15. Rybicki, E. P., A proposal for naming geminiviruses: A reply by the *Geminiviridae* study group chair. *Arch. Virol.*, 1998, **143**, 421–424.
16. Brown, J. K., Idris, A. M., Torres-Jerez, I., Banks, J. K. and Wyatt, S. D., The core region of the coat protein is highly useful for establishing the provisional identification and classification of begomoviruses. *Arch. Virol.*, 2001, **146**, 1581–1598.
17. Bendahmane, M., Schalk, H.-J. and Gronenborn, B., Identification and characterization of wheat dwarf virus from France using a rapid method for geminivirus DNA preparation. *Phytopathology,* 1995, **85**, 1449–1455.
18. Wyatt, S. D. and Brown, J. K., Detection of Subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology,* 1996, **86**, 1288–1293.
19. Altschul, S. F., Thomas, L. M., Alejandro, A. S., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J., Gapped BLAST and PSI-BLAST, a new generation of protein data base search programs. *Nucleic Acids Res.*, 1997, **25**, 3389–3402.
20. Thompston, J. D., Higgins, D. G. and Gibson, T. J., CLUSTAL W: Improving the sensitivity of multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 1994, **22**, 4673–4680.
21. Feinberg, A. P. and Vogelstein, B., A technique for radio-labelling DNA restriction endonuclease fragments to high specific activity. *Ann. Biochem.*, 1983, **150**, 6–12.
22. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, 2nd edn.
23. Harrison, B. D., Liu, Y. L., Khalid, S., Hameed, S., Otim-Nape, G. W. and Robinson, D. J., Detection and relationships of cotton leaf curl virus and allied whitefly transmitted geminiviruses occurring in Pakistan. *Ann. Appl. Biol.*, 1997, **130**, 61–75.
24. Khan, J. A., Siddiqui, M. K. and Singh, B. P., The association of begomovirus with bitter melon in India. *Plant Dis.*, 2002, **86**, 328.
25. Khan, J. A., Siddiqui, M. K. and Singh, B. P., The natural occurrence of a begomovirus in sunn hemp (*Crotalaria juncea*) in India. *Plant Pathol.*, 2002, **51**, 398.
26. Khan, J. A., Sohrab, S. S., Aminuddin and Gupta, R. K., Detection of a begomovirus affecting guar [*Cyamopsis tetragonoloba* (L.) Taub.] in India. *Z. Pflanzenkr. Pflanzenschutz.*, 2002, **109**, 68–73.

ACKNOWLEDGEMENTS. We thank D. P. Pushpangadan, Director, NBRI, Lucknow for providing necessary research facilities.

Received 13 September 2004; revised accepted 27 January 2005

Inheritance of thermosensitive genic male sterility in rice (*Oryza sativa* L.)

Li Rongbai[1], M. P. Pandey[2,*] and Pankaj Sharma[2]

[1]Guangxi Key Laboratory of Subtropical Bioresource Conservation and Utilization and Guangxi Crop Genetic Improvement and Biotenology Lab, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi 530 007, China
[2]Department of Genetics and Plant Breeding, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar 263 145, India

Thermosensitive genic male sterility (TGMS) in rice is a useful trait for exploitation of heterosis, especially of inter-subspecific kind using two-line system. Three pairs of independent recessive (*tms*) genes with additive effects were involved in TGMS expression in UPRI 95-140 TGMS. Expression of the trait in F_2 generation involving 44 different genetic backgrounds indicated monogenic (3F : 1S), digenic (15 : 1S) and trigenic (63F : 1S) inheritance with frequencies of 18.2, 52.3 and 29.5% respectively. No single pair of genes was capable of causing complete male sterility. Two pairs of major *tms* genes in UPRI 95-140TGMS, non-allelic to any of the known *tms* genes, were located on chromosomes 3 and 7, and tentatively designated as *tms6*(*t*) and *tms7*(*t*) respectively.

THERMOSENSITIVE genic male sterility (TGMS) is a useful genetic tool for the development of two-line hybrids in rice. At the thermosensitive stage of panicle development, the TGMS gene(s) cause/s male sterility under high environmental temperatures and result/s in fertility under low temperatures. A TGMS line can therefore be used for hybrid seed production as well as for its seed multiplication under different growing environments. The system has advantages in much simpler and economic hybrid seed production and broader choice of male parents for enhancing yield potential as the maintainer and restorer lines employed in the currently used three-line hybrid breeding system based on male sterility, are not required[1]. The trait has shown monogenic inheritance and three independent genes, *tms1*, *tms2* and *tms3* were reported[2–4]. Few other TGMS sources have also been reported[5,6]. Recently, a new TGMS source, UPRI 95-140TGMS was identified in our hybrid breeding programme[7]. The trait in the line has shown digenic inheritance[8]. However, further intensive investigations of the line revealed more complicated inheritance of the trait and therefore, these findings are reported in this communication.

The TGMS line, UPRI 95-140TGMS, a spontaneous mutant with known fertility–sterility transformation behaviour under different temperatures[7], was studied for inheritance of its TGMS expression. Forty-four male fertile indica rice lines/varieties (Table 1) were used as male parents to cross the

*For correspondence. (e-mail: matappandey@rediffmail.com)

Table 1. Patterns of spikelet fertility segregation in 44 F_2 populations derived from crosses between TGMS line UPRI 95-140TGMS and normal male fertile indica genotypes used as male parents

Male genotype	Number of plants in F_2			Segregation pattern (expected)	χ^2	*P*-value
	Total	Fertile	Sterile			
RL94179	113	83	30	3 F : 1 S	2.299	0.25–0.50
UPRI 95-124	149	113	36	3 F : 1 S	0.056	0.80–0.95
UPRI 95-141	224	162	62	3 F : 1 S	0.857	0.50–0.80
UPRI 95-145	125	95	30	3 F : 1 S	0.067	0.80–0.95
UPRI 95-151	123	100	23	3 F : 1 S	2.604	0.25–0.50
UPRI 95-160	118	93	25	3 F : 1 S	0.915	0.50–0.80
UPRI 95-161	144	109	35	3 F : 1 S	0.037	0.80–0.95
UPRI 95-162	123	94	29	3 F : 1 S	0.202	0.50–0.80
BPHR1	138	126	12	15 F : 1 S	1.409	0.25–0.50
BPH17	112	101	11	15 F : 1 S	2.366	0.25–0.50
DK25	155	148	7	15 F : 1 S	0.795	0.80–0.95
DK54	148	136	12	15 F : 1 S	0.872	0.80–0.95
HKR 86	126	118	8	15 F : 1 S	0.004	0.95–0.99
HKR217	131	121	10	15 F : 1 S	0.428	0.80–0.95
IR 58025B	123	112	11	15 F : 1 S	1.522	0.25–0.50
IR36	136	129	7	15 F : 1 S	0.282	0.80–0.95
Narendra 80	123	115	8	15 F : 1 S	0.013	0.80–0.95
Narendra 118	114	105	9	15 F : 1 S	0.526	0.80–0.95
OPRBB	119	110	9	15 F : 1 S	0.350	0.80–0.95
Pant Dhan 4	120	112	8	15 F : 1 S	0.036	0.80–0.95
Pant Dhan 6	132	123	9	15 F : 1 S	0.073	0.80–0.95
PMS2B	126	110	16	15 F : 1 S	0.915	0.50–0.80
Pusa Basmati 1	175	161	14	15 F : 1 S	0.701	0.50–0.80
IRBB21	126	120	6	15 F : 1 S	0.476	0.50–0.80
UPRI 95-117	297	280	17	15 F : 1 S	0.148	0.50–0.80
UPRI 95-123	155	143	12	15 F : 1 S	0.589	0.50–0.80
UPRI 95-129	431	411	20	15 F : 1 S	1.906	0.25–0.50
UPRI 95-148	174	161	13	15 F : 1 S	0.443	0.50–0.80
UPR1004-30-2-1	169	160	9	15 F : 1 S	0.246	0.50–0.80
UPR 109-10-1-1	107	103	4	15 F : 1 S	1.152	0.25–0.50
UPR1875-12-2	144	138	6	15 F : 1 S	1.067	0.25–0.50
DK50	147	144	3	63 F : 1 S	0.219	0.50–0.80
IET8585	147	146	1	63 F : 1 S	0.744	0.50–0.80
IR 64	158	156	2	63 F : 1 S	0.090	0.80–0.95
IR 74	141	140	1	63 F : 1 S	0.667	0.50–0.80
Manhar	98	96	2	63 F : 1 S	0.146	0.50–0.80
Pant Dhan 10	125	122	3	63 F : 1 S	0.570	0.50–0.80
RL 253-3	353	345	8	63 F : 1 S	1.137	0.25–0.50
Sarju 52	136	134	2	63 F : 1 S	0.007	0.95–0.99
UPRI 91-17	170	168	2	63 F : 1 S	0.165	0.80–0.95
UPRI 91-56	134	130	4	63 F : 1 S	1.763	0.25–0.50
UPRI 95-169	127	122	5	63 F : 1 S	4.656	0.25–0.50
UPRI 95-170	120	115	5	63 F : 1 S	5.291	0.25–0.50
UPRI 95-174	113	108	5	63 F : 1 S	6.019	0.25–0.50

F_1 plants of all crosses are normal and male fertile.

TGMS line for inheritance study. TGMS lines IR 71018-13-73-33 with *tms2* gene and IR 323624 with *tms3* gene and a set of primary trisomics in the genetic background of IR 36 provided by the International Rice Research Institute, Philippines were also included for test of *tms* gene allelism and location on chromosome.

F_1 seeds from UPRI 95-140TGMS crossed with 44 lines (Table 1) taken as male parents were obtained and grown in 1997 wet season (WS) and F_2 populations in 1998 WS at temperature 28.55–28.72°C of mean 15-day thermosensitive temperature (15-dTT). The15-dTT, recorded 5 days prior to heading, was the most thermosensitive period of TGMS lines. Among these 44 crosses, four from the crosses of UPRI 95-140TGMS with normal fertile genotypes UPRI 95-117, UPRI 95-141, RL 253-3 and IR 58025B were taken for inheritance study, while the other crosses were used to confirm the genetic segregation of the *tms* gene(s) and study the frequencies of different segregation patterns. The parental, F_1, F_2 and TC_1F_1 generations of these four crosses were grown under mean 15-dTT of 28.61°C, at which the female TGMS

parent exhibited complete male sterility. Total of 102 and 135 lines from two F_3 families derived from crosses UPRI 95-140TGMS/UPRI 95-117 and UPRI 95-140TGMS/UPRI 95-141 respectively, were also studied to confirm fertility segregations. In F_3 families, 60 plants in each line were grown. Relatively wider spacing of 30×30 cm was adopted to ensure maximum expression of each genotype and minimize any environmental variation of non-genetic nature. At heading, some of the panicles in each plant were removed to promote late tiller formation and heading at different dates to coincide with different environment temperatures.

Spikelet fertility for the parental and F_1 populations was recorded. For screening F_2 populations, 80% spikelet fertility was taken as the critical point to distinguish the normal male fertile plants from male sterile as the standard rice varieties usually show minimum spikelet fertility of 80% under normal conditions. The heading date of individual panicle was recorded. Plants in F_2 and TC_1F_1 generations were sampled thrice at different 15-dTTs. Each time, three main panicles with similar heading time were sampled. Pollens were stained with IKI (1%) solution and pollen fertility was recorded in three replications. For recording observations on spikelet fertility, the incompletely filled grains were grouped into fertile class, while the infertile spikelets enclosed by the leaf sheath were excluded as the incomplete heading of male sterile plants leads to lack of anthesis and fertility. F_3 families were classified into three groups to study fertility segregation: Group I derived from F_2 normal fertile (F) plants with spikelet fertility equal to or larger than 80%, Group II from F_2 partially sterile (PS) plants with fertility between 10 and 80% and Group III from F_2 completely sterile (CS) plants with fertility 0–10% (Table 3). The space-planted plants displayed heading at mean 15-dTT of 28.55°C, which was higher than critical sterility point (CSP) of the female parent, UPRI 95-140TGMS, with critical sterility point of 25°C in our earlier study. The spikelet fertility of individual plants was visually examined and the number of fertile and sterile plants recorded.

Data on daily mean and minimum temperatures were obtained from the University Meteorological Observatory located close to the experimental field. The 15-day mean and minimum daily temperatures (mean and minimum 15-dTTs) were analysed to study their relationship with male fertility and segregation pattern.

UPRI 95-140TGMS with unknown *tms* gene(s), IR 71018-13-73-3-3 with *tms2* and IR 32364 with *tms3* were crossed with each other. The CSP with both TGMS lines, IR 71018-13-73-3-3 and IR 32364, was lesser than 26°C in our earlier study. Parental, F_1 and F_2 generations were grown during 2000 WS at temperatures higher than CSP of all the three parental lines. Pollen and spikelet fertilities of individual plants were recorded for analysis of fertility–sterility segregation.

Identification of primary trisomics was done based on morphology described by Khush *et al.*[9]. As the identified trisomics were not confirmed cytologically, 5–10 possible trisomic plants for each primary triplo were crossed to ensure the involvement of at least one plant per triplo in the crosses. As the transmission of extra chromosome from male (pollen) is low[9], i.e. only 0–27.3% and the TGMS line UPRI 95-140TGMS was completely male sterile, the triplos were used as the female parent and the F_1 of the TGMS line crossed with a normal fertile line UPRI 95-141 was used as the male parent in crosses with individual triplos. The cross IR 36//UPRI 95-140TGMS/UPRI 95-141 was used as control for comparison. The segregation ratios were subjected to χ^2 test.

Results on per cent spikelet fertility of the parental lines and F_1 revealed male fertility/sterility transformation behaviour of female TGMS line, UPRI 95-140TGMS, and normal male fertility of male parents, viz. UPRI 95-117, UPRI 95-141, RL 253-3 and IR 58025B. All F_1 hybrids had normal fertility at different temperatures, indicating complete dominance of fertility over sterility. The fertility segregation in F_2 and TC_1F_1 populations (Table 2) in cross UPRI 95-140TGMS/UPRI 95-141 indicated distinct fertility (F) and complete sterility (CS) groups and the presence of a third partial sterile (PS) group with wide variation. The ratios of observed segregation in F_2 and TC_1F_1 revealed goodness-of-fit to the expected ratio of 3F : 1S and 1 : 1 respectively, indicating monogenic segregation with respect to male fertility and sterility. The male sterile plants in the cross were separated into partial sterile (PS, 11–80% of spikelet fertility) and complete sterile (CS, 0–10% of spikelet sterility) with segregation ratio of 3PS : 1CS in F_2 (Table 2). The three groups of F, PS and CS of cross UPRI 95-140TGMS/UPRI 95-141 were subjected to genetic test in F_3 generation. Data presented in Table 3 display lack of segregation in the progenies of Group III derived from F_2 CS plants in the cross, indicating recessive homozygous nature of F_2 plants. However, the progenies of Group II derived from F_2 PS plants segregated in the ratio of 2 segregating : 1 true breeding sterile progenies and were devoid of true breeding fertile progenies. It indicated the presence of an independent PS class in the F_2 population of the cross. It is controlled by genetic factor and is not due to environment. Progenies of Group I derived from normal fertile F_2 plants exhibited segregation in a ratio of 4 true breeding fertile : 8 segregating progenies. The segregation pattern in F_3 could be due to differential expression of two dominant genes for male fertility and clearly confirms a dominant epistatic gene interaction between two genes (Table 3).

The segregation of male fertility in the cross UPRI 95-140TGMS/UPRI 95-117 was discrete and two distinct classes were shown in the F_2 and TC_1F_1 populations. The F_2 population segregated in the ratio of 15F : 1CS for pollen and spikelet fertilities and the TC_1F_1 population in the ratio of 3F : 1CS. The results confirmed digenic segregation of F_2 in the cross (Table 2). The F_3 families derived from F_2 CS plants (Group III) were true-breeding, while those derived from fertile plants (Group I) segregated in the ratio of 7 true

Table 2. Segregation for per cent pollen (PF) and spikelet fertility (SF) in F_2 and testcross populations of crosses

Cross[a]	Genera-tion	Trait	No. of plants[b] Total	F	PS	CS	Expected ratio[c] (F : S)	χ^{2d}	Expected ratio[e] (F : PS : CS)	χ^{2d}
UPRI 95-	F_2	PF	225	166	44	15	3 : 1	0.179d	12 : 3 : 1	0.185b
140TGMS/		SF	224	162	46	16	3 : 1	0.857e	12 : 3 : 1	0.845d
UPRI 95-141	TC_1	PF	120	59	33	28	1 : 1	0.033c	2 : 1 : 1	0.495c
		SF	223	113	55	55	1 : 1	0.040c	2 : 1 : 1	0.040a
UPRI 95-	F_2	PF	120	114	–	6	15 : 1	0.320d	–	–
140TGMS/		SF	297	280	–	17	15 : 1	0.140d	–	–
UPRI 95-117	TC_1	PF	120	88	–	32	3 : 1	0.178d	–	–
		SF	106	80	–	26	3 : 1	0.013b	–	–
UPRI 95-	F_2	PF	355	337	14	7	15 : 1	0.068c	60 : 3 : 1	0.804d
140TGMS/RL		SF	467	435	21	11	15 : 1	0.026c	60 : 3 : 1	1.934e
253-3	TC_1	PF	135	100	17	18	3 : 1	0.062c	6 : 1 : 1	0.091a
		SF	260	190	34	36	3 : 1	0.513e	6 : 1 : 1	0.384c
UPRI 95-	F_2	PF	550	540	–	10	63 : 1	0.236d	–	–
140TGMS/		SF	550	540	–	10	63 : 1	0.236d	–	–
IR58025B	TC_1	PF	210	186	–	24	7 : 1	0.220d	–	–
		SF	210	186	–	24	7 : 1	0.220d	–	–

[a]F_1 plants of all crosses are normal and male fertile.
[b]F, Fertile; PS, Partial sterile, CS, Completely sterile.
[c]S = PS + CS, when only F and S are considered.
[d]Probability value: a, 0.95–0.99; b, 0.90–0.95; c, 0.70–0.90; d, 0.50–0.70 and e, 0.25–0.50.
[e]Three segregation classes F, PS and CS are considered.

Table 3. Segregation for spikelet fertility in F_3 progenies from two crosses

Cross	Group of families[a]	Number of lines Total	True breeding (fertile)	Segregating	True breeding (sterile)	Expected ratio	χ^2	*P*-value
UPRI 95-140TGMS/	I	88	38	50	0	7 : 8	0.357	0.50–0.75
UPRI 95-117	III	12	0	0	12	–	–	–
UPRI 95-140TGMS/	I	80	24	56	0	4 : 8	0.400	0.50-0.75
UPRI 95-141	II	40	0	28	12	2 : 1	0.312	0.50–0.75
	III	15	0	0	15	–	–	–

[a]Groups I, II and III are lines derived from F_2 normal fertile, partial sterile and complete sterile plants respectively.

breeding fertile : 8 segregating progenies (Table 3). Segregation results indicated that only such plants in F_2 which possessed both the recessive genes in homozygous condition were completely male sterile.

The segregation of the cross UPRI 95-140TGMS/RL 253-3 indicated three classes of F, PS and CS in F_2 populations in the ratios of 15 : 1 with respect to fertility (F)–sterility (PS + CS) segregation for pollen and spikelet fertilities both, and 60F : 3PS : 1CS on resolution of the sterile plants into PS and CS classes. The TC_1F_1 population segregated in the ratio of 3F : 1S (or 6F : 1PS : 1CS), which further confirmed the trigenic inheritance of male sterility in this cross with dominant epistasis (Table 2).

In the cross UPRI 95-140TGMS/IR 58025B, the large F_2 population of 550 plants showed only 10 male sterile individuals. The observed segregation fitted well to the expected trigenic segregation ratio of 63F : 1S. Similarly, the TC_1F_1 generation segregated in the ratio of 7F : 1S, confirming the trigenic nature of inheritance of male sterility in the cross with triplicate gene interaction (Table 2).

Perusal of the results from 44 crosses involving the common female TGMS parent, UPRI 95-140TGMS, with 44 normal male fertile parents revealed normal fertility in all F_1 plants (data not shown) and F_2 segregations for spikelet fertility with three kinds of inheritance patterns, viz. monogenic (3F : 1S), digenic (15F : 1S) and trigenic (63F : 1S) in the proportion of 18.2 (8 populations), 52.3 (23 populations) and 29.5% (13 populations) respectively (Table 1).

The allelic relationships between *tms2* in IR 71018-13-73-3-3, *tms3* in IR 32364 and the unknown *tms* gene(s) in UPRI 95-140TGMS were studied in all possible crosses

Table 4. Allelic relationship(s) of unknown *tms* genes in UPRI 95-140TGMS with *tms2* and *tms3*

Cross	Gene	F_1 fertility[a]	F_2 segregation		
			F : S (no. of plants)[a]	Expected ratio	χ^2
UPRI 95-140TGMS/ IR 71018-13-73-3-3	Unknown/*tms2*	F	128 : 22	27 : 37	114.49*
IR 32364/ IR 71018-13-73-3-3	*tms3/tms2*	F	41 : 30	9 : 7	0.065^{ns}
IR 32364/ UPRI 95-140TGMS	*tms3*/unknown	F	56 : 72	27 : 37	0202^{ns}
UPRI 95-140TGMS	Unknown	CS			
IR 71018-13-73-3-3	*tms2*	CS			
IR 32364	*tms3*	CS			

[a]F, Normal male fertile; S, Male sterile and CS, Completely male sterile.
*Highly significant; ns, Not significant.

among them. The hybrid F_1 plants from the three crosses were all fertile in contrast to complete sterility of the respective parents (Table 4). The F_2 population of cross *tms3/tms2* displayed digenic segregation in the ratio of 9F : 7S, while the cross between *tms3* and unknown *tms* gene(s) revealed trigenic (27F : 37S) segregation. However, the cross between unknown *tms* gene(s) and *tms2* showed highly significant χ^2 value, indicating observed segregation to be different from the expected trigenic segregation ratio of 27F : 37S. These results indicate complementary gene interaction between *tms* gene(s) in UPRI 95-140TGMS and *tms2* in IR 71018-13-73-3-3 or *tms3* in IR 32364 (Table 4).

The location of unknown *tms* gene(s) of UPRI 95-140 TGMS was analysed with the help of set of primary trisomics in the genetic background of IR 36 by three-way cross method. Triplos 2 and 3 were not available and triplo 8 was excluded from analysis, as most of its progenies were late in heading. Results of the analysis involving nine triplos are presented in Table 5. Two of the three-way crosses, viz. triplo 3//UPRI 95-140TGMS/UPRI 95-141 and triplo 7//UPRI 95-140TGMS/UPRI 95-141 exhibited two kinds of segregation. One of these segregated in the ratio of 7F : 1S, which was identical to control population of IR 36// UPRI 95-140TGMS/UPRI 95-141, while the other had fewer male sterile plants than expected and segregated significantly different from the expected ratio of 7F : 1S. Segregation of individual F_2 progenies in these crosses in the ratio of 7F : 1S can be explained by the fact that the female plants initially utilized for the cross were not trisomics but disomics only due to mis-identification of individual trisomic plants phenotypically. On the other hand, segregation in the F_2 progenies significantly different from 7F : 1S ratio occurred as the true trisomic plants were used as female and the respective extra chromosomes 3 and 7 in the cross modified the segregation ratios significantly. Results from these two critical crosses explicitly conclude that two of the *tms* genes in UPRI 95-140TGMS were present one each on chromosomes 3 and 7 respectively (Table 5). Comparatively, the magnitude of male sterility of segregating sterile plants in the cross involving triplo 7 (mean 82.8%) was higher than the cross involving triplo 3 (65.3%). It provided the possibility of the *tms* gene with larger effect on sterility to be located on chromosome 7. Seven of the three-way crosses involving triplos 1, 5, 6, 9, 10, 11 and 12 as the respective female parents and the F_1 of UPRI 95-140TGMS/UPRI 95-141 as male parent were non-critical crosses, as they produced F_2 populations segregating in the ratio of 7F : 1S and identical to IR 36// UPRI 95-140TGMS/UPRI 95-141. It indicated that the *tms* gene expression was disomic and not modified by primary trisomics. It revealed lack of any evidence for the *tms* genes to be located on the chromosomes 1, 5, 6, 9, 10, 11 and 12 (Table 5).

Earlier reports from our studies indicated digenic inheritance of the TGMS trait in UPRI 95-140TGMS[8,10]. However, results of the present study have displayed mostly digenic nature of inheritance of TGMS and in some cases trigenic inheritance was evident. The monogenic pattern of segregation for fertility–sterility (3F : 1S) in F_2 was indeed digenic in nature (12F : 3PS : 1CS), while some segregations (15F : 1S) were trigenic (60F : 3PS : 1CS). The segregation patterns were strongly related to the genetic background. Thus, the results of the present study indicate the involvement of three pairs of independent and major recessive genes in the inheritance of TGMS in UPRI 95-140TGMS. The expression of these genes was complicated due to genetic background. Further analysis revealed two of the unknown major *tms* genes to be located on chromosomes 3 and 7 respectively (Table 5). Reports indicate that *tms1* gene is located on chromosome 8, *tms2* on chromosome 7, *tms3* on chromosome 6 and a new *tms4*(*t*) gene[11–14] on chromosome 9. All these genes were located by mapping populations with molecular markers. Our results (Tables 4 and 5) indicated two major *tms* genes in UPRI 95-140TGMS present on chromosomes 3 and 7 and, therefore, non-allelic to *tms1*, *tms3* and *tms4*(*t*) as the latter were located at different chromosomes. On the other hand, study indicated one of the unknown *tms* genes to be present on the same

Table 5. Segregation for fertility and sterility in F_2 populations of crosses involving TGMS line with unknown *tms* genes and set of primary trisomics

Cross	Segregation in individual F_2 progenies (plant no.)			χ^2 (7 : 1)	*P*-value
	Total	Fertile	Sterile		
Triplo 1//UPRI 95-140TGMS/UPRI 95-141	300	267	33	0.617	0.25–0.50
	300	263	37	0.007	0.90–0.95
	300	268	32	0.922	0.25–0.50
	300	268	32	0.922	0.25–0.50
	450	402	48	1.383	0.25–0.50
Triplo 3//UPRI 95-140TGMS/UPRI 95-141	600	566	34	25.612	< 0.001
	450	404	46	2.134	0.10–0.25
	300	282	18	11.588	< 0.001
	300	284	16	14.087	< 0.001
	300	265	35	0.190	0.50–0.75
Triplo 5//UPRI 95-140TGMS/UPRI 95-141	300	266	34	0.373	0.50–0.75
	300	260	40	0.190	0.50–0.75
	300	258	42	0.617	0.25–0.50
	300	263	37	0.007	0.90–0.95
	300	267	33	0.617	0.25–0.50
Triplo 6//UPRI 95-140TGMS/UPRI 95-141	450	498	52	0.367	0.50–0.75
	300	262	38	0.007	0.90–0.95
	300	259	41	0.373	0.50–0.75
	300	264	36	0.068	0.75–0.90
	300	267	33	0.617	0.25–0.50
Triplo 7//UPRI 95-140TGMS/UPRI 95-141	529	469	60	0.648	0.25–0.50
	450	425	25	19.841	< 0.001
	300	284	16	14.087	< 0.001
	300	282	18	11.588	< 0.001
	300	265	35	0.190	0.50–0.75
Triplo 9//UPRI 95-140TGMS/UPRI 95-141	300	266	34	0.373	0.50–0.75
	300	268	32	0.922	0.25–0.50
	300	261	39	0.068	0.50–0.75
	300	263	37	0.007	0.90–0.95
	300	259	41	0.373	0.50–0.75
Triplo 10//UPRI 95-140TGMS/UPRI 95-141	300	263	37	0.007	0.90–0.95
	300	262	38	0.007	0.90–0.95
	300	262	38	0.007	0.90–0.95
	300	264	36	0.068	0.75–0.90
	300	260	40	0.190	0.50–0.75
Triplo 11//UPRI 95-140TGMS/UPRI 95-141	300	258	42	0.617	0.25–0.50
	300	265	35	0.190	0.50–0.75
	300	268	32	0.922	0.25–0.50
	300	264	36	0.068	0.75–0.90
	300	262	38	0.007	0.90–0.95
Triplo 12//UPRI 95-140TGMS/UPRI 95-141	300	266	34	0.373	0.50–0.75
	300	265	35	0.190	0.50–0.75
	300	262	38	0.007	0.90–0.95
	300	261	39	0.068	0.75–0.90
	300	266	34	0.373	0.50–0.75
IR 36//UPRI 95-140TGMS/UPRI 95-141 (control)	300	267	33	0.617	0.25–0.50

chromosome 7 as *tms2*. The allelic test results have however, proved the unknown gene to be non-allelic to *tms2* but located near it because in the cross of unknown *tms* gene(s) with *tms2*, the F_1 was fertile and the F_2 population segregated with significantly lower proportion of sterile plants than the expected segregation ratio of 27F : 37S.

Therefore, both these new genes on chromosomes 7 and 3 of UPRI 95-140TGMS were non-allelic to any known *tms* genes and proposed as *tms6*(*t*) and *tms7*(*t*), respectively.

1. Yuan, L. P., Hybrid rice in China. In *Advances in Hybrid Rice Technology*, *Proceedings of the 3rd International Symposium on Hybrid Rice* (eds Virmani, S. S., Siddiq, E. A. and Muralidharan, K.), 14–16 November 1996, Hyderabad, International Rice Research Institute, Manila, Philippines, 1998, pp. 27–33.
2. Yang, R. C., Liang, K., Wang, N. and Chen, S., A recessive gene in Indica rice 5406S for thermosensitive genic male sterility. *Rice Genet. Newsl.*, 1992, **9**, 56–57.
3. Maruyama, K., Araki, H. and Kato, H., Thermosensitive genic male sterility induced by irradiation. In *Rice Genetics II, Proceedings of the Second International Rice Genetics Symposium*, 14–18 May 1990, International Rice Research Institute, Manila, Philippines, 1991, pp. 227–235.
4. Borkakati, R. P. and Virmani, S. S., Genetics of thermosensitive genic male sterility in rice. *Euphytica*, 1996, **88**, 1–7.
5. Chen, S. H., Lu, H. R. and Yang, J. B., Allelism of thermosensitive genic male sterile genes of indica rice. *Sci. Agric. Sin.*, 1996, **29**, 27–33.
6. Reddy, O. U. K., Siddiq, E. A., Ali, J., Hussain, A. J. and Ahmed, M. I., Genetics of thermosensitive genic male sterile lines in rice. *Int. Rice Res. Notes*, 1998, **23**, 10.
7. Pandey, M. P., Rongbai, Li, Singh, J. P., Mani, S. C., Singh, H. and Singh, S., The identification and nature of a new thermosensitive genic male sterility source, UPRI 95-140TGMS in Rice. *Cereal Res. Commun.*, 1998, **26**, 265–269.
8. Rongbai, Li and Pandey, M. P., Genetics of the thermosensitive genic male sterility trait in rice. *Int. Rice Res. Notes*, 1998, **23**, 9–10.
9. Khush, G. S., Singh, R. J., Sur, S. C. and Librojo, A. L., Primary trisomics of rice: Origin, morphology, cytology and use in linkage mapping. *Genetics*, 1984, **107**, 141–163.
10. Rongbai, Li and Pandey, M. P., Genetics and breeding behavior of thermosensitive genic male sterility in rice (*Oryza sativa* L.). *J. Genet. Breed.*, 1999, **53**, 11–17.
11. Wang, B. *et al.*, Tagging and mapping the thermosensitive genic male-sterile gene in rice (*Oryza sativa* L.). *Theor. Appl. Genet.*, 1995, **91**, 1111–1114.
12. Yamaguchi, Y., Ikeda, R., Hirasawa, H., Minami, M. and Ujihara, A., Linkage analysis of thermosensitive genic male sterility gene, *tms2* in rice (*Oryza sativa* L.). *Breed. Sci.*, 1997, **47**, 371–373.
13. Subudhi, P. K., Borkakati, R. P., Virmani, S. S. and Huang, N., Molecular mapping of a thermosensitive genetic male sterility gene in rice using bulked segregant analysis. *Genome*, 1997, **40**, 188–194.
14. Reddy, O. U. K. *et al.*, Genetic analysis of temperature-sensitive male sterility in rice. *Theor. Appl. Genet.*, 2000, **100**, 794–801.

Received 17 July 2003; revised accepted 15 March 2005

Applying intra-specific protoplast fusion in *Streptomyces griseoflavus* to increase the production of Desferrioxamine B

R. Nazari[1,5], A. Akbarzadeh[2,*], D. Norouzian, B. Farahmand, J. Vaez, A. Sadegi[1], F. Hormozi[3], M. Kiani-rad[3] and B. Zarbakhsh[4]

[1]Department of Genetics, The Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Pilot Biotechnology and [4]Department of Biotechnology, Pasteur Institute of Iran, Tehran, Iran
[3]Pilot of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran
[5]Present address: Department of Medical Genetics and Microbiology, University of Toronto, Room 211, 150 College St., Toronto, Ontario, M5S 3E2, Canada

***Streptomyces griseoflavus* secretes Desferrioxamine B, which is used as a precursor for producing Desferal, a chelator that absorbs additional iron from the blood of thalassaemia patients. In order to obtain a higher rate of Desferrioxamine B synthesis, two UV-mutated strains of *S. griseoflavus*, which were selected upon their resistance to sodium azide or crystal violet, were used in intra-specific protoplast fusion with polyethylene glycol 1000. One of the fusants showed higher production rate compared to the wild type *S. griseoflavus* (81.8%), and was characterized. Desferrioxamine B extracted from mutants and fusants and wild type *S. griseoflavus* was compared with Desferal by paper chromatography to assess the correct identity of the product.**

BLOOD transfusion, which has an important role in the treatment of patients suffering from thalassaemia major, poses several problems to recipients, such as iron overloading of blood[1]. It is necessary to remove the additional iron to prevent its toxic effects on the heart, liver, kidneys, etc.[2].

Currently, the only prescribed medicine, which acts as an iron chelator to absorb and excrete additional iron, is Desferal (Desferrioxamine B mesylate)[3,4], being produced by Ciba-Geigy[5,6] through fermentation of a modified strain of *Streptomyces pilosus*[7]. About 3–4% of Iranians suffer from thalassaemia major[8], and the purchase and import of Desferal to treat such patients is expensive. In order to develop a cost-effective method, *Streptomyces griseoflavus* was modified by intra-specific protoplast fusion to overproduce Desferrioxamine B (Des B) in an economical manner on a large scale. The choice of technique is based on the premise that it is the most efficient recombination technique in the case of *Streptomycetes*, and that the genes involved in Desferrioxamine synthesis and their sequences are not known properly[9]. In protoplast fusion it is essential to have at least one strain or species with a unique screenable marker. Protoplast forma-

*For correspondence. (e-mail: azimakbarzadeh@institute.psteur.ac.ir)