTPP	G	0.3559	0.2081	-0.1035	-0.0211	0.4983	-0.4642	-0.1018	0.4386	-0.7344	-0.3410	0.6183	0.353
	P	0.9479	0.0895	-0.1038	0.0759	-0.0221	-0.1438	-0.5027	0.5163	0.0803	-0.8785	0.2930	0.352
DF50	G	0.1577	0.4696	0.0643	-0.0310	0.2988	-0.1127	-0.0434	0.2757	-0.4860	-0.3433	-0.1841	0.066
	P	0.2132	0.3981	-0.0442	0.2251	-0.0133	-0.0011	-0.2244	0.1651	0.0666	-0.7522	-0.0153	0.018
PE	G	-0.1089	0.0892	0.3382	-0.0324	0.2789	-0.3857	-0.0180	0.0694	-0.3154	-0.0400	0.6275	0.503**
	P	-0.1733	-0.0310	0.5680	-0.0332	-0.0076	-0.0853	0.0273	0.1447	0.0078	-0.1348	0.1706	0.453*
TA	G	0.1027	0.1989	0.1495	-0.0733	0.6189	-0.3270	-0.0878	0.4791	-0.7256	-0.4321	0.1358	0.039
	P	0.1113	0.1386	-0.0292	0.6463	-0.0226	-0.0345	-0.3379	0.3124	0.0792	-0.9791	0.0706	-0.045
ABO	G	0.1824	0.1443	0.0970	-0.0466	0.9724	-0.7430	-0.1319	0.6747	-1.1083	-0.3400	0.6965	0.398*
	P	0.4503	0.1138	0.0926	0.3137	-0.0466	-0.1975	-0.6863	0.7879	0.1465	-0.9651	0.3522	0.362*
PL	G	0.1772	0.0567	0.1399	-0.0257	0.7748	-0.9325	-0.1776	0.7768	-1.0633	-0.2000	1.1602	0.687**
	P	0.5004	0.0017	0.1780	0.0818	-0.0338	-0.2724	-0.8729	0.8964	0.1174	-0.4811	0.5493	0.665**
NSPP	G	0.1825	0.1027	0.0307	-0.0324	0.6457	-0.8339	-0.1986	0.8364	-0.9351	-0.1901	0.9123	0.520**
	P	0.4472	0.0838	-0.0146	0.2049	-0.0300	-0.2231	-1.0657	0.9631	0.1322	-0.4855	0.4786	0.491**
OC	G	0.1846	0.1531	0.0278	-0.0415	0.7759	-0.8566	-0.1964	0.8456	-1.0957	-0.2535	0.9483	0.492**
	P	0.4797	0.0644	0.0806	0.1979	-0.0360	-0.2393	-1.0061	1.0202	0.1393	-0.6765	0.4680	0.492**
CST	G	-0.2229	-0.1946	-0.0909	0.0453	-0.9188	0.8453	0.1583	-0.7899	1.1730	0.4113	-0.7595	-0.343
	P	-0.4322	-0.1505	-0.0252	-0.2905	0.0388	0.1815	0.7998	-0.8069	-0.1761	0.9451	-0.3741	-0.290
PH	G	0.2290	0.3042	0.0256	-0.0597	0.6237	-0.3518	-0.0712	0.4044	-0.9103	-0.5300	0.2983	-0.038
	P	0.5521	0.1985	0.0508	0.4196	-0.0298	-0.0869	-0.3430	0.4576	0.1104	-1.5083	0.1379	-0.041
SYPP	G	0.1832	-0.0720	0.1767	-0.0083	0.5638	-0.9006	-0.1508	0.6675	-0.7416	-0.1316	1.2013	0.788**
	P	0.4456	-0.0097	0.1555	0.0732	-0.0263	-0.2401	-0.8185	0.7661	0.1057	-0.3338	0.6232	0.741**

Resi = 0.0467 *, ** significant at 5 % and 1 % level, respectively

NTPP: No. of Tillers Per Plant, DF50: Days to 50 % Flowering, PE: Panicle Exsertion, TA: Time of Anthesis, ABO: Angle of Bloom/glume Opening (°), PL: Panicle Length (cm), NSPP: Number of Spikelets per Panicle, OC: Out Crossing, CST: Critical Sterility Temperature (°C), PH: Plant Height (cm), SYPP: Seed Yield per Plant (g), RYPP: Ratoon Yield per Plant (g) yield per plant (0.520 phenotypic, 0.49 genotypic), seed yield per plant (0.91 phenotypic, 0.47 genotypic), out-crossing rate (0.83 phenotypic, 0.96 genotypic), days to 50 percent flowering (0.10 phenotypic, 0.08 genotypic) and number of tillers per plant (0.18 phenotypic, 0.44 genotypic). It displayed an indirect negative effect on panicle length (-0.83 phenotypic, -0.22 genotypic) and plant height (-0.19 phenotypic, -0.48 genotypic). Data analyzed for path coefficient analysis depict that maximum positive effect on that seed yield per plant (1.201) was found to have a maximum positive effect on critical sterility temperature (1.17), angle of glume opening (0.972) and days to 50 % flowering (0.469) in the Fig.4 of Structural equation modelling (11).

Discussion

TGMS line seeds are commonly raised during winter, promoting fertility pollen since low temperature (< 23°C) favours fertility and above 30°C leads to sterility irrespective of photoperiod. In addition, these lines can be readily multiplied during autumn due to the onset of low temperatures (12). Evaluation of TGMS lines for morphological, flowering and quantitative traits is a prerequisite to finding commercially useable TGMS lines (13, 14). All the ten TGMS lines tended to transform from sterile to fertile phase as temperature was decreased in the field. Fertility restoration was more at lower temperatures for all ten lines. These critical sterility temperature points ranged from 24°C to 28.9°C for all studied TGMS lines. The morphology of these lines showed a significant variation. Floral biology, both in the sterile and fertile phase, showed varied behaviour. In the sterility phase, the time of anthesis ranged from 10 to 10:30 a.m. in PTG 5-1, PTG-102, PTG-103 and PTG-104 took maximum time to anthesis. The angle of glume opening ranges from 15 to 30° PTG-101, PTG-102, PTG-104 and PTG-106 showed 25° angle of glume opening while the maximum angle of glume opening is 30° *i.e.,* found in PTG-103. All the ten TGMS lines showed a great variation for grain types ranged from medium bold to long awned to awnless in the Fig.5. Panicle exertion percentage ranged from 85 % to 100 % (Partial Panicle exerted percentage to well exerted Panicle percentage). PTG-12 showed a poor panicle exertion percentage of 85 % while five lines, namely PTG 5-1, PTG-7, PTG-28, PTG-101 and PTG-103 showed complete Panicle exertion percentage means 100 %. The out-crossing rate varied from 25 to 43 %; PTG-105 and PTG-104 exhibited the highest out-crossing rate (40 and 43 %) in Table 1. These lines are most suitable for two-line hybrid development. Several workers emphasized that some other studies also reported that the TGMS lines with maximum anther length and stigma length may be beneficial to achieve higher out-crossing rate as they are positively correlated (15). High out-crossing rate is crucial for hybrid seed production, ensuring adaptability and genetic diversity (16). The positive environmental conditions, appropriate temperature regimes regarding TGMS lines and high pollen load for the restorer line allow having the best out crossing rates, resulting in potential hybrid seed yield. However, effective utilization of this unique male sterility system counts on the knowledge of fertility behavior of TGMS lines (17). Seed yield in hybrid rice seed production depends on the out-crossing potential of the parental lines. To successfully finish hybrid seed production, sufficient amounts of pollen grains should be deposited on stigma lobes of the spikelets of male sterile parents (12) and the higher stigma length increased the out-crossing ability of TGMS lines (18). Moreover, the most crucial stage is 4–8 days after panicle initiation (13). Differentiation of the secondary branch primordium and the filling stage of the pollen, that is, 24 to 5 days before heading were deemed sensitive to temperature (19). This study's critical stages occurred during panicle developmental stages and approximately 26 to 5 days before heading in most of the TGMS lines (20).

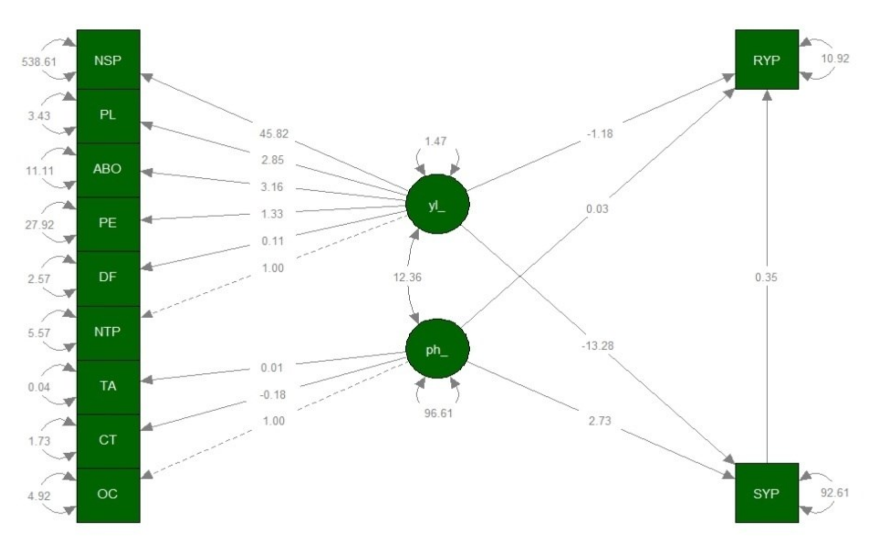

Fig. 4. Structural equation model depicts genotypic path coefficients, linking rice traits to yield with significance denoted by dashed/solid lines. Significance level (***p b 0.001; **p b 0.01; *p b 0.05).

Note: NTPP: No. of Tillers Per Plant, DF50: Days to 50 % Flowering, PE: Panicle Exsertion, TA: Time of Anthesis, ABO: Angle of Bloom/glume Opening(°), PL: Panicle Length (cm), NSPP: Number of Spikelets per Panicle, OC: Out Crossing, CST: Critical Sterility Temperature (°C), PH: Plant Height (cm), SYPP: Seed Yield per Plant (g), RYPP: Ratoon Yield per Plant (g).

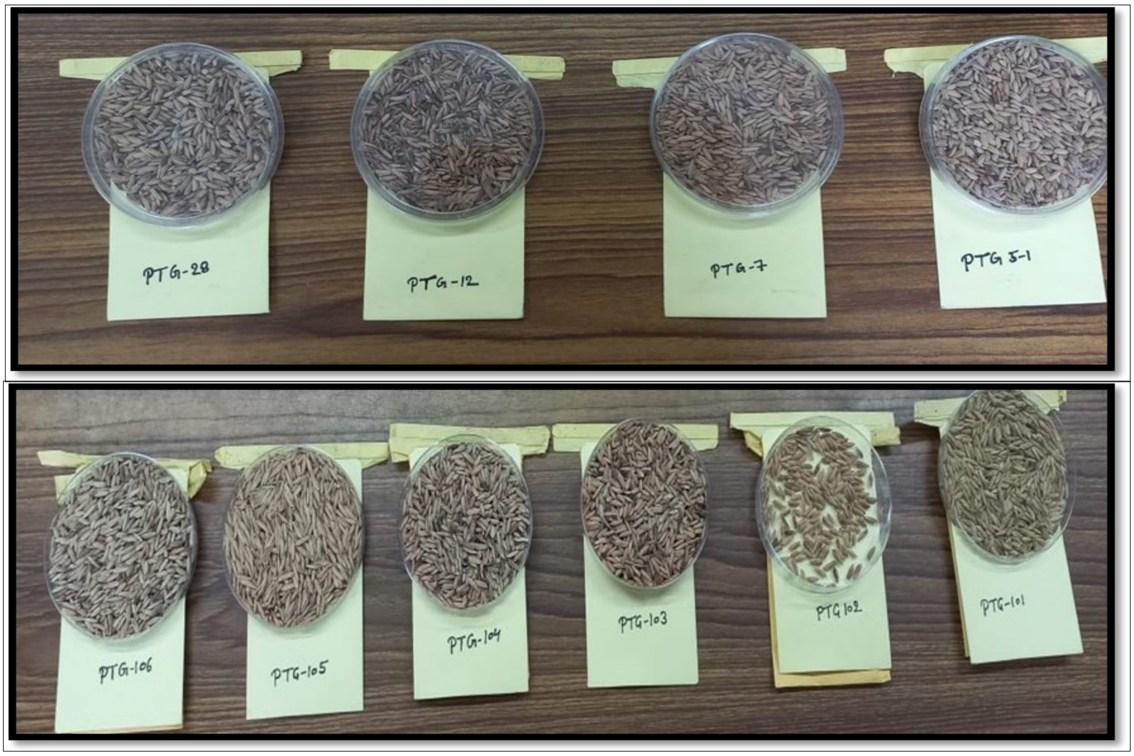

Fig. 5. Grain size and grain types of different TGMS lines of rice.

The evaluated phenotypic and genotypic coefficients of variability provided a better knowledge of the variation in the genotypes under study. Path coefficient analysis showed a maximum positive effect on genotypic correlation analysis. It showed the number of tillers per plant significantly positively correlated to the angle of glume opening (0.512), number of spikelets per panicle (0.513), outcrossing rate (0.519), plant height (0.643) and seed yield per plant (g) (0.515) also significantly negatively correlated to critical temperature (-0.626). Similarly, In the male-sterile parental line, out-crossing was directly associated with floral shape and flowering behaviour (21). Increased exsertion and stigma surface area, which resulted in a more significant percentage of seed-set, were associated with wider angles of the lemma and palea. There is effectively an increase in seed-set potency that can be identified using visual phenotypic selection (22, 23). female parents should exhibit a panicle exsertion rate of >70 %, have a very high out-crossing rate, have an early and short flowering period and have well-closed lodicules and lemmas after pollination (24). Conversely, pollen parents should have many anthers, abundant pollen and vigour (24). Male-sterile lines with higher panicle exsertion from the sheath would produce more spikelets for out-crossing than lines with less complete panicle exsertion (16, 25, 26). For the generation of hybrid rice, lines with a more significant percentage of panicle exsertion, together with increased seed set and spikelet fertility, may be well-suited since they affect out-crossing ability (27).

Days to 50 % flowering correlated positively to the time of anthesis (0.424) and with plant height (0.648) but negatively to critical temperature (0.414). The essential phases of panicle development sensitive to temperature could be determined from the stages that show a significant correlation with pollen sterility (17). It positively correlated significantly without crossing rate (0.919), plant height (0.377), seed yield per plant (g) (0.966) and ratoon yield per plant (g) (0.687) of panicle exerstion percentage. The length of the panicle is significantly positively correlated with the number of spikelets per panicle (0.894), out-crossing rate (0.984), seed yield per plant (g) (0.759) and ratoon yield per plant (g) (0.520). Number of spikelets per panicle (0.472) showed highly positive correlation with the out-crossing rate (0.984), seed yield per plant (g) (0.759) and ratoon yield per plant (g) (0.520). On similar lines, out crossing rate showed significant positive correlations with plant height (0.478), seed yield per plant (g) (0.789) and ratoon yield per plant (g) (0.492). The seed yield per plant showed a highly significant positive correlation with the ratoon yield per plant (g) (0.788).

Depending on the different characters, the coefficients of variability were sometimes low and at other times high or moderate in magnitude. This fluctuation indicates a high level of variability in the experimental material. Similar results for a greater phenotypic variation coefficient than a genotypic variance coefficient were obtained in rice by related supported discoveries (28-32). High heritability estimates for every character in the present study indicated that there is a good possibility that these traits can be enhanced through direct selection and that the environment has less influence on how they are expressed. Direct selection of a given character would be pointless due to low heredity, which indicates that environmental influences affect character expression. Instead, indirect selection should be employed. High heritability estimates were obtained for every character in the study (33-35). The highest heritability was recorded for the angle of opening (96.70 %), number of spikelets per panicle (95.82 %), seed yield per plant (95.76), panicle length (91.94 %), whereas moderate heritability for days to 50 % flowering (37.04 %), time of anthesis (41.63) and panicle exsertion indicating the effect of additive genes. The selection of these traits is pivotal for improvement, as additive genetic effects predominantly influence them. A similar heritability trend was also supported by other workers (8, 31, 36-39). The impact of additive genes has been demonstrated through significant genetic progress and high heritability for the number of spikelets per panicle (125.29 %) and seed yield per plant (27.64 %). Since these traits seem regulated by additive gene action, their selection will consequently have a more favourable effect on improvement.

While high heritability with low genetic advance was observed for traits such as the number of tillers per plant (5.48 %) and panicle length (7.83 %), this indicates that the action of additive genes may enhance these features through hybridization. The present study also found that traits such as tillers per plant and panicle length have high heritability but low genetic gain, which means that some environmental influence is present there. However, traits such as out-crossing rate and glume angle revealed high heritability and genetic gain, supporting improvement. Breeding programmes focus on increasing rice productivity by utilizing various genetic types and proper selection techniques to enhance productivity through yield-related traits (40). Correlation analysis identifies the relationship between yield and traits, while path analysis splits further into their direct and indirect effects (41, 42). The studies show that seed yield per plant is positively influenced by critical sterility temperature (1.17), angle of glume opening (0.972) and days to 50 % flowering (0.469). Therefore, selection should focus on critical sterility temperature and angle of glume opening to maximize yield.

Conclusion

The study reveals significant genetic and phenotypic diversity among ten TGMS lines across twelve qualitative traits, showing unique variations offering valuable insights for hybrid breeding. Variation in grain types panicle exertion and out-crossing rates highlights their potential for Two-line hybrid development, with PTG-104 and PTG-105 showing the highest out-crossing rates (40 % and 43 %). Key traits like the number of spikelets per panicle, seed yield and out-crossing rate exhibited high heritability and genetic advance, suggesting substantial additive genetic control. Path coefficient analysis confirmed their positive impact on yield, while critical sterility temperature, glume angle and flowering time emerged as essential selection targets. Significant genotype differences at 1 % and 5 % levels validate the relevance of these traits, providing a solid foundation for breeding strategies aimed at improving yield and adaptability in TGMS lines.

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Authors' contributions

MN contributed to the conceptualization of the study, the development of methodology and the investigation. DB was involved in study conceptualization, assisted in methodology design and participated in the investigation. LB was responsible for writing the original draft of the manuscript. SS reviewed and edited the manuscript to improve clarity and content. All authors read and approved the final version of the manuscript. All authors read and approved the final version.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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