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Marker Assisted Transfer of Thermo Sensitive Genic Male Sterile Gene to Red Rice (*Oryza sativa* L.) Lines

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Abstract: *Hybrid rice technology is the most promising approach to break yield barriers in rice, being practically feasible and readily exploitable. As rice is a self-pollinated species, use of male sterility is essential for hybrid rice breeding and seed production. Hybrid rice technology exploiting thermosensitive genic male sterility or TGMS is gaining much importance in declining rice production scenario. In this present study, we aim to identify the critical temperature requirements and thermosensitive period in selected TGMS lines. Two TGMS lines, EC720903 and EC720904 were imported from IRRI for the present study. Results have shown that the line EC720903 performed better with short stature, early maturity, more number of productive tillers, wider glume opening, higher panicle and stigma exsertion, more number of filled grains and longer panicle length with critical sterility period of 15-22 days before heading and the sterility inducing average temperature of 26.9 °C. Thermosensitive Genic Male Sterility is controlled by one or two recessive nuclear gene and hence the character can be transferred to any line of interest to us with respect to quality or yield. Hence in this present study we developed suitable hybrid rice by transferring the TGMS gene to red rice background using molecular marker assisted selection.*

Keywords: *Critical sterility, hybrid rice, thermosensitive genic male sterility, molecular marker.*

INTRODUCTION

The two-line system of hybrid rice breeding utilizing environment-sensitive genic male sterility (EGMS) is considered as an alternative to overcome the problems associated with the three-line breeding method and to surmount the yield plateau. The temperature sensitive genic male sterility (TGMS) system is considered more useful than the photo period-sensitive genic male sterility (PGMS) system in breeding two-line hybrids under tropical conditions, where day length differences are marginal (Virmani, 1996). This TGMS system offers many advantages in terms of simplifying hybrid seed production, removing the requirement for restorer genes, excluding maintainers, freedom of choice for pollen parents, and overcoming the negative effects of male sterility systems. It also avoids genetic vulnerability resulting from excess dependence on a single cytoplasmic source (WA cytoplasm). For successful exploitation of this novel male sterility system in heterosis breeding, more TGMS lines need to be developed (Shanker *et al* 2007).

The thermo-sensititve genic male-sterile (TGMS) gene in rice can alter fertility in response to temperature and is useful in the two-line system of hybrid rice production (Virmani *et al* 1996). TGMS trait is governed by a single recessive gene (Yang *et al*, 1992, Borkakti and Virmani, 1996, Reddy *et al*, 2000, Dong *et al*, 2000).

Hybrid rice production using TGMS system will be the ideal approach in tropical system as in Kerala where there is significant variation in temperature between seasons. The TGMS lines will be male sterile at higher temperature and become

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fertile at lower temperature. The climate is suitable for hybrid rice production in summer and multiplication of TGMS lines in winter season. TGMS hybrids yields more than 9-10 tons/ hectare and are having 10 – 20 % yield advantage than the high yielding CMS hybrids.

TGMS trait is controlled by a single recessive nuclear gene (Ali and Khan 1997) and depends on the environmental factors like temperature for its expression (Virmani 2003). Many TGMS sources were identified from worldwide. The success of the two line breeding depends on extend of maintaining stability in changing the fertility and sterility status. Each TGMS sources have their own specific temperature regimes in exhibiting the sterility/ fertility forms. So it is highly essential to know the specific temperature for inducing sterility and fertility in every line. So our present study was to identify the critical sterility period and temperature inducing sterility in selected TGMS lines. The sensitive stage usually exists between 15-24 days before heading (Ali *et al*. 1995; Ramakrishna *et al*. 2006) and it coincides with pollen mother cell development to secondary branch primordial formation.

Study of floral and morphological traits was found to be highly essential for the release of a commercially viable TGMS line (Kalaiyarasi and Vaidyanathan 2002). Since the male sterile plants can be identified only at anthesis stage, molecular markers are essential in crop improvement programme to identify the sterile plants and to expose them in fertility inducing condition for seed production. TGMS trait is controlled by '*tms*' genes located in different chromosomes in rice. To date there are 12 *tms* genes reported in rice. Some of the reported markers for TGMS are RM 239 for *tms1* (Wang *et al*., 1995) on chromosome 8, RM 11 for *tms2* (Maruyama *et al*., 1990) on chromosome 7, RM 257 for *tms4* (Dong *et al*., 2000, Reddy *et al*., 2000) on chromosome 2, and RM 3351, RM3476, and RM 440 for *tms6* (Lee *et al*., 2005, Wang *et al*., 2004) on chromosomes 5 and 3, respectively.

MATERIALS AND METHODS

The present investigation was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University. Two stable Thermosensitive Genetic Male Sterile lines IR75589-31-27-8-33 (EC 720903) and IR68301-11-6-4-4-3-6-6 (EC 720904) were imported from International Rice Research Institute, Philippines through a Standard Material Transfer Agreement. Two ruling red rice varieties Uma and Jyothi were used along with the TGMS lines for our study.

The stable TGMS lines IR 75589-31-27-8-33 and IR IR68301-11-6-4-4-3-6-6 along with ruling red rice varieties of Kerala Uma and Jyothi were sown sequentially at monthly interval to characterize the Phenological stages. The period of sowing when there is 100 % sterility were marked for hybrid seed production and the sowing dates when the fertility is maximum were selected for seed multiplication.

Each pot was labelled separately for making observation during different stages of plant growth. Five plants were selected at random for taking observation. During the time of anthesis, five spikelets from each plant were sampled and anthers were smeared in 1% Iodine Potassium Iodide (I-KI) solution to analyse the fertility and sterility status of pollen grains. The anthers were crushed using a needle and debris was removed. A cover slip was placed and observed under stereo microscope (Leica). Round and darkly stained pollen grains were taken as fertile and irregularly shaped lightly stained ones were taken as sterile pollen. Average of the pollen fertility value of five pots was expressed in percentage. Till maturity, each panicle was covered with paper bags to avoid cross pollination. Number of filled grains was counted and the percentage of spikelet fertility was also assessed.

The temperature sensitizing period was determined by physical cum morphological index method and through tracking method (Ali, *et al*. 1995). The day on which first spikelet emerges out was taken as the date of panicle emergence. The date of panicle emergence and fertility status of the pollen was recorded on daily basis. 15-24 days before heading was considered for the study as critical sterility period. Environmental parameters like maximum and minimum temperatures, relative humidity and sunshine hours during the period

were recorded. Focused dusting was more efficient in hybridization than the other two methods. Here pollen was directly applied to stigma. As anthesis commences in the morning a blooming panicle of the male parent was selected and pollen was gathered just before anthesis. Anthers were collected carefully by a clean forceps and were crushed open to release the pollen. Precisely collected the pollen by a brush and applied over the stigma. Care was taken to avoid injury to stigma. The date was marked on the bag with a wax pencil and the bag was placed over the panicle and the bottom edge was folded over and a paper clip was placed on the fold against the stem to hold it securely in place. The string of the identification tag was attached to the stem and tag was placed under the paper clip at the bottom of the glassine bag with the side showing the parents of the cross inward and the pollination date outward. The pots with female plants were placed in an area protected from wind, rain, and pests but with good exposure to sunlight.

RESULTS AND DISCUSSION

The two line hybrid technology revolutionized the production of rice in China. The adoption of the technique by other developing countries widened the horizon of hybrid rice scenario. But in India, the hybrids produced through TGMS is lacking. The TGMS tool is very much viable in a tropical country like India, where there is possibility of getting variable temperature regimes across seasons and along altitudes. The suitability of each TGMS lines under different agro-climatic region has to be studied before actually going for breeding program. Depending on the requirement of region, the genetic background may be selected, which is only possible through TGMS. Through repeated crossing (marker assisted breeding) it is now possible to transfer the trait from the recurrent parent.

The phenological characterization of the TGMS line is highly required for the breeding program. The synchronization of the flowering time of TGMS lines and that of the pollen parents is important for better crossing rate among the parental lines (Ramakrishna *et al*. 2006). Phenological study of two TGMS and non TGMS lines were conducted. The sensitive phase of the TGMS line was studied by physically split opening the primary tiller and observing the developing panicle during the specific intervals. Figure1 shows the panicle initiation in TGMS rice. The size of the panicle was correlated with the length of the flag leaf. The critical sterility point was determined by tracking method. The maximum, minimum and average temperature during the period of 15-24 days before heading was recorded.

In the TGMS line EC720903, 15-22 days prior to heading was obtained as the critical sterility period and the temperature for induction of sterility was found to be 30.6°C/ 23.3°C with an average temperature of 26.9°C (Table 1). On the contrary, Yao (1995) found that the critical sensitive stage is between 5-20 days before heading. According to Ali *et al*. (1995) the best Critical Sterility Point under tropical condition for a TGMS line is between 30-32°C. A study conducted by Yuan also found that the minimum temperature for inducing pollen sterility is between 23°C to 29°C, which is similar to our findings.

In the present study, the sterility inducing temperature regimes (30.6°C/23.3°C) show that this

Table 1
Phenological study of the TGMS lines and red lines

	Days taken to 50% flowering							
Lines used	*March-April sown*	*May-June sown*	*July-Aug sown*	*Sep-Oct sown*	*Nov-Dec sown*	*Jan-Feb sown*	*No. of tillers*	*Plant height (cm)*
TGMS03	113	95	97	100	83	110	3-5	44-59
TGMS04	127	123	102	110	84	83	2	60-68
Uma	115	121	115	113	113	115	2-4	52-63
Jyothi	95	102	95	95	91	105	2-4	58-67

Table 2
Characteristics of TGMS hybrids with Jyothi

Traits	*Pot 1*	*Pot 2*	*Pot 3*	*Pot 4*	*Pot 5*	*Pot 6*	*Pot 7*	*Pot 8*
Floral Traits								
Anther length(mm)	0.3	0.3	0.3	0.3	0.3	0.25	0.35	0.30
Anther colour	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Stigma length	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.3
Stigma colour	white	White	white	white	white	white	white	white
Pollen sterility	80%	100%	80%	70%	80%	100%	100%	100%
seed fertility %	100	100	100	100	100	100	100	100
Morphological Traits								
Basal leaf sheath colour	Light Green	Light Green	Green	Light Green	LightGreen	LightGreen	LightGreen	LightGreen
Leaf blade colour	Green	Green	Green	Green	Green	Green	Green	Green
Flag leaf angle	Erect	Erect	Erect	Erect	Erect	Erect	Erect	Erect
Auricle colour	Light Green	Light Green	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen
Legule length	18mm	15mm	17mm	20mm	20mm	20mm	25mm	12mm
Legule colour	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen	LightGreen
Legule shape	Truncate	Truncate	Truncate	Truncate	truncate	Truncate	truncate	Truncate
Collar colour	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
Panicle type	intermediate	Intermediate	intermediate	Intermediate	intermediate	Intermediate	intermediate	Intermediate
Apiculous colour	Creamy White	Creamy White	Creamy White	Creamy White	Creamy White	Creamy White	Creamy White	Creamy White

TGMS line belong to low CSP group, according to the classification given by Ali *et al*. (1995). ID 24, parent of EC720903 was reported to belong in the low CSP group (Siddiq and Ali 1999). At times, the expression of fertility alteration changes with genetic backgrounds (Mou *et al*. 1998, Viraktamath and Virmani 2001). The fertility and sterility status of EC720903 and EC720904 plants were represented in Figure 2 and 3. The TGMS sources should be

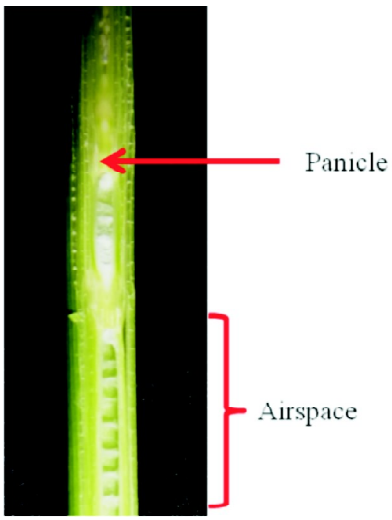

Figure 1: Figure showing the panicle initiation in TGMS line

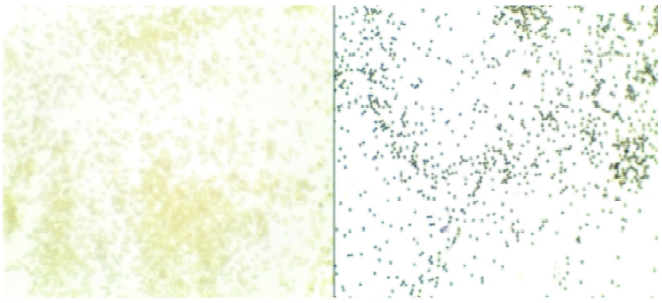

Figure 2: The sterility and fertility status respectively of EC720903 plants

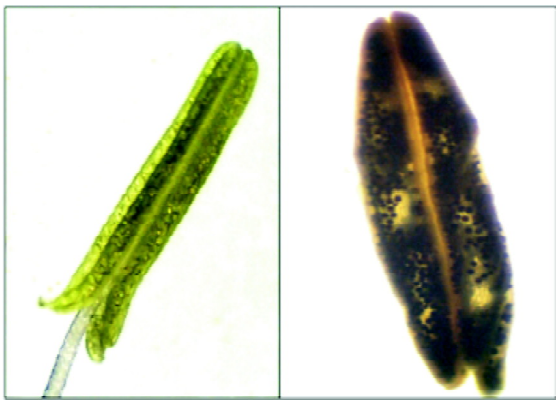

Figure 3: Sterile and fertile anthers of EC 720904

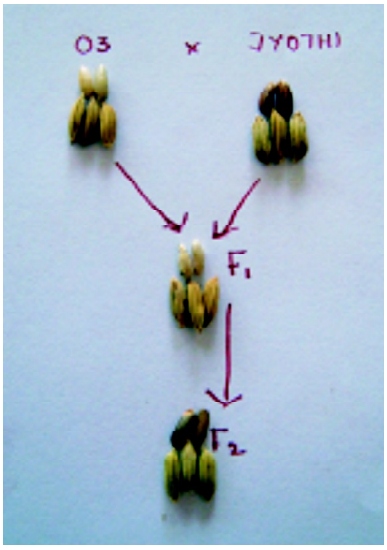

Figure 4: Morphology of Hybrids (TGMS-03 × Jyothi)

selected such a way that the seed setting of the line at low temperature is more than 30% and 100% pollen sterility at high temperature (Lu *et al*. 1994). For the identification of CSP in low-CSP TGMS lines, the characterization should be done ideally during the period of wet season (Sanchez and Virmani 2005). The temperature inducing fertility in the TGMS lines should remain consistent for certain period for the expression of fertility (Sanchez and Virmani 2005). Low-CSP TGMS line with high stability, however is a better option for two-line breeding in the tropics, where there are chances of sudden lowering of temperatures (Sanchez and Virmani 2005).

In SSR analysis RM 3351 differentiates TGMS from non TGMS with 6bp difference. Here the above primer gives a fragment of 173bp for male sterile plants and a fragment of 179bp for non TGMS plants.

The crucial factor under consideration behind any hybrid rice program is the knowledge about the sterility- fertility alteration behaviour of each TGMS source. There will be a different critical sterility and fertility inducing temperatures and periods in each line. The TGMS lines used in the present study yielded a temperature regime of 30.6°C/23.3°C with an average temperature of 26.9°C. The critical sterility inducing period of 22 days before heading coincided with the secondary branch primordial formation to stamen pistil primordial formation stage. Critical stage in most TGMS lines ranged between 15-24 days before heading (Ali *et al*.1995). The above results are in line with previous studies. The importance of maximum and average temperatures in inducing sterility is evident in the present study. It is inferred that the maximum temperature above 30.6 °C can induce sterility. The line EC720903 is a better candidate for selection as a TGMS source for developing a male sterile red rice parent for hybrid seed production in Kerala.

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