

Research Article**Fertility alteration behaviour of Thermosensitive Genic Male Sterile lines in Rice *Oryza sativa* L.****R.Latha and K.Thiyagarajan****Abstract**

The utilization of thermosensitive genic male sterility system (TGMS) has great potential for revolutionizing hybrid rice production in tropical countries through simple, less expensive and efficient seed production technology without any limitation in fertility restoration which have been the hindrance for commercial exploitation of heterosis in rice using cytoplasmic genic male sterility system (CMS). However for successful utilization of this novel male sterility system in hybrid rice breeding, knowledge on the fertility behaviour of TGMS lines, critical fertility and sterility temperatures and critical stages of thermosensitivity are highly essential. In this study six TGMS lines viz., TS 6, TS 16, TS 18, TS 29, TS 46 and TS 47 were characterized for their fertility behaviour. The lines were screened for pollen and spikelet fertility by raising the plants at fortnightly interval. All the lines had stable sterile phase with 100 per cent pollen sterility for more than 50 consecutive days during high temperature condition ($>30/20^{\circ}\text{C}$ max/min) and they reverted to fertile with more than 60 per cent pollen and spikelet fertility during low temperature condition ($<30/20^{\circ}\text{C}$ max/min). All the lines except TS 29 showed one distinct sterile phase from March to June, whereas TS 29 had two sterile phases from mid February to mid June and from second week of September to first week of November. Since all the lines were completely sterile for more than 30 consecutive days during sterile phase, hybrid seed production utilising these lines can be taken up by raising the lines in such a way that flowering coincides with sterile phase. All the lines reverted into fertile in two phases. The first fertile phase was short (13-17 days) during July for all the lines except TS 29, for which it was observed during August. The second fertile phase was longer in duration from late November to early February for more than 30 days in all the lines except TS 29, in which it was only for 16 days during December. The maximum pollen and spikelet fertility recorded during this period was 63 to 85 per cent and 58 to 70 per cent, respectively. The correlation analysis between pollen sterility and weather parameters revealed that the daily maximum and mean temperature were the primary factors influencing fertility alteration, whereas sun shine hours, relative humidity and photo period were the secondary factors. The panicle development stages from meiotic division of pollen mother cell (S6) to pollen ripening (S8) were found to be sensitive in TS 16 and TS 29. For TS 18 Stamen and Pistil primordia differentiation to pollen ripening (S4 to S8) were identified as sensitive stages for temperature. The critical temperature inducing fertility alteration in these lines was found to be between 24 and 26°C. These lines satisfied the requirement of stable fertility behaviour for commercial exploitation of these lines in two line hybrid rice breeding.

Key words:

Rice, TGMS, fertility behavior, CST, CFT

Introduction

Today, rice is synonymous to food security in most parts of Asia, which produce more than 91 per cent of global harvest. Rice accounts upto half of Asia's farm income and makes up nearly 80 per cent people's daily calories. Rice is also the staple food in West Africa, the Caribbean and tropical regions of Latin America, where it is often the most important source of protein for the poorest 20 per cent of the population. The green revolution in many rice producing countries enabled global rice production to meet the demand of the world's increasing population and saved millions of people in several developing countries from starving. However, the potential

Centre for Plant Breeding and Genetics, Tamil Nadu
Agricultural University, Coimbatore-3

yields of the modern semi-dwarf rice varieties released after IR 8 are more or less the same as if not lower than that of IR 8 showing a yield plateau or ceiling. Recent progress in plant breeding research indicated that a significant shift in the yield frontiers could be possible through hybrid rice. China's success in hybrid rice technology has generated interest in other Asian countries. However, outside China, no tropical country could exploit the hybrid rice technology 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 three-line hybrids. Although effective, the three-line system of hybrid rice breeding, is expensive and cumbersome. Maintaining the CMS lines and choosing appropriate restorer lines for developing the fertile hybrids are

major limiting factors. Additionally, the availability of only a few CMS lines for hybrid development narrows the genetic base and increases the risk of genetic vulnerability (Yuan, 1997).

The two-line system of hybrid breeding utilizing environment sensitive genic male sterility (EGMS) is considered as an alternative to overcome the problems associated with three-line breeding and to surpass the yield plateau. For breeding two-line hybrids 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 sterile (PGMS) system (Virmani, 1996). For successful exploitation of this novel male sterility system in heterosis breeding, more TGMS lines need to be developed and characterized for their sterile and fertile phases and critical stages and temperature for fertility alteration. In this context, the present study was undertaken with the specific objective of characterizing a set of promising TGMS lines for their fertility behaviour and their potential use in hybrid rice breeding.

Materials and methods

The putative TGMS lines identified at Tamil Nadu Agricultural University (TNAU), Coimbatore, India and IRRI-bred lines obtained from the Directorate of Rice Research, Hyderabad were evaluated during summer at Coimbatore (High temperature condition - 36/22°C) and Hybrid Rice Evaluation Centre, Gudalur, India (Low temperature condition - 30/13°C) simultaneously. At the time of heading, top ten spikelets were collected from ten random plants of each line and the pollen fertility was observed using 1% Iodine potassium iodide (IKI) stain. Among 16 lines evaluated, six lines *viz.*, TS 6, TS16, TS18, TS29, TS46 and TS47 were completely sterile with 100 per cent pollen sterility at Coimbatore and the same lines were fertile with 50 to 90 per cent pollen fertility at Gudalur. These lines were further characterized for fertility behaviour, critical stages and temperature for fertility alteration.

Sterile and fertile phases

TGMS lines were sown at fortnightly interval. 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 than 50 per cent pollen fertility was considered as fertile phase. The period of partial sterility was considered as the phase of fertility transition.

Relative influence of weather factors on fertility

The influence of weather factors 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 working out simple correlation coefficient between the weather factors and pollen sterility.

Critical stages of fertility alteration

The correlation coefficients between sterility and the average of daily mean temperature at differentiation of first bract primordium (S1) to pollen ripening (S8) stages were calculated to determine the thermo sensitive stage of fertility alteration. The eight panicle development stages and their duration were considered as given by Rangaswamy *et al* (1993). The panicle development stages exhibiting significant correlation between temperature and pollen sterility were recorded as the critical stages for fertility alteration.

Critical sterility and fertility temperatures

The critical sterility temperature (CST) is the temperature, at which the line becomes sterile from fertile condition and critical fertility temperature (CFT) is the temperature, at which the line becomes fertile from sterile condition. The dissemination figures were charted out between pollen sterility at fertile and sterile phases (X) and the corresponding daily mean temperature during thermo-sensitive stage of fertility alteration (Y). The lowest mean temperature among the temperatures inducing sterility was considered as the critical sterility temperature and the highest mean temperature inducing fertility was recorded as critical fertility temperature.

Results and Discussion

Fertile and sterile phases

The multiplication of TGMS lines and seed production of two-line hybrids is not a difficult task as that of three-line hybrids, which require a maintainer line to multiply sterile line. TGMS lines behave 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. The fertility behaviour of TGMS lines at different dates of heading is depicted in Fig.1 to 6. Except TS29, other lines showed one distinct sterile phase during summer months, when the plants headed from March to June, whereas TS29 had two sterile phases from mid February to mid June and from second week of September to first week of November. The sterile period of all the lines was more than 50 days, which is a favourable feature for successful utilization of these lines. The sterile period was the longest in TS18 (156 days) followed by TS16 (141 days) and TS29 (122 days). The maximum/minimum temperature during the panicle development stages inducing sterility was around 30/20°C in most of the flowering months. Since all the lines were completely sterile for more than 30 consecutive days during sterile phase, hybrid seed production utilizing these lines can be taken up in Coimbatore by raising the lines in such a way that flowering coincides with the sterile phase. At Coimbatore, the lines TS16, TS18 and TS29 can be sown from December to March for hybrid seed production. For TS46 and TS47, sowing can be taken up during January to March for hybrid seed production. Lu *et al.* (1998) suggested that for successful utilization of TGMS lines, the sterile and fertile phases should be atleast for 30 consecutive days.

All lines reverted into fertile in two seasons. The first fertile phase was short (13-17 days) during July in TS16, TS18, TS46 and TS47. In TS29, it was observed during August. These lines recorded 52 to 72 per cent pollen fertility and 51 to 70 per cent spikelet fertility during fertile phase but the duration of fertile period was less than a month. The multiplication of TGMS lines with purity standards will be difficult during this season because of the very short duration of fertile phase. The late tillers may convert into sterile and favour outcrossing, which may affect the purity of TGMS lines. Lu *et al.* (1998) suggested that for successful utilization of TGMS lines, the sterile and fertile phases should be atleast for 30 consecutive days.

The second fertile phase was longer in duration from late November to early February for more than 30 days in all the lines except TS29, in which it was only for 16 days during December. The maximum pollen and spikelet fertility recorded during this

period was 63 to 85 per cent and 58 to 70 per cent, respectively. For seed multiplication, sowing of these lines could be undertaken during the months of September and October. Since the fertile phase of TS29 was short in both the seasons and it requires lower temperature for fertility reversion, seed multiplication of this line could be done in high altitude areas like Gudalur. In Gudalur TS29 was fertile with more than 60 per cent pollen fertility and seed set when the mean temperature was 22°C (27/17°C) and below from June to November.

Influence of weather factors on fertility

The transition of fertility in TGMS lines is mainly induced by temperature but the effect of other factors such as light, humidity, solar radiation, rainfall, etc., might be subordinate and varies in lines with different genetic background (Liu *et al.*, 1997). In the present investigation, all lines were observed with complete sterility at temperatures more than 30/20°C and were fertile at the temperatures less than 30/20°C. But occasionally sterility and fertility were noticed at temperatures below and above this limit, which forced to find out the influence of other factors on fertility alteration.

The results of correlation analysis between pollen sterility and weather parameters (Table 1) revealed that maximum temperature and mean temperature were the primary factors influencing fertility alteration. In TS16, the degree of influence of weather factors was in the order of mean temperature > maximum temperature > relative humidity. The influence of relative humidity was in the negative direction. In TS18 and TS29, the influence of maximum temperature was more than mean temperature and TS18 was found to be influenced by sunshine hours also. In TS46 and TS47, maximum, mean and minimum temperatures were identified as the primary factors influencing pollen sterility and the subordinate factor was photoperiod in TS46 and sunshine hours in TS47. Influence of sunshine hours and relative humidity on fertility alteration have been reported by Liu *et al.* (1997) in the TGMS line, 5460S.

Critical stages of panicle development for fertility alteration

There is certain amount of risk in exploiting rice heterosis by means of TGMS, if temperature fluctuation occurs at critical stages of panicle development (Wu, 1997). Therefore, knowledge on critical thermo-sensitive stage for fertility alteration

is useful to determine the most suitable time of sowing the 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 required temperature. The stages of panicle development sensitive to environmental factors varied among the lines (Table 2). In this study, the later stages were found more sensitive than the early stages. All panicle developmental stages from differentiation of primary bract primordium (S1) to pollen ripening (S8) were sensitive to temperature in TS46 and TS47. These lines were sterile relatively for a shorter period than the other lines. This might be due to the effect of temperature at all stages of panicle development inducing fertility alteration. If low temperature prevails even for a short period during any stage of panicle development, it will affect the sterility. The panicle developmental stages from stamen and pistil primordia differentiation to meiotic division of pollen mother cell have been reported as critical stages for different TGMS lines in other studies (Borkakati and Virmani, 1997).

Critical sterility and fertility temperatures

In the present study, the mean temperature inducing fertility alteration during the sensitive stages of panicle development was 24 to 26°C (Table 2). 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. The CST and CFT of TS16 and TS18 were lower than that of TS46 and TS47, even though all the four lines had derived TGMS gene from the same source, Norin PL 12. It might be due to the influence of different genetic backgrounds. Wu (1997) has reported similar case of varying CST and CFT in the lines with different genetic backgrounds. He found differences in CST among the four lines derived from Annon S-1.

The critical temperature inducing sterility must be relatively low i.e., 23°C in temperate zone and 24°C in subtropics (Yuan (1998)). The lines with more critical temperature (>26°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. The critical temperature for fertility alteration of TS29, TS46 and TS47 was more than 25°C and it was 24 to 25°C for TS16 and TS18. 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°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 for sterility but the seed yield will be low, as it requires very low temperature for transition from sterile to fertile, which can be multiplied only in high altitude areas.

Conclusion

The results of this study showed that all TGMS lines have clearly defined fertile and sterile phases. They were completely sterile under high temperature condition and exhibited acceptable level of pollen and spikelet fertility under low temperature condition. The CST and CFT of all the lines are 24 to 26°C. All these lines satisfied the requirements for commercial exploitation.

References

- Borkakati, R.P., Virmani, S.S., 1997. Determination of critical stage of fertility alteration in two thermo-sensitive genic male sterile mutants of rice. In : Proc. Int. Symp. on Two-Line System of Heterosis Breeding in Crops. Sep. 6-8, 1997, China National Hybrid Rice Research and Development Center, Changsha, China. pp.188-192.
- Liu, Y., He, H., Shun, Y., Rao, Z., Pan, X., Huan, Y., Geo, J., 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. Sep. 6-8, 1997, China National Hybrid Rice Research and Development Center, Changsha, China. pp. 49-58.
- Lu, X.G., Virmani, S.S., Rencui, Y., 1998. Advances in two line hybrid rice breeding. In: Virmani, S.S., Siddiq, E.A., Muralidharan, K. (Eds.), Advances in Hybrid Rice Technology, International Rice Research Institute, P.O. Box 933, Manila, Philippines. pp. 89-98.
- Rangaswamy, M., 1993. Guidelines and techniques of Hybrid seed production in Rice. In: Training Manual of Hybrid Rice Seed Production Technology, Directorate Rice Research, Hyderabad.
- Virmani, S.S., 1996. Hybrid rice. Adv. Agron. 57, 377-462.
- Wu, X.J., 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. Sep. 6-8, 1997,



China National Hybrid Rice Research and Development Center, Changsha, China. pp. 121-131.

- Yuan, L.P., 1997. Exploiting crop heterosis by two-line system hybrids: Current status and future prospects. In : Proc. Int. Symp. on Two-Line System of Heterosis Breeding in Crops. Sep. 6-8, 1997, China National Hybrid Rice Research and Development Center, Changsha, China. pp. 215-220.
- Yuan, L.P., 1998. Hybrid rice breeding in China. In: Virmani, S.S., Siddiq, E.A. Muralidharan, K. (Eds.), Advances in hybrid rice technology. International Rice Research Institute, P.O. Box 933, Manila, Philippines. pp. 27-33.

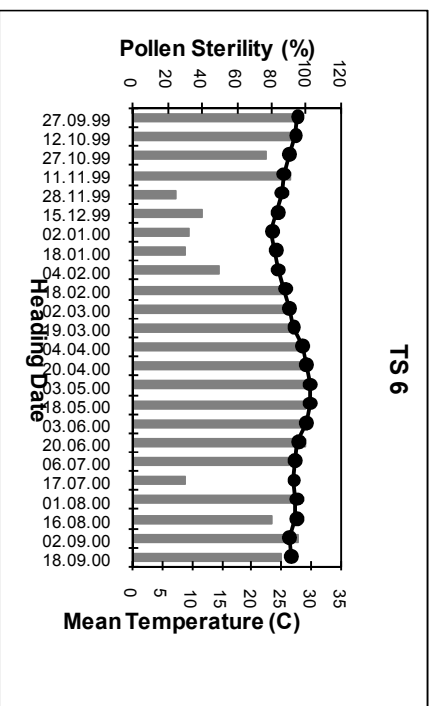


Fig. 1 Fertility behaviour of TS6

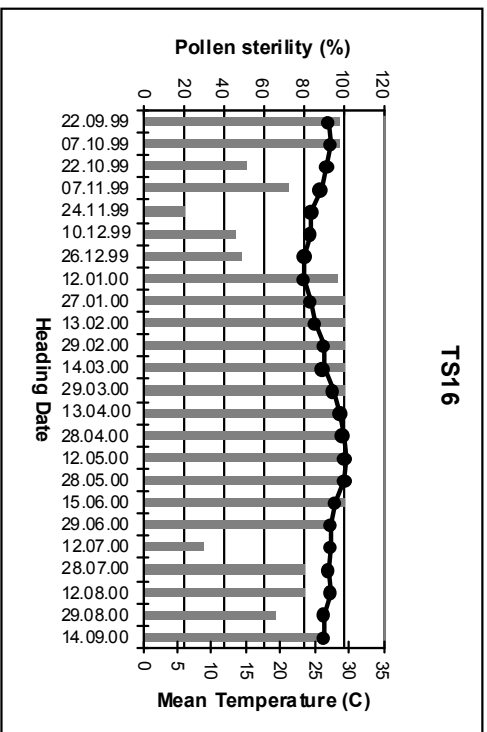


Fig. 2 Fertility behaviour of TS16

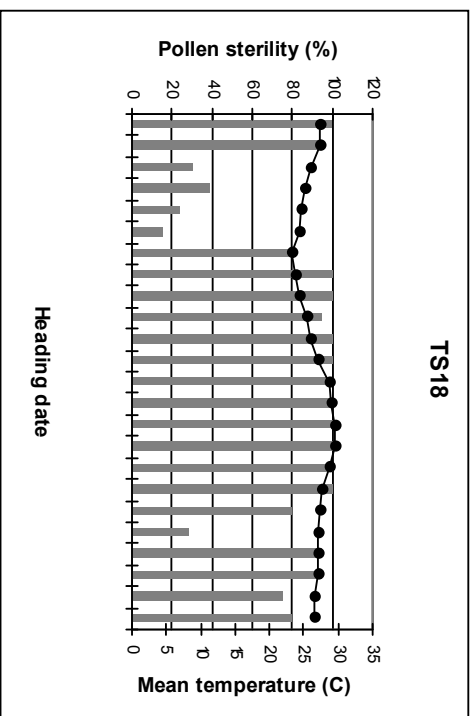


Fig. 3 Fertility behaviour of TS18

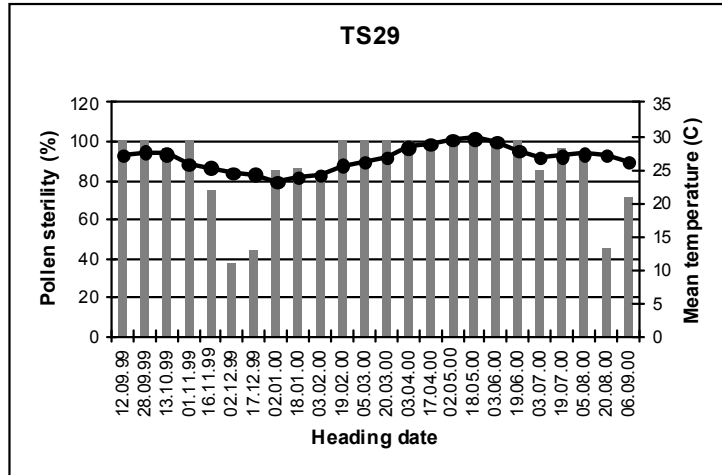


Fig. 4 Fertility behaviour of TS29

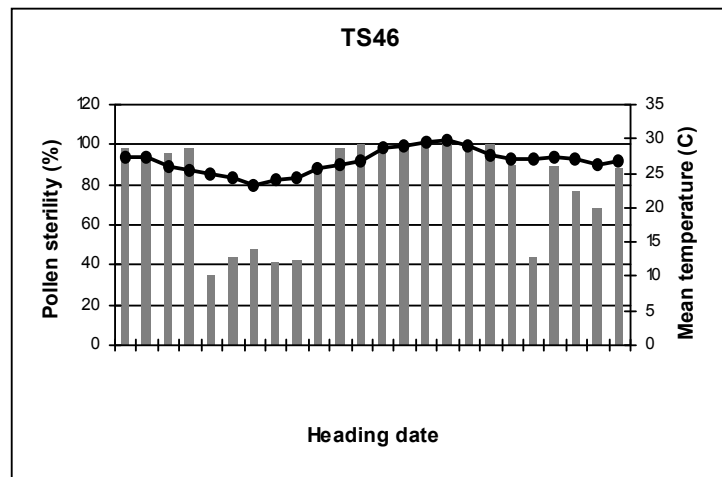


Fig. 5 Fertility behaviour of TS46

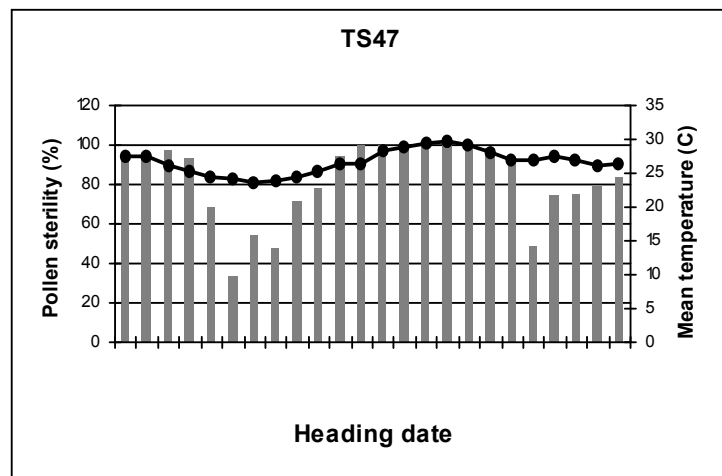


Fig. 6 Fertility behaviour of TS47



Table 1. Correlation coefficient of pollen sterility with mean weather factors over a period of 26 days before heading

Lines/ parameter	TS 6	TS 16	TS 18	TS 29	TS 46	TS 47
Maximum temperature	0.597**	0.487*	0.598**	0.557**	0.701**	0.751**
Minimum temperature	0.456*	0.013	0.200	0.342	0.661**	0.574**
Mean temperature	0.568**	0.489*	0.463*	0.488*	0.725**	0.722**
Relative humidity	-0.375	-0.423*	-0.619**	-0.475*	-0.284	-0.328
Sun shine hours	0.378	0.314	0.522**	0.301	0.378	0.437*
Photo period	0.341	0.058	0.326	0.323	0.454*	0.390

n = 24

* p = 0.05

** p = 0.01