Chapter 6

Development of Thai rice TGMS variety

6.1 Introduction

In Thailand research work on hybrid rice has been since 1977 (Sommai, 1978). There were many problems against hybrid rice development project. The big problem was low yield in hybrid seed production so it was not worthwhile in commercialization. At that time, Rice Research Institute, Thailand, tried to produce hybrid rice by developing three-line system that was cytoplasmic male sterile (CMS). In the present study, a breeding program on two-line system, using thermo-sensitive genic male-sterility (TGMS) for producing hybrid rice variety is proposed.

This chapter is aimed to develop TGMS line of KDML 105 which is one of the most popular aromatic Thai rice varieties. KDML 105 possesses very good cooking quality but low yield production (515 kilograms per rai). In order to improve yield of KDML 105, transferring TGMS gene into KDML 105 for developing TGMS KDML 105 lines to produce two-line rice hybrid is the main purpose of this study. Results obtained from the studying in inheritance of TGMS trait of T29s variety in Chapter 4, and use of molecular markers to assist screening of TGMS line in BC_1F_2 population in Chapter 5 are useful for developing Thai rice TGMS lines which will be studied in this chapter.

The study on developing Thai rice TGMS variety has the following objectives:

1. To inherit TGMS gene from T29s, a TGMS variety to Thai rice KDML 105 variety.
2. To evaluate agronomic characters of progenies in back cross generation.
3. To develop promising Thai rice TGMS lines.

Location and experimental period: The experiments were conducted at the evaporation green house of Maejo University, Chiang Mai, at net and plastic houses of Almatha Seeds Co., Ltd. Maesuay district, Chiang Rai province, during December 2004 to April 2006**.**

6.2 Materials and methods

Thai rice variety, KDML 105 was assigned as male parent and crossed to T29s, a TGMS variety to develop F_1 hybrid seeds. Backcross method was used to generate backcross progenies by crossing each BC_nF_1 to KDML 105, its recurrent parent. In each backcross generation, male sterile trait of individual progeny was detected by growing under temperature higher than 26 ^oC. Backcross generations were made to develop BC_1F_1 to BC_4F_1 generation for studying agronomic traits of their backcross progenies. As well, BC_nF_2 were generated by selfing each BC_nF_1 generation. In order to develop Thai rice TGMS lines, seeds of BC_3F_1 were grown under temperature lower than 24 ^oC to produce BC_3F_2 selfed fertile seeds. These BC_3F_2 seeds were planted under temperature higher than 26 ^oC in the subsequent growing season for developing segregants of male sterile and male fertile plants. BC_3F_2 male sterile plants were screened at seed development stage and their plant stems were cut out about one half to remain their ratoons. These ratooning plants then were re-planted under temperature lower than 24 ^{o}C to produce fertile selfed seeds. In order to develop homozygous Thai rice TGMS lines, male sterile lines which were screened from ratooning plants were grown and selfed for two consecutive generations. Besides screening the TGMS trait, some desirable agronomic characters such as earliness, short plant height, tillering ability, grain shape and so on were also evaluated in each backcross generation and among the selected KDML 105 TGMS lines.

To evaluate the sterility percentage of selected KDML 105 TGMS lines, plants were grown under temperature higher than 26 ^{o}C and 1% KI_2 solution was used to stain the pollens and counted the normal and abnormal pollens under the microscope as described in Chapter 4.

6.3 Results

6.3.1 Evaluation of agronomic characters of progenies in backcross generations

6.3.1.1 Plant height of BC_nF_1 and BC_nF_2 generations

Figure 6.1 shows the development of plant height of parents, (KDML 105 and T29s), their F_1 hybrid and BC_nF_1 progenies. Results indicated that plant height of parents, F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 were slightly different from early stage to booting stage (85-95 days) but at maturing stage (119 days), plant height within BC_nF_1 generations was differently observed. BC_1F_1 showed highest plant height followed by BC_4F_1, BC_3F_1, BC_2F_1 and F_1 with 100.29, 93.79, 112.0 and 65.18 cm respectively. It was observed that plant height of BC_nF_1 generations was slightly different from their KDML 105 parent but different obviously from their TGMS parent (T29s). For F_1 hybrids, plant height was shorter than both parents and their succeeding backcross progenies at each stage of growth.

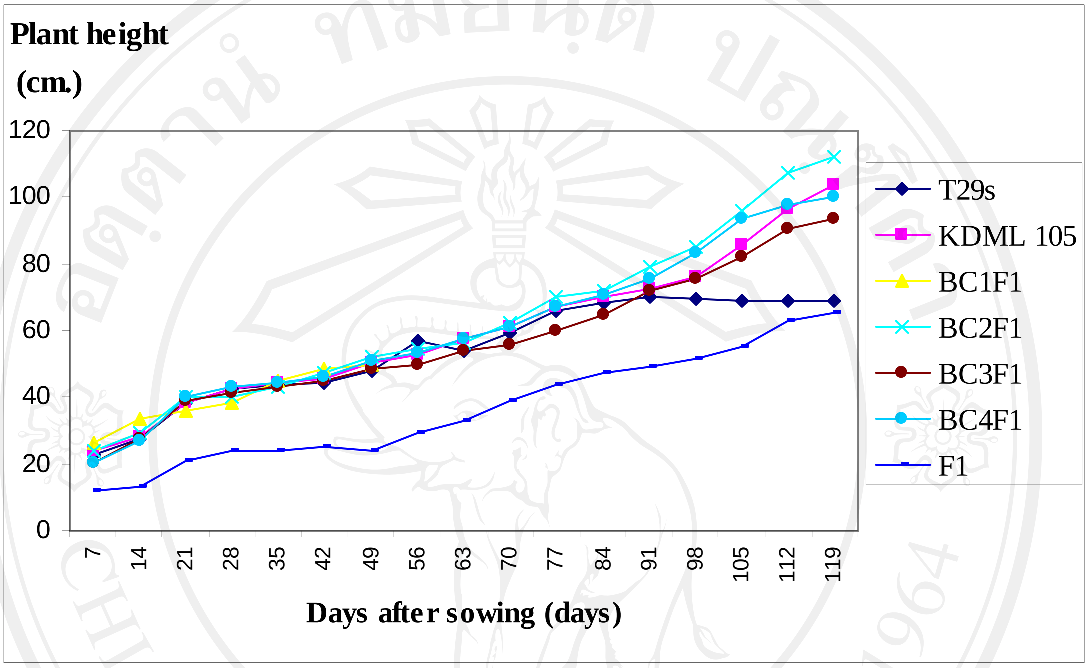

Figure 6.1 Plant height of T29s, KDML 105, F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 populations.

Figure 6.2 shows the development of plant height of parents, F_2 and BC_nF_2 generation. Results indicated that plant height of F_2 and BC_nF_2 progenies were slightly different at maturing stage and their plant heights were between their parents with average of 80-90 cm.

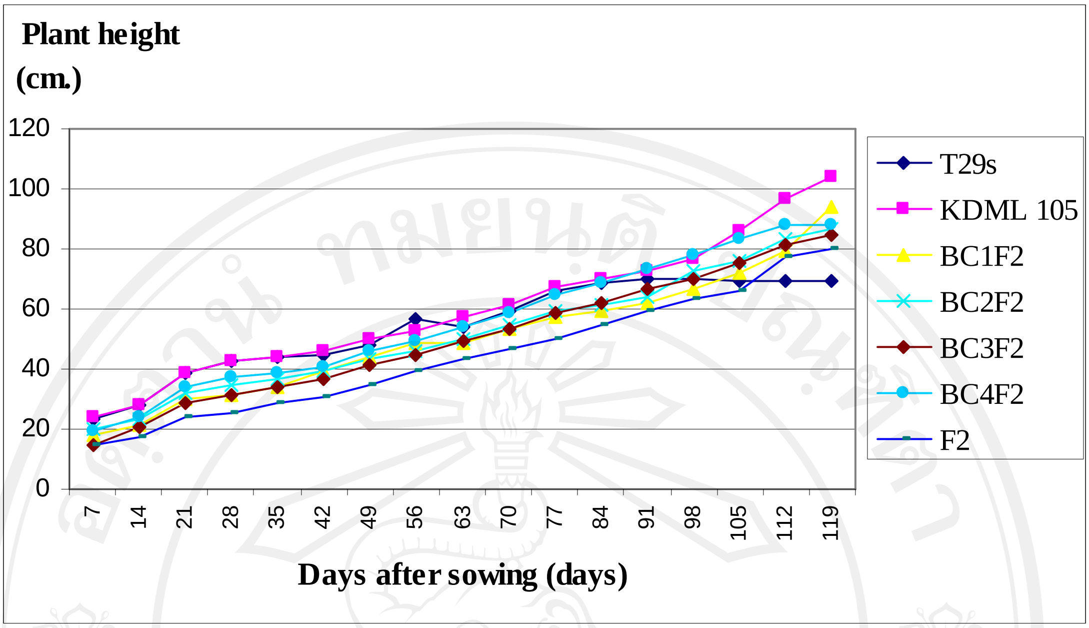

Figure 6.2 Plant height of T29s, KDML 105, F_2, BC_1F_2, BC_2F_2, BC_3F_2 and BC_4F_2 populations.

6.3.1.2 Tillering ability of BC_nF_1 and BC_nF_2 generations

Figure 6.3 shows tillering ability of parents, F_1 and BC_nF_1 generations at each stage of growth. Results indicated that at maturing stage, BC_3F_1 produced highest tiller / plant (32 tillers / plant) and were different from their parents, F_1 and other BC_nF_1 progenies. It should be noted that the number of tiller/plant produced by F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 were between their respective parents, averaged 14-22 tillers / plant. TGMS parent (T29s) could produce more tillers than KDML 105 parent (24 VS 9 tillers / plant).

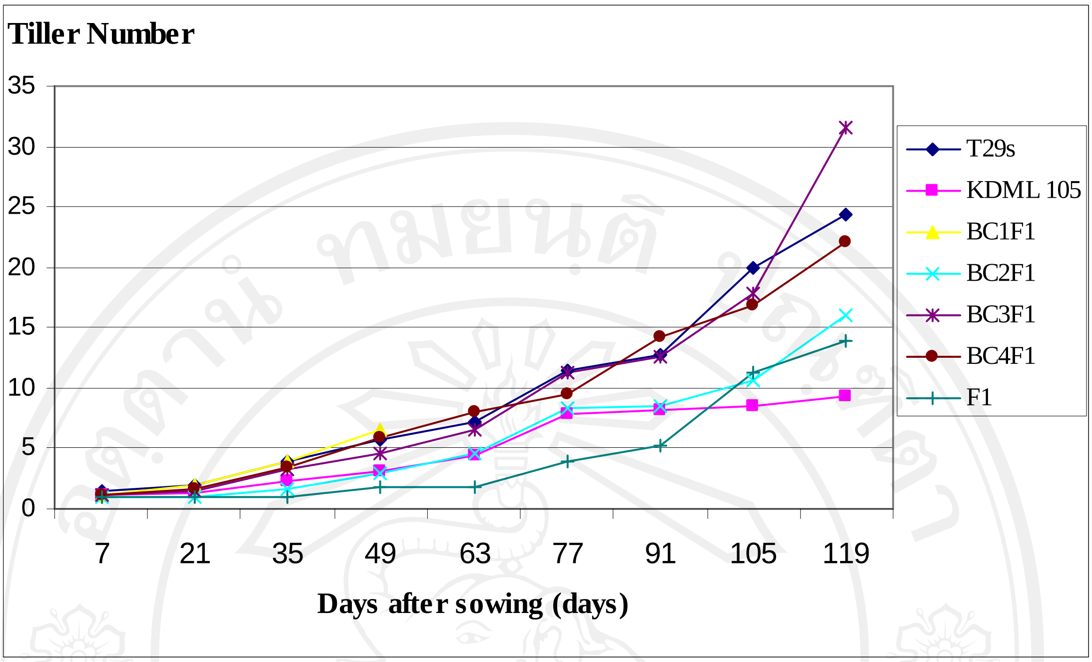

Figure 6.3 Average tillers per plant of F_1, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 populations.

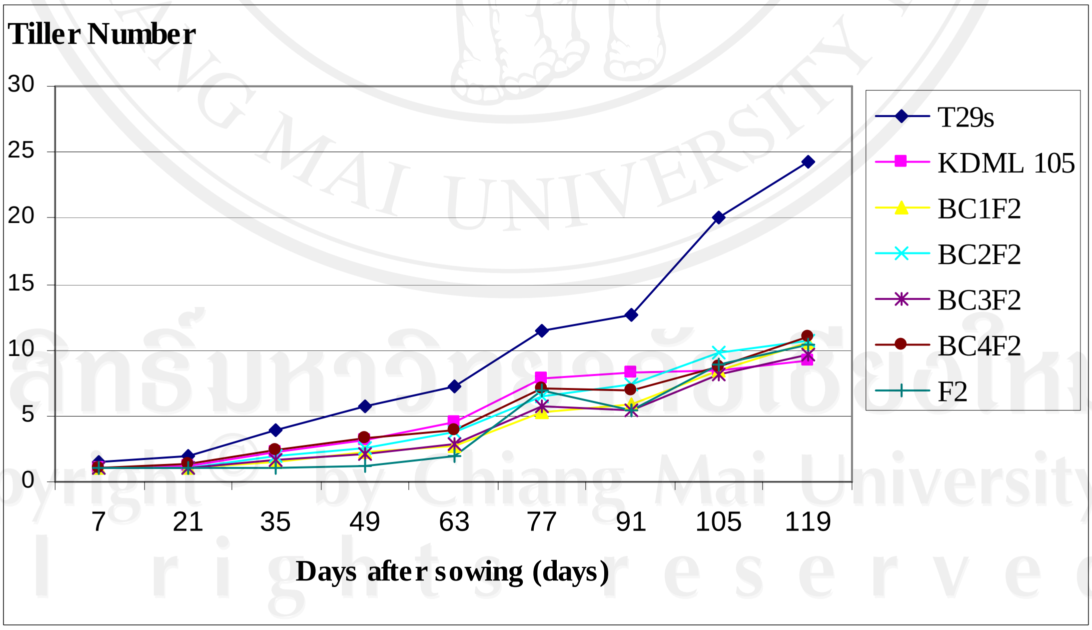

Figure 6.4 Average tillers per plant of F_2, BC_1F_2, BC_2F_2, BC_3F_2 and BC_4F_2 populations.

Fig 6.4 shows tillering ability of parents, F_2 and BC_nF_2 generations. It was found that at maturing stage, both F_2 and BC_nF_2 progenies could produce 10-14 tillers per plant and were between their parents. The same results as BC_nF_1, TGMS parent could produce more tillers / plant than KDML 105 parent (24 VS 8 tillers / plant).

6.3.1.3 Grain dimensions of BC_nF_1 and BC_nF_2 generations

Table 6.1 shows the distribution of paddy grain length of parents, selfed F_1, selfed F_2 and BC_nF_1 generations. Results indicated that paddy grain length of selfed F_1, selfed F_2, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 were slightly different from each other and were between their parents with average of 0.857-0.919 cm, but were shorter than BC_2F_1 and KDML 105 parents which averaged 0.991 and 0.973 cm, respectively.

Table 6.1 Variation in paddy grain lengths of parents, selfed and backcross generation.

Paddy grain length (cm)	T29s	selfed F_1	selfed F_2	selfed BC_1F_1	selfed BC_2F_1	selfed BC_3F_1	selfed BC_4F_1	KDML 105
0.6	3		1	1		1		
0.7	76	16	4	9	3	8	53	1
0.8	291	182	212	121	17	123	268	32
0.9	30	186	176	227	187	231	71	207
1.0		16	6	42	169	37	7	152
1.1			1		24		1	7
1.2								1
Mean	0.834	0.898	0.887	0.919	0.991	0.919	0.857	0.973
Std	0.046	0.058	0.049	0.060	0.064	0.060	0.056	0.058

Table 6.2 shows the dimensions of paddy grain and brown grain of parents, F_2 and BC_nF_2 generations. Results of study indicated that paddy grain dimensions were slightly different among parents, F_2 and BC_nF_2 generations. Average paddy grain width, length and thickness for parents, F_2 and BC_nF_2 ranged 0.22-0.23 cm, 0.86-0.99 cm and 0.16-0.17 cm, respectively. These paddy grain dimensions were comparable to their recurrent parent, KDML 105 which possessed grain width, length and thickness of 0.22, 0.97 and 0.16 cm, respectively.

For brown grain dimensions, average grain width, length and thickness among parents F_2 and BC_nF_2 generation were slightly different, as well. Grain width, length and thickness of F_2 and BC_nF_2 generations averaged 0.19, 0.64 and 0.15 cm, respectively, compared with their respective parents, T29s and KDML 105 which possessed grain length, width and thickness of 0.19, 0.59 and 0.15 and 0.18, 0.67 and 0.14 cm, respectively.

It should be noted that brown grain dimensions of F_2 and BC_nF_2 were close to their respective KDML 105 Thai rice parent, especially, grain length which was an important character for considering the grain quality of rice production.

Table 6.2 Dimension of paddy grain and brown grain of parents, F_2 and BC_nF_2 generation.

Generation	Paddy grain (cm)			Brown grain (cm)		
	Width	Length	Thick	Width	Length	Thick
F_2 seed	0.23	0.9.	0.17	0.2.	0.65	0.15
BC_1F_2 seed	0.22	0.92	0.17	0.19	0.65	0.15
BC_2F_2 seed	0.22	0.99	0.17	0.19	0.69	0.15
BC_3 F_2 seed	0.22	0.92	0.16	0.18	0.64	0.14
BC_4 F_2 seed	0.22	0.86	0.16	0.18	0.59	0.14
Mean	0.22	0.92	0.17	0.19	0.64	0.15
Std	0.00	0.05	0.01	0.01	0.04	0.01
T29s	0.22	0.83	0.17	0.19	0.59	0.15
KDML 105	0.22	0.97	0.16	0.18	0.67	0.14
Mean	0.22	0.90	0.17	0.19	0.63	0.15
Std	0.00	0.10	0.01	0.01	0.06	0.01

Results of study on grain dimensions of both paddy and brown grain clearly indicated that these two kinds of grain size traits could be completely inherited from their respective recurrent parents to their descendent progenies within four backcross generations.

6.3.1.4 Grain weight of BC_nF_2 generation

1,000 paddy grains were randomly selected from parents, F_2 and BC_nF_2 generations for measuring their paddy grain weight, brown grain weight and husk weight. Results are presented in Table 6.3 which indicated that 1,000 paddy grains were slightly different within F_2 and BC_nF_2 generations, ranged 20.00-24.925 gm. (averaged 23.060 gm.) compared with their respective recurrent parent, KDML 105 which averaged 23.450 gm.

For 1,000 brown grains weight, the results were quite similar to 1,000 paddy grains weight. Brown grain weights were not different within F_2 and BC_nF_2 generations, ranged 15.777-19.825 gm. (averaged 18.195 gm.) compared to respective recurrent parent, KDML 105 which averaged 18.675 gm.

The same results were obtained from 1,000 paddy husk weight that the husk weight was not different within F_2 and BC_nF_2 generations, as well; ranged 4.225-5.200 gm. (averaged 4.865 gm) and was slightly higher than their recurrent parent which averaged 4.775 gm.

Results of paddy and brown grain weight evaluation clearly indicated that these two important yield traits could be completely inherited from their recurrent parent to their descendent progenies within four backcross generations.

Table 6.3 Weight of paddy grain, brown rice grain and husk of parents, F_2 and BC_nF_2 generation.

Generation	Paddy grain (gm)	Brown grain(gm)	Husk (gm)
F_2 seed	24.925	19.825	5.100
$BC_1 F_2$ seed	23.600	18.525	5.075
BC_2F_2 seed	24.700	19.500	5.200
$BC_3 F_2$ seed	22.075	17.350	4.725
$BC_4 F_2$ seed	20.000	15.775	4.225
Mean	23.060	18.195	4.865
Std	2.049	1.661	0.400
T29s	21.950	19.075	2.875
KDML 105	23.450	18.675	4.775
Mean	22.700	18.875	3.825
Std	1.061	0.283	1.344

6.3.2 Development of Thai rice TGMS lines

In order to develop complete homozygous TGMS lines, seeds of BC_3F_2 were planted and allowed to self for advancing to BC_3F_3 and BC_3F_4 generations. Then, three TGMS lines were detected by growing under temperature higher than 26 ^oC. Three promising TGMS lines were selected and named: KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3, respectively.

Table 6.4 shows the agronomic characters of three KDML 105 TGMS lines. Results indicated that KDML 105 TGMS-2 gave 178 spikelets / panicle which was higher than KDML 105 TGMS-1 and KDML 105 TGMS-3 which gave 158 and 177 spikelets / panicle, respectively. For panicle / plant and tiller / plant, KDML 105 TGMS-1 gave highest for these two characters, followed by KDML 105 TGMS-2 and KDML 105 TGMS-3.

There were 51, 34 and 23 panicles / plant and 75, 56 and 35 tillers / plant KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3, respectively. Plant heights were observed differently among these TGMS lines. KDML 105 TGMS-1 possessed short plant height of 85.0 cm. while KDML 105 TGMS-2 and KDML 105 TGMS-3 were classified as medium and tall plant height with 113.0 cm and 130.0 cm, respectively. For days to flowering, it was slightly different among the TGMS lines. They were 80, 77 and 75 days after sowing for KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3, respectively.

It was interesting to observe that these three KDML 105 TGMS lines possessed some important yield components such as spikelet/panicle, tiller/plant and panicle/plant which were higher their recurrent KDML 105 parent. At the same time, plant heights of these TGMS lines were shorter than KDML 105 parent which should be beneficial to prevent yield losses due to stem lodging. As well, days to flowering was shorter than KDML 105 parent and this character would be important for avoiding drought effect and could be cultivated for more growing seasons a year for hybrid rice production (Table 6.4 and Figure 6.5).

Table 6.4 Agronomic characters of Thai rice TGMS lines.

TGMS lines	Plant height (cm.)	Days to flowering	Tiller per plant	Panicle per plant	Spikelet per panicle
KDML 105 TGMS-1	85	80	75	51	158
KDML 105 TGMS-2	113	77	56	34	178
KDML 105 TGMS-3	130	75	35	23	177
Average	109	77	55	36	171
KDML 105	140	98	9	8	134

Male sterility plants were detected by planting the TGMS lines under temperature higher than 26 ^{o}C. Results indicated that promising TGMS lines of KDML 105 TGMS-1, KDML 105 TGMS-2, KDML 105 TGMS-3 and their TGMS female parent (T29s) gave 100 percent of pollen sterility (100 percent pollen sterility of lines was classified as complete male sterile lines as described by Dong *et al*. (2002) and presented in Table 4.1). For restoring male fertility of these TGMS lines, remnant seeds of these TGMS lines were grown under cool weather on high land at Doi Chang where average temperature was lower than 24 ^{o}C. (elevation of Doi Chang is about 1,300 m above mean sea level). High average percent seed set, 91.2-92.7 percent was obtained from these three TGMS lines compared with their respective male sterile parent (T29s) and male fertile parent (KDML 105) which gave high seed set as well, averaged 93.4 and 97.7 percent, respectively (Table 6.5)

(a) (b) (c)

Figure 6.5 Three KDML105 TGMS lines which showed differences in agronomic characters when grown under low temperature (below 24 ^{o}C), (a) KDML105 TGMS-1 (b) KDML 105 TGMS-2 and (c) KDML 105 TGMS-3.

Table 6.5 Average seed set percentage of TGMS lines compared with their parents when planted under temperatures lower than 24 ^{o}C and higher than 26 ^{o}C.

TGMS lines	Average seed set percentage	
	Planted at lower than 24 ^{o}C	Planted at higher than 26 ^{o}C
KDML 105 TGMS-1	92.1	0
KDML 105 TGMS-2	92.7	0
KDML 105 TGMS-3	91.2	0
Average	92.0	0
T29s	93.4	0
KDML 105	97.7	98.5

6.4 Discussion and Conclusion

The main objective of study in this chapter was to develop Thai rice TGMS lines for producing two line rice hybrids. To achieve this goal, backcross method was chosen in breeding program. Some important agronomic characters as well as TGMS gene were evaluated in each succeeding backcross generations, until the agronomic characters were almost completely identified with their recurrent parent and TGMS gene was fully transferred or added. In Chapter 4, results of study obviously revealed that TGMS gene of donor parent was controlled by a single recessive gene so that it was advisable to grow backcross generation to BC_nF_2 to permit the identification of the homozygous recessive TGMS genotype (Briggs and Knowles, 1967). After intense selection was made for the recurrent types of KDML 105 in the early backcross generations (BC_1F_2 to BC_2F_2), it was found that agronomic characters under evaluation such as days to flowering and maturity, grain dimensions and grain weight of backcross progenies were much alike to their respective recurrent parent. When two additional backcrosses (BC_3F_2 and BC_4F_2) were made, almost the agronomic characters were of complete identity with KDML 105, recurrent parent (Tables 6.2-6.4 and Figure 6.4). Due to these observations, selection and evaluation of TGMS lines were stopped after four backcrosses had been conducted.

As suggested by Briggs and Knowles (1967), if the character under transfer was a single or a few recessive genes control, with clear expression in progenies generations, three to four backcrosses were sufficient. Selection might be ineffective after the fourth backcross because all the plants to be selected would be so much alike. Again, as suggested by Briggs and Knowles (1967), the backcross method has been most useful in transferring of character with high heritability. In case of TGMS gene which was controlled by a single recessive gene and was highly heritable to their progenies as results were reported in Chapter 4.

In order to obtain the homozygous recessive TGMS lines, breeding material of TGMS lines of BC_3F_2 were advanced through BC_3F_3 and BC_3F_4 generations and tested for their TGMS trait and restoring of male fertility by growing alternatively under low and high temperature growing season. At the end of BC_3F_4, three promising of Thai rice TGMS lines were successfully developed, namely, KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3. These Thai rice TGMS lines reconstituted the KDML 105 parent in most aspects and TGMS gene had been completely added.