Chapter 4

Inheritance of thermo-sensitive genic male sterile characteristic

4.1 Introduction

There were several studies on inheritance of TGMS characteristic which had been reported. The studies reported that one recessive gene controlled TGMS characteristic, by studying in F_2 population, fertile to sterile ratio was 3:1. (Lang *et al*., 1997; Dong *et al*., 2000; Jia *el al*., 2001; Lopez *et al*., 2003). Haohua *et al*. (2006) studied on fertility behavior of rice (*Oryza sativa*) lines with dominant male sterile gene and inheritance of sterility and fertility restoration in Pingxiang male-sterile rice (PMSR) by using progeny populations created between PMSR and 11 fertile lines. It was found that the male sterility was determined by two interacting (epistatic) dominant nuclear genes, one for sterility and another one for fertility restoration. The dominant sterile gene expressed as male-sterility when existing solely, but as normally fertile when coexisting with the restoration gene. The individuals with only the restoration gene are normal and fertile. There were several papers studying in this trait but not any paper reported about T29s variety.

Thus, experiment in this chapter was aimed to study the inheritance of TGMS characteristic in TGMS lines of T29s rice variety.

Location and experimental period: This experiment was conducted at the experimental field of Almatha Seeds Co., Ltd., Maesuay District, Chiang Rai Province and at the evaporated glass house and laboratory of Maejo University, Sansai District, Chiang Mai Province, during November 2003 to September 2005.

4.2 Materials and methods

The inheritance patterns of sterility in TGMS lines were studied by crossing between T29s (TGMS line) with Thai rice KDML 105 variety (non-TGMS line) during the summer season in 2003. The F_1 progenies were raised along with their parents. The pollen and spikelet fertilities were recorded on 10 random plants. The F_1 progenies were selfed to obtain an F_2 population. There were 150 plants in F_2 population. Studied materials including F_2 and their both parents were planted in the field under short day duration. The data were collected such as plant number of TGMS plants and morphological plant characteristics such as color of culm, auricle, epiculi and stigma. The F_2 populations were evaluated for their pollen and spikelet fertilities under high-temperature condition. The segregating F_2 plants were grouped into sterile and fertile. The goodness of fit to Mendelian segregation of fertility and sterility in segregating population was tested by using Chi-square test.

Chi-square (χ^2 - test) parameter is calculated by using formula:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e}$$

where o_i = observed value
e_i = expected value
i = 1, 2, …, n

The experiment was conducted under short day length duration because KDML105 variety is sensitive to short day length but segregating F_2 plants exhibited both flowering and non-flowering character in long day length. In order to obtain all flowering of F_2 plants, genetic materials then were grown in short day length condition.

4.2.1 Pollen viability test

Pollen viability test was made by staining pollen with 1% iodine–potassium (KI_2) solution just before counting pollens under microscope. There were three random fields of microscope on cover slip for counting stained pollens and unstained pollens for averaging sterile percentage. Round and dark brown-stained pollen was scored as fertile, and irregular-shaped, small and yellowish or light brown pollen colored grains as sterile (Figure 4.1). About 200 to 300 pollen grains were scored from three randomly-chosen fields on each slide (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 pollens and/or seed set were considered as sterile, whereas plants having more than 50% stained pollens and/or seed set were classified as fertile plants (Dong *et al*., 2000).

(a) Sterile pollens (b) Fertile pollens

Figure 4.1 (a) sterile pollen (pale color) compared to (b) fertile pollen (blue color) after staining with 1% KI_2 solution.

Scale description for fertility and sterility levels is presented in Table 4.1. χ^2-tests was calculated to determine the fitness of the F_2 generation to a 3:1 ratio. The clearly sterile and fertile individual plant was observed from the original population of individual bases on the fertility behavior and F_2 segregation pattern.

Table 4.1 Male sterility grouping scale as described by Dong *et al*. (2000).

Scale Description	Pollen sterility (%)
Complete sterile	100
Highly sterile	99.0-99.9
Sterile	95.0-98.9
Partially sterile	70.0-94.9
Partially fertile to fertile	<70

4.2.2 Correlation test

T29s (TGMS line) possessed some special morphologies such as red culm, red auricle, dark purple stigma and red apiculi. These characters were also morphological markers in TGMS trait. Thus, parents, F_1, F_2 and BC_1F_1 populations were used for studying the association between these morphological markers with TGMS trait.

4.3 Results

The results indicated that F_2 plants segregated to TGMS characteristic (sterile plant) and normal character (fertile plant) as shown in Table 4.2. KDML 105 exhibited all fertile plants but T29s were all sterile plants. F_2 plants exhibited 2.16 for

male fertile: 1 male sterile ratio which fitted significantly by with the Mendelian ratio of 3:1 by chi-square test ($\chi^2_{0.01,1}$ = 6.635). This implied that TGMS characteristic of T29s variety was controlled by one recessive gene.

Table 4.2 Amount of fertile and sterile plants of parental parents and their F_2 generation derived from crossing between T29s and KDML105 parents, materials were grown under high temperature above 26 ^oC and short day length condition.

Population	Fertile	Sterile	Total	χ 2	Ratio of fertile : sterile plants
T29s (female line)	0	40	40		
KDML 105 (male line)	40	0	40		
F_2	97	45	142	3.390	2.16 : 1

$\chi^2_{0.01,1}$ = 6.635

The morphological characters of T29s and KDML 105 parents were also examined in F_2 plant population. The results indicated that in F_2 population, the segregation ratio of red culm of T29s parent: white culm of KDML 105 parent was 2.55:1 and 1:1 ratio for BC_1F_1 to T29s population. These segregation ratios of both populations fitted significantly to the Mendelian ratio of 3:1 and 1:1, respectively, with chi-square test of χ^2 = 6.635. These identifications further revealed that red culm

and red epiculi of T29s were controlled independently by single dominant gene (Tables 4.3 and 4.4).

Population in F_2 plants were observed in terms of relationship between red culm and red auricle, red apiculi and purple stigma character because these characters were always expressed together within the same plant. Results indicated that there were no relationships between these morphological characters with TGMS trait of T29s rice variety.

Table 4.3 Number of red culm with red auricle plants and white culm with white auricle plants in F_1, F_2 and BC_1F_1 population.

Line	White culm and auricle	Red culm and auricle	Total	χ^2	Ratio of red culm:white culm
T29s (female line)	-	126	126		
KDML 105 (male line)	125	-	125		
F_1	-	16	16		
F_2	62	158	210	0.006	2.55:1
BC_1F_1 to KDML 105	41	48	89	0.551	1.1:1

$\chi^2_{0.01,1} = 6.635$

Table 4.4 Number of red apiculi with purple stigma plants and white apiculi with purple stigma plants in F_1, F_2 and BC_1F_1 population.

Line	white apiculi and purple stigma	Red apiculi and purple stigma	Total	χ^2	Ratio of red apiculi: white apiculi
T29s (female line)	-	126	126		
KDML 105 (male line)	125	-	125		
F_1	-	16	16		
F_2	62	158	210	0.006	2.55:1
BC_1F_1 to KDML 105	41	48	89	0.551	1.1:1

$\chi^2_{0.01,1} = 6.635$

4.4 Discussion and Conclusion

Since KDML 105 is a short-day photosensitive rice variety, if crossed with T29s, a non-photosensitive TGMS line, F_2 progenies of this cross will give an interaction of photosensitive with male sterility and 4 groups of progenies will be observed involving (1) non-photosensitive male fertile (2) short-day photosensitive male fertile (3) non-photosensitive male sterile and (4) short-day photosensitive male sterile. These results are supported by the works of Ku *et al*. (2001); Wu *et al*. (2003); Singh and Virmani (1990) that environmental genic male sterility of rice might be caused by temperature and photoperiod factor and interaction of temperature and photoperiod, as well.

In order to avoid photosensitive effect and to evaluate male sterility effect due to temperature factor solely, F_2 plant population was grown under short day and high temperature during hot growing season (January to April). The results revealed that F_2 plants segregated to fertile plants and sterile plants with the ratio of 2.16:1 which fitted significantly to the 3:1 Mendelian ratio. This result implied clearly that TGMS trait of T29s variety was controlled independently by one recessive gene. This study results gave the same effect as reported by Jia *et al*. (2001) that J207s, an TGMS line was completely sterile when temperature was lower than 31 ^{o}C and the genetic analysis indicated that male sterility of J207s was controlled by a single recessive gene which was first named as rtms1. Latha *et al*. (2004) also reported in TS6MRTS9 and TS6IB, two lines of TGMS lines which were developed at Tamil Nadu Agricultural University, India, that male sterility of these two lines was inherited as a monogenetic recessive in F_2 and BC_1F_1 population.

Evaluation of morphological traits of F_2 and BC_1F_1 to KDML 105 populations which were inherited from both parents indicated that red culm and red epiculi which were inherited from T29s parent were controlled independently by monogenic dominance. These dominance traits might be used as morphological markers for selecting male sterile plants in segregating population.

However, the studied morphological characters did not show any association with male sterility in T29s variety since this individual character might be independently controlled by different genes. So that, inheritance of these morphological character was independent from TGMS trait.