Chapter 5

Study of molecular marker linked TGMS character

5.1 Introduction

Within the last twenty years, molecular biology has revolutionized conventional breeding techniques in all areas. Biochemical and molecular techniques have shortened the duration of breeding programs from years to months, weeks, or eliminated the need for them altogether. The use of molecular markers in conventional breeding techniques has also improved the accuracy of crosses and allowed breeders to produce strains with combined traits that were impossible before the advent of DNA technology (Stuber *et al.*, 1999).

In recent years, marker-assisted selection (MAS) has been reported to be an useful and reliable method for the improvement of disease and insect pest resistance in crop plants (Hanada and Hirai, 2003). In hybrid rice breeding, the transfer of TGMS genes to different backgrounds will be highly facilitated by using the MAS technique in a simple and accurate way with those identified molecular markers linked to TGMS genes. Several TGMS genes have been identified in rice genomes, such as *tms1* to *tms5*, by using different marker techniques (Jia *et al.*, 2001).

The study of molecular marker linking TGMS characteristic of T29s variety is aimed to identify the appropriate molecular markers for selecting TGMS lines in the segregating progeny populations.

Location and experimental period: This study was conducted at the experimental field of Almatha Seeds Co., Ltd., Maesuay District, Chiang Rai Province, at the Biotechnology Laboratory of Maejo University, Sansai District, Chaing Mai Province,

at DNA Technology Laboratory Unit, BIOTECH of Kasetsart University Khamphaengsaen Campus, Nakornpathom and Plant Molecular Genetic Laboratory BIOTECH, NSTDA, Prathumthani Province, during November 2003 to June 2008.

5.2 Materials and methods

5.2.1 Plant materials

The F_2 populations derived from crossing between T29s variety (TGMS line) and KDML 105 (Thai rice variety) were used for phenotyping. Individual plants were prepared for identifying fertile or sterile phenotype. Since KDML 105 is photo-sensitivity variety, 150 F_2 plants were planted at the experimental field both under short day season (less than 10.5 hours a day) and long day season (more than 11 hours a day). Pollen fertility was determined at the anthesis stage by staining with 1% potassium iodide (KI_2) solution. Round and dark brown-stained pollen was scored as fertile, and irregular-shaped, small and yellowish or light brown colored pollen as sterile. 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 spikelets divided by the total number of spikelets. Plants with less than 5% stained pollen and/or seed set were considered sterile, whereas plants having more than 50% stained pollens and/or seed set were classified as fertile (Dong *et al*., 2000). Scale description for fertility and sterility levels is presented in Table 4.1. A sub set of 30 F_2 plants which showed clearly sterile and fertile individual were selected from the

original population of 150 individual bases on the fertility behavior and F_2 segregation pattern and were prepared for identification of the molecular markers linked to TGMS gene of T29s variety.

5.2.2 DNA extraction

DNA of selected F_2 individuals and the parents were extracted by following protocol described by Doyle and Doyle (1987). To identify putative markers linked to TGMS genes, DNA bulk from individuals of two phenotypic extremes as suggested by Michelmore *et al*. (1991). DNA from 30 sterile and fertile individuals will aliquot to constitute fertile and sterile bulks, respectively.

DNA was extracted from approximately 0.6 g of frozen leaf tissue as generally described by Weining and Henry (1995) for F_2 individual genotypes for 30 fertile and 30 sterile. The following modifications were included: 1 ml of 2X CTAB extraction buffer and 600 µl of chloroform / isoamyl alcohol (24:1) were used; DNA was precipitated with 400 µl of 100 % cool ethanol in addition to 0.3 volume of 7.5 M ammonium acetate and then washed twice with 70 % ethanol. The DNA was re-suspended in 200 µl of Tris-EDTA (TE) buffer. DNA preparations were diluted with TE buffer to a final concentration of approximately 50 to 100 ng per µl.

Reagents and chemicals

1. Solution 1: 2 % (w/v) hexadecyltrimethylammonium bromide (CTAB), 100 mMTris-Cl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl
2. Solution 2: 2-Mercaptoethanal
3. Extraction buffer: Solution 1 and solution 2 at a ratio of 500:1 (v/v)

4. 7.5 M ammonium acetate
5. Isoamyl alcohol, chloroform, ethanol (AR grade)

DNA isolation protocol

1. Grind the samples with liquid nitrogen in mortar.
2. Add 600 µl of extract solution and keep the tube in a 37°C water bath for 45 min.
3. Add 600 µl of chloroform / isoamyl alcohol (24:1) and mix for 10 s by Vortexer BR-2000 (BIORAD); keep the tube at room temperature10 min.
4. Centrifuge plate at 14,000 rpm for 1.5 min at room temperature.
5. Carefully transfer 100 µl of supernatant to new tube addition to 0.3 volume of 7.5M ammonium acetate.
6. Add 400 µl of 100 % cool ethanol and mix by inversion several times.
7. Centrifuge plate at 14,000 rpm for 2 min at room temperature.
8. Discard the supernatant and wash the pellet with 70% ethanol (v/v).
9. Dry the pellet by twice centrifugation at 14,000 rpm for 2 min at room temperature then discard the supernatant and evaporation at room temperature in clean bench 2 – 3 hours.
10. Dissolve in 200 µl of TE buffer and directly use as template for polymerase chain reaction (PCR) amplification.

5.2.3 Simple sequence repeat (SSR) markers analysis

A set of 35 SSR primers (Table 5.1) mapping on chromosome 2 was used for bulked segregant analysis. Amplification reactions were performed in 0.2 ml. thin-walled PCR tubes in a thermal cycle programmed for 35 cycles. PCR amplified

products would be subjected to electrophoresis on 8 % polyacrylamide gel. The electronic image of silver nitrate stained gel would be captured using gel documentation (model Syngene Gene Genious).

Master mix preparation for PCR reaction

1. 10x PCR buffer	1.00	µl
2. 25 mM $MgCl_2$	0.80	µl
3. dNTP (1 µm)	2.00	µl
4. Primer forward	0.30	µl
5. Primer reverse	0.30	µl
6. Taq DNA polymerase (1U)	0.20	µl
7. DNA template (10 ng)	2.00	µl
8. deionized water	3.40	µl
Total	10.00	µl

PCR reaction by PCR model MJ Research DYADALD 1244 setting follows:

Predenaturation	94 °C : 3.00 min	1 cycle
Denaturation	94 °C : 0.30 min	35 cycles
Annealing	55 °C : 0.30 min	35 cycles
Extension	72 °C : 2.00 min	35 cycles
Post extension	72 °C : 7.00 min	1 cycle

Table 5.1 Simple sequence repeat (SSR) primers mapping for molecular marker linked TGMS characters.

Entry	Primers	Chromosome number	Annealing Temp (^{o}C)	Expected size (bp)
1	RM106	2	55	297
2	RM110	2	55	156
3	RM112	2	55	128
4	RM138	2	55	233
5	RM145	2	67	215
6	RM154	2	61	183
7	RM166	2	61	321
8	RM174	2	67	208
9	RM203	3,2	55	203
10	RM207	2	55	118
11	RM208	2	55	173
12	RM211	2	55	161
13	RM213	2	55	139
14	RM221	2	55	192
15	RM233A	2	55	162
16	RM236	2	55	191
17	RM240	2	55	132
18	RM250	2	55	153
19	RM254	3,6	55	165
20	RM259	1,2	55	162

Table 5.2 Simple sequence repeat (SSR) primers mapping for molecular marker linked TGMS characters (continue).

Entry	Primers	Chromosome number	Annealing Temp (^{o}C)	Expected size (bp)
21	RM262	2	55	154
22	RM263	2	55	199
23	RM266	2	55	127
24	RM27	2	55	150
25	RM279	2	55	174
26	RM29	2	55	250
27	RM324	2	55	175
28	RM341	2	55	172
29	RM48	2	55	204
30	RM482	2	55	188
31	RM498	2	55	213
32	RM53	2	55	182
33	RM535	2	55	138
34	RM6	2	55	163
35	RM204	6	55	169

PCR products were resolved by electrophoresis in 8 % acrylamine gel containing 4x TBE buffer. Gel electrophoresis was run for 4.5 hours by 3 watt 196 volt and 10-20 mA. After that, gel was photographed by Gel Documentation (model Syngene Gene Genious).

5.2.4 Random amplified polymorphic DNA (RAPDs) marker analysis

OPAC-10 primer was reported for using as molecular marker selection assisted for TGMS trait in population derived from crossing between T29s variety with Suphanburi 1 and T29s variety with RD 11 (Jindasingh, 2006). Thus, this primer was used to identify molecular marker linked TGMS gene in T29s lines.

Master mix preparation for PCR reaction included:

1. 10x PCR buffer	2.50	µl
2. 50 mM $MgCl_2$	1.90	µl
3. dNTP (100 µm)	0.25	µl
4. Primer (10 µm)	1.00	µl
5. Taq DNA polymerase (1U)	0.15	µl
6. DNA Template (5 ng)	5.00	µl
7. deionized water	14.20	µl
Total	25.00	µl

PCR reaction setting as follow:

Pre-denaturation	95 °C : 5 min	1 cycle
Denaturation	95 °C : 1 min	45 cycle
Annealing	35 °C : 1 min	45 cycle
Extension	72 °C : 2 min	45 cycle
Post extension	72 °C : 7 min	1 cycle

The PCR products were resolved by electrophoresis in 1.4 % agarose gel containing 1x TBE buffer. Gel electrophoresis was run for 3 hours by 120 volts and 100 A. After that, gel was photographed by Gel Documentation (model Syngene Gene Genious).

5.2.5 Single-strand conformation polymorphism (SSCP) markers analysis

The SSCP primers were designed by Primer Tree Program (program for design primer) on www.ncbi.nlm.nih.gov in clone AP004039 base on location of RM154 and RM279 (personal communication Dr. Amornthip Mungphrom, 2008). The 7 SSCP primers (Table 5.3) located on chromosome 2 were used for detecting TGMS gene of T29s variety.

Master mix preparation for PCR reaction included:

1. 10x PCR buffer	1.00	µl
2. 50 mM $MgCl_2$	0.40	µl
3. dNTP (100 µm)	1.00	µl
4. Primer forward	1.00	µl
5. Primer reverses	1.00	µl
6. Taq DNA polymerase (1U)	0.05	µl
7. DNA Template (5 ng)	2.00	µl
8. deionized water	3.55	µl
Total	10.00	µl

Table 5.3 SSCP primers (gene) position, melting temperature (TM: ^{o}C), description, PCR product size (bp), single nucleotide polymorphisms (SNP) and original clone.

Primer	Position	TM ^{o}C	Description	PCR product size (bp)	SNP	Clone
Os02g12290	6,397,342-6,399,236.	62.33	nuclear ribonuclease Z, putative	317	9	AP004039
Os02g12300	6,399,355-6,402,186.	61.02	pectate lyase precursor, putative, expressed	275	lot	AP004039
Os02g12310	6,404,018-6,405,879.	62.30	NAC domain-containing protein 18, putative, expressed _ candidate gene	271	4	AP004039
Os02g12350	6,424,555-6,430,620.	61.51	histone deacetylase, putative, expressed	230	lot	AP004039
Os02g12370	6,443,131-6,445,362.	61.63	expressed protein	290	7	AP004039
Os02g12400	6,468,567-6,472,845.	61.22	receptor-like protein kinase precursor, putative, expressed	231	11	AP004039
Os02g12420	6,476,660-6,480,097.	61.70	receptor-like protein kinase precursor, putative, expressed	304	7	AP004039

PCR reaction by PCR model MJ Research DYADALD 1244 setting as follow:

Pre-denaturation	94 °C : 5.00 min	1 cycle
Denaturation	94 °C : 0.45 min	35 cycle
Annealing	55 °C : 0.45 min	35 cycle
Extension	72 °C : 1.00 min	35 cycle
Post extension	72 °C : 7.00 min	1 cycle
Hold	15 °C	

Denature for single stain conformation:

After PCR reaction was finished, adding 20 µl 2x dye in each PCR product tube, then take them on PCR machine again. Setting as follow:

Denaturation	94 °C : 10.00 min
Express cold	4 °C : 8.00 min

The denatured PCR products were resolved by electrophoresis in 8 % acrylamine gel containing 6x TBE buffer. Gel electrophoresis was run for 4.5 hours by 3 watt 196 volts and 10-20 mA. After that, gel was photographed by Gel Documentation (model Syngene Gene Genious).

Gene linkage analysis was determined in the following manners (Kittipat, 2006):

1. Recombinant frequency = ((Fertile plant + Heterozygous plant)/2) / sterile plant number of F_2
2. Linkage percentage (cM) = Recombinant frequency x 100

5.3 Results

5.3.1 Phenotyping and DNA extraction

Evaluation phenotyping trial of F_2 population was conducted during short day length and high temperature in order to avoid day length photosensitivity effect. After finishing testing of male sterillty in F_2 population, it was found that F_2 progenies showed 45 sterile plants from total 142 plants. 35 sterile plants and 30 fertile plants of this population were selected to extract DNA for using as template for polymerase chain reaction (PCR) amplification.

This DNA extractions, were used as template for testing primers; SSR, RAPD and SSCP. Choomsai *et al.* (2006) reported that tms2 gene for TGMS trait of T29s variety located on chromosome 7. But Quiyun Deng (2003, personal communication) at Hunan Rice Research Institute reported that tms5 located on chromosome 2 for controlling TGMS trait in T29s variety. So, above primers were prepared for optimal linked range tms5 gene regions (Figure 5.5).

Since TGMS trait was controlled by one recessive gene and did not express in BC_nF_1 generation, so it needs co-dominant inheritance for detecting this trait. This study was started firstly with SSR analysis.

5.3.2 Simple sequence repeat (SSR) markers analysis

35 SSR primers were analyzed with both parents for testing polymorphisms of F_2 populations. The results indicated that there were 20 SSR primers (Table 5.4) showed polymorphisms between parental lines (Figure 5.1). In Figure 5.1, the labeled lane 5 and the labeled lane 21 belonged to T29s and KDML 105 variety, respectively.

Table 5.4 20 SSR primers showed polymorphisms in F_2 population between their parental varieties.

Entry	Primers	Chromosome number	Annealing Temp (^oC)	Expected size (bp)
1	RM110	2	55	156
2	RM154	2	61	183
3	RM204	6	55	169
4	RM207	2	55	118
5	RM208	2	55	173
6	RM211	2	55	161
7	RM213	2	55	139
8	RM233A	2	55	162
9	RM254	3,6	55	165
10	RM259	1,2	55	162
11	RM262	2	55	154
12	RM263	2	55	199
13	RM266	2	55	127
14	RM279	2	55	174
15	RM324	2	55	175
16	RM341	2	55	172
17	RM48	2	55	204
18	RM482	2	55	188
19	RM53	2	55	182
20	RM6	2	55	163

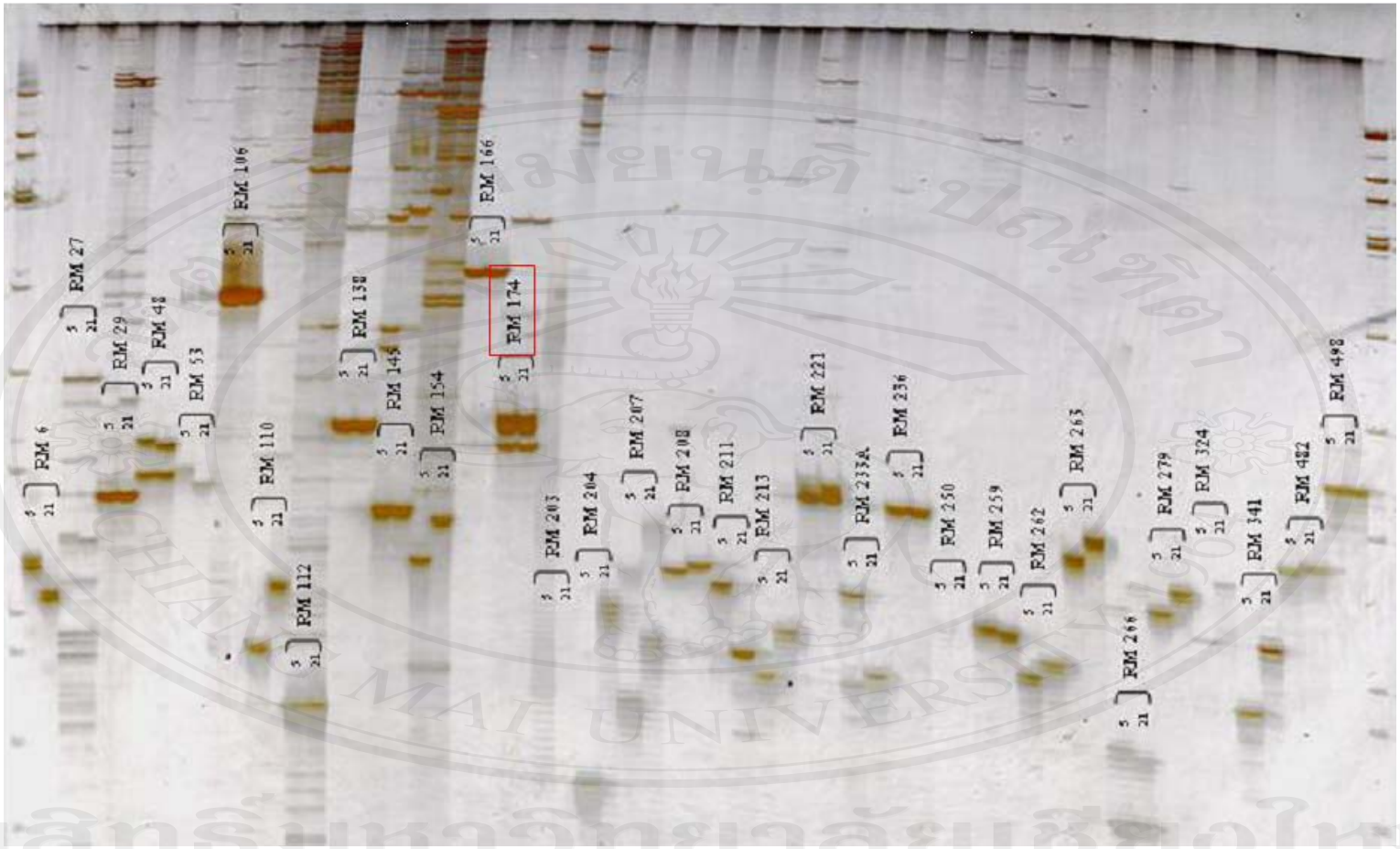


Figure 5.1 SSR primers showed different polymorphisms between parents, labeled lane 5 and lane 21 belonged to T29s and KDML 105 variety, respectively.

After that 10 polymorphism primers including RM110, RM145, RM174, RM204, RM211, RM259, RM263, RM279, RM324 and RM341 were selected to identify the region of tms5 gene which followed morphology map in www.grameme.org . The results indicated that polymorphisms were present between parental lines in RM279 and RM324 primers, but it did not show polymorphisms between parental lines in RM174, even though analysis was made among parental lines, F_1 and F_2 progenies. The remaining primers did not show clearly polymorphisms between fertile progeny group and sterile progeny group (Figure 5.2 and Figure 5.3). Polymorphisms did not show either in any population when analyzed by RM145, RM174 and RM259 primers. This study disagreed with Wang *et al*. (2003) that four SSR primers; RM279, RM327, RM324 and RM492 were closely linked to tms5 gene. Jia *et al*. (2003) also reported that RM174 primer was 0.0 cM linked with tms5 gene.

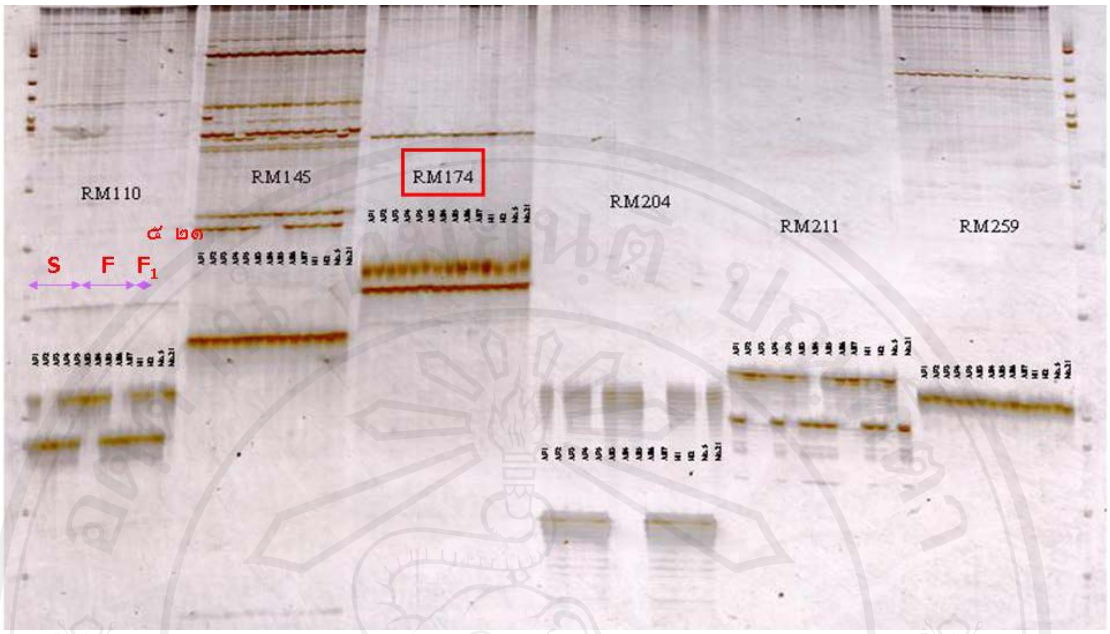


Figure 5.2 Testing of primers for analyzing polymorphisms between sterile progenies (S) and fertile progenies (F) of F_1, F_2 and parental lines.

Figure 5.3 Testing of primers for analyzing polymorphisms between sterile progenies (S) and fertile progeny (F) for F_1, F_2 and parental lines.

5.3.3 Random amplified polymorphic DNA (RAPDs) markers analysis

Results obtained from SSR analysis revealed that all of SSR markers could not detect TGMS trait. So that, RAPD markers was proposed to detect TGMS trait of T29s variety. OPAC-10, a RAPD primer was successful in analysis of TGMS trait since this type of primers is dominant marker, as reported by Jindasingh (2006). In order to solve this problem, OPAC-10 primer was selected to analyze polymorphisms between parents and their progenies. The SCAR marker was further developed following RAPD marker results.

The OPAC-10 primer was analyzed in individual parents and their progenies. Results indicated that polymorphisms did not show in any population. In Figure 5.4, bands of T29s parent (Lane F_1-F_7), pointed by red arrow, were similar to KDML 105 parents (band M1 – M6) and TGMS lines (Lane R1-R6 and L1-L6). Results of this study indicated that OPAC-10 primer could not be used as molecular marker in linking to TGMS trait of T29s variety, since SCAR markers could not develop polymorphisms in the studied populations.

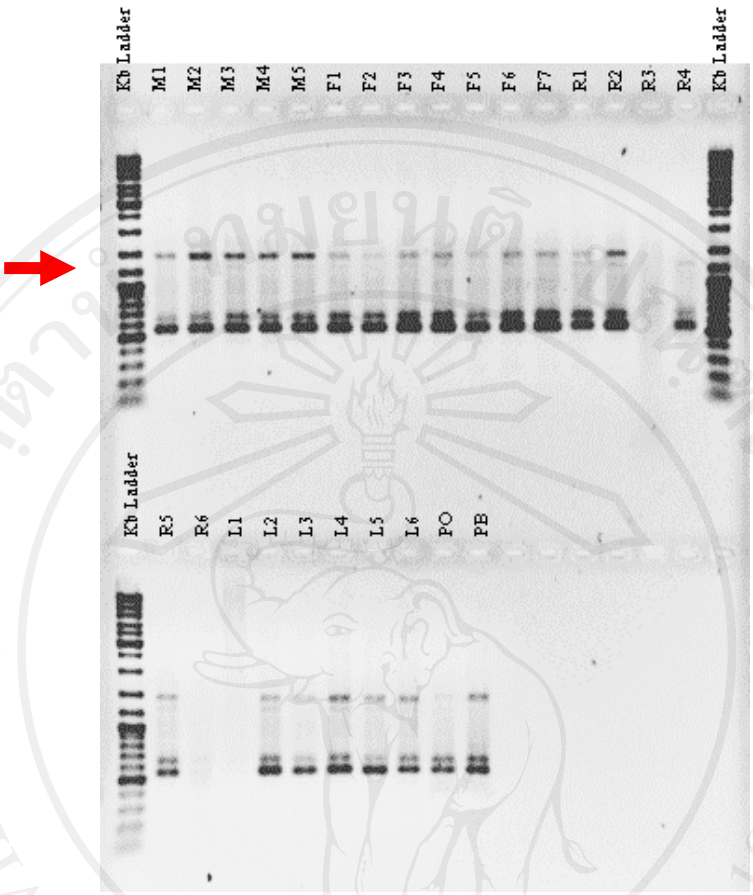


Figure 5.4 Bands of OPAC-10 primer.

Lane M1 – M5 were KDML 105

Lane F1 – F7 were T29s

Lane R1 – R6 and L1 – L6 were TGMS lines

Lane PO and PB were Prathumthani 1

kb Ladder were standard molecular weight marker

5.3.4 Single-strand conformation polymorphism (SSCP) analysis

RM154 and RM279 markers were located for genetic map on chromosome 2 as reported by Boonjaroen (2008). Figure 5.5 shows genetic map of TGMS gene on chromosome 2 based on www.grammene.org. RM154 and RM279 markers linked 24.57 cM and 21.77 cM with TGMS gene, respectively. So that TGMS gene was located in this region. Dr. Amornthip Maungphrom and Miss Chuenjit Boonjaroen (Plant Molecular Genetic Laboratory, BIOTEC), pointed out that RM154 and RM279 markers linked TGMS gene which located in region AP004039 prove. Therefore 7 SSCP markers which were designed by Primer Tree Program (Table 5.3) were prepared to analyze molecular marker linking to TGMS gene for T29s variety.

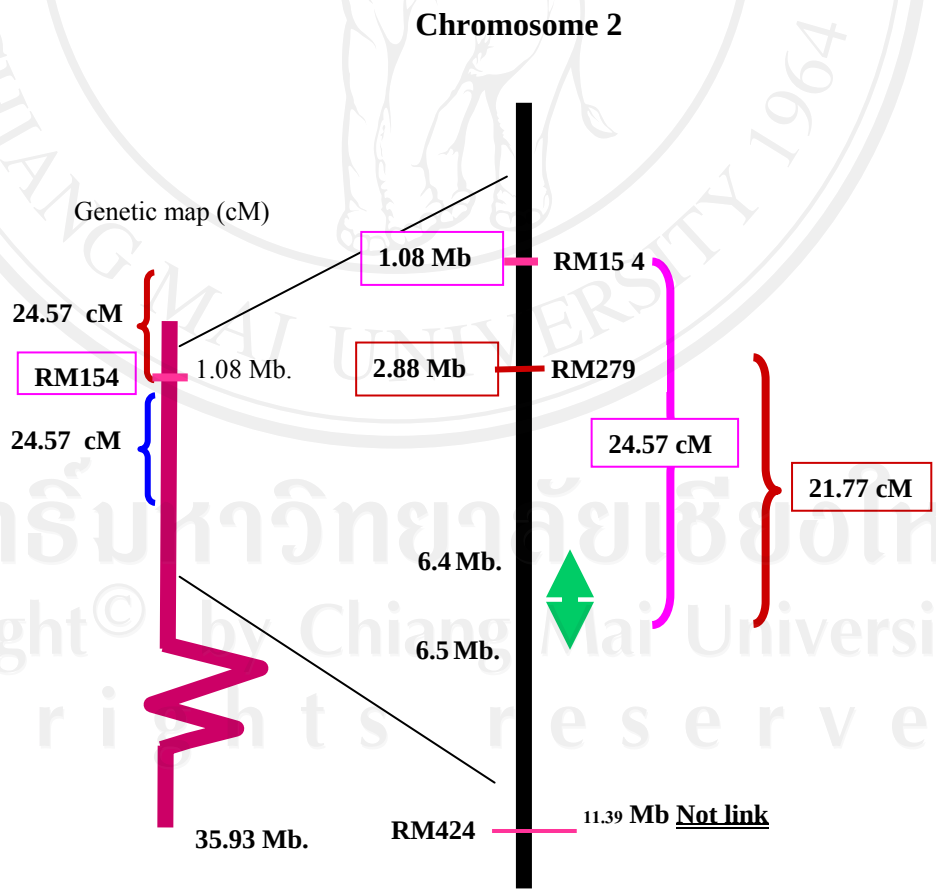
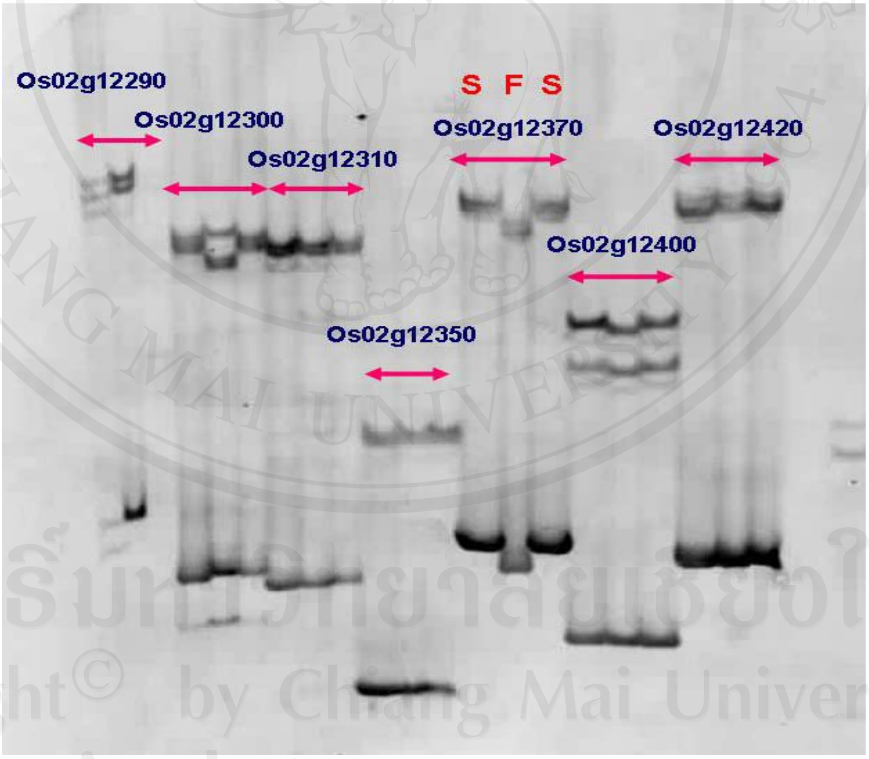


Figure 5.5 Genetic map base on www.grammene.org followed Boonjaroen (2008).

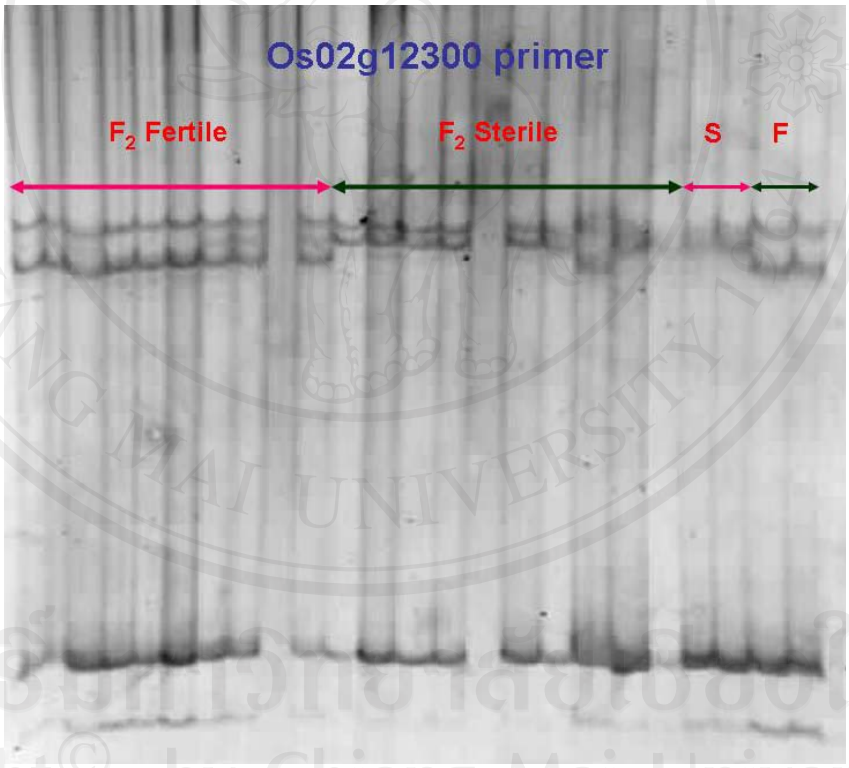
All selected SSCP markers were screened to detecting polymorphisms between parents in each marker. Figure 5.6 shows polymorphisms among parental lines and their TGMS line progenies. First, second and third lanes were T29s, KDML 105 and TGMS line (nucleotide checks), respectively (TGMS line which could detect TGMS trait). Results clearly indicated that Os02g12300 and Os02g12370 primers showed polymorphisms between parents. Band in lane 1 (T29s) and band in lane 3 (nucleotide checks) of these two primers were similar and different markedly with band in lane 2 (KDML 105). Therefore, both primers were selected for screening the TGMS traits in F_2 population.



S = Sterile parent (T29s) and TGMS lines, F = Fertile parent (KDML 105)

Figure 5.6 SSCP primers screening for polymorphisms test among parents and TGMS lines.

There were 10 individual fertile plants and 10 individual sterile plants which were subjected to detecting TGMS gene of T29s parent by using Os02g12300 and Os02g12370 primers. Figure 5.7 presents polymorphisms of Os02g12300 primer for F_2 fertile plants in lanes 1 – 10 and F_2 sterile plants in lanes 11 – 20 compared to lanes 21 – 22 and lanes 23 – 24 which were their respective parents, T29s and KDML 105 ,respectively. This primer showed different polymorphisms between F_2 fertile and F_2 sterile plants. However, polymorphisms of these fertile plants and sterile plants were quite similar.

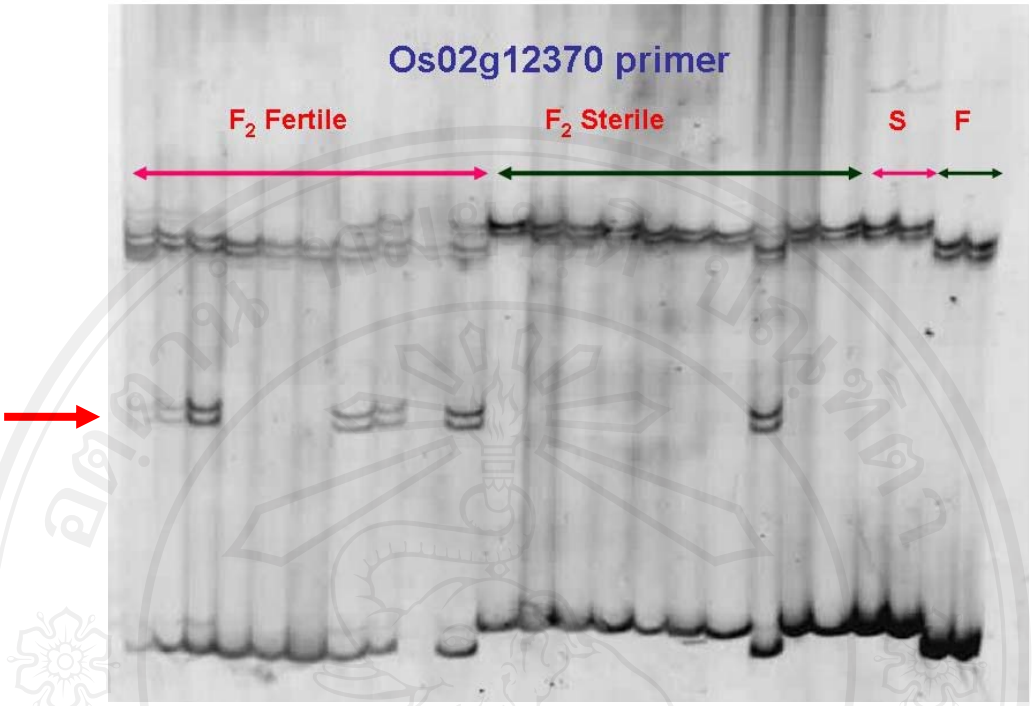


S = Sterile parent (T29s), F = Fertile parent (KDML 105)

Figure 5.7 Os02g12300 primer screening for polymorphism test between individual fertile plants and individual sterile plants in F_2 population compared to their respective parents.

Figure 5.8 presents polymorphisms among F_2 plants and their respective parents which were analyzed by Os02g12370 primer. Results revealed that F_2 fertile plants in lanes 1 – 10 differed from F_2 sterile plants in lanes 11 – 20 and compared with to lanes 21 – 22 and lanes 23-24 which were their parents, T29s and KDML 105. It was found that this primer could develop different bands as observed from F_2 fertile and F_2 sterile plants group. Therefore, Os02g12370 primer should be considered as more effective in analyzing TGMS trait of T29s than Os02g12300 primer. However, Os02g12370 primer showed an alien band (black arrow) in F_2 fertile plants. This band probably belonged to mixed segregating plants. One recombinant band was observed in lane 18 which might be exhibited by recombinant wild type of T29s variety.

In order to confirm whether Os02g12370 primer was actually able to detect the specific region of the TGMS gene location of T29s variety, F_2 progenies derived from T29s x KDML 105 cross were randomly selected and used to screen both sterile F_2 plants and F_2 fertile plants in F_2 population. Results revealed that Os02g1230 primer was able to effectively to screen both sterile and fertile of F_2 plants.

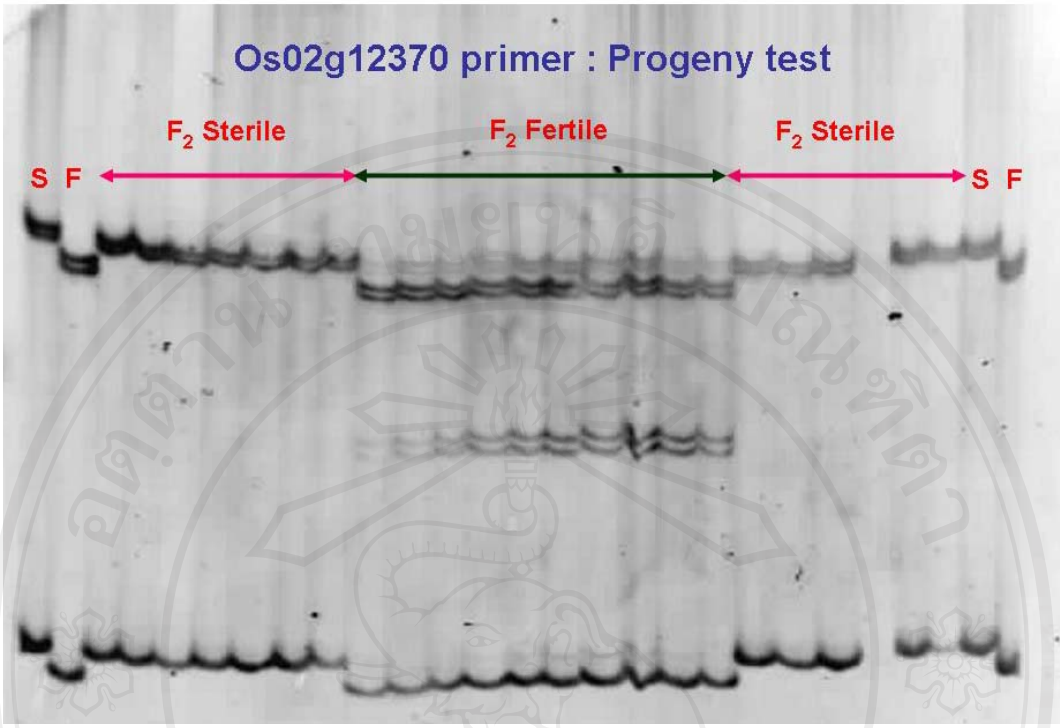


S = Sterile parent (T29s), F = Fertile parent (KDML 105)

Figure 5.8 Os02g12370 primer screening for polymorphism test between individual fertile plants and individual sterile plants in F_2 population compared to their respective parents.

Figure 5.9 shows polymorphisms for random individual sterile and individual fertile plants in F_2 population compared with their respective parents. Lane 1 and lane 26 were sterile parent (T29s), lane 2 and lane 27 were fertile parent (KDML 105), lanes 9-19 were F_2 fertile plants and lanes 3-18 and 20 - 25 were F2 sterile plants.

In conclusion, the accuracy of selecting sterile plants with Os02g12370 primer was 100 percent.



S = Sterile parent (T29s), F = Fertile parent (KDML 105)

Figure 5.9 Os02g12370 primer screening for polymorphism test between random individual fertile plants and individual sterile plants in F_2 population compared to with their respective parents.

Linkage analysis of Os02g12370 primer was calculated to determine molecular marker linked to TGMS gene as follow:

1. Recombinant frequency = ((Fertile plant + Heterozygous plant)/2) / sterile plant number of F_2

   Recombinant frequency = ((1 + 3)/2) / 35 = 0.0571

2. Linkage percentage (cM) = 0.0571 x 100 = 5.71 cM

This result revealed that Os02g12370 primer could bind with the molecular marker and was closely linked to TGMS gene of T29s variety with a recombination frequency of 0.0571 and linked to TGMS gene with a genetic distance of 5.71 cM. Linkage of Os02g12370 primer with TGMS gene of T29s variety was located on chromosome 2 and TGMS gene was tms5 gene as suggested by Maungphrom (2008) and Boonjaroen (2008).

5.4 Discussion and Conclusion

Results obtained from Chapter 4 studying the inheritance of male sterility of T29s clearly indicated that TGMS trait was controlled by a single recessive gene. Since this TGMS trait was controlled by a single recessive gene, thus selection of male sterile plants could not be made in $BCnF_1$ generation. By using backcross method, breeders have to delay for one generation or one growing season in order to develop BC_nF_2 generation which homozygous recessive male sterile plants can be observed.

Molecular marker linking to TGMS gene of T29s rice variety was used for selecting TGMS lines in the segregating population. The advantages of molecular marker to detect the TGMS gene are that it is superior to other forms of MAS because it is relatively simple to detect, abundant through out the genome even in highly-bred cultivars, completely independent of environmental conditions and can be detected at virtually any stage of plant development. In case of SSR analysis, 35 primers could not be used as molecular marker assisted in TGMS lines selection in BC_nF_2 generation derived from crossing between T29s and KDML 105 because this SSR primer is simple sequence nucleotides which these selected 35 SSR primers could not

be detected probably due to the fact that they could not bind the target region of TGMS gene locus and the molecular markers. In addition, molecular marker to be identified by these primers may distantly link to the target region where the TGMS gene of T29s rice variety is located. Thus, polymorphisms among the sterile and fertile plants in segregating population did not develop. These results did not agree with Wang *et al*. (2003) reported that four SSR primers: RM 279, RM 324, RM 327 and RM 492 were closely linked to tms5 gene (TGMS gene). Jia *et al*. (2006) also reported that RM 174 primer linked very tightly with tms5 gene with a genetic distance of 0.0 cM.

For RAPD primer, OPAC-10 could not develop the polymorphisms and could not assist in selecting TGMS lines in the segregation population. Interpretations of these results were that RAPD analysis was used for random amplification of polymorphic DNA's segments but in this case of study, the RAPD primer used did not show any polymorphisms in the segregating population which was probably due to failure to amplify the target area of DNA segments where TGMS gene is located.

In addition, RAPD markers used for this study might produce repetitive sequences rather than producing polymorphic DNA of the target regions. Wang *et al*. (1995) studied in bulked segregant analysis of F_2 population to identify RAPD markers linked to the TGMS gene for the 400 RAPD primers screened for polymorphisms, and reported that most of RAPD primers were of repetitive sequences, only two single-copy sequences were found. In contrast with Jindasingh (2006)'s work that OPAC-10, a RAPD primer was successful in analysis of TGMS trait derived from two crosses; T29s x Suphanburi and T29s x RD10, since this type of primer is dominant marker type.

In order to find out the problems, seven SSCP primers were selected to screen TGMS trait in F_2 bulk population of T29s x KDML 105 cross. Details of these 7 SSCP primers are listed in Table 5.3. Result of study revealed that two primers; Os02g12300 and Os02g12370 could assist to screen TGMS trait in BC_1F_2 population. However, Os02g12370 primer was considered as more effective in analyzing TGMS trait than Os02g12300. It could distinguish clearly sterile from fertile plants with 100 percent accuracy. This os02g12370 primer could link molecular markers closely to TGMS gene of T29s rice variety with the random recombination rate of 0.0571 and linked to TGMS gene with a genetic distance of 5.71 cM. As well, this TGMS gene was tms5 gene and located on chromosome 2 as suggested by Maungphrom (2008) and Boonjaroen (2008).

In conclusion, Os02g12370 primer could precisely screen TGMS trait in BC_1F_2 generation of T29s x KDML 105 cross. Thus, this primer will be profitable in improving and developing two-line rice hybrid varieties by employing TGMS gene.