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Changes in the Activity of Enzymes Associated with Fertility Alteration in Thermosensitive Genic Male Sterile (TGMS) Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT. *Thermo sensitive genic male sterility (TGMS) in rice is found to be efficient in the two-line method of hybrid rice production especially in a tropical country like India. Thermo sensitive genic male sterility in rice is controlled by recessive nuclear genes expressed at specific temperature and reverts back to fertility at another temperature. An investigation was carried out in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2001-2002 using three stable Thermo sensitive genic male sterile lines in mini-Phytotran. The variation in physiological and biochemical parameters was analyzed in fertile and sterile TGMS plants. Activities of free radical scavenging enzymes were reduced significantly in sterile plants compared to the fertile plants and a reduction in the activities of IAA oxidase, nitrate reductase, malate dehydrogenase, glutamate dehydrogenase and acid phosphatase under sterility inducing high temperature treatment was also observed. The major cause of male fertility alteration into male sterility during high temperature condition might be due to the accumulation of activated oxygen species in TGMS lines. The results revealed that some physiologically active compounds were produced as signals or messengers in response to temperature affecting pollen abortion. Key words: Rice, Thermo sensitive genic male sterility (TGMS), Enzymes activity.*

INTRODUCTION

One of the most important achievements in modern agricultural science is the development of male sterile lines for commercial exploitation of heterosis especially in self- pollinated crop like rice. The relative complexity of the existing three-line system using cytoplasmic male sterile (CMS) lines in rice hybrid seed production and seed multiplication made it inefficient, cumbersome and less popular (Ali, 1995). The discovery of thermosensitive genic male sterility (TGMS), where plants become male sterile at one temperature and fertile at another temperature is an important step favored in the two-line system of hybrid rice production. India being a tropical country with significant variation in temperature between seasons and between altitudes, TGMS system will be highly suitable (Ilyas Ahmed, 1996). Another advantage of this system is the wide restoring spectrum with broad choice of parents.

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For the efficient and stable performance of the TGMS system, the fertility transformation should be quite clear and distinct, which necessitates the understanding of the physiological and biochemical mechanisms of male sterility in TGMS rice. Knowledge of the changes in the activity of enzymes during fertile and sterile stages of TGMS lines will help in exploring the possibility of altering the fertility to sterility during the hybridization programme and also designing the systematic application of growth regulators for chemical manipulation of male sterility in TGMS rice. Where further success and sustenance of hybrid rice in India depends primarily on the TGMS system, an investigation was carried out to analyze the association of changes in the activity of various enzymes in deciding the fertility / sterility alteration during thermosensitive stage in TGMS rice.

MATERIALS AND METHODS

Three stable TGMS lines viz., TS 6, TS 16, and TS 29 supplied from the Centre for Plant Breeding and Genetics, TNAU, Coimbatore formed the basic material for the study. The experiments were conducted in the glass house of the Department of Crop Physiology, TNAU, Coimbatore during 2001-2002. Two hills per pot of 20 cm diameter were maintained with six replication for each variety in each set. Two mini-Phytotrans (Conviron E15-Conviron Products Company, Manitoba, Canada) available in the Department were utilized simultaneously. One was programmed in sterility inducing condition (average day/night temperatures 35/23°C) and the other in fertility inducing condition (average day/night temperatures 28/20 °C) to have 100% fertile and sterile plants separately. The plants were transferred to Phytotran 25 days before flowering and exposed to respective treatments for 20 days. The high temperature treatment for the induction of male sterility was fixed based on the critical sterility temperature (CST) of TGMS lines (Table1). The critical sterility temperature (CST) is decided by imposing different day and night temperatures at different stages of growth. The leaf samples were collected at three stages of panicle development i.e. Stage III (i.e. 22-26 days before heading -DBH), Stage V (16-19 DBH) and Stage VIII (2 DBH) and analysed various biochemical parameters. Data were given only for the Stage V, because this is the critical stage of thermosensitivity when pollen mother cells are formed. The data were statistically analyzed with five replications using the factorial completely randomized block design (FCRD) proposed by Panse and Sukhatme (1969).

The enzyme nitrate reductase activity was determined as per the method of Nicholas *et al.*,(1976) and IAA oxidase activity was estimated calorimetrically by adopting the method of Parthasarathy *et al.*,(1970). The enzyme ascorbic acid oxidase activity was determined by following the method of Oberbacher *et al*., (1963).

Activities of the free radical scavenging enzymes *viz.,* peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) were measured as per the procedure of Luck (1974), Malik and Singh (1980) and Beau Champ and Fridovich (1971) respectively. The activity of enzymes Malate dehydrogenase (MDH), Glutamate dehydrogenase (GDH) and Acid phosphatase (AP) were analysed by using the method proposed by Sadasivam and Manickam (1996).

Table 1. Thermo period conditions in Phytotran.

Time (hrs)	Treatment temperatures (°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 – 800µmoles m^{-2} s^{-1}

RESULTS AND DISCUSSION

Changes in the activity of Enzymes

IAA oxidase (IAAO)

During the reproductive phase of any crop plants, the activity of IAAO generally declines, so that the available auxin content can be utilized for the flowering process (Paulas and Shanmugavelu, 1988). Hamdi *et al.* (1987) proved that the genes controlling maleness was correlated with auxin content and the sterility determinants modified the IAA content in male sterile lines. In the present study, the IAAO activity increased upto 30.0 per cent in leaves of sterile plants compared to fertile plants (Table 2). Similar results were also reported by Roystephen and Thangaraj (2000) that the higher activity of IAAO depleted the auxin upto 33 per cent in sterile TGMS lines. Shen and Gao (1992) suggested that deletion of IAA might be one of the reasons for male sterility in photo sensitive genic male sterile (PGMS) lines. Increase in IAAO activity under sterile condition was also reported by the He and Xiao (1993) in TGMS lines. This increased enzyme activity causes deletion of IAA, which led to pollen abortion. In fertile TGMS lines, reduction in the activity of enzymes made available more auxin in the reproductive stage. The increase in activity was from primary and secondary rachis branch primordia differentiation of panicle development. A difference between IAAO in fertile and sterile conditions during the differentiation of stamen and pistil primordia observed by He and Xiao (1993), which corroborated the findings of the present study. Increase in IAAO activity of TGMS lines under sterile conditions found in the present experiment led to the deletion of IAA content which was associated with male sterility in TGMS lines by causing pollen abortion under high temperature (Lakshmi Praba and Thangaraj, 2000).

Nitrate reductase (NRase)

NRase is highly sensitive to environmental fluctuations and it is an important enzyme of nitrogen metabolism and is involved in the conversion nitrate to nitrite using assimilatory powers from chloroplasts. Jzochanjong and Chenchuang Jann (1982) related the higher NRase activity with flowering in higher plants. In the present investigation, NRase activity reduced upto 42.3 per cent in leaves under sterile condition in TGMS lines (Table 2). This was in accordance with the results of Roystephen and Thangaraj (2000). They reported that the reduction in NRase activity reduced the nitrogen assimilation rate and synthesis of amino acids, thus acting as a cause for reduced free amino acid content in cytosol during sterility in TGMS lines, as observed in the present experiment.

Ascorbic acid oxidase (AAO)

The activity of AAO also varies in response to environment. The regulation of levels of oxidised and reduced glutathione, NADPH and ascorbate content is the major role of AAO in plants. AAO activity increased upto 2 fold in leaves of sterile TGMS lines compared to fertile lines (Table 2). Lakshmi Praba and Thangaraj (2000) found that the activity of AAO increased due to the induction of critical temperature during the thermosensitive stage in sterile TGMS lines. The present study also revealed the constant reduction of AAO activity in fertile lines, which was in accordance with the results of Liang *et al.* (1995) who reported an increase in ascorbic acid and glutathione content with reduced AAO activity in anthers under fertile condition. Higher AAO activity under sterility might be the cause of reduced ascorbate content under sterile conditions and thus be associated directly with pollen sterility (Roystephen, 1998).

Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) occurs in almost all living organisms. In higher plants it exists in chloroplast and mitochondria as two different forms. In this experiment, the GDH activity reduced under sterile lines during panicle development stages, but it gradually increased in fertile TGMS lines (Table 3). Tang *et al.* (1994) opined that the reduction in GDH activity was due to a marked decline of the photochemical activity of chloroplasts (reduction in PSII activity) leading to the impaired NADP reduction specifically required for the activity of GDH present in the chloroplast. Zhu and Yang (1992) found that the reduction in activities of dehydrogenases (ADH and GDH) affected the fertility percentage under long day with high temperature condition. This result agreed with the results of the present investigation.

Table 2. Changes in certain enzyme activities in thermosensitive genic male sterile rice genotypes during fertility alteration under critical temperature conditions (Stage V).

Parameters	TGMS Lines	Fertile plants	Sterile plants	% Change
IAA oxidase activity *(µ moles of unoxidised auxin h^{-1} g^{-1})*	TGMS6	16.07	11.57	29
	TGMS16	16.92	11.77	31
	TGMS29	15.72	11.15	30
	Mean	16.23	11.49	30.0
	CD (P=0.05)	L	T	L x T
		0.115	0.094	0.168
NRase activity *(µ moles of NO_2 produced g^{-1} h^{-1})*	TGMS6	0.548	0.312	44
	TGMS16	0.587	0.346	42
	TGMS29	0.542	0.322	41
	Mean	0.559	0.326	42.3
	CD (P=0.05)	L	T	L x T
		0.043	0.035	0.061
Ascorbic acid oxidase (AAO) activity *(Enzyme units min^{-1} g^{-1})*	TGMS6	4.05	6.36	37
	TGMS16	3.19	6.40	51
	TGMS29	3.18	6.12	47
	Mean	3.47	6.29	45.0
	CD (P=0.05)	L	T	L x T
		0.128	0.164	0.181

L: TGMS lines; **T**: Treatments (Fertile/Sterile); **LxT**: Interaction effect

Malate dehydrogenase (MDH)

Malate dehydrogenase (MDH) is one of the enzymes involved in the TCA cycle. MDH activity declined to 26.0 per cent in leaves of sterile TGMS lines but the activity increased gradually in fertile lines (Table 3). Zhang *et al.* (1994) reported the same results in the male sterile lines of photo and thermosensitive genic male sterile (PTGMS) rice. LiPeng *et al.* (1997) found a change in MDH activity in TGMS lines during PMC formation, reduction division and pollen ripening stages and suggested that the abortion of pollen grains might be related to the decrease in MDH activity in spikelets.

Table 3. Changes in certain enzyme activities in thermosensitive genic male sterile rice genotypes during fertility alteration under critical temperature conditions (Stage V).

Parameters	TGMS Lines	Fertile plants	Sterile plants	% Change
Glutamate dehydrogenase activity (GDH) *(nano moles of NADH oxidised min^{-1} mg^{-1} protein)*	TGMS6	1.982	1.012	49
	TGMS16	2.086	1.046	50
	TGMS29	2.003	1.038	48
	Mean	2.061	1.032	49.0
	CD (P=0.05)	L	T	L x T
		0.026	0.022	0.038
Malate dehydrogenase activity (MDH) *(µ moles of NADH oxidised min^{-1} ml^{-1} of extract)*	TGMS6	0.121	0.092	26
	TGMS16	0.128	0.096	25
	TGMS29	0.130	0.096	27
	Mean	0.129	0.094	26.0
	CD (P=0.05)	L	T	L x T
		0.014	0.011	0.019
Acid phosphatase activity *(µ moles p-nitrophenol released min^{-1} mg^{-1} protein)*	TGMS6	627.8	482.9	24
	TGMS16	662.6	547.1	18
	TGMS29	688.7	523.8	24
	Mean	654.5	517.3	22
	CD (P=0.05)	L	T	L x T
		0.239	0.195	0.339

L: TGMS lines; T: Treatments (Fertile/Sterile); LxT: Interaction effect

Acid phosphatase (AP)

Acid phosphatase is involved in the liberation of inorganic phosphate from organic phosphate esters in a plant system. In TGMS lines the activity of acid phosphatase significantly under high temperature induced sterile condition. But in fertile lines there was no such reduction in acid phosphatase activity (Table 3). This was in accordance with the results reported by Wang and Weicheng (1996): that the acid phosphatase activity decreases during sterile conditions compared to fertile conditions in TGMS lines. Chen and Zhou (1997) also reported similar results in photo and thermosensitive genic male sterile (PTGMS) lines during high temperature long days. LiPeng *et al*. (1997) found the change in activity of acid phosphatase in TGMS lines during pollen mother cell (PMC) formation, reduction division and pollen ripening stages and opined that the abortion of pollen grains was closely related to the decrease in acid phosphatase activity in spikelets.

Activity of free radical scavenging enzymes

Catalase (CAT)

Catalase (CAT) is mainly involved in the destruction of hydrogen peroxide and oxidation of hydrogen donors. The enhanced production of most reactive hydroxyl radicals, which cause peroxidation of unsaturated lipids of cell membrane and damaging the structural integrity of the membranes, change the structure and functions of proteins including enzymes and nucleic acids, is due to the impaired activity of catalase. Catalase activity was reduced in sterile TGMS lines under high temperature. The reduction in the activity was upto 65 per cent in leaves compared to fertile plants during different stages of panicle development (Table 4). Zhu and Yang (1992) reported that the reduction in CAT activity was one of the causes for reduction in the fertility percentage. Raj and Siddiq (1986) and Liang and Chen (1993) opined that the weaker oxygen scavenger system coupled with a high level of lipid peroxidation in anthers was responsible for male sterility in CMS and TGMS lines. Shen and Cao (1992), Zou *et al.* (1993) and Zhang *et al.* (1994) also reported a reduction of CAT activity in sterile PTGMS lines. The findings of the present experiment confirmed the results of Roystephen and Thangaraj (2000) who reported the role of active oxygen species in causing pollen abortion in TGMS lines. They observed a reduction in photochemical efficiency and disturbance in protein and nucleic acids metabolism besides lower enzyme activities due to lower CAT activity under sterile condition. Similar reasons were also suggested by Zou *et al.* (1993) for pollen sterility in photo sensitive genic male sterile (PGMS) lines.

Peroxidase (POX)

Peroxidase is an important enzyme involved in morphogenesis and auxin oxidation. It is the enzyme, which is very sensitive to environmental fluctuations and is considered as the measure of a plant's resistance to stress. The major function of POX is the catalytic conversion of H_2O_2 produced by SOD to water. In sterile TGMS lines, there was upto 37 per cent reduction of POX activity in leaves compared to fertile TGMS lines (Table 4). The reduction in POX activity was marked even at the beginning of secondary branch primordia differentiation. He and Xiao (1993), Zhang *et al.* (1994) and Zhu and Cao (1997) also confirmed the increased POX activity under fertile conditions during sensitive stages. Chen and Zhou (1997) found decreased activity of POX in PTGMS lines under long days with high temperature. Roystephen and Thangaraj (2000) reported similar results in TGMS lines under high temperature and opined that the abnormal activity of POX might be associated with male sterility through strengthening the role of active oxygen species, which causes pollen abortion in TGMS lines.

Table4. Changes in activities of free radical scavenging enzymes in thermosensitive genic male sterile rice genotypes during fertility alteration under critical temperature conditions.

Parameters	TGMS Lines	Fertile plants	Sterile plants	% Change
Catalase activity *(Enzyme units x 10^4 g^{-1} min^{-1})*	TGMS6	3.33	1.26	70
	TGMS16	3.31	1.32	61
	TGMS29	3.25	1.21	63
	Mean	3.29	1.26	64.6
	CD (P=0.05)	L	T	L x T
		0.079	0.064	0.112
Peroxidase activity *(Enzyme units l^{-1} hr^{-1})*	TGMS6	184.7	123.0	34
	TGMS16	185.5	116.0	38
	TGMS29	181.2	111.2	39
	Mean	183.8	116.3	37.0
	CD (P=0.05)	L	T	L x T
		0.079	0.064	0.112
Superoxide dismutase (SOD) activity *(Enzyme units mg^{-1} protein hr^{-1})*	TGMS6	7.99	5.08	37.0
	TGMS16	8.19	5.30	35.0
	TGMS29	8.35	5.33	37.0
	Mean	8.17	5.23	36.3
	CD (P=0.05)	L	T	L x T
		0.318	0.287	0.502

L: TGMS lines; T: Treatments (Fertile/Sterile); LxT: Interaction effect

Superoxide dismutase (SOD)

The biological role and significance of SOD as a protective enzyme against oxygen toxicity are reported in numerous higher plants (Bowler *et al*., 1992). The increase in SOD activity is induced as a defence to tolerate adverse environmental factors. In the present study, the SOD activity because reduced drastically during CST treatment at different stages of panicle development in TGMS lines (Table 4). Shen and Cao (1992) observed the same results in sterile PTGMS lines under high temperature with long day conditions. He and Xiao (1993) suggested that the change in SOD activity was closely related the transformation of fertility in TGMS lines.Zhang *et al.* (1994) also reported higher activity of SOD in fertile phase than in sterile phase of TGMS panicles. The results of the present experiment confirmed the findings of Roystephen (1998) who had suggested that higher temperature was an adversity for TGMS lines where it produced more superoxide radicals causing membrane damage and that this might be one of the most likely reasons for temperature induced pollen sterility in TGMS lines. Zhang *et al.* (1994) suggested that the low reduction potential of male sterile lines might have caused uncontrolled activated oxygen molecules and hence anther sterility.

CONCLUSION

The results of the present experiment revealed that the activities of free radical scavenging enzymes were reduced significantly in sterile plants compared to the fertile plants and a reduction in the activities of IAA oxidase, nitrate reductase, malate dehydrogenase, glutamate dehydrogenase and acid phosphatase under sterility inducing high temperature treatment was also observed. The better status of biochemical molecules, greater activity of enzymes and the resultant better assimilation potential and effective partitioning of assimilates to the reproductive part enhanced good seed setting in the TGMS lines grown under fertile conditions. In the sterile TGMS lines, reduced enzymes activity revealed that some physiologically active compounds were produced when plants sensed the higher temperatures and acted as signals or messenger molecules affecting other physiological and biochemical processes leading to pollen abortion and eventually, sterile seeds. The major cause of sterility was found to be the accumulation of active oxygen species due to an impaired scavenging system in the sterile plants of TGMS lines.

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