Chapter 3

Critical temperature testing for thermo-sensitive genic male sterility induction

3.1 Introduction

The present plant improvement investigation is aimed at inventing a new and innovative approach for quick screening of breeding lines by applying cytological techniques. A comparison of sterile and fertile lines for variation in the developmental pattern of anthers has been attempted in the TGMS rice lines. Studying of this chapter is going to investigate the stage at which sterility occurs during anther development in TGMS lines.

Thermo-sensitive genic male sterile (TGMS) lines will show male sterility or male fertility by temperature control. The point that controls these characters is called critical temperature. Cause of environmental control character, TGMS lines may have some effects with genetic by environment. Haohua *et al*. (2006) reported a homozygous sterile line which was developed from Pingxiang male-sterile rice (PMSR) by repeated selfing and plant was characterized for its fertility behavior under controlled and field conditions. Male fertility was affected by both temperature and photoperiod factor but temperature being more important. The critical temperature for inducing fertility was 27–28 °C. Below this critical temperature, plants remained sterile, but became partially fertile at higher temperature. The panicle development stages that were sensitive to temperature were from differentiation of the secondary branch primordium to the meiotic division of the pollen mother cells. Continuous high temperature (>30 °C) during these sensitive stages is necessary to maintain male fertility. Long photoperiod (15 h) induced partial fertility even under

low temperature, which could induce sterility. In practice, this line can be regarded as thermo-sensitive. In the sub-tropical zone, this line has completed sterile phase longer than 4 weeks and thus is suitable for hybrid production using the two-line system (a pair of pure sterile and fertile lines). Male sterile line required low temperature and short photoperiod to express male sterility, like the one derived from Pingxiang male-sterile rice (PMSR), has never been reported in any crop species. This line is also the first dominant nuclear male-sterile line that could be exploited for hybrid seed production using the two-line system.

Knowledges of the male sterility mechanism in TGMS lines are very important for hybrid rice development through a two-line breeding approach. Conventional approaches are tedious and time-consuming, especially for identifying appropriate lines in an easy and quick manner. The study on critical temperature testing for TGMS line has the following objectives:

1. To study critical temperature for inducing male sterility of T29s variety (TGMS line).
2. To study the effective timing after seed sowing for inducing male sterility of T29s variety (TGMS line).

Location and experimental period: The experiments were conducted at the evaporation green house and modifier mini-phytotron of Maejo University, Chiang Mai province, during March 2004 to February 2005.

3.2 Materials and methods

The experiment was set for two factors of study which included four ages of plant: 40, 50, 60 and 70 days after transplanting and three levels of critical temperatures: 22, 24 and 26 ^{o}C for inducing male sterility. The treatments were carried out in modifier mini-phytotron. 25 – 30 seeds of T29s variety were germinated by between-paper methods. After seed germination, seedlings were transplanted to plastic tray (104 holes per tray) which contained complete plant nutrition media. After 21 days of emergence or 3rd – 4th leaf blade stages, 20 seedlings were transplanted to plastic pots (size 12 inches diameter), planting one plant per pot. The critical temperatures were set for three temperature levels: 22, 24 and 26 °C. Control temperature or room temperature was also included as the control treatment. At each temperature level, 4 plants of different ages: 40, 50, 60 and 70 days after emergence were treated. At each temperature testing, modifier mini-phytotron was set for 11 hours with 22,000 lux light density. Data were recorded as follow: flowering date, tillers per plant (at harvesting date), panicles per plant, spikelet per panicle, percent seed set.

Pollen sterility test

Pollen test was examined by staining pollens with 1% iodine–potassium iodide (KI_2) solution just before pollen counting under the microscope. There were three random fields of microscope on cover slip counting stained pollens and unstained pollens for averaging sterility percentage. Round and dark brown-stained pollen was scored as fertile, and irregular-shaped, small and yellowish or light brown colored was scored as sterile pollen (Figure 3.1). About 200 to 300 pollen grains were scored from

three randomly-chosen fields on each slide counting stained pollens and unstained pollens for averaging sterile percentage (Subudhi *et al*., 1997). To evaluate spikelet fertility, two panicles per plant emerging from leaf sheath were bagged with glassine paper bags prior to anthesis to prevent cross-pollination. The bagged panicles were harvested 25–30 days after anthesis. Seed set of bagged panicles was calculated by number of filled grains divided by the total number of grains. Plants with less than 5% stained pollen and/or seed set were considered sterile, whereas plants having more than 50% stained pollen and/or seed set were classified as fertile (Dong *et al*., 2000).

The details of the experimental treatments of critical temperature for inducing male sterility were set as shown in Table 3.1.

Table 3.1 The experimental treatments of critical temperature for inducing male sterility of TGMS line.

Activity	Level of critical temperatures		
	22 °C	24 °C	26 °C
Sowing date	30 Mar 04	5Aug 04	20 Sep 04
Transplanting date	2 Apr 04	9 Aug 04	27 Sep 04
Planting date	22,26 Apr 04	31 Aug 04	11 Oct 04
40 days after sowing date	20 May 04	12 Oct 04	19 Nov 04
50 days after sowing date	31 May 04	26 Oct 04	29 Nov 04
60 days after sowing date	9 Jun 04	5 Nov 04	13 Dec 04
70 days after sowing date	21 Jun 04	15 Nov 04	22 Dec 04
Harvesting date	12 Oct 04	7 Feb 05	7 Feb 05

3.3 Results

3.3.1 Critical temperature and plant development stage including pollen sterility

Table 3.1 and Fig 3.1 show the pollen fertility percentage of TGMS line when plants were moved to the growth chamber at four different growth stages; 40, 50, 60 and 70 days after seed sowing and grown under three different temperatures; 20, 24 and 26 ^{o}C.

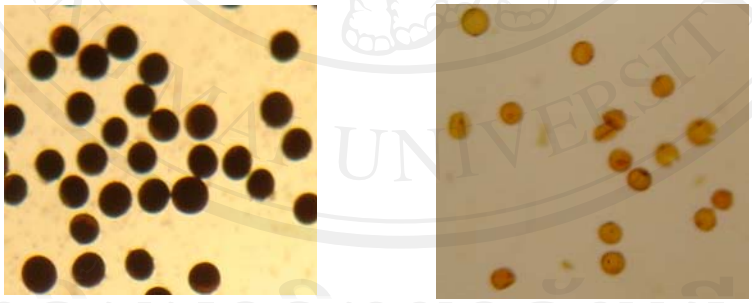
The results indicated that 26 ^{o}C temperature should be the critical temperature for inducing male sterility of TGMS line since all treatments for days after sowing exhibited pollen sterility, ranged 0.00-0.77 percent (averaged 3.25 percent). In contrast with 22 ^{o}C temperature growing condition, all treatments of TGMS line were still producing high pollen fertility, ranged 81.5-82.5 percent (averaged 83.83 percent).

Stage of plant development for inducing pollen sterility evaluation clearly indicated that 60 to 70 days after seed sowing should be the most effective time for inducing male sterility of TGMS line since both 40 and 50 days after sowing of 26 ^{o}C treatments did not show any pollen sterility percentage. As these 40 and 50 days after seed sowing were probably still in seedling stage and floral initiation had not place, thus, high temperature could not affect pollen sterility because the treatments were imposed too early.

Table 3.2 Average fertile pollens (%) of TGMS line grown under different temperatures in mini-phytotron condition.

Days after sowing	Average fertile pollens (%)		
	22 °C 1/	24 °C	26 °C
40	-	84.3	0.0
50	-	85.2	0.0
60	-	84.3	7.1
70	-	81.5	5.9
Average	-	83.83	3.25

1/ Data were not available due to out of control of growth chamber during booting stage.



(a) Stained pollen (male fertile) (b) unstained pollen (male sterile)

Figure 3.1 (a) Fertile pollens (blue color) compared with (b) Sterile pollens (pale color) after staining with 1% KI_2 solution.

3.3.2 Seed set evaluation

Table 3.3 shows the ability of seed set of TGMS line when different stages of plant were grown at different temperatures under control conditions. Results obviously agreed with 3.3.1 topic that TGMS line when grown under high temperature of 26 ^{o}C exhibited complete male sterile pollens and did not produce any seed at maturing stage. In contrast with plants growing under low temperature of 24 ^{o}C which was the critical temperature for inducing pollen fertility, a small amount of seed sets was obtained from different plant development stages, ranged 13.5 to 19.4 percent (averaged 17.2 percent). Low seed set percentages obtained from critical temperature inducing pollen fertility (24 °C) were probably due to epidemic of some leaf diseases on TGMS plants during the grain filling period and caused abortive grains even though disease control was made intensively.

Table 3.3 Seed set evaluation of TGMS line grown under different temperatures in mini-phytotron condition.

Days after sowing	Seed set (%)		
	22 °C 1/	24 °C	26 °C
40	-	13.5	0.0
50	-	19.4	0.0
60	-	17.4	2.4
70	-	18.6	0.0
Average	-	17.2	0.5

1/ Data were not available due to out of control of growth chamber during booting stage.

3.3.3 Tillering, booting and flowering evaluation

The experiment on pollen fertility evaluation of TGMS line was further studied for some agronomic character developments as responded to different temperature growing conditions. Tables 3.4 to 3.6 show the development of tillering, booting and flowering stage of TGMS line grown under three different temperatures; 22, 24 and 26 ^{o}C.

Results indicated that all treatments of seedling stages of TGMS line (40, 50, 60 and 70 days) took 23, 25 and 25 days for tiller development when grown under 22, 24 and 26 ^{o}C, respectively.

For booting stage development, it should be observed that seedling growth stage of 40 days and 50 days took longer times than 60 days and 70 days seedling stages for booting stage development when grown under 22 ^{o}C (Table 3.4). But times for booting stage developments were not different among the seedling growth stages for 24 ^{o}C growing condition. In contrast with 26 ^{o}C growing condition, seedling growth stage of 40 days and 50 days took shorter times than 60 days and 70 days for booting stage development (Table 3.6).

For flowering date development, it was found that seedling growth stages of 40, 50 and 60 days grown at 22 ^{o}C showed slight difference for days to flowering, ranged 86-94 days but markedly differed from 70 days of seedling stage which took 73 days (Table 3.4).

It should be noted that all treatments of seedling growth stages when grown at 24 ^{o}C took longer times for flowering development, ranged 114-118 days (averaged 115 days). Comparing to growing at 26 ^{o}C, seedling growth stages of 40 and 50 days

were slightly different, ranged 84-87 days but different obviously from 60 and 70 days of seedling growth stages which took 97 and 103 days (Table 3.6).

Table 3.4 Tillering, booting and flowering date of TGMS line grown at 22 °C in mini- phytotron condition.

Days after sowing	Tillering date(days)	Booting date(days)	Flowering date(days)
40	23	81	94
50	23	91	93
60	23	71	86
70	23	72	73
Average	23	79	87

Table 3.5 Tillering, booting and flowering date of TGMS line grown at 24 °C in mini-phytotron condition.

Days after sowing	Tillering date(days)	Booting date(days)	Flowering date(days)
40	25	111	118
50	25	109	115
60	25	109	114
70	25	109	114
Average	25	110	115

Table 3.6 Ttillering, booting and flowering date of TGMS line grown at 26 °C in mini-phytotron condition.

Days after sowing	Tillering date(days)	Booting date(days)	Flowering date(days)
40	25	74	87
50	25	81	84
60	25	94	97
70	25	99	103
Average	25	87	93

3.3.4 Plant height development

Table 3.7 shows the plant height development of TGMS line of different seedling growth stages grown under different temperature conditions. Results indicated that 40 days and 50 days seedling growth stages gave slight difference in plant height, ranged 73.3-66.7 cm. but were different obviously from 60 days and 70 days of seedling growth stages which gave 82.5 and 91.7 cm., respectively.

TGMS line which was grown at 24 ^{o}C also showed slight difference in plant height development among the four seedling growth stages, ranged 90.0-105.0 cm. but different results obtained from growing at 26 ^{o}C suggested that plant height development among the seedling growth stages were obviously different. Plant height of 40, 50 and 60 days showed slight difference, ranged 90.0-106.7 cm. but higher than plant height of 70 days which gave only 77.5 cm.

By comparing the development of plant height of TGMS line when grown under different levels of temperatures, it suggested that TGMS line growing under cool condition (22 ^{o}C) developed shorter plant height than when grown under warm condition (24 ^{o}C and 26 ^{o}C). Average plant height obtained from growing at 22 ^{o}C, 24 ^{o}C and 26 ^{o}C were 78.5, 96.3 and 94.0 cm, respectively.

Table 3.7 Plant height of TGMS line grown under different temperatures in mini-phytotron condition.

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Days after sowing	Plant height (cm.)			
	22 °C	24 °C	26 °C	Average
40	73.3	90.0	106.7	90.0
50	66.7	100.0	101.7	89.5
60	82.5	90.0	90.0	87.5
70	91.7	105.0	77.5	91.4
Average	78.5	96.3	94.0	89.6

3.3.5 Tillers per plant development

Table 3.8 shows the tillers per plant development of TGMS line at harvest when grown under different temperature conditions. Results indicated that each seedling growth stage gave varying tiller per plant development within temperature level growing conditions. Growing at 22 ^{o}C condition, TGMS line gave tillers per plant which ranged 12.7 to 20.5 tillers per plant comparing to growing at 24 ^{o}C and 26

^{o}C growing conditions which gave 3.0 to 11.5 tillers per plant and 3.5 to 8.0 tillers per plant, respectively.

It should be noted that TGMS line when grown under cool temperature (22 ^{o}C) developed tiller per plant more than when grow under warm temperature (24 ^{o}C and 26 ^{o}C) conditions. Average tillers per plant obtained from growing at 22 ^{o}C, 24 ^{o}C and 26 ^{o}C were 15.7, 5.8 and 6.1 tillers per plant, respectively.

Table 3.8 Tiller per plant of TGMS line at harvest when grown under different temperatures in mini- phytotron condition.

Days after sowing	Tillers per plant			
	22 °C	24 °C	26 °C	Average
40	16.7	3.0	7.0	8.9
50	13.0	6.0	8.0	9.0
60	20.5	3.0	3.5	9.0
70	12.7	11.5	6.0	10.1
Average	15.7	5.8	6.1	9.3

3.3.6 Panicles per plant development

Table 3.9 shows the ability of panicle per plant production of TGMS line at harvest when grown under different temperature conditions. Results were quite similar to tillers per plant development that each seedling growth stage gave varying number panicles per plant within temperature level growing conditions. Growing at

22 ^{o}C gave 9.0 to 18.0 panicles per plant compared to growing at 24 ^{o}C and 26 ^{o}C which gave 1.0 to 10 panicles per plant and 3.5 to 8.0 panicles per plant, respectively.

As well, TGMS line when grow under cool temperature (22 ^{o}C) condition was able to produce panicles per plant higher than when grow at warm temperature (24 ^{o}C and 26 ^{o}C) conditions. Average panicles per plant produced from growing at 22, 24 and 26 ^{o}C were 13.8, 3.7 and 6.2 panicles per plant, respectively.

Table 3.9 Panicles per plant of TGMS line at harvest when grown under different temperatures in mini- phytotron condition.

Days after sowing	Panicles per plant			
	22 °C	24 °C	26 °C	Average
40	16.0	1.0	6.0	7.7
50	9.0	3.0	7.3	6.4
60	18.0	1.0	3.5	7.5
70	12.3	10.0	8.0	10.1
Average	13.8	3.7	6.2	7.9

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3.4 Discussion and Conclusion

Study on the critical temperature and stage of growth for inducing TGMS trait in T29s rice variety revealed that TGMS trait was induced if plants were grown under the higher than 24 ^{o}C. The critical growth stage for TGMS trait to be induced was about 60-70 days after seed sowing or 10-15 days after floral initiation. These results agreed with Latha *et al*. (2004) that fertility of TGMS trait was highly influenced by daily maximum temperature, followed by average minimum temperatures. The results were further reported that TGMS trait of T29s to be induced for male sterility was about 60-70 days after seed sowing or about 10-15 days after floral initiation. These works were also supported by Nguyen Tri Hoan (2005) that 26 ^{o}C temperature was the critical temperature for expressing TGMS trait of T29s variety. Plants could restore male fertility if grown under 24 ^{o}C and transform to male sterility if temperature became higher than 26 ^{o}C. Maruyama *et al*. (1990) also reported that T29s variety possessed TGMS trait and could induce male sterility about 60 days after seed sowing if plants were grown under high temperature.

Apart from studying the critical growth stage and critical temperature inducing TGMS trait in T29s variety, some important agronomic characters such as tillering, panicle production abilities, plant height, booting stage and flowering stage as influenced by different temperatures in control environments were also evaluated. Results obviously indicated that TGMS line if grown under cool temperature (22 ^{o}C) was able to produce more tillers per plant and panicles per plant than when grown under warm temperatures (24 ^{o}C and 26 ^{o}C) (Tables 3.8 and 3.9). Plants grown under cool temperature condition had ability to develop more tillers and panicles per plant since this TGMS line had its origin in cool area in China (Yuan Long Pin, 2005,

personal contact) and cool temperature had an influence on delaying of plant growth and development, resulting in more tillers and panicles per plant developed. However, cool temperature might have an effect on reducing plant height (Table 3.7) but prolonged days to flowering of TGMS line (Table 3.4). Thus, for maintaining the TGMS line, cool areas where temperature during the floral initiation was lower than 24 ^{o}C should be selected for planting. In Thailand, paddy field on the highland areas where weather is cold during the winter season will be the suitable places for maintaining male fertility of TGMS line and also is suitable for developing plant growth and development in providing good selfed-seed production.