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Characterization of Thermo sensitive Red Rice Line for its Suitability in the State of Kerala, India

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Hybrid rice technology has guaranteed the food supply for the ever growing population in the world. The identification of male sterility in rice was a breakthrough in the development of rice hybrids since hybrids have a yield advantage over conventional varieties. The hybrid seed production utilizing cytoplasmic male sterility is the widely adopted method in which a cytoplasmic male sterile source, a maintainer and a restorer line should be maintained for successful hybridization. The two line breeding system exploiting thermo-sensitive genic male sterility (TGMS) has a great potential to overcome the difficulties in three line system of hybrid rice development and can successfully be utilized in tropical countries where significant variations in temperature exist between season and between altitudes. TGMS lines are responsive to the temperature during a specific stage between panicle initiation and flowering for the expression of their single recessive nuclear male sterility gene(s). This trait can be transferred to any line of interest by backcrossing. TGMS lines will remain sterile when temperature is above Critical Sterility Temperature (CST) and it will become fertile if the temperature is below the CST. Stable TGMS line EC720903 was imported from International Rice Research Station (IRRI), Philippines and transferred male sterile gene to popular red rice variety Jyothi through backcross breeding. The seeds were sown at monthly interval for twelve months to evaluate the environmental conditions required for complete male sterility during 2017-2018. The TGMS red rice developed was found to be completely pollen sterile with pollen free anthers. On the other hand it was fully fertile at high altitude region with 82.08% seed setting. Therefore the new TGMS red rice line can be considered as a better choice as a female parent for Kerala condition for commercial hybrid rice seed development.

Key words: TGMS Rice, Male sterility, Pollen sterility

Rice is an important staple food of more than half of the world's population. It is reported that rice is cultivated in an area of 43.385 million ha with the production of 104.317 million tonnes contributing a major share on world's rice production (NFSM, 2017). However, it is not sufficient to meet the increasing food requirement of the growing population. Two line system of hybrid rice development exploiting thermosensitive genic male sterility has significantly bridged the gap between the increasing demands. Also hybrids possess improved characteristics and it can produce 15-20% yield gain over conventional rice varieties. Moreover it is a simple system compared to the complex three line system of hybrid rice development. In addition it is suitable for the tropical countries like India where significant variations in temperature exist between season and between altitudes. Hybrids released for commercial cultivation in India were developed using CMS system with white slender grain which is not suitable to the state of Kerala since the people prefer to consume red bold rice.

Male sterility expression is controlled by single recessive *tms* gene which is influenced by temperature and it is transferable to the next generation (Borkakati and Virmani, 1997). Around ten *tms* genes were reported in TGMS lines developed so far (Sheng *et al.,* 2013; Huang *et al.*, 2014). Reverse TGMS genes exhibiting sterility at low temperature and fertility at high temperature were also identified (Dora *et al.,* 2017). TGMS lines will remain sterile when temperature is above Critical Sterility Temperature (CST) and it will become fertile if the temperature is below the CST. The critical stages of panicle development would be exposed to appropriate temperature conditions for the fertility alterations while we utilise TGMS lines for hybridisation programmes. Therefore characterisation of TGMS lines is essential for the successful utilisation of TGMS system since the fertility behaviour is influenced by the existing weather conditions.

Stability of sterility in TGMS lines determines the success of two line hybrid technology. The TGMS 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 lines for commercial exploitation (Lu et al., 1994). The temperature conditions existing during panicle developmental stages especially from stamen

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and pistil primordial differentiation to meiotic division of pollen mother cell greatly influence the fertility of different TGMS lines (Borkakati and Virmani, 1997). The TGMS rice lines when exposed to maximum temperature higher than 30ºC one to two week after panicle initiation showed complete pollen and spikelet sterility. These lines reverted back to 85.5% spikelet fertility when exposed to 26ºC to 29ºC during the critical stage (Lopez and Virmani, 2000). The period four to eight days after panicle initiation was the stage most responsive stage to temperature fluctuations (Viraktamath and Virmani, 2001). Otherwise 11 to 15 days before heading were also considered as the critical stages of TGMS lines (Pham *et al.*, 2004).

Although maximum, minimum and mean temperatures were the primary factors influencing pollen sterility, relative humidity and sunshine hours were also found to influence fertility behaviour (Latha *et al.,* 2005). The maximum and minimum temperatures ranging from 30-360C and 24-260C were ideal for hybrid seed production. The TGMS lines GD 98014, GD 99017, GD 98029 and GD 98049 showed maximum pollen and spikelet fertility of 69.47 to 79.44 per cent and 60.00 to 68.50 per cent under two temperature regimes, respectively (Chandirakala et al., 2008). The critical temperatures of TGMS were found to be around 23°C to 29°C with variation from line to line (Wongpatsa *et al*., 2014). The TGMS line EC720903 was found to be a better option for Kerala condition with critical sterility period of 15 to 22 days before heading and the sterility inducing average temperature of 26.9°C (Celine *et al*., 2014). Knowledge on the fertility/sterility expression pattern is needed for the successful exploitation of TGMS system. Hence, the present study was undertaken with an objective of characterizing the new TGMS red rice for its suitability in the state of Kerala.

Material and Methods

The stable Thermosensitive Genetic Male Sterile line (EC720903) was imported from International Rice Research Station (IRRI), Philippines through Standard Material Transfer Agreement (SMTA) through National Bureau for Plant Genetic Resources (NBPGR), New Delhi. The critical sterility temperature and the critical stage of thermo sensitivity of the TGMS line were identified and phenological characterisation was done. This TGMS line was used as the donor plant for transferring male sterile gene to popular red rice variety Jyothi. The F_1 plants obtained was selfed to obtain F_2 generation and the sterile F_2 plants were backcrossed to yield BC_1F_2. This was sown at monthly interval for twelve months starting from May, 2017 to April, 2018 in order to characterise the new TGMS red rice by observing pollen sterility and spikelet sterility in the open field and Rain Out Shelter (ROS). The climatic parameters were monitored throughout the crop period. Each monthly sowing was considered as a treatment.

The pollen sterility was observed by staining pollen grains with 1 % IKI solution of five florets from three selected plants of each treatment. The number of pollen grains unstained to the total number of pollen grains was visually counted under compound microscope Leica DC 7.5 V (10 X) and expressed as percentage. The panicles were collected from three plants and number of spikelets unset to the total number of spikelets was counted and the spikelet sterility was expressed as percentage. The weather parameters were observed throughout the growing period from both open field and rain out shelter.

Results and Discussion

The new TGMS red rice line was stable for sterility expression throughout the study period at both experimental locations (Table 1; Fig. 1). Spikelet sterility at the time of maturity was observed and it was without any seed set. Anatomical studies showed that anthers were pollen free similar to donor parent EC720903 and the same line showed fertility at high altitude zone with 82.08% seed setting. Analysis of weather data shows that mean temperature during the critical thermosensitive stage of all the monthly sown TGMS red rice were above the critical sterility temperature of 26.9ºC under both conditions resulting in male sterility. The critical thermosensitive stage is considered as 15 to 22 days before flowering as reported by Viraktamath and Virmani, 2001; Pham

Table 1. Pollen and spikelet sterility of TGMS red rice as influenced by time of sowing

Month	Open field		Rain out shelter		Mean temperature during the critical thermosensitive stage
	Pollen sterility	Spikelet sterility	Pollen sterility	Spikelet sterility	
May	100	100	100	100	28.5
June	100	100	100	100	28.1
July	100	100	100	100	28.3
August	100	100	100	100	27.8
September	100	100	100	100	27.4
October	100	100	100	100	27.0
November	100	100	100	100	27.3
December	100	100	100	100	28.2
January	100	100	100	100	30.0
February	100	100	100	100	30.0
March	100	100	100	100	28.1
April	100	100	100	100	28.0

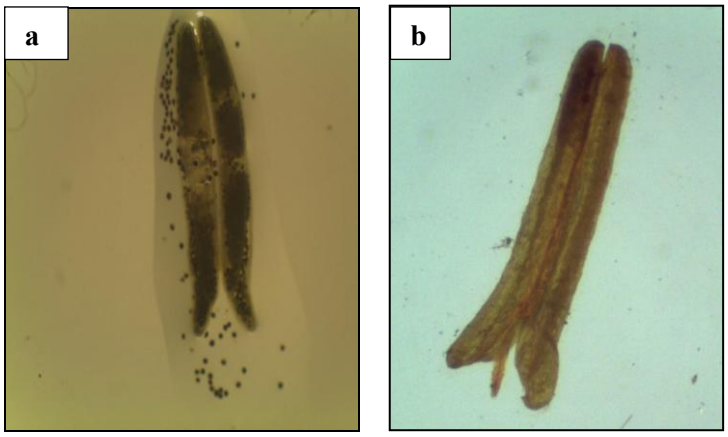

Fig. 1. a) Fertile anther with stained pollen grains
b) Sterile anther of TGMS red rice (pollen free)

et al., 2004; Celine et al., 2014. The TGMS lines will show sterility behaviour when exposed to CST during its thermosensitive stage. Earlier reports of Lopez and Virmani, 2000; Chandirakala et al., 2008; Celine et al., 2014; Wongpatsa et al., 2014 confirms the present study. The TGMS red rice line is performing similar to the donor parent in this genetic background also hence confirms the transfer of male sterile gene to next generation. Therefore it is suitable as a female parent development of red rice hybrids suitable to the state of Kerala.

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