Tropical Agricultural Research Vol. 15: 68-76 (2003)

Physiological Responses Associated with Thermosensitive Genic Male Sterility (TGMS) in Rice (*Oryza sativa* L.)

A. Senthil, R. C. Babu[1] and M. Thangaraj[2]

Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore - 641 003. India

ABSTRACT. *An investigation was carried out with three Thermosensitive Genic Male Sterile (TGMS) rice lines viz., TGMS 6, TGMS 16 and TGMS 29 and a normal variety ADT 36 to study the physiological changes during fertility/sterility transformation and to understand the exact mechanism responsible for male sterility in TGMS lines under high temperature conditions. The physiological aspects such as, gas exchange parameters, hill reaction rate, ethylene release rate and nutrient status were determined during different stages of panicle development in fertile and sterile TGMS lines to identify their association, if any with male sterility. Photosynthetic and hill reaction rates of TGMS lines reduced under sterile condition. The status of calcium, copper and boron, which play major roles in reproductive growth of plants, was changed in TGMS lines during fertility alteration. The concentration of ethylene released from young panicles also showed a significant difference between fertile and sterile conditions. Increased ethylene release rate under sterile condition revealed its possible role in determining male sterility in TGMS lines.*

INTRODUCTION

Rice is the major food crop in the world and cultivated in about 148 million ha with a production of 560 million tons of grains annually. It is the staple food providing about two-thirds of the calories for more than two billion people in humid and sub humid Asia and one-third of the calorie intake of the nearly one billion people in Africa and Latin America. Achieving self-sufficiency in rice production and maintaining price stability are important in low-income countries because of the importance of this crop in providing food security and generating employment and income for low-income people (Hossain, 1995). Most Asian countries have done remarkably well in meeting the food needs of the growing population over the last quarter of the century. However, the future poses even more challenging and ambitious tasks. The yield ceilings of rice varieties of the green revolution era must be lifted yet again, because by 2020 the world must produce 350 million tons more of rice than it produced in 1992, to meet the demand created by increasing populations and rising incomes. This production increase must be achieved on less land, with less labour, less water, and less pesticide and it must be sustainable (Anonymous, 1993). Using hybrid rice is one of the strategies to meet this immense challenge (Virmani and Dedolph, 1993).

In hybrid rice development, two line breeding system is one of the possible alternatives, which comprises of environmental sensitive genic male sterility (EGMS)

1 Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

2 Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641003, India.

and chemical induction of male sterility (Ali, 1995). Hybrids evolved through two line system based on EGMS are capable of producing 9-10 t ha^{-1}, which have a yield advantage of 10-20% over the best existing hybrids (Yuan, 1996). EGMS comprises of photosensitive genic male sterility (PGMS) and thermosensitive genic male sterility (TGMS), which are based on the variation in day length and temperature, respectively. In tropics, TGMS system is more useful where day length differences are marginal and high and low temperatures prevail on plains and high altitudes, respectively.

Though considerable information is available on the heterosis and combining ability of two line hybrids using TGMS, the change of fertility to sterility and vice-versa in TGMS lines is not quite clear and distinct. This insists the need of understanding the physiological, biochemical and molecular basis of male sterility caused by high temperature. Studying the physiological, biochemical and molecular mechanisms of fertility/sterility transformations of TGMS lines would open new frontiers for possible external application of some chemicals or metabolites in manipulating deviation in fertility status under unexpected weather conditions. Therefore, the objective of this study was for identify the physiological changes associated with high temperature induced male sterility in TGMS lines in rice.

MATERIALS AND METHODS

This experiment was done in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 1998-2001. Three TGMS lines (TGMS 6, TGMS 16, and TGMS 29) along with a normal variety ADT 36 formed the basic material for this experiment. Sowing date of each TGMS line was adjusted so that the lines came to panicle initiation stage during 1st week of December. The mean maximum and minimum temperature for December and January were 29.4 and 17.9°C respectively. Five pots each with three plants were kept for each variety to have sufficient samples for analysis. One set of TGMS lines with ADT 36 which was used to compare with TGMS lines, were kept in the glass house for obtaining fertile plants and another set of plants which were grown in the glass house was transferred to the growth chamber, when the plants were in stage III of panicle development (25 days before heading). The temperature treatment given in the growth chamber (Phytotran-Conviron E15-Conviron Products Company, Manitoba, Canada) to imitate the diurnal variation of summer season for induction of male sterility is given below:

Time (h)	Temperature (°C)	Relative Humidity (%)	Light intensity ($\mu M\ m^{-2}\ s^{-1}$)
08.00-10.00	34	80	600
10.00-12.00	35	70	800
12.00-16.00	36	60	800
16.00-18.00	35	60	600

The plants were kept inside the growth chamber upto stage VII of panicle development. Leaf samples and young panicles were collected five days before heading for physiological and biochemical analysis and the data were statistically analyzed with five replications using factorial completely randomized block design (FCRD) proposed by Panse and Sukhatme (1969).

Gas exchange parameters *viz.*, photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$), transpiration rate (µg H_2O m^{-2} s^{-1}) and diffusive resistance (s cm^{-1}) were recorded using a portable CO_2 gas analyzer (model CL-CID Inc., USA). The readings were taken during 11:00 to 12:00 noon on a clear sunny day when the photosynthetically active radiation (PAR) was more than 1000 µ moles m^{-2} s^{-1}. Relative humidity (RH) was also maintained at a steady state level equal to the ambient RH to simulate a condition very similar to that of the ambient air. Hill reaction was determined by recording the reduction of 2, 6- Dichlorophenol indophenol (DCIP) as described by Fleischhacker and Senger (1978).

Ethylene release rate was measured by Gas chromatograph technique, in which the young panicles of sterile and fertile TGMS lines were covered with 200 gauge thick polythene covers fitted with a septum, which was used to draw the ethylene sample from the covers. Samples (2 ml) were drawn after 24 hours from the plastic covers, injected to Gas chromatograph and compared with standard sample for retention time, height of the peak, and area covered by the peak. The ethylene concentration was computed using the formula adopted by Sadasivam and Manickam (1996).

Calcium content of leaves and young panicles was analysed by versanate titration method proposed by Piper (1966) and expressed in µg g^{-1} on dry weight basis. Copper was estimated by Atomic absorption spectrophotometer using triple acid extract (Lindsay and Norvell, 1978) and the content of copper was expressed in µg g^{-1} of sample on dry weight basis. The content of boron in leaves and young panicles was estimated by the method proposed by Banuleos *et al.* (1992) using azomethine-H and was expressed in µg g^{-1} of sample on dry weight basis.

RESULTS AND DISCUSSION

Gas exchange measurements

The rate of photosynthesis of TGMS lines reduced significantly in high temperature induced sterile condition. Table 1 presents the changes in major gas exchange parameters *viz.*, photosynthetic rate (P_n), transpiration rate (E) and stomatal diffusive resistance (r_s) of TGMS lines under fertile and sterile conditions.

Photosynthetic rate reduced down to 25% in sterile condition compared to fertile condition and the highest percentage of reduction was observed in TGMS 29 (30.0%). The percent change in transpiration rate and stomatal diffusive resistance was 9.6% and 12.3%, respectively and was lesser when compared to the percent change in photosynthetic rate. The increase in stomatal diffusive resistance was associated with corresponding decrease in transpiration rate. The results indicated that the photosynthetic rate and transpiration rate decreased but stomatal diffusive resistance increased under sterile condition in TGMS lines. At lower or higher temperatures carbon exchange rate was reduced, thereby the net photosynthetic rate was affected. Kabede *et al.*, (1989) reported that the limitation in the supply of reducing power was the main cause for the reduction in photosynthesis than reduced stomatal conductance. A change in the content or activity of the mesophyll enzyme Rubisco will also alter the photosynthetic rate in plants. Maruyama *et al.* (1990) also reported that the synthesis of

Rubisco and several thylakoid proteins responsible for photosynthetic electron transport and photo phosphorylation were inhibited at high temperatures.

Table 1. Gas exchange parameters of TGMS lines and ADT 36 under fertile and sterile conditions.

Parameters	TGMS Lines	Fertile condition	Sterile condition	% Change
	TGMS6	20.5	15.9	-23.0
Photosynthetic rate	TGMS16	20.0	15.2	-24.0
(Pn)	TGMS29	21.5	15.1	-30.0
(μ mol CO_2 m^{-2} s^{-1})	ADT36	21.7	-	-
	Mean	20.6	15.4	-25.6
	TGMS6	8.3	7.1	-15.0
Transpiration rate	TGMS16	7.1	6.8	-5.0
(E)	TGMS29	8.5	7.8	-9.0
(μg H_2O m^{-2} s^{-1})	ADT36	8.3	-	-
	Mean	7.9	7.2	-9.6
	TGMS6	0.58	0.65	11.0
Stomatal diffusive	TGMS16	0.63	0.70	10.0
resistance (r_s)	TGMS29	0.55	0.65	16.0
(s cm^{-1})	ADT36	0.61	-	-
	Mean	0.58	0.66	12.3
CD (P=0.05)		Treatments	Lines	T x L
	P_n	1.06	1.28	1.76
	E	0.41	0.37	0.54
	r_s	0.35	0.46	0.62

Hill reaction rate

The photochemical efficiency leading to the production of assimilatory power measured as Hill reaction was reduced to 24% in sterile TGMS lines compared to fertile plants. The normal variety ADT 36 recorded the highest value for hill reaction rate which was on par with TGMS lines under fertile condition (Table 2).

Similar results were reported by Tang *et al.* (1994) that sterility inducing condition decreased the photochemical activity and efficiency of light energy transformation in PS II and markedly reduced the electron transmission of chloroplast in TGMS lines. This might be the cause for lower ATP and NADPH in the leaves of long day with high temperature treated sterile TGMS lines observed by Xia *et al.* (1993). Reduction in ATP content and carboxylation rate could be associated with impaired photochemical efficiency or excitation energy transfer to the reaction centre protein complex. The decrease in photochemical activity during high temperature might have decreased the concentration of photosynthetic products affecting male sterility as proposed by Tang *et al.* (1994).

Table 2. Hill reaction rate of TGMS lines and ADT 36 under fertile and sterile conditions.

TGMS Lines	Hill reaction rate (μ moles DCIP reduced mg^{-1} Chlorophyll h^{-1})		
	Fertile condition	Sterile condition	Per cent reduction
TGMS6	52.37	41.11	22.0
TGMS16	48.40	40.23	17.0
TGMS29	50.45	39.30	33.0
ADT36	53.47	-	-
Mean	50.40	40.21	24.0
	Lines (L)	Treatments (T)	T x L
CD (P=0.05)	0.982	0.767	1.548

Ethylene release rate

Ethylene is a gaseous hormone that is synthesized in higher amounts during stress conditions in plants. In the present experiment, the ethylene release rate of TGMS lines under fertile and sterile conditions was estimated by Gas chromatography techniques. The concentration of ethylene released from young panicles showed a significant difference between fertility and sterility. The fertile panicles of TGMS lines recorded low ethylene concentration than sterile panicles (Table 3). ADT 36 recorded the lowest value for ethylene concentration under fertile condition.

Table 3. Ethylene release rate (ERR) in panicles of TGMS lines and ADT 36 under fertile and sterile conditions.

TGMS lines	Height of peak (ml)		Area (ml s^{-1})		Ethylene release ate (μ mol m^{-1})	
	F	S	F	S	F	S
TGMS 6	0.106	0.263	1.203	2.690	2.047	3.787
TGMS 16	0.127	0.378	1.629	5.479	2.268	5.645
TGMS 29	0.138	0.399	1.990	5.531	2.403	5.784
ADT 36	0.081	-	1.012	-	1.723	-
Mean	0.123	0.346	1.607	4.566	2.239	5.072
CD (P=0.05)						
Lines	0.164		0.017		0.441	
Treatments	0.116		0.012		0.312	

F: Fertile condition; S: Sterile condition

The ethylene release rate was negatively correlated with pollen fertility and was low in long days with low temperature and high under short days with high temperature (Li DeHong *et al.*, 1996). In the present investigation, all the TGMS lines under fertile condition showed lesser concentration of ethylene compared to that of sterile condition in young panicles. This was in conformity with the results of Luo *et al.* (1993). Treatment with amino ethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis, resulted in a marked increase in pollen fertility in male sterile lines. This indicated the key role of ethylene in the process of pollen sterility (Tian, 2000). The release of comparatively more ethylene from sterile panicles of TGMS lines and comparatively less ethylene from panicles of fertile plants of TGMS lines and normal variety revealed the role of ethylene in fertility alteration under high temperature condition. This can be used as a potential parameter for identifying the TGMS character in rice genotypes.

Nutrient content

The contents of calcium, copper and boron, which play a major role in reproductive growth of plants, were lower in leaves and young panicles of sterile TGMS lines compared to fertile plants (Table 4). There was no significant variation in calcium, copper and boron contents between genotypes. The nutrient status of ADT 36 was on par with fertile TGMS lines.

Table 4. Nutrient status in leaves and panicles of TGMS lines and ADT 36 under fertile and sterile conditions.

TGMS lines	Calcium (μg g^{-1})				Copper (μg g^{-1})				Boron (μg g^{-1})			
	Leaves		Panicles		Leaves		Panicles		Leaves		Panicles	
	F	S	F	S	F	S	F	S	F	S	F	S
TGMS 6	201	165	170	140	104	81	90	65	38	22	31	16
TGMS 16	198	162	173	132	110	80	90	69	36	23	30	15
TGMS 29	208	162	186	126	106	81	92	66	39	22	32	15
ADT 36	203	-	180	-	119	-	93	-	38	-	31	-
Mean	202.3	163.0	176.3	132.6	106.6	80.6	90.6	66.6	37.6	22.3	31.0	15.3

CD	Lines (L)	Nutrients (N)	Treatments (T)	L x N	Interaction effect L x T	T x N	L x N x T
(P=0.05)	3.416	2.902	3.021	6.124	5.772	5.432	7.462

F: Fertile condition; S: Sterile condition

Wu and Li (1993) and Tian *et al.* (1998) suggested the importance of calcium in leaves and young panicles in determining pollen fertility. In sterile anthers, calcium precipitates were less in anther walls, pollen grain surfaces and these anomalies in the distribution of calcium accumulation caused the failure of pollen development and pollen abortion. Graham, (1975) and Agarwala *et al.* (1979) found that boron and copper deficiencies induced male sterility in many cereals. They indicated that the deficiency of these nutrients exhibited the reduction in anther size and pollen abortion. The pollen grains were poorly developed, small, shrivelled, and highly vacuolated with lack of proper exine and cytoplasmic contents. The reduction in boron content in sterile lines in the present study was in accordance with the results of Kaul (1988) who reported that the pollen structure development and viability were highly affected by boron deficiency. Dell (1981) opined that the reduction in copper content causes minute anthers with scanty, dented, small, starchless, abortive pollen grains. From the present study, it is evident that the deficiency of these nutrients causes failure of pollen development or pollen abortion, which leads to male sterility in TGMS lines.

CONCLUSION

Photosynthetic and hill reaction rates of TGMS lines reduced under sterile condition induced by high temperature. The status of calcium, copper and boron, which play a major role in reproductive growth of plants, was changed in TGMS lines during fertility alteration. The concentration of ethylene released from young panicles also showed a significant difference between fertile and sterile conditions. Thus the reduction in photosynthetic products, micronutrients especially calcium, copper and boron and increased ethylene release rate under sterile condition revealed their association with male sterility in TGMS lines under high temperature condition.

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