Short communication

Evaluation of thermosensitive genic male sterile lines in rice suitable to Kerala through marker assisted selection

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Abstract

An investigation was carried out at College of Agriculture, Vellayani, Trivandrum, Kerala, India to identify a suitable thermosensitive male sterile line for Kerala to be used as a donor parent to transfer the TGMS trait to popular varieties of Kerala. Two TGMS lines introduced from IRRI namely EC 720903 and EC 720904 were evaluated along with Uma and Jyothi, two popular male fertile rice varieties of Kerala at monthly intervals from January 2012 to December 2013. The male sterile lines showed 100% male sterility during April- May, September - October and January- March months indicating the suitability of the lines as donors of male sterility. Male sterile and fertile lines differed morphologically. TGMS and non TGMS lines differed at molecular level for TGMS gene specific marker RM 3351. The study proves the potential of the TGMS lines to be used as donor parent to transfer male sterility to popular varieties of Kerala by marker assisted back cross breeding.

Keywords: Hybrid rice, Molecular marker, SSR primer, Thermosensitive Genic Male Sterility

Rice is a staple food for over half of the world's population. Over 90 percent of the world's rice is produced and consumed in Asia. The area under rice in India has decreased drastically from the year 1985.With increasing population, demand for increased production of rice must be met from less land with limited resources. Hybrid rice technology is the best option for increasing the production of rice in tropics (Ikehashi et al., 1995). In India as many as 65 hybrids have been developed and released by public and private sectors, covering 2 m ha. However, none of these hybrids are popular in Kerala owing to the specific quality requirement.

TGMS system involves only two parents and can be easily exploited. No rice hybrid has been released in Kerala. The yield potential of thermosensitive genic male sterile (TGMS) hybrids is more than 10-12 t/ha (Li et al., 2009). The floral and morphological characterization of TGMS lines is a pre requisite for screening of commercially useable TGMS lines (Virmani et al., 1997; Kalaiyarasi and Vaidyanathan, 2002). Studies conducted by Lopez et al. (2003) indicated the use of microsatellite markers for identification of TGMS lines in the early stage of the crop, without the exposure of materials to required temperature. In 2012, Hussain et al. mapped another *tms* gene, *tms8*. Recently, a *tms* gene, *tms9* was identified from a line, Zhu 1S (Sheng et al., 2013). The present study was conducted at Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala to evaluate the TGMS lines and fertile lines at phenotypic and genic levels to test the suitability of using the male sterile lines as donor parents.

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Two TGMS lines, EC720903 and EC720904(denoted as 03 and 04 respectively), introduced from International Rice Research Institute (IRRI), Philippines were evaluated along with two popular rice varieties, Uma and Jyothi, from Kerala**.** The lines were sown at monthly intervals continuously from January till December during 2012-'13 for studying flowering behaviour. The fertility status of the pollen grains was assessed every day during April-May, September-October and January-March months. Pollen sterility/ fertility was determined using 1% potassium iodide solution.

A few qualitative traits of the plants as well as floral parts were observed in TGMS and non TGMS lines during April- May months**.** Total genomic DNA was isolated from fresh-frozen leaf tissue of TGMS lines and red rice lines using GenElute Plant Genomic DNA Miniprep Kit (Sigma). Quality of DNA was checked by performing Agarose gel electrophoresis (0.8%). Microsatellite analysis was done to find the SSR markers polymorphic to the *tms* gene. The SSR markers used are presented in Table 1.

The results on the morphological characterisation of the male sterile and fertile lines during April-May months are presented in Table 2. Pollen sterility of TGMS lines, EC720903 and EC720904, was 100 % under sterility inducing condition with maximum, minimum and average temperatures of 30.5^0C, 23.3^0C and 26.9^0C respectively. All four parents produced similar stigma colour, basal leaf sheath colour, collar colour, ligule shape and auricle colour. Panicle type was compact for Uma, whereas intermediate for the other three parental lines. Ligule colour varied between white (EC 720903 and EC 720904) and light green (Uma and Jyothi). EC 720903 showed a white apiculous colour while all the other lines produced light green colour. The study revealed that sterility inducing temperature at 22 days before heading induced complete sterility of pollen grains of TGMS lines making it a viable male sterile donor line suitable for transfer of male sterility genes to popular rice varieties. Among the 20 SSR markers used, one RM primer namely, RM 3351, was found polymorphic to the *tms* gene present in both the TGMS lines.

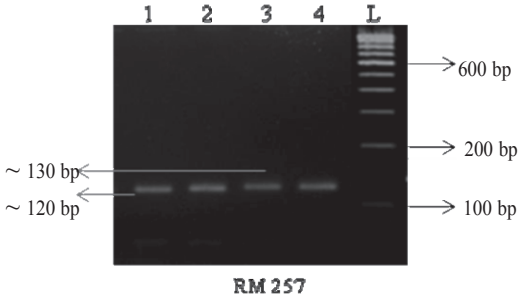
Table 1. List of SSR markers used for genotyping the male sterile lines

Sl. No.	Primers	Forward sequence	Reverse sequence
1	RM3351	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
2	RM5862	TTAGTACCTCATCATAGCTG	CTCTAATCTTCTCTCATTATCA
3	RM230	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG
4	RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
5	RM253	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
6	RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
7	RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
8	RM6776	AGCCCGGACATGCAAAAC	GAAGCAGGCGAAATCTCCTC
9	RM7121	GGAGATGGCACACGTCAAAC	AGGATCCCGTTTTGTAGCAG
10	RM21194	GTGATGCAGTGGGCGAAGTGG	GGGATGTTTGTTTGGTGGGAAGG
11	RM21197	CGGTGAGAATGGTACTCTGTCCTAGC	ATGGGCAAGGGCAATTAAAGG
12	RM3859	TTGCAGATCGGTTTCCACTG	GGTCCTGGATTCATGGTGTC
13	RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
14	RM2	ACGTGTCACCGCTTCCTC	ATGTCCGGGATCTCATCG
15	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
16	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
17	RM27	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG
18	RM29	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG
19	RM214	TTTTCCTTCTCACCCACTTCA	TCTTTGACAAGAGGAAAGAGGC
20	RM2020	ACACGTCTTTTCTGCAAGAT	GACGAACGTTATCGTGAAAT

Table 2. Floral biology and morphological characterization of TGMS and non TGMS lines in April- May months

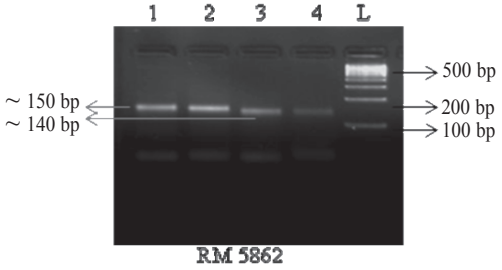
Traits	Pedigree	Anther colour	Stigma colour	Pollen sterility (%)	Basal leaf sheath colour	Collar colour	Ligule colour	Ligule shape	Auricle colour	Apiculus colour	Panicle type
EC 720903	D 24 x IR 65469-I61-2-3-2-3-2-2	White	White	100	Green	Light green	White	Truncate	Light green	White	Intermediate
EC 720904	IR 32364-28-1-3-2 x IR 68	White	White	100	Green	Light green	White	Truncate	Light green	Light green	Intermediate
Uma (MO 16)	MO6 X Pokkali	Yellow	White	0	Green	Light green	Light green	Truncate	Light green	Light green	Compact
Jyothi (PTB 39)	Ptb-10 x IR-8	Yellow	White	0	Green	Light green	Light green	Truncate	Light green	Light green	Intermediate

When RM 257 primer was used, Uma and Jyothi amplified at ~ 120 bp and 03 and 04 at ~130 bp (Fig. 1). According to Hussain (2001), '*tgms*' gene located on chromosome 9 from SA2 rice line has a linked marker RM 257 which is 6.2 cM near to the gene. Uma and Jyothi amplified at ~ 140 bp and 03



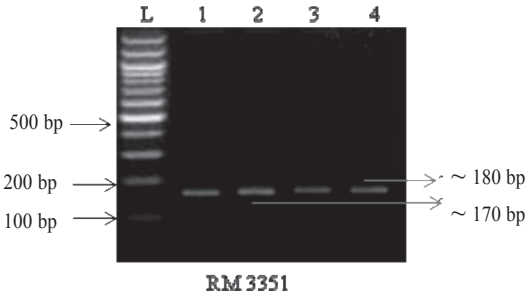
L - 100bp ladder, 1. Uma, 2. Joythi, 3. 03, 4. 04

Figure 1. PCR amplification products of SSR primer, RM 257 in 3% agarose



L - 100bp ladder, 1. 03 2. 04, 3. Uma, 4. Joythi

Figure 2 . PCR amplification products of SSR primer, RM 5862 in 3% agarose gel



L - 100bp ladder, 1. Uma, 2. Joythi, 3. 03, 4. 04

Figure 3. PCR amplification products of SSR primer, RM 3351 in 3% agarose gel

and 04 at ~150 bp when RM 5862 primer was used for PCR (Fig. 2). Yang et al. (2007) constructed a physical map covering the *tms5* gene and found that RM 5862 closely linked with the gene at a distance of 1.43 cM. SSR marker, RM 3351 primer amplified Uma and Jyothi at ~ 170 bp and 03 and 04 at ~180 bp (Fig. 3). Lee et al. (2005) fine mapped the *tms6* gene and found a closely linked marker, RM 3351 at a distance of 0.1 cM. It was located in

Table 3. Allele sizing of microsatellite marker RM 3351(in base pairs)

Sample Name	RM 3351	
Uma	173	173
Jyothi	173	173
03	179	179

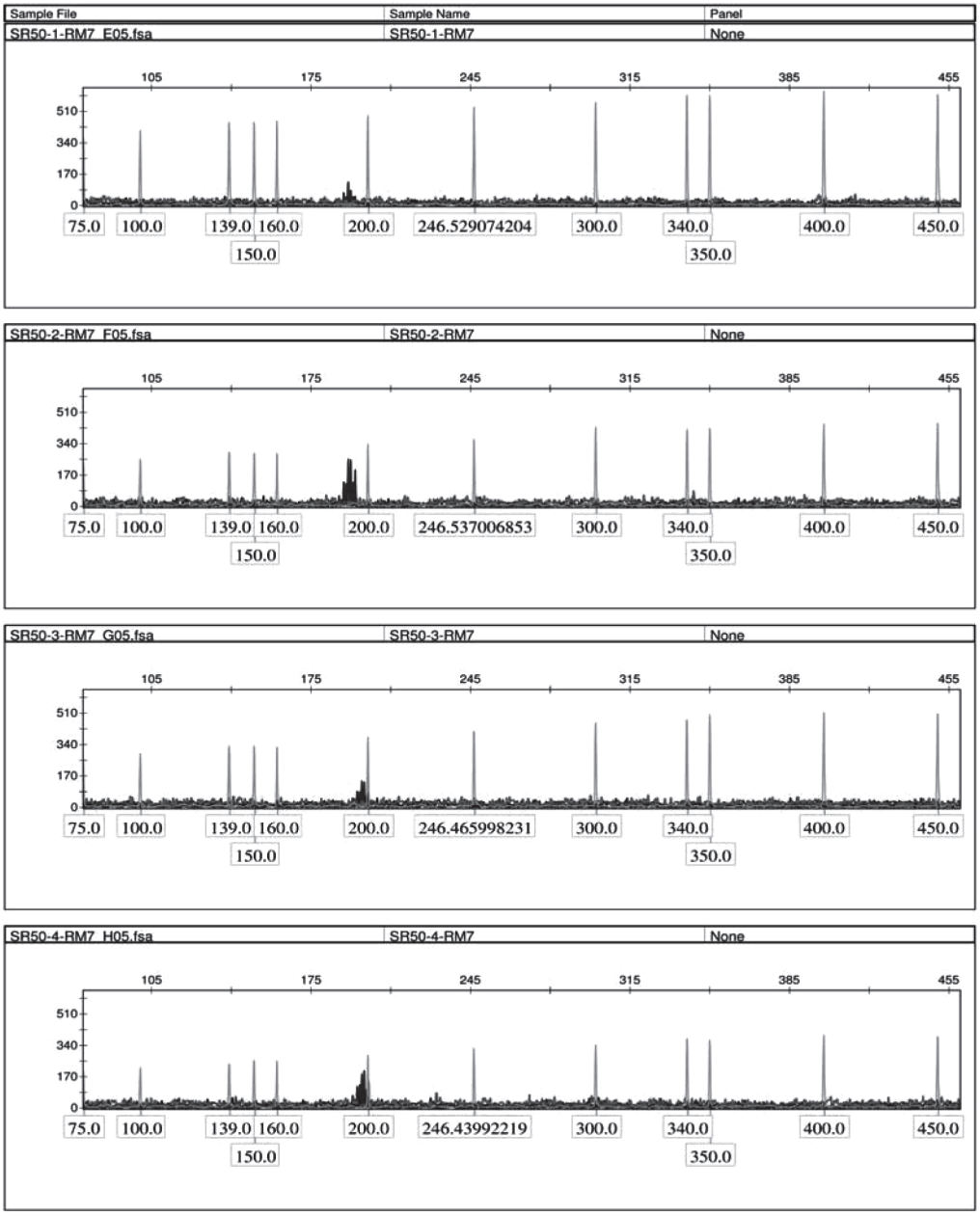


Figure 4. Electropherogram showing the differential amplification of Uma, Jyothi, 03 and 04 using RM 3351

chromosome number 5. Further analysis using capillary electrophoresis has shown its significance. TGMS lines got amplification at 179 bp, while the non TGMS parents amplified at 173 bp (Fig.4 and Table 3). The other two primers did not work. The present study concluded that the TGMS lines (EC720903 and EC720904) and non TGMS lines (Uma and Jyothi) can be well differentiated using SSR marker RM 3351 which was found to be polymorphic with *tms* gene. As the TGMS lines were expressing 100 percent male sterility during April- May, September- October and January-

March periods, and they have gene specific markers, these lines can be used to transfer male sterility genes into popular rice varieties of Kerala by marker assisted back cross breeding.

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