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Determination of fertility behaviour of thermo sensitive genic male sterile lines in rice

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ABSTRACT

Knowledge on fertility behavior of thermo-sensitive genic male sterile (TGMS) line is highly essential for increasing hybrid rice production. A set of four promising TGMS lines viz., GD 98014, GD 99017, GD 98029 and GD 98049 was characterized for their fertility behaviour under field conditions. The pollen and spikelet fertility data revealed that all lines had stable sterile phase with 100 per cent pollen sterility for more than 50 consecutive days during high temperature condition (30/20 ^{0}C maximum/minimum temperature) and they reverted to 60% pollen and spikelet fertility during low temperature condition (less than 30/20 ^{0}C). The critical panicle developmental stages sensitive to temperature were ascertained for each line. The daily mean temperature, relative humidity and photoperiod appeared to influence fertility alteration in some lines. The environmental conditions influencing fertility alteration varied among the lines. However, all the lines satisfied the requirement of stable fertility behaviour to the level of commercial exploitation in two-line hybrid breeding and found as potential TGMS donors to develop new TGMS lines.

Key words: *Rice, TGMS, critical temperature, sterility, fertility behaviour*

Recent progress in plant breeding research indicated that a significant shift in the yield frontiers could be possible through hybrid rice. But the hybrid rice technology has not been exploited successfully to the extent expected. This may be partially due to the inherent limitations associated with cytoplasmic genic male sterility (CMS) system used in the development of rice hybrids. In this context, environmental sensitive genic male sterility (EGMS) is considered as potential alternative to overcome the problems associated with three-line breeding and to surpass the yield plateau. Under tropical conditions, where day length differences are marginal, temperature sensitive genic male sterile (TGMS) system is considered more useful than the photoperiod sensitive genic male sterility (PGMS) system. However, successful exploitation of this novel male sterility system relies on the knowledge on fertility behaviour of TGMS lines. Hence, the present study was undertaken with the specific objective of characterizing a set of promising TGMS lines for their fertility behaviour so as to use them in two-line heterosis breeding.

MATERIALS AND METHODS

A set of four putative TGMS lines identified at Tamil Nadu Agricultural University (TNAU), Coimbatore, India and TS-29 bred lines, *viz.*, GD 98014, GD 99017, GD 98029 and GD 98049 obtained from the Hybrid Rice Evaluation Centre, Gudalur, Tamil Nadu were evaluated for pollen and spikelet fertility during summer 2004 at Coimbatore (High temperature condition – 36/22 ^{0}C and low temperature condition – 30/16 ^{0}C) to characterize the fertility behaviour of TGMS lines.

The TGMS lines were raised at fortnightly interval from July 2003 to June 2004. At the time of heading, pollen and spikelet fertility were observed on ten random plants. From the 24 sets of data on pollen fertility, the fertile and sterile phases of each TGMS line, the duration of each phase and the fertility transition phase were identified. The flowering period, in which the lines were completely sterile (100% pollen sterility) was taken as sterile phase. The period, in which the plants recorded more then 50 per cent pollen fertility was considered as fertile phase. The period of partial sterility was considered as the phase of fertility transition.

The influence of weather factors *viz.*, maximum temperature, minimum temperature, mean temperature, relative humidity, sunshine hours and photoperiod (of each day from 26 to one day before heading, average of each factor at different stages of panicle development and the overall mean of each factor throughout the panicle development) on pollen sterility was assessed by simple correlation analysis. The critical stages of panicle development sensitive to temperature were determined from the stages exhibiting significant correlation with pollen sterility. The lowest mean temperature among the temperature inducing sterility was considered as the critical sterility temperature (CST) and the highest mean temperature inducing fertility was recorded as critical fertility temperature (CFT).

RESULTS AND DISCUSSION

The preliminary step in exploitation of two-line system of hybrid rice breeding on a large scale is the identification of TGMS lines with stable fertility transformation behaviour. The lines with complete pollen sterility under high temperature condition and more than 30 per cent self seed set under low temperature condition are considered as promising TGMS lines for commercial exploitation (Lu *et al.*, 1994). The multiplication of TGMS lines and seed production of two-line hybrids is not difficult as that of three-line hybrids, which require a maintainer line to multiply sterile lines whereas TGMS lines are fertile during certain temperature regime, in which it can be multiplied by mere selfing. However, the sterile, fertile and the fertility transition phases of TGMS lines need to be determined in different ecological areas, so that the proper seasons and locations for sterile line multiplication and hybrid seed production can be recommended.

In this study, the fertility among the plants of a line varied during the fertility transition phase, whereas complete sterility and relatively same degree of fertility were observed among the plants during their sterile and fertile phases, respectively. All the lines except GD 98014 had two distinct sterile phases during summer months when the panicle emerged from April to May and July to September. GD 98014 had one distinct sterile phase when the panicle emerged from March to May. The sterile period of all the lines was more than 60 days, which was favorable for successful utilization of these lines. The sterile period was the longest in GD 99017 (96 days) followed by the other three lines (64 days). Since all these lines were completely sterile for more than 30 consecutive days during sterile phase, hybrid seed production using these lines can be taken up by raising the lines in such a way that flowering coincides with the sterile phase. These lines can be sown from January to March for hybrid seed production, in areas where mean temperature is more than 26 ^{0}C during summer season.

The line GD 98049 had two fertile phases, while all other lines had one fertile phase. The longest fertile phase was observed in the line GD 98014 (110 days) followed by GD 99017 (77 days), GD 98029 (66 days) and GD 98049 (63 days). All these lines recorded more than 50 per cent pollen and spikelet fertility in their fertile phase (Table 1). The fertile period of all the lines was in the months of October to February and the short fertile phase was in the months June-July. The second fertile phase of GD 98049 (18 days) was of very short duration. Hence, this season is not preferable for multiplication of TGMS lines. The plains with maximum and minimum temperatures ranging from 30-36 ^{0}C and 24-26 ^{0}C are ideal for hybrid seed production. Care should be taken such that the heading does not coincide with the monsoon months, because the low temperature and cloudiness prevailing during these months might induce fertility and affect the purity of the hybrid seed.

Table 1. Sterile and Fertile phases of TGMS lines

Lines	Sterility duration (Days)	Pollen sterility (%)	Fertility duration (Days)	Maximum fertility (%)	
				Pollen	Spikelet
GD 98014	64	100	110	77.74	68.50
GD 99017	96	100	77	77.68	66.80
GD 98029	64	100	66	69.47	62.70
GD 98049	64	100	63	79.44	60.00

The maximum pollen and spikelet fertility recorded during this period was 69.47 to 79.44 per cent and 60.00 to 68.50 per cent as mentioned above under two temperature regimes, respectively.

The results of correlation analysis between pollen sterility and weather factors revealed that maximum and mean temperatures were the primary factors influencing fertility transition whereas minimum temperature, sunshine hours and relative humidity were secondary factors influencing the pollen sterility. The influence of maximum temperature, mean temperature and sunshine hours on pollen fertility was observed in the line GD 98014. But the influence of sunshine hours was in negative direction. Minimum temperature and relative humidity had no influence on pollen fertility in this line. This is in accordance with the findings of Liu *et al.* (1997). The fertility of GD 99017, GD 98029 and GD 98049 was influenced by maximum, minimum and mean temperatures (Table 2). Relative humidity and sunshine hours had no influence on pollen fertility in these lines. These results are in conformity with the findings of Latha *et al.* (2004). The significant influence of maximum temperature on pollen sterility was reported by Elsy (1997). The results obtained from this study revealed that the effect of temperature was the major one while other factors played a minor role in pollen sterility/fertility transition.

There is certain amount of risk in exploiting rice heterosis by means of TGMS, if temperature fluctuation occurs at critical stages of panicle development. Therefore, knowledge on critical thermo-sensitive stages for fertility alteration is useful to determine the most suitable time of sowing dates of TGMS lines for seed multiplication and hybrid seed production. The appropriate sowing dates of TGMS lines should be determined in such a way that the critical stages of panicle development would be exposed to the desired temperature. The stages of panicle development sensitive to environmental factors varied among the lines (Table 3). The results of the study indicate that all the eight panicle development stages *i.e.* from differentiation of first bract primordium (S_1) to pollen ripening stages (S_8) of the lines, GD 98014 and GD 99017 were sensitive to temperature. This is in conformity with the findings of Elsy (1997), Latha *et al.* (2004 and 2005). If short-term low temperature occurs in the sensitive development stage, it results in impurity of the hybrid. The sensitive stages of fertility alteration in GD 98029 were from differentiation of primary branch primordium (S_2) to pollen ripening (S_8) stages. The stages from differentiation of first bract primordium (S_1) to pollen filling stage (S_7) were found to be sensitive stages for GD 98049. The critical stages of panicle development were reported by Yozhioka (2001). Sun *et al.* (1993) reported that PMC formation to late uninucleate stages were critical stages for temperature. If low temperature prevails even for a short period during any stage of panicle development, it will affect the sterility.

Table 2. Correlation coefficient of pollen sterility with mean weather factors over a period of 26 days before heading

Lines/parameters	GD98014	GD99017	GD98029	GD98049
Maximum temperature	0.762**	0.478*	0.520**	0.435*
Minimum temperature	0.251	0.746**	0.548**	0.724**
Mean temperature	0.682**	0.768**	0.667**	0.730**
Relative humidity	0.324	-0.216	-0.068	-0.336
Sunshine hours	-0.426*	0.088	-0.143	0.240

n = 24 *p = 0.05 **p = 0.01

Table 3. Critical panicle development stages and temperature for fertility alteration

Lines	Critical stages of thermosensitivity	CST (°C)	CFT (°C)
GD 98014	Differentiation of first bract primordium (S_1) to Pollen ripening (S_8)	26.0	25.8
GD 99017	Differentiation of first bract primordium (S_1) to Pollen ripening (S_8)	27.1	26.9
GD 98029	Differentiation of primary branch primordium (S_2) to Pollen ripening (S_8)	26.5	25.8
GD 98049	Differentiation of first bract primordium (S_1) to Pollen filling stage (S_7)	27.2	26.9

In the present study, the mean temperature inducing fertility alteration during the sensitive stages of panicle development was 24 to 26 ^{0}C. The critical temperature was found to vary in different TGMS lines as the TGMS genes of these lines are from various sources or transferred into different genetic backgrounds (Table 3). Wu (1997) has reported similar case of varying CST and CFT in the lines developed from single TGMS source (Annong S-1) on different genetic backgrounds. The critical temperature inducing sterility must be relatively low *i.e.*, 23 ^{0}C in temperate zone and 24^{0}C in subtropics (Yuan, 1998).

The lines with more critical temperature (>26^{0}C) are not favourable for commercial exploitation, since even a short fall in temperature during summer months may cause fertility reversion in TGMS lines, which may lead to self seed set. All these lines satisfied the requirements for commercial exploitation. Multiplication of these TGMS lines will also be an easy task as the critical fertility temperature of these lines was 24 to 25^{0}C and this temperature will prevail during the months of November, December and January in most parts of India. But low critical temperature poses problem in sterile line multiplication. The line may be stable to fertile, which can be multiplied only in high altitude areas.

In this study, all the lines had CST above 25°C, while the highest being recorded by GD 98049 (27.2°C). The CST of 28.5°C was reported by Sun *et al.* (1993) and 28°C by Viraktamath and Virmani (2001). In the present study, the CFT ranged between 26 and 27°C for all the lines. The lines GD 98014 and GD 98029 had CFT of 25.8°C while GD 99017 and GD 98049 had CFT of 26.9°C. The effect of CST and CFT was also reported by Elsy (1997) and Latha *et al.* (2004). The fertility behavior of all the four lines was represented in the Figures 1 and 2.

The difference between CST and CFT of all the lines was very narrow. This narrow difference is undesirable, since there is a possibility of fertility reversion due to unusual occurrence of low temperature during hybrid seed production. However, Wu and Yin (1992) opined that low temperatures that would

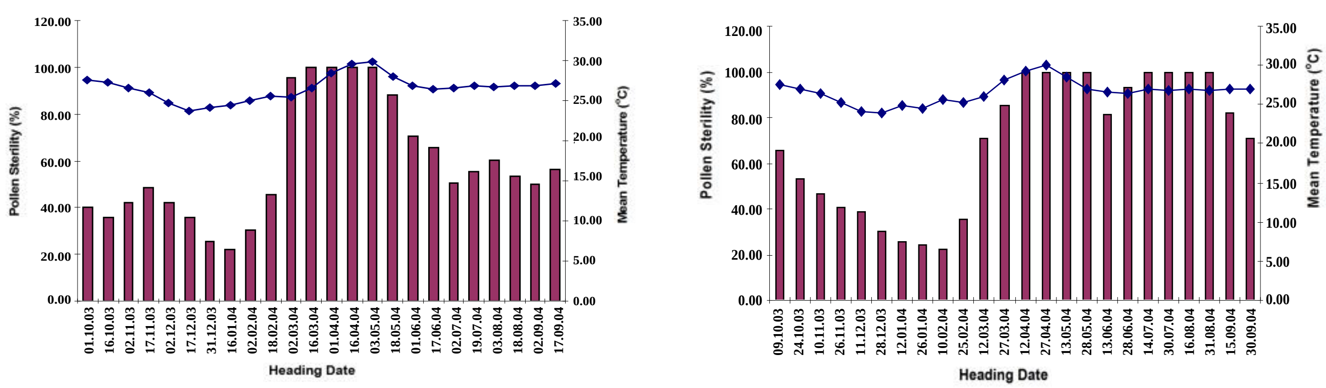

Fig. 1. Fertility behaviour of GD 98014 and GD 99017 in field condition

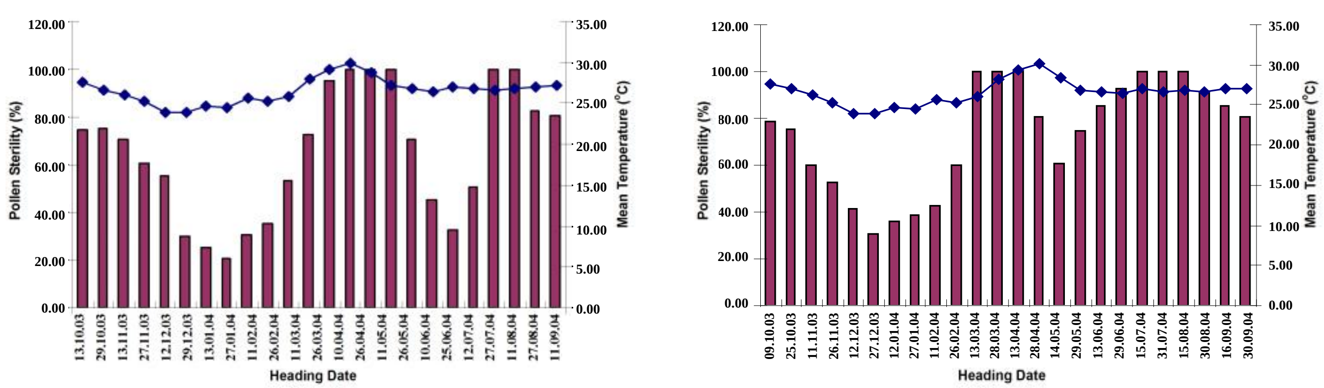

Fig. 2. Fertility behaviour of GD 98029 and GD 98049 in field condition

transform TGMS lines into fertile ones will not occur frequently during high temperature seasons. Even if it occurs, it would last for only a few days, thus the purity of hybrid seed will not be affected. In the present study also, the same trend was observed.

It is assumed that the conditions influencing fertility alteration in EGMS lines vary among different lines due to different sources of male sterile genes and the genetic background (Wu *et al.*, 1991and Zhang *et al.*, 1991). The results of this study showed that all TGMS lines have clearly defined fertility expressing temperature regimes, which are frequently available in tropical rice growing countries. They were completely sterile under high temperature condition and exhibited acceptable level of pollen and spikelet fertility under low temperature condition. They can be better exploited as TGMS donor for developing new TGMS lines.

All the TGMS lines had a stable sterile period of more than 60 days. More or less all the other lines had two distinct sterile phases when panicle emerged from April to May and July and September. All the TGMS lines had a fertile phase of more than 40 days in the months of December to February. The pollen sterility of all the lines was influenced by maximum temperature and mean temperature. Sunshine hours and relative humidity had minimum influence on the fertility in all the lines. The critical sterility temperature (CST) and critical fertility temperature (CFT) of all the TGMS lines were above 25°C.

The effect of temperature was the major one while other factors play a minor role in pollen sterility/ fertility transition. Since many factors associated with temperature directly or indirectly influenced the fertility of TGMS lines, the influence of any single factor should be determined by evaluating the lines in phytotron by keeping other factors under control.

REFERENCES

Elsy CR 1997. Identification and characterization of TGMS lines for heterosis breeding in rice (*Oryza sativa* L.). Ph.D. Thesis, Tamil Nadu Agri. University, Coimbatore

Latha R, Thiyagarajan K and Senthilvel S 2004. Genetics, fertility behavior and molecular marker analysis of a new TGMS line, TS 6 in rice. Plant Breeding 123: 235-240

Latha R, Senthilvel S and Thiyagarajan K 2005. Critical temperature and stages of fertility alteration in Thermo-sensitive Genic Male Sterile lines of rice. 4th International Crop Science Congress pp 1-6

Liu Y, He H, Shun Y, Rao Z, Pan X, Huan Y, Geo J and He X 1997. Light and temperature ecology of photo-thermo-sensitive genic male sterile rice and its application in plant breeding. In: Proc. Int. Symp. On Two-Line System of Heterosis Breeding in Crops. China National Hybrid Rice Research and Development Centre, Changsha, China. pp 49-58

Lu XG, Zhang ZU Maruyama K and Virmani SS 1994. Current status of two line method of hybrid rice breeding in Hybrid rice technology-New Development and future prospects. IRRI, Philippines pp 37-49

Sun ZX, Cheng XH and Si HM 1993. Determination of critical temperature and panicle development stage for fertility change of thermo sensitive genic male sterile rice 5460S. Euphytica 67: 27-33

Viraktamath BC and Virmani SS 2001. Expression of thermosensitive genic male sterility in rice under varying temperature situations. Euphytica 122: 137-143

Wu XJ 1997. Genetic strategies to minimize the risk in exploiting heterosis in rice by means of thermosensitive genic male sterility system. In: Proc. Int. Symp. On Two-Line System of Heterosis Breeding in Crops China National Hybrid Rice Research and Development Centre, Changsha, China. pp 121-131

Wu XJ and Yin HQ 1992. Genetics and stability of thermo-sensitive genic male sterile rice. Chinese J of Rice Sci 6 (2): 63-69

Wu XJ, Yin HQ and Yin H 1991. Preliminary study of temperature effect on Annong S1 and W6154S. Crop Research 5: 4-6

Yozhioka H 2001. Effects of day length on heading of early-season-culture in Miyazaki prefecture. Japan J Crop Sci 70: 54-58

Yuan LP 1998. Hybrid rice breeding in China. In: Advances in Hybrid Rice Technology. IRRI, Philippines. pp. 27-33

Zhang ZG, Yuan SC, Zen HL, Li YZ, Li ZC and Wei CL 1991. Preliminary observation of fertility changes in the new type temperature sensitive male sterile IV A. Hybrid Rice 1: 31-34