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Identification of critical sterility temperature (CST) and thermo sensitive stage in selected TGMS lines in rice (*Oryza sativa* L.)

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Abstract : Thermosensitive genic male sterility (TGMS) is a type of genetic male sterility expression regulated by certain temperature conditions. The knowledge of the critical sterility temperature and the critical thermosensitive stage for fertility alteration of TGMS lines is useful to determine most suitable time of their planting for multiplication or hybrid seed production. The experiment was conducted in mini phytotron. The critical sterility temperature of three TGMS lines *viz.* TGMS 6, TGMS 16 and TGMS 29 was assessed by subjecting them to different temperatures during reproductive stage. The pollen sterility percentage increased as the daily mean temperature increased and the genotypes behaved differently before they attain complete pollen sterility. The results that the CST of TGMS 6 was 28.0°C, TGMS 16 was 27.0°C and TGMS 29 was 27.5°C with 34.0, 33.0°C and 34.0°C the maximum temperatures respectively. Three TGMS lines were exposed to CST for different durations coinciding the various stages of panicle development to understand the critical stage of thermosensitivity for fertility alteration. In this study, 10-25 DBH was identified as critical thermosensitive stage for fertility alteration, which corresponds to differentiation of secondary branch primordiam, differentiation of pistil and stamen, formation of PMC and early meiosis stages (Stage 111 to IV).

Key words: Rice, TGMS lines, CST, Critical stage of Thermosensitivity.

Introduction

The hybrid technology in a strictly self-pollinated crop like rice with a yield advantage of 2-3 t/ha over the best conventional varieties is a major landmark in the history of rice breeding. The technology now being adopted in over 58 per cent of its rice area has helped China during the last 10 years to advance its rice production by 40 per cent. Using the improved parental -lines especially the male sterile lines better adapted to tropical environment, India has planted commercial hybrids in 1994 and brought over 2 million hectares under hybrid rice by the turn of 20th century. Inspite of their potential and promise, the conventional three-line hybrids are not without limitations. The limitations have promoted to look for alternative approaches to exploit hybrid vigour.

One of the possible alternatives is the two line breeding system, which comprises of Environmental Sensitive Genic Male Sterility (EGMS) and chemical induction of male sterility (Ali, 1995). EGMS comprises of Photosensitive Genic Male Sterility (PGMS), which is based on the variation in day length, and Thermosensitive Genic Male Sterility (TGMS), which is caused by high temperature.

In tropics, TGMS system is more useful where day length differences are marginal and high and low temperature prevails on plains and high altitudes respectively. India, being a tropical country with significant temperature variation at different altitudes and different seasons, where in the genotypes become male sterile at particular temperature and become fertile at some other temperature (Borkakati and Virmani, 1996). Successful use of TGMS lines in hybrid rice breeding depends on their stability and adaptability across environments, since the nuclear sterile gene reacts differently to temperature under different genetic backgrounds. With this background, the study was formulated

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Table 1. Thermo period conditions in phytotron

Time (hrs)	Treatment temperature (°C)								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
06-10	34	33	32	32	32	31	30	30	28
10-16	36	35	34	34	34	33	32	32	30
16-20	32	31	30	30	30	29	28	28	26
20-06	24	23	23	22	21	21	21	20	20
Max (°C)	36	35	34	34	34	33	32	32	30
Min (°C)	24	23	23	22	21	21	21	20	20
Daily mean (weighted average)	30.0	29.0	28.5	28.0	27.5	27.0	26.5	26.0	25.0

Day length : 10.00 h day^{-1} (08:00-18:00 h)
RH : 60-80 per cent
Light : 400-800 µmoles m^{-2} s^{-1}

Table 2. Standardization of CFT and CST of TGMS lines

Temperature Max/Min (°C)	Mean temperature (°C)	Pollen sterility (%)			Mean
		TGMS 6	TGMS 16	TGMS 29	
35 / 24	30.0	100	100	100	100
34 / 23	29.0	100	100	100	100
33 / 23	28.5	100	100	100	100
32 / 22	28.0	100	100	100	100
31 / 21	27.5	97.3	100	100	99.1
30 / 21	27.0	92.6	100	96.3	96.3
29 / 21	26.5	87.0	90.3	92.0	89.7
29 / 20	26.0	67.3	58.3	63.3	62.9
28 / 20	25.5	28.6	34.3	37.0	33.3
27 / 20	25.0	25.3	24.3	30.6	26.7
26 / 20	24.5	21.0	24.3	26.3	23.8
25 / 20	24.0	15.3	13.0	14.3	14.2

Table 3. Pollen sterility (%) under different thermo sensitive stage during pollen development

Treatment (DBH)	TGMS 16	TGMS 16	TGMS 29	Mean
25-30	100.00	100.00	100.00	100.00
20-25	100.00	100.00	100.00	100.00
15-20	90.73	90.16	92.20	91.01
10-15	25.46	27.0	31.93	28.13
15-20	78.70	83.93	84.46	82.36
20-25	100.00	100.00	100.00	100.00
25-30	25.30	32.16	36.20	31.22
Mean	71.61	74.47	76.61	

DBH : Days before heading
CD (P=0.05)

Treatment	1.58
Lines	1.03
LxT	2.73

to identify Critical Sterility Temperature (CST) and critical stage of thermo- sensitivity for selected TGMS lines in rice.

Materials and Methods

Critical Sterility Temperature (CST) and Critical stage of thermo sensitivity

Three TGMS lines *viz.* TGMS 6, TGMS 16 and TGMS 29 possessing stable sterility were supplied by the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore formed the basic material for the study. Experiments were conducted in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 1999-2001. The TGMS lines were sown sequentially once in 20 days in plastic pots of 20 cm diameter, keeping two plants per pot in the glass house and four replications were maintained for each line in each set. After studying the various developmental stages and growth duration of the TGMS lines, sowing date was adjusted so that the lines had similar reproductive stage.

The sensitive phase was the differentiation of secondary rachis branches to the formation of pollen mother cells (Yuan, 1990) and Yao *et al.* (1995) found it as 5 to 20 days before flowering. With this information, the TGMS lines were exposed to sterility inducing temperature in the present investigation with different duration during reproductive stage. The pollen sterility at flowering was noted for identifying the critical stages of thermo sensitivity. The TGMS lines grown in plastic pots were maintained in glass house during winter season 1999 and transferred to the Phytotron, when they were in stage III of panicle development (25 days before heading). The plants were kept inside the phytotron upto stage VII of panicle development. The mini-Phytotron (E15-Conviron Products Company, Manitoba, Canada) available in the Department of Crop Physiology was used in this experiment. The temperature regimes at which the growth chambers were maintained to get the diurnal variation of daily mean temperature for the induction of male sterility are given in the Table 1. Four replications were maintained in each set for each variety. Package of practices as recommended in Crop Production Guide (1999) of Tamil Nadu Agricultural University for rice crop were followed for maintenance of the crop.

The male sterility was assessed by collecting the anthers from fully developed spikelets before opening of the spikelets and observed visually for fertility/sterility. Fertile anthers were yellow and plumpy while the sterile ones looked white, shrivelled, irregular and kidney shaped. The anthers were crushed and pollen grains were collected in a clean glass slide. It was stained with iodine-potassium iodide (HU) (Nelson, 1968) and observed under microscope. The pollen grains that were stained fully were considered as fertile while unstained shrivelled and empty pollen grains were considered as sterile.

Treatments :

T_1 - 0-30 DBH
T_2 - 20-30 DBH
T_3 - 5-25 DBH
T_4 - 5-20 DBH
T_5 - 5-15 DBH
T_6 - 10-25 DBH
T_7 - 10-20 DBH

(DBH - Days Before Heading)

The Phytotron was set to an average temperature of 29°C. Mean of the pollen sterility data was used to identify the critical stage of thermosensitivity.

Results and Discussion

Critical sterility temperature (CST) of TGMS lines

Thermosensitive genic male sterility (TGMS) is a type of genetic male sterility expression regulated by certain temperature conditions. Generally TGMS lines have 100 per cent pollen sterility at 30-34°C / 22-23°C and partial to normal pollen sterility at 24-27°C / 18-19°C (Virmani and Voc, 1991). In the present experiment, the pollen sterility percentage of TGMS 6, TGMS 16 and TGMS 29 at different daily mean temperatures is given in Table 2. The pollen sterility percentage increased as the daily mean temperature increased and

genotypes behaved differently before they ain complete pollen sterility. TGMS 6 attained per cent sterility at 28.0°C (34/22°C), TGMS at 27.0°C (33/21°C) and TGMS 29 at 27.5-34/21°C). The anthers were pollen free above aily mean temperature of 29°C. Thus there a significant varietal difference in the critical ility temperature (CST) and was 28.0°C for MS, 27.0°C for TGMS 16, and 27.5°C for MS 29 with maximum temperatures of 34.0, 0 and 34.0°C, respectively.

Yuan (1992) suggested that the CST- the imum temperature for complete pollen sterility aried from 23°C to 29°C depending on the M.S lines. In the present study, the maximum imum temperature for the complete pollen ility for TGMS 6 was 34/22°C, 33/21°C TGMS 16 and 34/21°C for TGMS 29 firming the findings of Borkakati and Virmani 96). The first TGMS line, Annong S had CST of 27.0°C (Lu *et al.* 1994). Virmani 92) have reported the CST of Norin PL - which is the source of male sterility TGMS 16 and TGMS 18, as a mean daily perature of 27.5°C (31°/24°C). Similar findings re reported by Gong *et al.* (2000) and ystephen and Thangaraj (2000) under phytotron dition.

Critical stage of thermosenstivity

All the lines were 100 per cent sterile en exposed from 0 to 30 days before heading BH), 5-25 DBH and 10-25 DBH (Table 3). posing for 5-20 DBH and 10-25 DBH recorded per cent sterility whereas 10 days exposure the later (20-30 DBH) or early stage (5-DBH) of panicle development did not increase pollen sterility percentage of the TGMS s. Thus, the critical stage of thermosensitivity fertility alteration in TGMS lines was 10 25 days before heading, as it was the minimum iod of exposure to CST to get complete len sterility.

Conditions inducing fertility alteration in MS lines vary among different lines due different source of mate sterile genes and the effect of genetic backgrounds. (Zhang *et al.* 1992). Yuan *et al.* (1988) identified the stage from secondary rachis branching and spikelet primordia differentiation to pollen mother cell formation in the process of panicle development as the sensitive stage for fertility alteration in Nongken 58S. Zhang and Lu (1992) reported that pollen sterility of some TGMS lines was positively correlated with the daily air temperatures at around 10 to 16 days before heading.

Zhang *et al.* (1993) also reported the stages between the formation of secondary rachis branch and the formation of PMC as the critical stage of thermosensitivity in TGMS lines. Maruyama *et al.* (1990) found that the critical stage for Norin PL 12, the source of male sterility for TGMS lines 16 and 18, as 22 to 26 DBH and that even high temperature for an hour would adversely affect the seed setting. Zeng (1991) reported that the daily mean temperature of >28°C during 13 to 25 DBH and during 10 to 18 DBH were crucial for complete pollen sterility.

In this study, 10-25 DBH was identified as critical thermosensitive stage for fertility alteration, which corresponds to differentiation of secondary branch primordium, differentiation of pistil and stamen, formation of PMC and early meiosis stages (Stage 111 to IV). This corroborates the findings of Laskhmi Praba and Thangaraj (2000) and Gong et al. (2000). Yao *et al.* (1995) reported that 5-20 DBH was critical stage in TGMS lines. But in the present study, the sterility was only 90 percent when exposed to 5-20 DBH. This might be due to the variation in thermosensitivity between TGMS lines as reported by Ali *et al.* (1995) that sensitive stage varied according to the genotypes.

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