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New Temperature Sensitive Genic Male Sterile Lines with Better Outcrossing Ability for Production of Two-Line Hybrid Rice

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Abstract: An investigation was carried out with three newly developed temperature sensitive genic male sterile (TGMS) lines for their floral traits, seed production potential and outcrossing ability in ten cross combinations. In the TGMS lines, fertile pollens had an average diameter of 0.89 mm while the sterile pollens was with 0.02 mm diameter.TS-29-150GY produced the biggest fertile pollens with 0.92 mm and other two lines produced relatively smaller pollens with 0.91 and 0.85 mm. Pollen fertility during the fertility reversion period was an average of 60.7%. TS-29-150GY had the maximum of 66.9% spikelet fertility whereas other two lines (TNAU18S and TNAU60S) had relatively lower spikelet fertility of 27.8% and 26.7%, respectively. Average of 17.00 g of seed yield was obtained in the TGMS lines during the fertility reversion period. TS-29-150GY had the highest value of 21.20 g of seed yield while TNAU18S and TNAU60S produced 16.6 g and 13.2 g of seed yield, respectively. The low seed production ability of these three TGMS lines was attributed only to the environmental conditions prevailing during the period. All three TGMS lines had considerable outcrossing potential of 41.2%, 24.6% and 25.0%, respectively. The cross combinations *viz*. TNAU18S/IET21508 (36 g/plant), TNAU18S/IET21044 (13 g/plant), TNAU18S/IET21009 (26.5 g/plant), TNAU60S/CB-09-106 (26.2 g/plant), TNAU60S/IET21009 (14 g/plant) and TS29-150-GY/DRR 3306 (39.2 g/plant) showed perfect synchronization with acceptable hybrid seed yield, indicating suitability of TGMS system under Indian condition. Based on the outcrossing related traits *viz*. panicle exertion, angle of glume opening, stigma length and pollen size, TNAU18S was identified as the best, followed by TS-29-150GY.
Key words: temperature sensitive genic male sterile line; outcrossing; floral trait; hybrid rice

Heterosis in rice was observed as early as in 1926 (Jones, 1926; Ramaiah, 1933). However, attempts for the adoption of hybrid technology were initiated only in 1966 by Yuan Long-ping, father of hybrid rice in China (Yuan, 1977). The identification of wild abortive (WA) type cytoplasmic male sterility (CMS) in 1970 was a breakthrough in exploiting heterosis in rice breeding. Success in the use of hybrid rice depends on the extent of heterosis and efficiency of the seed production techniques. One of the major anticipated constraints to the sustenance of heterosis breeding in rice is the availability of quality seed within an affordable price range. The use of male sterility is a prerequisite for commercial exploitation of heterosis since rice is a self-pollinating crop. The widely employed cytoplasmic male sterility-fertility restoration system based on three-line breeding is often difficult to maintain. Problems with the maintenance of A lines, the lack of diversity in A and R lines, and the presence of minor fertility genes in B lines have to constantly be addressed in breeding programs, ultimately leading to low heterotic potential and high seed production costs.

Following the landmark finding of rice genotypes reversibly turn male fertile to male sterile and vice versa with changes in environmental factors, such as temperature and/or day length during critical phases of plant growth, the concept of two-line breeding emerged as an alternative to the three-line approach in China (Yuan, 1997). The main advantages of two-line heterosis breeding include the ability to use a wide range of genotypes as male parents, absence of negative effects associated with sterility-inducing cytoplasm and no need for maintainer lines. Male sterility in temperature sensitive genic male sterile (TGMS) lines is heritable. Higher temperature (> 30 °C) results in sterility while lower temperature (< 23 °C) results in fertility. These characteristic features of TGMS facilitate the hybrid seed production and subsequently it was demonstrated that the TGMS line was more effective in increasing grain yield and

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seed production efficiency (Yuan, 1990). International Rice Research Institute has shown that two-line hybrids derived from TGMS lines had higher frequency of heterotic combinations than three-line hybrids derived from CMS lines (Lopez and Virmani, 2000). Seed production efficiency using TGMS line is higher than that using CMS line because of its inherent nature of eliminating the need of a maintainer line.

TGMS lines remain selectively male sterile at a specific range of temperature and turn self-fertile at certain other temperatures. The prevailing wide range of temperature differences in Tamil Nadu favors both hybrid seed production and the maintenance of TGMS in different locations (Siddiq and Ali, 1999). Thus, TGMS system has great potential for revolutionizing hybrid rice production through simple, less expensive and more efficient seed production technology. For the successful utilization of this novel male sterility system, knowledge of the breeding and fertility behavior of a TGMS line is essential.

An investigation was carried out to study the flowering traits, seed production capacity and outcrossing potential of three recently developed TGMS lines from Tamil Nadu Agricultural University, Coimbatore, India.

MATERIALS AND METHODS

Rice materials

An investigation was carried out with three new TGMS lines *viz.* TNAU60S, TNAU18S and TS-29-150GY developed through recombination breeding and mutation breeding. The above stabilized lines were studied for their floral traits, seed production potential and outcrossing ability with 10 male parents at the Paddy Breeding Station, Coimbatore, India, during 2010–2011. Seeds of TGMS lines were produced in a cooler season during August to December, 2010. Seed production plot was raised by planting the stubbles of the previous season's crop. The biometrical data on the flowering traits and spikelet fertility were studied. Individual plants covered with butter paper bags and seeds were obtained.

Seed production and outcrossing potential

Seedlings of the respective parents of 10 cross combinations involving three TGMS lines *viz.* TNAU18S, TNAU60S and TS-29-150GY and corresponding male parents were raised in a nursery. Three staggered sowings were done in weekly intervals. Planting was done at two different stages. Seedlings of the first and second staggering sowings were mixed together for the first planting in a wide spacing of 40 cm × 40 cm. In the second planting, the seedlings from the third staggering sowing were planted in the space between the first planting, thus the final spacing was attained as 20 cm × 20 cm with one plant per hill. In these plantings, single row of TGMS lines were accommodated in three rows between male parents either side. Each cross combination was maintained separately as individual plots. These mini hybrid seed plots were separated with high density polyethylene barriers to six feet height during the entire flowering stage to avoid possible pollen contamination. Rouging was done carefully to eliminate the shedder plants from TGMS lines during flowering. Supplementary pollination was done, twice daily, manually at the time of peak anthesis using bamboo sticks. The stick was used gently to dust the pollen from the male parents onto the female parents. Tagged five TGMS plants were used to record flowering traits viz., panicle exertion percentage, outcrossing potential and microscopic studies on anther length, anther width, style length and angle of glume opening. Apart from these traits, biometrical observations were also recorded and associated with seed production ability. Synchronization between the male and female parents was studied and their duration was calculated based on its anthesis period. All these traits were monitored at the flowering to maturity stage of a male sterile line grown in the field where pollen supply at its flowering time is abundant. Seed-setting rate on the out pollinated primary panicles was observed.

RESULTS

In this study, the established field with previous crop's stubbles was also used to generate some useful information on the flowering traits and potentials of self-seed multiplication abilities of three TGMS lines (Table 1).

Panicle exertion influences the number of spikelet and grains during the favourable seasons. The highest panicle exertion was obtained in TNAU18S with 92.8%, followed by TNAU60S with 91.8% and TS-29-150GY with 90.6%. TNAU60S produced the maximum anther length of 2.21 mm, followed by TS-29-150GY and TNAU18S with 1.92 and 1.84 mm, respectively. Anther breadth was also highest in TNAU60S, and the other two lines TNAU18S and TS-29-150GY produced similar anther breadths with 0.37 and 0.38 mm, respectively. In

Table 1. Outcrossing related traits of temperature sensitive genic male sterile (TGMS) lines.

TGMS line	PE (%)	PF (%)	SO (%)	SPF (%)	AL (mm)	AB (mm)	FPD (mm)	SPD (mm)	SL (mm)	AGO (°)	SY (g)
TNAU18S	92.8	60.0	20.2	26.6	1.84	0.37	0.91	0.03	2.01	23.0	16.60
TNAU60S	91.8	61.0	15.5	27.8	2.21	0.41	0.85	0.01	1.75	18.5	13.20
TS-29-150GY	90.6	61.0	15.3	66.9	1.92	0.38	0.92	0.01	1.85	20.2	21.20
Mean	91.7	60.7	17.0	40.4	1.99	0.39	0.89	0.02	1.87	20.6	17.00

PE, Panicle exertion percentage; PF, Pollen fertility rate; SO, Glume opening rate; SPF, Spikelet fertility rate; AL, Anther length; AB, Anther breadth; FPD, Fertile pollen diameter; SPD, Sterile pollen diameter; SL, Style length; AGO, Angle of glume of opening; SY, Single plant yield.