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Characterization of Thermo Sensitive Genetic Male Sterile Lines for Temperature Sensitivity, Morphology and Floral Biology in Rice (*Oryza sativa* L.)

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Abstract: Thermo sensitive genetic male sterile lines were screened for temperature sensitivity and their morphology and floral biology were studied. All the six TGMS lines clearly showed a tendency of transformation from sterile to fertile phase, when temperature was decreased in the field as well as under growth chamber conditions. Fertility restoration was more at lower temperature (28/21°C and 24/21°C) for all the six TGMS lines. All the TGMS lines exhibited critical sterility point at temperature more than 30°C. Critical sterility point ranged from 30°C (DRR 1S) to 35.9°C (IR 73827-23S). For all morphological traits except ligule shape variation was observed. Floral biology both in sterile and fertile phase showed varied behavior. In sterile phase the time of anthesis ranged from 9:05AM to 9:40 AM. For duration of anthesis there was clear cut difference among the TGMS lines. DRR 5S took minimum time (165 min) and UPRI 95-140S (270 min) took maximum time. Blooming duration varied from two to four days. Angle of gloom opening ranged from 25 to 36°. DRR 5S showed less exerted stigma (25%). High stigma exertion was observed in UPRI 95-167S (70%). Out crossing ranged from 20 to 41%. Maximum out crossing rate was recorded in DRR 1S. In fertile phase time of anthesis ranged from 9:35 AM to 10:30 AM. Duration of anthesis varied from 140 min in DRR 5S to 215 min in UPRI 95-67S. Blooming duration varied from 3-7 days. Angle of glume opening ranged from 15 to 25°. Stigma exertion was highest in UPRI 95-167S (62.4%) and lowest in DRR 5S (20%).

Key words: TGMS, floral biology, characterization

INTRODUCTION

Hybrid rice technology has played a major role in increasing the yield of rice over the last two decades. However the narrow genetic base of the parental lines used in three line system limited the full harnessing of the potential of heterosis. The possibilities of further exploitation of heterosis by utilizing intersubspecific variability (indica and japonica) is lower as less frequency of restorers in japonica rices and difficulties in transfer of restorer genes from indica to japonica rice lines.

Shi (1981) discovered a novel genetic male sterility in rice. In which sterility depends upon the length of photoperiods, it was called photoperiod sensitive genetic male sterility (PGMS). Later, Yang and Wang (1989) induced a mutant (5460 S) in the indica variety IR 54, that was either pollen sterile or pollen fertile depending on the change of temperature. This was called temperature sensitive genetic male sterility (TGMS). These pioneering findings led to put forward a new strategy of hybrid rice breeding, which involves exploitation of environment sensitive genetic male sterility (EGMS). It was named two-line heterosis breeding, as it requires only male and female lines to produce F_1 seed.

Male sterility in a TGMS system is heritable and is regulated by temperature. At sensitive stage, higher temperature (>30°C) results in sterility, whereas lower temperature (<23°C) results in fertility. This characteristic feature of TGMS line eliminates the need of maintainer line for its multiplication. There is no need for fertility restorer lines. Since the cytoplasm is not involved in sterility expression and single recessive nuclear gene controls sterility. It is possible to develop hybrids with diverse cytoplasmic and nuclear backgrounds. The TGMS lines remain selectively male sterile at a specific range of temperature or day length conditions and turn self-fertile at certain other temperature or photoperiod. Wide range of temperature difference occurs in India across locations,

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regions and seasons. Therefore, Thermosensitive genetic male sterility system for developing two line hybrids appears to be a feasible approach in breaking the yield barriers in rice.

The present study is an attempt to identify and utilize TGMS lines in developing two line-hybrids. The objectives of the study were, screening of the TGMS lines for temperature sensitivity, morphological characterization and study of floral biology.

MATERIALS AND METHODS

The present investigation was undertaken at the Directorate of Rice Research, Rajendranagar, Hyderabad, India. The material consisted of six TGMS lines, two each from Directorate of Rice Research, Pantnagar and International Rice Research Institute (IRRI, Philippines) (Table 1). These lines were characterized for critical sterility/fertility transformation period by screening under natural and artificially controlled temperature conditions. The sensitive stage for sterility/fertility transformation was determined during panicle development phase. The morphological and floral traits were recorded as per standard evaluation systems of International Rice Research Institute (IRRI).

For field screening, six TGMS lines were raised during two different seasons viz., post rainy 2002 (October-December) and pre rainy 2003 (February-April). Post rainy season (October-December) was chosen to evaluate the fertile phase of the TGMS lines, since temperature during panicle initiation stage was low (25.5/16.1°C). Pre rainy season (February-April) facilitated evaluation of sterile phase, since temperature was high (35.7/23.8°C) during panicle initiation. TGMS lines were planted in three staggered sowings at an interval of 10 days. Individual tillers of five hills were labelled for making observations through different stages of panicle development. Various stages of panicles development were determined by relating flag leaf length periodically. At flowering stage, five plants were chosen randomly for pollen studies. Five spikelets were sampled from each plant during anthesis and their anthers were smeared in 1% Iodine Potassium Iodide (IPI) solution. Round and dark stained pollen were scored as normal fertile and irregular shaped, yellowish or light brown colored pollen as sterile. Pollen fertility, averaged over all the five hills, was expressed as percent. At maturity, panicles covered with paper bags were taken for recording spikelet fertility.

The TGMS lines were raised under controlled temperature and light in growth chamber. Thirty-day-old seedlings of TGMS lines were transplanted in pots. In order to avoid bias pots were placed randomly in growth chambers, the hills were properly labelled and maintained uniformly with required nutrient and water. Treatments were continued for 20 days after which pots were shifted to net house. At the time of flowering three panicles from each plant were labelled and date of emergence of panicle was noted down. About five spikelets were taken from each panicle and studied for pollen sterility-fertility. At the time of maturity labelled panicles from each hill were harvested. Numbers of filled grains were counted and spikelet fertility was recorded in percentage. The growing conditions provided in the two chambers are given in the Table 2.

Table 1: TGMS lines used as experimental material for field screening

TGMS line	Abbreviated as	Source	Plant habit
DRR IS	S1	DRR	Semi-dwarf
DRR 5S	S5	DRR	Semi-dwarf
IR 73827-23S	S18	IRRI	Semi-dwarf
IR 73834-21S	S19	IRRI	Semi-dwarf
UPRI 95-140S	S20	Pantnagar	Semi-dwarf
UPRI 95-167S	S21	Pantnagar	Semi-dwarf

Table 2: Details of temperature and light duration maintained in two growth chambers

	Treatment		
	1	2	3
Regime	1 (10 h)	2 (12 h)	3 (14 h)
Maximum temperature	32°C	28°C	24°C
Minimum temperature	26°C	21°C	21°C
Mean	29°C	24.5°C	22.5°C

The sensitive phase of each line was determined by split opening its primary tillers at different intervals and correlating the developing panicle size with flag leaf length. When the flag leaf on the primary tiller was about 8-10 cm long, panicle development stage was found to be between pollen mother cell formation and meiosis, i.e., between stage IV and V. When the main/primary tiller was at the stage between V and VI, secondary tillers, were found to be at stamen-pistil primordial developmental stage (stage IV) with panicle length measuring about 2 to 4 cm. When the test plants approached the sensitive stage (stage IV) each of the six TGMS lines were made into three sets of single plants and transferred to growth chamber.

Five plants from each line were taken at random to study the distinguishable morphological characters for six TGMS lines. Observations were recorded at appropriate growth stage of the crop based on standard evaluation system for rice.

RESULTS

Screening of TGMS lines in field: TGMS lines were characterized based on sensitive stage of panicle development and critical sterility-fertility points. Sensitive

stage was determined by the use of the physical cum morphological method and the tracking technique. Critical fertility point is the temperature at or below which the TGMS line produces high proportion of fertile and partially fertile pollen grains. Critical sterility point is the temperature at or above which complete or maximum sterility can be induced in a TGMS line during its sensitive stage. The lowest among the maximum mean temperatures of three critical temperatures was taken as CSP.

For determining critical temperatures (CSP-Critical sterility point and CFP-Critical fertility point) and sensitive stage the criterion given by Ali *et al.* (1995) was followed. In DRR 1S, 24 days prior to heading was showing highest (35.10°C) maximum mean temperature and it was taken as critical temperature days coinciding with stamen-pistil primordial stage.

The sensitive stage for fertility was different among the lines studied. It was found to be 24 days prior to heading in DRR 1S and UPRI 95-140S. In IR 73834-21S and UPRI 95-167S sensitive stage was 21 days and in DRR 5S and IR 73827-23S it was 15 and 19 days, respectively (Table 3).

All the six TGMS lines clearly showed a tendency of transformation from sterile to fertile phase (Fig. 1) when temperature was decreased particularly in the 3rd week of November (29/13°C) as well as under growth chamber studies (Fig. 2). Fertility restoration was more at lower temperature (28/21°C) and (24/21°C) for all the six TGMS lines (Table 4). With regard to critical sterility point all the six TGMS lines exhibited high CSP (> 30°C). CSP ranged from 30°C (DRR 1S) to 35.9°C (IR 73827-23S).

Table 3: Critical Sterility temperature of different TGMS lines post rainy season 2003

TGMS line	Date of Complete Sterility	Critical temperature (°C) Max	Min	Days Before Heading
DRR 1S	1-Apr	34.30	19.70	24
	4-Apr	36.50	21.50	
	7-Apr	34.50	18.70	
DRR 5S	5-Apr	34.40	20.80	15
	9-Apr	37.30	24.30	
	11-Apr	37.50	22.00	
IR73827-23S	29-Mar	35.00	19.20	19
	30-Mar	36.50	21.50	
	31-Mar	36.30	22.20	
IR73834-21S	31-Mar	35.00	19.20	21
	1-Apr	36.50	21.50	
	2-Apr	36.30	22.20	
UPRI95-140S	1-Apr	34.30	19.70	24
	4-Apr	36.50	21.50	
	7-Apr	34.50	18.70	
UPRI95-167S	31-Mar	35.00	19.20	21
	1-Apr	36.50	21.50	
	2-Apr	36.30	22.20	

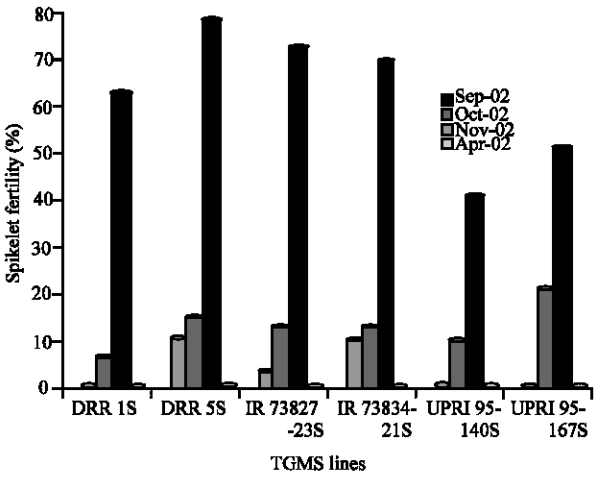

Fig. 1: Transformation of TGMS lines from sterile to fertile phase

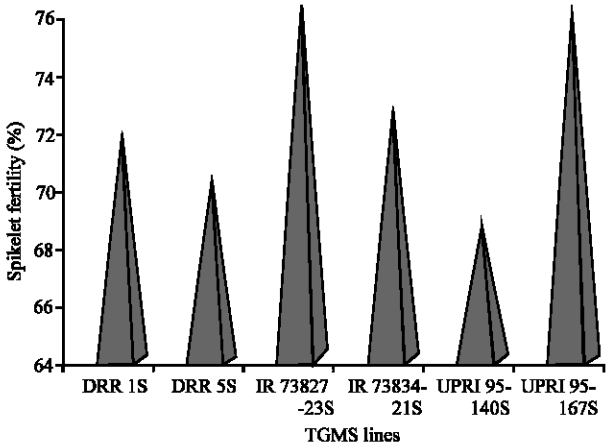

Fig. 2: TGMS lines showing varying levels of panicle exertion

Table 4: Pollen and spikelet fertility of TGMS lines screened in Growth Chambers

	Treatments					
	32/26°C		28/21°C		24/21°C	
TGMS line	F	SF	F	SF	F	SF
DRR 1S	0	100	86	14	84	16
DRR 5S	10	90	73	27	71	29
IR73827-23S	0	100	84	16	80	20
IR73834-21S	0	100	89	11	85	15
UPRI95-140S	0	100	86	14	82	18
UPRI95-167S	0	100	79	21	78	22

F - Fertile and SF - Spikelet Fertility (%)

Screening of TGMS lines in growth chambers: The six TGMS lines, which showed sterility, were further screened under growth chambers to study their fertility/sterility response under different temperature regimes during post rainy 2002-03. Three temperature treatments were 32/26°C

(10 h), 28/21°C (12 h) and 24/21°C (14 h). In the first treatment i.e., 32/26°C with 10 h of day length, four lines viz., DRR 1S, IR 73827-23 S, IR 73834-21S and UPRI 95-140 S exhibited complete pollen and spikelet sterility. Whereas, in DRR 5S (10%) and UPRI 95-167S (4%) there was some amount of pollen fertility and spikelet fertility was observed only in DRR 5S (2%) (Table 5). In the second treatment i.e., 28°C/21°C with 12 h of day length all the TGMS lines showed pollen and spikelet fertility. IR 73834-21 S, IR 73827-23 S and UPRI 95-140 S showed maximum spikelet fertility of 78, 75 and 75% respectively, followed by DRR 1S (74%) and minimum spikelet fertility was shown by DRR 5S (60%) although its pollen fertility was 73%. In the third treatment (24/21°C) and 14 h of day length all TGMS lines showed pollen and spikelet fertility, which were almost similar as in the 2nd treatment (28/21°C). DRR 1S (76%) showed maximum pollen and spikelet fertility followed by IR 73834-21 S (72%). DRR 5S exhibited a minimum spikelet fertility of 58%.

From the results given above, it is clear that when the temperature is high (>30°C) most of the lines exhibited sterility, and when the temperature is brought down there is increase in fertility level, which is clearly shown in 2nd and 3rd treatments.

Morphological characterization of TGMS lines: Six TGMS lines were studied by using nine stable morphological characters according to guidelines of standard evaluation system for rice (SES, 1996). Morphological characters were recorded at appropriate growth stage of the crop and results are presented in Table 6.

Variation was observed for leaf sheath color among six TGMS lines. DRR 1 S, UPRI 95-140 S and UPRI 95-167 S had purple leaf sheath color. Two IRRI lines viz., IR 73834-21 S and IR 73827-23 S had green leaf sheath color. Only one line DRR 5S exhibited light green leaf sheath. All lines were semi-dwarf. Based on the panicle type six TGMS lines were grouped into two categories. The lines which showed intermediate panicle type were DRR 1S, DRR 5S, IR 73834-21S, IR 73827-23S, UPRI 95-160S and only one line UPRI 95-140S exhibited open type. Panicle exertion (Fig. 2) ranged from 68.5 to 76%. Maximum panicle exertion was seen in IR 73834-21S (76%) followed by UPRI 95-167S (75.8%) and minimum in UPRI 95-140S (68.5%). Auricles were Purple in DRR 1S, UPRI 95-140S and UPRI 95-167S and the remaining three lines exhibited light green color. Ligule shape was monotypic trait, all the lines had truncate ligule shape. Ligule color was purple in DRR 1S, UPRI 95-140S and UPRI 95-167S remaining lines were white in pigmentation. Based on the apiculus pigmentation TGMS lines were grouped into two categories. The lines DRR 1S, UPRI 95-140S and UPRI 95-167S had purple apex and remaining lines had no apiculus pigmentation. Stigma color for six TGMS lines varied from white to purple color. In DRR 1S, UPRI 95-140S and UPRI 95-167S stigma color was purple where as, in DRR 5S, IR 73834-21 S and IR 73827-23 S lines it was white in color.

Floral biology: All the six TGMS lines were studied for floral characteristics during sterile (Table 7a) and fertile phases (Table 7b).

Table 5: Evaluation of TGMS lines for pollen fertility and spikelet fertility in different seasons

	September 2002 (32.3°C/21.5°C)			October 2002 (32.3°C/19.8°C)			November 2002 (29°C/13°C)			April 2003 (37.9°C/23.1°C)		
TGMS lines	S	F	Spikelet Fertility (%)	S	F	Spikelet Fertility (%)	S	F	Spikelet Fertility (%)	S	F	Spikelet Fertility (%)
DRR 1S	100	0	0	93	7	5	31	69	62	100	0	0
DRR5S	98	2	10	79	21	14	19	81	77	100	0	0
IR73827-23S	90	10	3	83	17	12	30	70	72	100	0	0
IR73834-21S	96	4	9	79	21	12	20	80	69	100	0	0
UPRI95-140S	100	0	0	86	14	9	44	56	40	100	0	0
UPRI95-167S	100	0	0	75	25	20	52	48	50	100	0	0

S-Sterile pollen, F-Fertile pollen

Table 6: Morphological characters among six TGMS lines in rice

Lines	Leaf sheath color	Plant height	Panicle type	Panicle exser -tion (%)	Auricle color	Ligule shape	Ligule color	Apiculus pigmentation	Stigma color
DRR 1S	Purple	Semi dwarf	Intermediate	71.5	Purple	Truncate	Purple lines	Purple apex	Purple
DRR 5S	Light green	Semi dwarf	Intermediate	70.0	Light green	Truncate	White	Absent	White
IR 73834-21 S	Green	Semi dwarf	Intermediate	76.0	Light green	Truncate	White	Absent	White
IR 73827-23 S	Green	Semi dwarf	Intermediate	72.4	Light green	Truncate	White	Absent	White
UPRI 95-140S	Purple	Semi dwarf	Open	68.5	Purple	Truncate	Purple lines	Purple apex	White
UPR 95-167 S	Purple	Semi dwarf	Intermediate	75.8	Purple	Truncate	Purple lines	Purple apex	Purple

Table 7a: Flowering behavior of TGMS lines in sterile phase (pre rainy season 2003)

Line	Time of anthesis (hr : min)	Duration of anthesis in a panicle (min)	Peak time of anthesis (hr : min)	Duration of blooming in a panicle (days)	Angle of glume opening (0)	Stigma exertion (%)	Out crossing (%)
DRR 1S	9 : 05	180	10 :15	2-3	36	45	41
DRR 5S	9 : 20	165	10 : 26	4	25	25	20
IR 73834-21 S	9 : 15	220	10 : 35	3-4	34	34	39
IR 73827-23 S	9 : 18	235	10 : 30	3-4	36	30	40
UPRI 95-140S	9 : 35	270	10 : 40	4	27	62	29
UPRI 95-167S	9 : 40	235	10 : 40	3	26	70	26

Table 7b: Flowering behaviour of TGMS lines in fertile phase (post rainy season 2002)

Line	Time of anthesis (hr : min)	Duration of anthesis in a panicle (min)	Peak time of anthesis (hr : min)	Duration of blooming in a panicle (days)	Angle of Glume opening (0)	Stigma exertion (%)
DRR 1S	9:35	155	10:45	3-4	20	39
DRR 5S	10:00	140	11:15	4	15	20
IR 73834-21S	9:37	195	11:10	4-7	25	32
IR 73827-23 S	9:40	190	11:00	5	25	30
UPRI 95-140S	10:10	210	10:50	3-4	24	58
UPRI 95-167S	10:30	215	11:05	3-5	20	62.4

Floral biology in sterile phase: Time of anthesis in six TGMS lines showed varied behavior which started from 9: 05 am in DRR 1S followed by IR 73834-21S (9: 15 am). Maximum time taken for opening of first glume was seen in UPRI 95-167S which was at 9: 40 am. For duration of anthesis in a panicle there was a clear-cut difference among the TGMS lines. DRR 5S took minimum time (165 min) and maximum time was taken by UPRI 95-140S (270 min). Peak time of anthesis was observed in DRR 1S at 10:15 am followed by DRR 5S (10:26 am). UPRI 95-140S and UPRI 95-167S took maximum time for peak anthesis, which was at 10:40 am. Blooming duration in all the TGMS lines varied from two to four days. DRR 1S took two to three days for opening of all the spikelets in a panicle followed by UPRI 95-167 S took three days. DRR 5S, IR 73834-21S, IR 73827-23S and UPRI 95-140S took four days for blooming. Angle of glume opening ranged from 25° to 36°. DRR 5S showed minimum 25° of glume opening followed by UPRI 95-167S (26°). In DRR 1S and IR 73827-23S high glume angle (36°) was observed.

DRR 5S showed less exerted stigma (25%) followed by IR 73827-23S (30%). High stigma exertion was observed in UPRI 95-167S (70%). Out-crossing ranged from 20 to 41%. Maximum out crossing rate was recorded in DRR 1S (41%) followed by IR 73827-23S (40%) and minimum out crossing rate was observed in DRR 5S (20%).

Floral biology in fertile phase: During fertile phase, time of anthesis in DRR 1S started at 9:35 am followed by IR 73834-21S (9:37 am). Maximum time taken for opening first glume was seen in UPRI 95-167S at 10: 30 am. For duration of anthesis in a panicle DRR 5S took minimum time (140 min) from opening to closing in a spikelet and maximum time taken by UPRI 95-167S (215 min). Peak time of anthesis in DRR 1S and UPRI 95-140S were at 10:45 am and 10:50 am respectively. IR 73834-21S took maximum time for peak anthesis, which was at 11: 10 am. Blooming duration in all the TGMS lines varied from 3-7 days. DRR 1S and UPRI 95-140S took 3-4 days for opening all the spikelets in a panicle followed by DRR 5S (4 days). UPRI 95-167S (3-5 days), IR 73827-23S (5 days) and IR 73834-21S took 4-7 days for blooming. Angle of glume opening ranged from 15° to 25°. DRR 5S showed minimum 15° of glume opening followed by DRR 1S and UPRI 95-167 S (20°), respectively. In IR 73834-21S and IR 73827-23S high glume angle (25°) was observed. High stigma exertion was seen in UPRI 95-167S (62.4%) followed by UPRI 95-140S (58%), whereas DRR 5S (20%) showed less exerted stigma.

DISCUSSION

Unitary source of cytoplasmic sterility, narrow genetic base of the maintainer and restorer lines causes the genetic vulnerability in the hybrids. One of the ways to widen the horizon of rice hybrids is to develop inter-sub specific hybrids of indica/japonica cross combinations. Nevertheless, low frequency of restorers in japonica rice's and difficulty in transfer of restorer genes from indica to japonica rice lines limit the approach. When hybrid breeders in China and elsewhere were looking for alternate means of exploiting hybrid vigor. Shi (1981 and 1985) discovered a novel genetic male sterility as a spontaneous mutation in rice. In which fertility and sterility were determined by length of the photoperiod (PGMS). Later, Yang and Wang (1989) induced a mutant (5460 S) in the indica variety IR 54, that was either pollen sterile or pollen fertile depending on the change of temperature. This was called temperature sensitive genetic

male sterility (TGMS). Subsequently many more EGMS lines were developed and they were of similar nature in fertility alterations with changing temperature or day length. (Ali *et al*., 1995; Lu and Wang, 1986; Maruyama *et al.,* 1990; Zhang *et al*., 1993; Mou *et al*., 1996). The TGMS lines remain selectively male sterile at a specific range of temperature or day length conditions and turn self-fertile at certain other temperature or day length. Chinese scientists have already developed commercially viable two-line hybrid technology using both PGMS and TGMS system.

Field screening of six TGMS lines during post rainy 2002 and pre rainy 2002-03 indicated that, Out of the six TGMS lines, three were found completely sterile (DRR 1S, UPRI 95-140S and UPRI 95-167S) during September-02. While three lines DRR 5S, IR 73827-23S and IR 73834-21S exhibited low levels of pollen and spikelet fertility. In the month of October-02, there was a transition between sterility and fertility and all the lines exhibited low levels of pollen and spikelet fertility. All the TGMS lines were transformed to fertile phase in 3rd week of November-02, when the temperatures were relatively low. In March-April-03, all the lines turned to sterile phase and during this period the mean maximum temperature was found to be 37.9°C and a minimum of 23.1°C. It is clear from the mean temperature data and pollen studies that all the TGMS lines responded to high temperature as well as low temperatures in expressing sterility and fertility, which is in agreement with the earlier findings (Zhang *et al*., 1993; Wan and Deng, 1990; Wu *et al*., 1991; Yang *et al*., 1990; Tan *et al*., 1990; Lohithaswaa, 2001; Soundarrajan *et al*., 2002). Success of two-line breeding depends on characterization of TGMS lines for stability in sterility-fertility alteration, stage of panicle development which is sensitive to temperature and identification of suitable lines for hybrid seed production and seed multiplication of TGMS lines, before using them in commercial breeding programs.

The sensitive stage (stamen-pistil primordia) in TGMS lines, which is influenced by the temperature, was found to be 15 and 19 days prior to heading in DRR 5S and IR 73827-23S, respectively. In IR 73834-21S and UPRI 95-167S sensitive stage was 21 days. The onset of sensitive stage was 24 days prior to heading in DRR 1S and UPRI 95-140S. Hence, from the above results it is clear that sensitive stage differs from line to line and has to be precisely determined. The present findings are in unison with earlier studies (Ali *et al*., 1995; Lohithaswa *et al*., 2001; Reddy *et al*., 1997).

Two-line hybrid technology depends on the stability of expression of sterility and fertility of TGMS lines respectively at their CSP and CFP. CSP and CFP vary from source to source. Zhang *et al*. (1993) studied the critical temperature and photoperiod requirements and intensity of their interaction for TGMS sources to become Completely Pollen Sterile (CSP) and to Express Maximum Fertility (CFP), such basic information being important to determine the relative utility of TGMS lines in commercial hybrid seed production. It is the CSP that causes fluctuation in the level of sterility, which in turn determines the level of genetic purity of hybrid seed. Equally important in CFP, fluctuation of which affects the multiplication of TGMS line. If CSP is not high enough, for instance, sudden drop in temperature would seriously affect hybrid seed production. Similarly, if the CFP is not low enough TGMS multiplication would be hampered.

As for the physiological features, all the test sources differed in their CSP and CFP on the expected lines. The TGMS lines IR 73834-21S, IR 73827-23S and UPRI 95-167S required a maximum temperature of 35°C for complete sterility induction and for the remaining three lines it ranged from 34.3°C (DRRIS and UPRI 95-140S) to 34.4°C (DRR 5S). Critical temperature for fertility induction was determined on the basis of proportion of fertile and partially fertile pollen grains. All the TGMS lines above 24 to 28°C remained fertile. Based on classification suggested by Ali *et al*. (1995), the test sources were grouped into High CSP-High CFP category.

The fertility-sterility transformation behavior of six TGMS lines under growth chambers at three temperature regimes viz., 32/26°C (10 h), 28/21°C (12 h) and 24/21°C (14 h); showed a tendency of high temperature sterile and low temperature fertile. Four TGMS lines DRR 1S, IR 73834-21S, IR 73827-23S and UPRI95-140S were completely sterile at high temperature of 32/26°C and 10 h of day length. But in DRR 5S and UPRI 95-167S there was some degree of pollen fertility that may be due to short day length (Zhang *et al*., 1994). When the TGMS lines were put under 28/21°C (12 h) and 24/21°C (14 h) all the lines fertility. The present findings are in confirmation with that of earlier reports. (Ali *et al*., 1995; Maruyama *et al*., 1990; Soundarrajan *et al*., 2002).

Characterization of morphological traits is essential for distinguishing cultivars; grow out test, seed certification and gene bank deposition. Therefore, characterization based on phenology for six TGMS lines was carried out. A thorough understanding of the floral biology is essential in TGMS system as it switches over to fertility/sterility conditions based on temperature influence. Saran *et al*. (1971) reported that duration of floret opening was positively correlated with the percentage of sterility. High frequency of exerted stigma facilitates stigma reception of naturally out crossed pollen grains and higher seed set. Oka (1998) stated that out

crossing in rice depended on the capacity of stigma to receive alien pollen and the capacity of anthers to emit pollen to pollinate other plants in the proximity

In all the TGMS lines, variation was observed for all characters like leaf-sheath color, panicle type, panicle exertion, auricle color, ligule color, apiculus pigmentation and stigma color. No variation was observed for ligule shape. TGMS lines DRR 1S, UPRI 95-140S and UPRI 95-167S can be easily be identified based on leaf sheath color, auricle color, ligule color, apiculus pigmentation and stigma color as all the traits have purple pigmentation. Whereas DRR 5S, IR 73834-21S and IR 73827-23S have light green to green leaf sheath color, light green auricle color, white ligule color, white stigma color and absence of apiculus pigmentation. As for panicle type, all the lines are of intermediate panicle type except for UPRI 95-140S which has an open type panicle. All the TGMS lines showed varying levels of panicle exertion ranging from 68.5 to 76%. Maximum panicle exertion was observed in IR 73834-21S (76%) followed by UPRI 95-167S (75.8%) and minimum in UPRI 95-140S (68.5%). This trait is useful in hybrid seed production and self-seed multiplication of TGMS lines.

The observations of the present study could be used in broad classification of TGMS lines on the basis of qualitative characters like pigmentation of auricle, leaf sheath color, ligule color, stigma color and apiculus pigmentation. Similar type of work was carried out earlier also. Singh *et al.* (1998) characterized rice germplasm lines according to standard evaluation system for rice and observed varietal differences for most of the characters studied. Subba Rao *et al.* (2001) also characterized 123 native rice germplasm lines based on various qualitative characters.

For a successful hybrid seed production it is essential to ensure perfect synchronization with respect of flowering and time and duration of anthesis between parental lines. Results from the present study on flowering behavior of TGMS lines in sterile and fertile phase indicate that the environmental conditions particularly temperature and humidity plays major role in governing the flowering process. For instance, the time of anthesis in sterile phase (post rainy 2003) ranged from 9:05 (DRR 1S) to 9:40 am (UPRI 95-167S), duration of anthesis in a panicle ranged from 165 min in DRR 5S to 270 min in UPRI 95-140S, peak time of anthesis was from 10:15 am (DRR 1S) to 10:40 am (UPRI 95-140S and UPRI 95-167S), duration of blooming ranged from 2-3 days in DRR 1S to 4 days in UPRI 95-140S and glume angle varied from 25° (DRR 5S) to 36° (DRR 1S and IR 73827-23S). In fertile phase, time of anthesis ranged from 9:35 am in DRR 1S to 10:30 am in UPRI 95-167S, duration of anthesis in a panicle ranged from 120 min (DRR 5S) to 215 min (UPRI 95-167S), peak time of anthesis was from 10:45 am (DRR 1S) to 11:15 am (DRR 5S), duration of blooming was from 3-4 days (DRR 1S) to 4-7 days (IR 73834-21S) and glume opening ranged from 15° (DRR 1S) to 25° (IR 73834-21S and IR 73827-23S). The results suggest that when temperature is high (sterile phase) flowering process is early, duration of anthesis takes more time, blooming completes fast and glume opening is wider. In fertile phase, however the condition is exactly reverse of sterile phase. The present findings are in confirmation with earlier reports (Virmani and Athwal, 1973; Parmar *et al.*, 1979; Wang and Gao, 1989; Chen *et al.*, 1996; Xu *et al.*, 1998). However, there was no much variation was observed in stigma exertion in both the phases. Out crossing rate in TGMS lines is largely influenced by glume angle, panicle exertion and stigma exertion. For example, in DRR 1S (41%) maximum out-crossing rate was observed, which can be ascribed to wider glume angle (36°), panicle exertion (71.5%) and stigma exertion (45%). Similar was the observation in IR 73834-21S and IR 73827-23S. The observations are in agreement with earlier reports (Chen *et al.*, 1996; Ganeshan, 2000; Banumathy *et al.*, 2002). However, DRR 5S, UPRI 95-140S and UPRI 95-167S though showed good stigma and panicle exertion, poor glume angle was perhaps the cause for low out-crossing. Therefore characterization of TGMS lines with respect to their sterility and fertility offers better understanding of TGMS lines for their efficient utilization in the development of two line hybrids.

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