Chapter 7

General discussion and Conclusion

Study on the critical temperature and stage of growth for TGMS trait expression of T29s rice variety was carried out under control condition of evaporation greenhouse and modifier mini-phytoton during March 2004 - February 2005. Results obviously indicated that TGMS trait expressed male fertility when plant development was lower than 24 ^{o}C but expressed male sterility if plants were grown in warm condition with temperature higher than 26 ^{o}C. The results further indicated that the growth stage of plants when TGMS trait started to express for male sterility of T29s variety was about 60-70 days after sowing or about 10-15 days after floral initiation. These results agreed with Hoan (2005) that temperature lower than 24 ^{o}C and above 26 ^{o}C were the critical temperature for expressing both male sterility and male fertility of T29s variety, respectively, but critical stage of growth for expressing male sterility was not reported. Maruyama *et al*. (1990) also supported the study that T29s variety had critical stage of growth for TGMS trait expression for male sterility at about 5-10 days after floral initiation or about 60 days after seed sowing.

Inheritance study of TGMS gene of T29s variety was conducted by crossing T29s, a non-photosensitive variety to KDML 105, photosensitive variety. Thus, segregants of F_2 generation from this cross provided four groups, responded to TGMS trait interacted with photosensitive trait as follow: (1) TGMS, male fertile – photosensitive group (2) TGMS, male fertile - non photosensitive group (3) TGMS, male sterile –photosensitive group (4) TGMS, male sterile - non photosensitive group. In order to avoid the interaction of these two traits for determining the inheritance of

TGMS gene, male sterile with non photosensitive group was selected for inheritance evaluation.

Results of evaluation indicated that TGMS trait of T29s variety was controlled by a recessive gene since the segregation ratio between male fertile: male sterile in F_2 population was 3:1 and significantly fitted to the Mendelian ratio with χ^2-test of 3.39 ($\chi^2_{0.01}$ = 6.63). This obtained results agreed with works reported by Lang *et al*. (1997); Dong *et al*. (2000); Jia *et al.* (2001) and Lopez *et al*. (2003). Further study also found that some unique phenotypic trait of T29s variety such as red culm, red epiculi and purple stigma did not show any association with TGMS trait since these phenotypic traits were probably controlled independently by different genes or did not link to TGMS gene. Thus, these phenotypic traits could not be used as phenotypic markers for TGMS traits selection in segregation progenies derived from T29s parent.

Works of molecular markers assisted for TGMS trait selection of T29s variety was still few in Thailand. Hence, name of gene control and it's location on chromosome is unknown. Lopez *et al*. (2003) reported that TGMS trait of T29s variety was controlled by tms2 gene and located on chromosome 2. In addition, M.L. Anothai Chumsai (2006) (personal communication) received informations from Professor Yuan Long Ping at China National Hybrid Research and Development Center, Hunan, China that T29s rice variety was developed at this center.

At this institute, Professor Quiyun Deng (2005) (personal communication) provided more informations that TGMS trait of T29s variety was controlled by tms5 gene and located on chromosome 2. Since TGMS trait was controlled by a recessive gene (Jia *et al*., 2003 and Wang *et al*., 2003), thus, co-dominant primers were needed to identify molecular markers of TGMS gene in BC_nF_1 population. From these

preliminary informations, different molecular markers were selected for detecting the region of TGMS gene on chromosome 2 of T29s variety. Thirty-five SSR primers which bind with chromosome 2 were used to amplify polymorphisms in parents and F_2 population. It was found that there were only 20 SSR primers which could perform polymorphisms in parents population but not in F_2 plants. Results did not agree with Wang *et al*. (2003) reported that four SSR primers, including RM279, RM324, RM327 and RM492, were closely linked to tms5 gene. Jia *et al*. (2003) also confirmed that two SSR primers, RM174 and R394, were closely linked to tms5 gene with genetic distances of 0.00 cM and 2.5 cM, respectively.

However, results of this study did not agree with Jia *et al*. (2003) since RM174 primer could not amplify and perform the polymorphisms of parents and F_2 population. The similar results reported by Jindasingh (2006) that OPAC-10, a SSR primer could not be used as a molecular marker assisted to screen TGMS trait in segregant population derived form two crosses; T29s x Suphanburi 1 and T29s x RD21. Fortunately, in early 2008, good informations were obtained from Boonjaroen (2008) (personal communication) that she could select two SSR primers from 100 primers included RM154 and RM27 which their locations in AP00439 region probe on chromosome 2 could detect molecular marker linked to TGMS gene in her TGMS breeding materials. Thus, SSCP markers which were designed by Primer Tree Program in website www.grammene.org were used for screening parents and F_2 plants.

The results obviously indicated that two SSCP primers; Os02g12300 and Os02g12370 were able to perform polymorphisms in both parents and F_2 plants and also could distinguish between male sterile and male fertile of F_2 plants. Especially,

Os02g12370 primer could perform polymorphisms more effectively than Os02g12300 primer. This Os02g12370 could distinguish male sterile from male fertile plants with 100 percent accuracy. As well, this primer could link molecular markers closely to TGMS gene of T29s variety with the random recombination frequency of 0.0571 and with the genetic distance of 5.71 cM. This TGMS gene was tms5 gene and located on chromosome 2 as suggested by Maungphrom (2008) and Boonjaroen (2008) (personal communication). Results obtained from the study could be concluded that Os02g12370 primer could precisely screen TGMS trait in BC_nF_2 generation of T29s x KDML 105 cross. Hence, this primer will be profitable in improving and developing two-line rice hybrid by employing TGMS gene.

To develop Thai rice lines for producing two line rice hybrid variety was another important objective of this study. Results obtained from Chapter 4 suggested that TGMS trait of T29s variety was cleary controlled by a recessive gene. Thus, backcross method was used to develop TGMS lines. It was advisable to grow backcross generation to BC_nF_2 in order to permit the identification of the homozygous recessive TGMS genotypes, as well, with clear expression in the progenies, three to four backcross generations were sufficient to transfer the TGMS gene from T29s to KDML 105 parent as suggested by Briggs and Knowles (1967). Two additional selfed generations of BC_3F_3 and BC_3F_4 were made after BC_3F_2, three promising Thai rice TGMS lines were selected namely, KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3. These three Thai rice TGMS lines reconstituted the KDML 105 parent in most aspects, especially grain dimensions and incorporated some desirable agronomic traits inherited from T29s, their donor parent, such as good tillering ability, non-photosensitive response, medium plant height, extrusion of stigma (a necessary

trait for trapping pollens from male fertile parent in producing commercial F_1 seeds) together with TGMS gene which was completely added.

Summary and Recommendation

Study on selection of thermo-sensitive genic male sterile (TGMS) line in Thai rice by using molecular marker was carried out during 2003 to 2008 growing seasons at the evaporation green house and modifier phytotron of Maejo University, Chiang Mai. The experimental field research works were conducted at the Almatha Seed Co., Ltd., Chiang Rai. Laboratory works for molecular marker analysis were conducted at DNA Technology Laboratory Unit, BIOTECH at Kasetsart University, Khamphaengsaen Campus, Nakornpathom.

The objectives of this study were to invest Tate the inheritance of TGMS gene of T29s rice variety, using molecular marker to assist TGMS line selection and transfer TGMS trait to Thai rice varieties for developing Thai rice TGMS-lines for two-line rice hybrid production.

Results of study could be summarized as follow:

1. Critical temperature to induce male fertility of T29s rice variety was lower than 24 °C and male sterility was higher than 26 °C. The stage of plant development to induce male sterility was about 60-70 days after seed sowing.
2. TGMG trait of T29s rice variety was controlled by a recessive gene. Unique traits of T29s variety such as red culm, red auricle, purple stigma and red apiculi did not show any association with TGMG trait. Hence, these traits were not able to be used as phenotypic markers to identify TGMG trait in segregating progeny populations.

3. Results obtained from molecular marker analysis for assisting TGMS line selection indicated that two SSCP primers; Os02g12300 and Os02g12370 primer could be used as molecular marker assisted in TGMS line selection in segregating population derived from T29s x KDML 105 cross. Os02g12370 primer could link molecular markers closely to TGMS gene of T29s rice variety with the random recombination frequency of 0.0571 and linked to TGMS gene with a genetic distance of 5.71 cM. This TGMS gene was proposed to be tms5 gene and located on chromosome 2.
4. TGMS line selection was finished after three backcross generations of T29s x KDML 105 cross and three promising TGMS lines were developed namely, KDML 105 TGMS-1, KDML 105 TGMS-2 and KDML 105 TGMS-3. These Thai rice TGMS line possessed TGMS gene which were able to exhibit complete male fertility if plants were grown below 24 ^{o}C and complete male sterility if temperature was above 26 ^{o}C.

Recommendations:

Results of the study gave some useful recommendations to the research works which relate to the areas of this study:

1. Identify male-parents which must have good specific combining ability with these three Thai rice TGMS line in order to produce maximum standard yield heterosis, approximately expected to get 15-25 percent higher than traditional varieties.
2. There are many problems which may involve in F_1 hybrid seed productions. Such problems are due to weather variation to induce male sterility of female parent, alien plant inspection, labour skills as well as post harvest technologies

to handle seed processing. All of these mentioned problems should be appropriately studied in order to produce high potential of F_1 seed production.

3. Breeders should keep in mind that reasons to encourage farmers to grow hybrid rice varieties are good grain quality, high acceptable yield and profit. Thus, adaptability, yield stability and grain quality of hybrid varieties exhibited from these three Thai TGMS lines development should be well estimated prior to releasing and extending to farmers for production.