Kasetsart J. (Nat. Sci.) 38 : 183 - 189 (2004)

Adaptability of Thermosensitive Genetic Male Sterile Rice Lines in Thailand

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ABSTRACT

To determine the performance of thermosensitive genetic male sterile (TGMS) rice lines in Thailand, field experiments were conducted in Chiang Mai, Chiang Rai and Kamphaeng Phet. Six TGMS lines were planted during hot and cool seasons, and the results showed that TGMS lines respond to the prevailing temperatures at the time of the critical stage of TGMS lines at specific location. At Kamphaeng Phet, the six TGMS lines were all male sterile when planted in June 2002 and January 2003. Cool season planting in November 2003 that flowered in January to first week of February produced fertile pollen, and produced filled spikelets up to about 76.8%. At Chiang Mai, spikelet fertility of TGMS lines was observed as high as 77.4%. Kamphaeng Phet during cool season, and the high altitude areas in Chiang Mai are suitable locations for seed increase of TGMS lines. However, more experiments should be conducted to determine the suitable planting dates that could give highest spikelet fertility for seed multiplication of specific TGMS line. TGMS lines producing self-seeds under sufficient low temperature and showing sterility under temperature above 30°C indicated that TGMS lines could be used as parental line for developing two-line hybrid rice in Thailand.

Key words: thermosensitive genetic male sterility (TGMS), cytoplasmic genetic male sterile line, hybrid rice, heterosis, *tms2* gene

INTRODUCTION

Initial success in the utilization of hybrid rice in increasing grain yield in China generated considerable interests in adapting this technology in tropical countries. Several Asian countries such as Bangladesh, India, Indonesia, Malaysia, Myanmar, Philippines, South Korea, Sri Lanka, Vietnam, and Thailand started their own hybrid rice research and development (Virmani, 1998). Experiences of these countries had shown that the success in the use of hybrid rice depends on the extent of heterosis and efficiency of the seed production techniques. Heterosis or hybrid vigor is described as the tendency of the first generation offspring of genetically diverse parents to perform better than the better parent, or better than the best cultivated variety. The use of male sterility is a prerequisite for commercial exploitation of heterosis since rice is a self-pollinating crop. In the tropics, the cytoplasmic genetic male sterility (CMS) and the thermosensitive genetic male

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Received date : 26/02/04 Accepted date : 31/05/04

sterility (TGMS) are the two male sterility systems that can be used. The CMS with wild abortive (WA) cytoplasm is the method used extensively; however, later studies had shown that TGMS system was more effective in increasing grain yield and seed production efficiency (Yuan, 1998; Lu *et al*., 1998; Lopez and Virmani, 2000). The experiments at the International Rice Research Institute (IRRI) had shown that two-line hybrids derived from TGMS lines showed higher frequency of heterotic combinations than the three-line hybrids derived from CMS lines (Lopez and Virmani, 2000). Seed production efficiency using TGMS line is higher than using CMS line because of its inherent nature. TGMS is a genetic male sterility wherein the expression of the male sterility gene is influenced and regulated by prevailing temperature. At present, five single recessive TGMS genes located on different chromosomes have been reported (Sun *et al*., 1989; Maruyama *et al*., 1991; Subudhi *et al*., 1997; Dong *et al*., 2000; Reddy *et al*., 2000). Exposure of TGMS line to a certain temperature like <30ºC for some lines at the critical stage (varying from 15 to 25 days before heading or 5 to15 days after panicle initiation) can cause male sterility to revert to fertility. TGMS lines can self-pollinate so seeds are multiplied like any other varieties by growing them under temperature conditions lower than about 30°C. On the other hand, seeds of CMS line are multiplied by pollinating it with its corresponding maintainer line; and pollinating it with the restorer line produces the hybrid. A TGMS line is sterile when the prevailing temperature is higher than 30°C at its critical stage, so it can be used as the female parent in hybrid rice seed production. In the hybrid seed production using TGMS method, only the pollen parent (any variety or line) and the TGMS lines are needed, so the hybrid is called a two-line hybrid. While the hybrid produced using CMS method is called a three-line hybrid for it involves three lines: the CMS, the maintainer and the restorer.

Although Thailand has sufficient rice, and the number one exporter of rice in the world, the goverment sector started hybrid rice research in 1993 to improve grain yield per unit area of land. Three-line hybrids developed using parental lines from another countries were found not adapted to Thailand conditions. The breeders tried to develop parental lines such as the CMS or A, the maintainer or B, and restorer or R lines. To develop CMS lines, wild abortive (WA) cytoplasm was transferred to several selected local varieties or lines. Twelve CMS lines were developed such as RD21A, RD25A, BS4A, Sonalee A, IR21845A, IR17492A, KDML 105A, SPRLR 75001-68-2-2A, SPRLR 76102-26-1-1A, CNT 82001-3-2-1-1-2-1A, CNT 86003-2-1-1-1-2-1-1-1A, and BKN 6-18-3-2A. Good restorers were identified from a large number of high-yielding local lines. Unfortunately, many of the CMS and restorer lines available did not meet the desired grain quality standards. Hybrid rice evaluations were done to a limited extent. In the 1993 wet season, hybrids derived from locally-bred CMS lines and restorer lines were planted at Kasetsart University, Kamphaeng Saen, Nakhon Pathom. Some IRRI-bred hybrids were also evaluated in two yield trials conducted at Pathumthani Rice Research Center and at Bangkhen Rice Research Institute in the 1995 wet season. Results of the yield trials were not sufficiently encouraging because most of the hybrids performed poorly (Amornsilpa, 1998). Subsequently, there was no significant progress reported on the hybrid rice research. To find ways on how to ensure an appropriate impact of hybrid rice technology on the farm level in Thailand, hybrid rice research and improving seed production techniques must be done simultaneously.

This study was conducted to determine the performance of locally bred and introduced TGMS lines in selected locations during cool and hot seasons in Thailand in order find the better method to attain high heterosis and more efficient hybrid rice seed production techniques.

MATERIALS AND METHODS

1. Selection of locations for the experiments

Temperature data of the northern provinces of Thailand (Table 1) were taken from the Meteorological Department to determine the suitable locations and period of the year to plant TGMS lines. A high altitude area in Chiang Mai was selected; Kamphaeng Phet was selected for hot and cool season experiments; and Chiang Rai for cool season experiments. In selecting location, minimum and maximum temperature data were given important considerations.

2. Plant materials and seeding dates

GD1, a Chinese-bred TGMS line; IRRI-bred TGMS lines, IRRI201S and IRRI202TGMS; and three newly developed TGMS lines with Thai variety background designated as KP205S, KP206S and KP207S were planted to evaluate the performance at three different locations during cool and hot seasons in Thailand. The TGMS gene present in all TGMS lines used were identified, except for GD1. IRRI201S, KP205S, KP206S and

Table 1 Mean monthly maximum and minimum temperatures in northern region of Thailand for the period of 22-25 years.

Province		Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Ave.
Chiang Rai	1978-2002	28.5	31.3	34.0	35.1	33.2	32.0	31.0	30.9	30.9	30.1	28.5	27.0	31.0
		12.4	13.2	16.5	20.3	22.4	23.4	23.3	23.2	22.5	20.7	17.1	13.3	19.0
Chiang Mai	1978-2002	29.7	32.5	35.2	36.4	34.3	32.7	31.9	31.5	31.7	31.3	30.0	28.5	32.1
		14.7	16.1	19.4	22.8	23.9	24.1	23.9	23.7	23.2	22.1	19.2	15.7	20.7
Kamphaeng Phet	1981-2002	32.3	34.5	36.1	37.4	35.4	33.6	33.1	32.6	32.7	32.1	31.4	30.7	33.5
		18.3	20.0	22.5	24.7	25.1	25.0	24.8	24.6	24.3	23.6	21.4	18.3	22.7
Mae Hong Son	1978-2002	30.2	33.4	36.8	38.6	36.0	33.1	32.1	31.9	32.7	32.6	30.9	29.2	33.1
		13.5	14.2	17.6	22.4	23.6	23.6	23.4	23.2	22.9	21.8	18.9	15.3	20.0
Phayao	1981-2002	29.2	32.1	35.0	36.0	33.5	32.4	31.4	31.3	31.2	30.5	28.9	27.5	31.6
		13.6	15.3	19.5	22.8	23.5	24.0	23.6	23.4	22.9	21.6	18.1	13.9	20.2
Lampang	1978-2002	31.3	34.1	37.1	38.3	35.6	33.8	33.1	32.8	32.6	32.0	31.0	29.9	33.5
		14.8	16.3	19.8	23.1	24.1	24.3	24.1	23.9	23.4	22.1	18.9	15.1	20.8
Lamphun	1981-2002	30.7	33.7	36.7	37.9	35.2	33.3	32.8	32.3	32.0	31.4	30.1	29.0	32.9
		14.1	15.5	19.2	22.9	23.8	24.2	23.9	23.7	23.3	22.2	19.0	15.0	20.6
Phrae	1978-2002	31.3	33.7	36.5	37.7	35.4	33.5	32.6	32.3	32.3	32.0	31.1	30.0	33.2
		15.5	17.3	20.9	24.1	24.6	24.5	24.3	24.2	23.9	22.7	19.4	15.6	21.4
Nan	1978-2002	30.6	33.1	36.2	37.0	35.0	33.3	32.2	32.0	32.5	32.2	30.9	29.4	32.9
		14.2	15.5	18.8	22.3	23.7	24.3	24.0	23.8	23.4	21.8	18.4	14.3	20.4
Uttaradit	1978-2002	32.4	34.6	36.9	38.2	36.1	34.1	33.2	32.8	33.1	33.1	32.2	31.1	34.0
		17.6	19.0	21.7	24.4	24.9	24.9	24.6	24.4	24.2	23.2	20.7	17.7	22.3
Tak	1978-2002	32.5	35.2	37.7	38.6	35.5	32.9	32.4	32.1	32.4	31.6	30.8	30.4	33.5
		16.8	19.7	23.9	26.2	25.6	25.2	24.9	24.6	23.9	22.7	20.1	16.6	22.5
Maesot	1978-2002	31.6	33.7	35.8	36.8	34.2	31.3	30.4	30.0	31.3	32.0	31.2	30.3	32.4
		15.0	16.5	19.6	22.9	24.0	23.5	23.2	23.0	23.0	22.2	19.0	15.2	20.6
Phitsanulok	1978-2002	31.9	33.9	36.0	37.4	35.7	33.9	33.1	32.4	32.4	32.2	31.6	30.8	33.4
		18.8	21.1	23.8	25.5	25.3	25.1	24.9	24.7	24.7	24.0	21.6	18.5	23.2
Phetchabun	1978-2002	32.4	34.7	36.6	37.2	35.0	33.2	32.6	32.0	32.1	32.1	31.6	30.9	33.4
		17.5	19.8	22.4	24.5	24.8	24.6	24.3	24.1	23.9	22.9	20.1	17.1	22.2

KP207S possess *tms2* gene, while IRRI202S possesses TGMS gene located in chromosome 9. All TGMS lines require a temperature below 30° C at its critical stage to revert to fertility. The right time to sow the seeds of the TGMS lines was inferred from the 25-year mean monthly maximum and minimum temperature data of each location. To determine the performance of TGMS lines under high temperature conditions, experimental materials were sown in June 2002 and in January 2003 at Kampheng Phet. Cool season experiments were sown on 15 November 2002 at Kamphaeng Phet; on 1 November 2003 and on 8 September 2003 at Chiang Rai; and on 12 August 2003 at Chiang Mai.

3. Cultural management

Seeds were pre-germinated in petri dish and planted in a mixture of soil and coconut husk in seed boxes with 2 × 2 cm spacing. One hundred to 500 plants of each TGMS lines were transplanted 21 days after germination at 25 × 20 cm spacing. Fertilizers were applied at the rate of 150 kg N, 100 kg P and 100 kg K per hectare throughout the growing period, and pesticides were applied whenever necessary.

4. Pollen and spikelet observations

Observations of the pollen sterility or fertility were taken from the first and second panicles. Anthers of each plant were observed visually. Observation of pollen under the microscope was also done to confirm visual observation of the anther color. Five to ten flowers that would open the following day were collected and stored in 70% ethanol until the time for observation. Pollen sterility was determined by staining pollen grains with 1% IKI solution. Pollen grains were classified based on their shape and extent of staining. The unstained withered or spherical pollen grains and the lightly stained round pollen grains were classified as sterile. The fertile pollen grains were black and round. Plants were classified based on the extent of pollen sterility as follows: pollen-free (no pollen grains), completely sterile (100%), sterile (91-99%), partially sterile (71-90 %), partially fertile (31-70%), and fertile (0-30%) (Virmani *et al.*,1997). Panicles were harvested at maturity. Spikelet fertility was taken from two primary panicles. Spikelet fertility was monitored by counting the number of filled grains and total spikelets per panicle and converted into percentage.

RESULTS AND DISCUSSION

Performance of TGMS lines during hot season

All TGMS lines planted at Kamphaeng Phet in June 2002 and in January 2003 were pollen- and spikelet-sterile (Table 2). All the TGMS lines were observed to have completely sterile pollen or pollen-free anthers (Figure 1). This data indicated that the maximum and minimum temperatures prevailing during the critical growth stage (1-2 weeks after panicle initiation) were suitable enough to maintain sterility of all TGMS lines. The performance of six TGMS lines in two planting seasons show that TGMS lines can be used as female parent in hybrid rice seed production. From the results and the temperature data available, it can be deduced that sowing TGMS line to produce two-line hybrids in Kamphaeng Phet can be done from January to September. The panicles of the TGMS lines will be exposed to sterility-inducing temperatures at the critical stage if planted in that period.

Performance of TGMS lines during cool season

TGMS lines sown in 1 November 2003 at Kamphaeng Phet had yellow anthers. Microscopic observation showed that pollen grains were partially fertile to fully fertile (Table 2). Fertile pollen grains were round and stained black with I_2KI when observed under the microscope (Figure 1). Exposure of the TGMS lines to fertility-inducing temperatures in mid-January to first week of

Table 2 Number of days to 50% flowering, pollen observation at Kamphaneng Phet, and spikelet fertility data taken at Kamphaeng Phet, Chiang Mai and Chiang Rai of six TGMS rice lines.

TGMS line	Days to 50% Flowering	Pollen observation at Kamphaeng Phet*			Spikelet fertility (%) in 2003		
		June 2002	Jan 2003	Nov 2003	Kamphaengphet	Chiangmai	Chiangrai
GD 1	75	NP	NP	PF - F	60.2	19.1	- **
IRRI201S	96	NP	NP	F- FF	34.4	-	0
IRRI202S	84	NP	NP	F- FF	63.1	51.5	44.1
KP205S	95	NP	NP	PF - F	69.0	32.4	0
KP206S	96	NP	NP	F - FF	68.1	68.8	0
KP207S	105	NP	NP	PF - F	76.8	77.4	0

* NP = pollen-free, PF = partially fertile, F = fertile, FF = fully fertile
** - = missing data

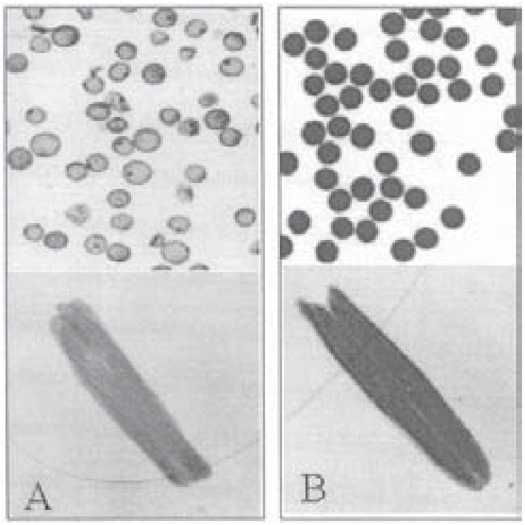

Figure 1 (A) Sterile pollen grains and pollen-free anther sac of TGMS lines exposed to temperature higher than 30°C. (B) Fertile pollen grains and pollen-filled anther of TGMS lines exposed to temperature below 30°C.

February induced TGMS lines to produce fertile panicles. Seed setting was observed 34.6% in IRRI201S to as high as 76.8% in KP207S. GD1, a TGMS line originated from China that may have different TGMS gene had spikelet fertility almost same as the TGMS lines possessing *tms2* gene. The late-flowering panicles of all TGMS lines were exposed to higher temperatures prevailing at the time of their development resulting in the development sterile panicles. These results indicate that to produce self-seeds of TGMS lines in Kamphaeng Phet, planting should be done in the first to second week of November so that the panicle initiation will be before the beginning of cool season and the panicles will be exposed to the temperature below 30°C at their critical stage. The monthly variations of temperature prevailing at Kamphaeng Phet made it possible to get fertile and sterile TGMS lines. Similar results were also obtained at IRRI farm in the Philippines. TGMS lines seeds can be produce in November planting and two-hybrid seeds can be produced in the dry season (Lopez and Virmani, 2000).

Experiments were planted twice in Chiang Rai. All TGMS lines were sterile in November 2002 experiment. The experimental materials were exposed to low minimum temperature starting the middle of December 2002. The prevailing minimum temperature in the area dropped down to 10°C inducing the plants to prolong the vegetative stage and delayed flower initiation. Maximum temperature started to rise in February 2003, so the TGMS lines were exposed to the high temperature at the critical stage. TGMS lines developed panicles after the cool period and flowered in March 2003 so the panicles that developed were all pollen- and spikelet-sterile.

The second experiment was seeded two months earlier than the previous year to avoid very cool periods. Soaking of the seeds was done on 8 September 2003. The earliest flowering TGMS line (IRRI202S) flowered in the first week of January 2004, had yellow anthers and produced 43.8% spikelet fertility (Table 2). This TGMS line was exposed to 15-20°C minimum temperatures in December 2003 coinciding to its critical stage about 15-25 days before heading, hence producing fertile pollen and filled panicles. However, all late maturing TGMS lines were sterile because they were exposed to the lower (<15°C) minimum temperature during reproductive stage. For rice that has no cold tolerance, temperature lower than 15°C was known to be stressful to the development of panicles that causes them to be sterile.

Performance of TGMS lines in high altitude area

Five TGMS lines showed acceptable spikelet fertility (32.4-77.4%) when sown in 12 August 2003 and planted in high altitude area in Chiangmai (Table 2). The prevailing minimum-maximum temperature in the area during the critical stage of the five TGMS lines in the third week of October to middle of November 2003 was sufficient to induce fertility. The spikelet fertility (32.4-77.4%) obtained in this high altitude area is comparable to the spikelet fertility (34.6-76.8%) obtained in Kamphaeng Phet during cool season. In the Philippines, similar spikelet fertility was obtained from the TGMS lines planted in high altitude areas (Lopez and Virmani, 2000). The results indicate that high altitude area is an alternative place to produce self-seeds of TGMS lines if cool season TGMS line seed multiplication failed in lower altitude areas.

CONCLUSION

The results indicate that TGMS method can be used in developing two-line hybrids in a tropical country like Thailand where there is a distinct cool and hot seasons, and where lower minimum and maximum temperatures can be attained in high altitude areas in other period of the year. Seed production of TGMS lines would be simple and easy. The key is to determine the period of the year of a particular location based on the existing climatic data that can induce maximum fertility. A temperature ranges from 20 to 29°C is enough to induce TGMS lines to become fertile. Sequential soaking of a TGMS line at 15 days interval can be done in each location to determine the possible period of the year when TGMS lines can produce maximum spikelet fertility. Based on the results in three locations, maximum fertility can be attained if the critical stage of panicles coincides to the occurrence of required temperatures. Likewise, seed production of two-line hybrids would be simple for it can be done in all rice growing areas in Thailand during hot season. To make sure that the TGMS lines are sterile, planting of hybrid seed production should be done in January until September to avoid a lower temperatures occurring in December to February. The date of planting of TGMS lines and the corresponding male parent to produce two-line hybrids can be determined based on the temperature data available. The period of the year of a particular location where the maximum temperature is higher than 30°C can be a suitable time to plant TGMS lines. TGMS method in developing hybrid rice in the tropics would be simpler and less expensive compared with the commonly used CMS method. It is more advantageous to use because it has diverse cytoplasmic backgrounds, no negative effects associated with sterility-inducing cytoplasm, no need for maintainer lines, and can give higher frequency and extent of heterosis. The different TGMS genes available can be incorporated using conventional or by molecular marker aided breeding methods (Lopez *et al.*, 2003) into any adapted lines and varieties. These wide varieties of TGMS lines crossed with any selected lines or varieties can produce more diverse hybrid rice that can be adaptable to any rice growing conditions.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Sant Singh Virmani of the Plant Breeding, Genetics and Biochemistry Division, IRRI, Philippines for providing the seeds of TGMS lines used in this study. The senior author gratefully acknowledged Mr. Montri Congtrakultien and Dr. Anek Silapapun of Charoen Pokhaphand Group, Crop Integration Business for the support in conducting field experiments.

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