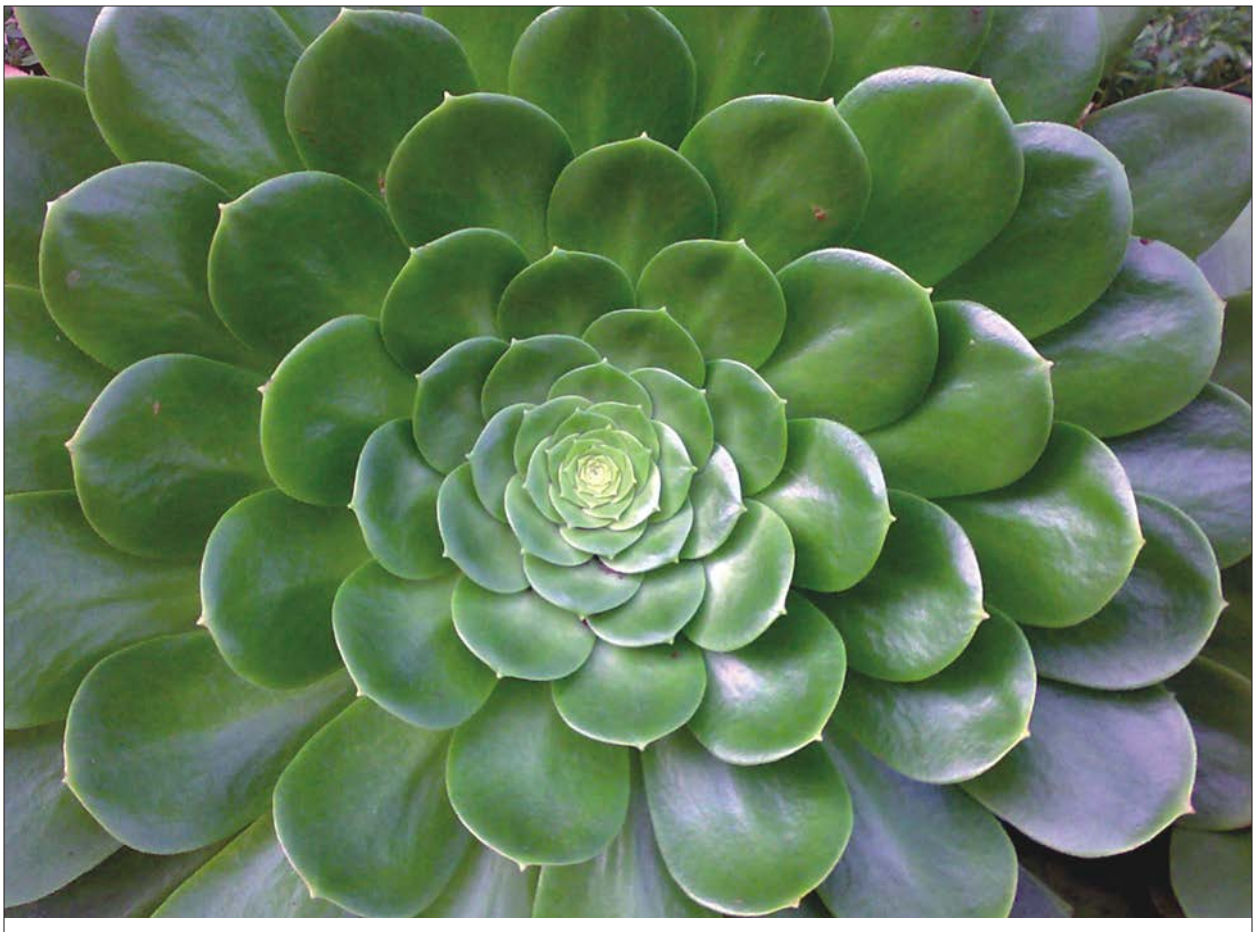

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Research Article
Genetic Background Screening to Accelerate Backcross Breeding Program for TGMS Lines Development

[1]Chonlawat Pongsri, [1]Prapa Sripichitt, [2]Fisseha Worede and [1]Tanee Sreewongchai

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
[2]Amhara Regional Agricultural Research Institute, Sirinka Agricultureal Research Center, Bahia Dar, Ethiopia

Abstract

Background: Male sterile line regulated by temperature is called Thermo Sensitive Genetic Male Sterile (TGMS) line. The TGMS line can be reproduced under temperature condition below the critical temperature. **Objective:** This study aims to develop TGMS lines of rice using Marker Assisted Backcrossing (MAB). **Methodology:** One TGMS line, ANT 2-5-2-4-4, was used as donor parent. This line has *tmsX* gene which is located on rice chromosome 2. The SSR markers were applied in each backcross generation for assisting selection of rice plants having *tmsX* gene. **Results:** Two of the 32 lines were selected from BC_2F_1 generation. Both of them were identified as TGMS lines with highest genetic background. They had 97.22% genetic background of their recurrent parent based on 36 SSR markers which are located across all the chromosomes of rice. **Conclusion:** They will be further utilized as female parent for two-line hybrid rice production.

Key words: Hybrid rice, marker assisted backcrossing, thermo-sensitive genetic male sterile line, two-line hybrid

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Corresponding Author: Tanee Sreewongchai, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice has been feeding half of the ever increasing world population. As a result of the Green Revolution technologies developed at the International Rice Research Institute (IRRI) in the 1960s, yield of rice has increased significantly. However, rice productivity remained stable for years. Hybrid rice varieties increase rice production by 15-20% over the commercial cultivars (Virmani *et al*., 1982; Hwa and Yang, 2008). China started hybrid rice research in 1969 and has been using it in commercial rice production since, 1976 (Yuan, 1998). Hybrid rice production methods, including two-line and three-line systems are developed from photo/thermo-sensitive genic male sterility (PGMS/TGMS) and Cytoplasmic Male Sterility (CMS), respectively. The application of TGMS has made great contribution to hybrid rice production because CMS line requires specific maintainer and restorer lines but TGMS line does not require restorer line, it is possible to use two-line hybrid instead of three-line hybrid (Tanee *et al*., 2014).

The TGMS was first developed in China (Yuan, 1987). Subsequently, other countries including Japan (Maruyama *et al*., 1990) and Philippines at IRRI (Virmani and Voc, 1991) started their TGMS development programs. The TGMS gene express pollen sterility or fertility by single recessive gene which interacts with temperature (Maruyama *et al*., 1990; Borkakati and Virmani, 1996). In TGMS, the non-pollen abortion type is more stable than typical abortion under the same Critical Sterility Point of Temperature (CSPT). Developing TGMS line is important step for two-line hybrid while Marker Assisted Selection (MAS) can be used for selecting the target gene in breeding population and applied for genetic background selection and combining with conventional breeding programs (Jena and Mackill, 2008). The transfer of TGMS gene to different genetic backgrounds was successfully implemented by using MAS technique (Tanee *et al*., 2014).

This study focuses on using MAS for accurate selection of plants and speeding up of breeding programs by identifying *tmsX* which is the chromosomal gene location and by the other Simple Sequence Repeat (SSR) markers that are available in rice (Matthayatthaworn *et al*., 2011). This study aimed at finding the way to accelerate the selection process and reduce the required labor, area and breeding population.

MATERIALS AND METHODS

Rice materials: The IR64, a rice variety well adapted to Thailand and resistant to broad diseases and insects, was nominated to be germplasm for hybrid rice. While, the non-pollen type TGMS line, ANT 2-5-2-4-4, derived from AnxiangS variety possessing a single recessive *tmsX* gene which is located on rice chromosome 2 (Matthayatthaworn *et al*., 2011), was used as the other parent. In the breeding program, backcross method was used. In this study, IR64 was used as recurrent parent, while the TGMS line was used as donor parent.

Methods

Markers for genotyping: The TGMS gene expression non-pollen type called *tmsX* gene which is located on rice chromosome 2 were reported by Peng *et al*. (2010). Nine markers (T1-T9) that are tightly linked to the *tmsX* gene developed by Matthayatthaworn *et al*. (2011) were used for screening polymorphism between the two parents. The T9 marker which showed polymorphism between the parents was used in genotyping the offsprings. The amplified products were analyzed on 6% PAGE gels and detected by silver staining (Benbouza *et al*., 2006).

Markers for identifying genetic similarity to recurrent parent: Rice genomics has now made it possible to map existing DNA markers. Molecular markers are now widely used to track loci and genome regions (Phillips and Vasil, 2001; Jain *et al*., 2002; Gupta and Varshney, 2004). A large number of molecular markers across rice chromosome (260 markers) were used for screening polymorphism between the two parents. After screening, 36 markers were found to be polymorphic. The 36 markers showing polymorphism were used for genotyping the offsprings. The amplified products were analyzed on 6% PAGE gels and detected by silver staining (Benbouza *et al*., 2006). Those offsprings that showed more bands like the recurrent parent were selected.

Selection for male sterile: To determine the CSPT in new TGMS lines, rice plants at the elongation stage were grown in a growth chamber, in which daily mean temperatures were set for 11.5 h day-length at 20, 25 and 30°C. Three tillers from each genotype were sampled for each treatment separately to examine the type of pollen abortion. Pollen and spikelet fertility were determined at the flowering stage using 1% I_2-KI straining solution. Plants with no stained pollen were considered to be completely male sterile, whereas plants having more than 95% darkly stained pollens were classified as male fertile. At the same time, self-pollination was tested to confirm the sterility and fertility (Wang *et al*., 2003; Tanee *et al*., 2014).

RESULTS

Screening marker for MAS: As the result showed that no marker can be used as MAS for foreground selection in the breeding program for TGMS/IR64 cross because no marker showed polymorphism between TGMS lines and IR64. From the 260 markers distributed throughout the rice genome, 36 markers (Table 1) were selected based on polymorphism between the two parents for genetic background selection.

Phenotyping and MAS in BC_1F_1, BC_2F_1 and BC_2F_2 generations: The BC_1F_1 population were planted and backcrossed with the recurrent parent at flowering phase. At the same time a separate panicle from the same plant was selfed to check plants carrying *tmsX* gene from the segregating BC_1F_2 plants and choose BC_2F_1 seeds from the same parent that have pollen sterile segregation. Then, screening of selected plants was done by 18 markers (Table 2) across rice genome for genetic background selection. All markers were classified into homozygous for IR64 and heterozygous for IR64 and TGMS. The plants with high number of homozygous in those loci were selected for backcrossing with IR64. The *tmsX* carrier plants were selected from BC_2F_1 as the same in BC_1F_1 generation. After that repeat primer for screen the selected plants by markers which shown heterozygous in the same place of previous generation. In addition, the carrier plants was done by 18 markers for genetic background selection (Table 3). The seeds from the highest number of homozygous in 36 markers like recurrent parent were harvested. The BC_2F_2 plants were grown under a temperature higher than 30°C. The segregation of BC_2F_2 plants was classified into homozygous dominant, heterozygous and homozygous recessive. The result of phenotypic observation showed that homozygous dominant and heterozygous genotypes showed fertile pollen and homozygous recessive showed sterile pollen.

Performance of new TGMS lines in different temperature regimes: The new TGMS lines with IR64 genetic background there are no pollen produced (Fig. 1a) at temperature higher than 30°C. At 25°C, these plants produced unstained pollen (Fig. 1b) which are classified to be male sterile and completely male fertile pollen (Fig. 1c) at daily mean temperature 20°C (Fig. 2) with the same result of Tanee *et al*. (2014). These results indicate the success of introgression of TGMS trait to IR64 cultivar.

Table 1: Polymorphic SSR markers between IR64 and TGMS lines

Chromosome	Markers
Chr-1	RM1 and RM443
Chr-2	RM211, RM71, RM521, RM6 and RM48
Chr-3	OSR16, RM251, RM16 and RM55
Chr-4	RM551, RM518, RM471 and RM280
Chr-5	RM413, RM437, RM188 and RM334
Chr-6	RM510, RM225 and RM8226
Chr-7	RM1132 and RM172
Chr-8	RM2819 and RM544
Chr-9	RM444, RM105 and RM215
Chr-10	RM222 and RM590
Chr-11	RM286, RM332 and RM536
Chr-12	RM19 and RM463

Table 2: Eight lines with higher percentages of the genetic background of the recurrent parent in BC_1F_1 identified by 18 markers via MAS

Line	No. of marker similar to IR64	No. of marker similar to TGMS line	No. of heterozygotes	Total	IR64 (%)	TGMS line (%)
1	14	0	4	18	88.89	11.11
2	13	0	5	18	86.11	13.89
3	12	0	6	18	83.33	16.67
4	11	0	7	18	80.56	19.44
5	11	0	7	18	80.56	19.44
6	10	0	8	18	77.78	22.22
7	9	0	9	18	75.0	25.0
8	7	0	11	18	69.44	30.56
Mean	10.87	0	7.12	18	80.21	19.79

Table 3: Eight lines with higher percentages of the genetic background of the recurrent parent in BC_2F_1 identified by 36 markers via MAS

Line	No. of fixed marker from BC_1F_1	No. of markers similar to IR64	No. of markers similar to TGMS line	No. of heterozygotes	Total	IR64 (%)	TGMS line (%)
1	14	20	0	2	36	97.22	2.78
2	14	20	0	2	36	97.22	2.78
3	14	19	0	3	36	95.83	4.17
4	14	19	0	3	36	95.83	4.17
5	14	19	0	3	36	95.83	4.17
6	14	14	0	7	36	90.28	9.72
7	14	14	0	8	36	88.89	11.11
8	14	11	0	10	36	86.11	13.89
Mean	14	17	0	4.75	36	93.4	6.6

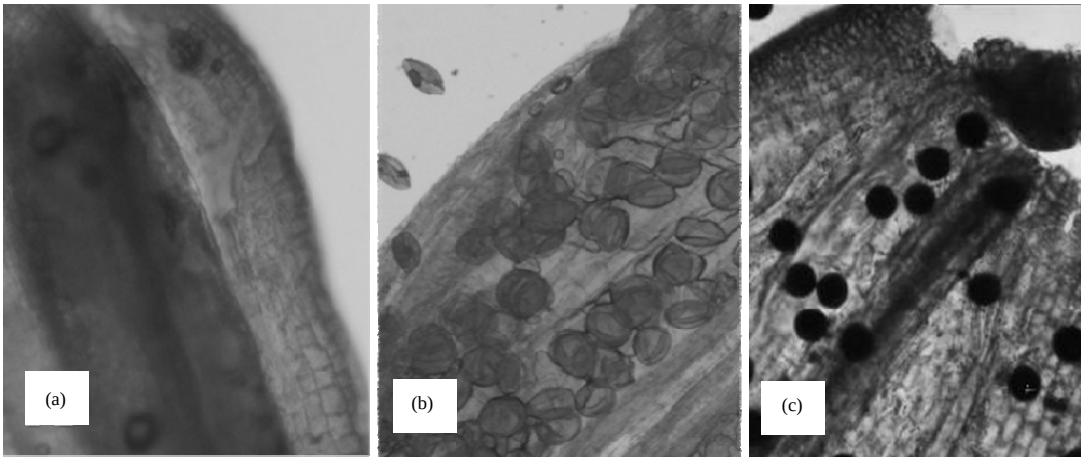

Fig. 1(a-c): Mature anthers of new thermo-sensitive genic male sterile line when grown in different daily temperature conditions and was squashed with 1% I_2-KI solution, (a) 30°C no pollen was found in anther, (b) 25°C irregular shaped and unstained pollen and (c) 30°C round and dark stained pollen

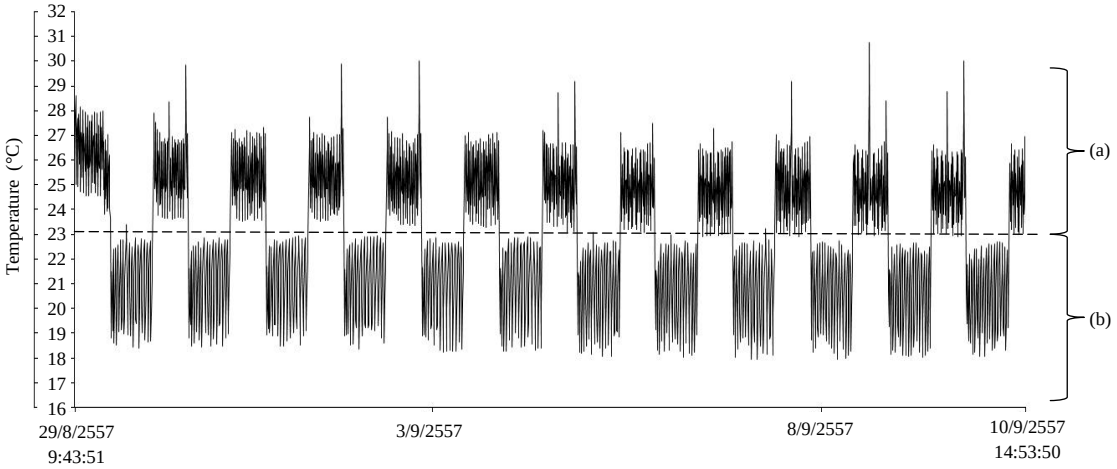

Fig. 2: Temperature graph in growth chamber that induce fertile pollen in TGMS lines, a: Day time temperature and b: Night time temperature

Table 4: Number of individual rice plants selected in BC_1F_1 and BC_2F_1 generations

Generation	Evaluated plants	Selected plants	Methods
BC_1F_1	78	28	Phenotyping
	28	1	Genetic background selection
BC_2F_1	60	32	Phenotyping
	32	2	Genetic background selection

DISCUSSION

Two-line hybrid rice production system mainly use TGMS lines as female parent (Wang *et al*., 2003). However, TGMS germplasm is hardly found in Thailand and introduced TGMS lines lack good traits. The efficient way to develop TGMS lines for Thailand is to introgress TGMS gene into desirable genetic backgrounds.

Totally, 78 BC_1F_1 plants were developed and the individual plants were evaluated for *tmsX* gene controlling TGMS characteristic by conventional breeding and 28 plants (Table 4) were selected. The MAS, using closely linked markers, can reduce the number of plants and experimental area than conventional breeding. Unfortunately, the closely linked markers of the *tmsX* gene cannot provide the opportunity to select the plants at early stage. The way to solve this problem is to develop specific markers for *tmsX* (Collard *et al*., 2005; Sreewongchai *et al*., 2010). In order to maintain genetic background of the recurrent parent, 18 SSR markers covering the 12 rice chromosomes were employed to select the outstanding genetic background for backcrossing.

At BC_2F_1 generation, 60 plants were screened for *tmsX* gene by phenotyping, subsequently 32 selected plants (Table 4) were screened by added more 18 SSR markers for genetic background. From theory, plants in the BC_1F_1 and BC_2F_1 generation carry 75 and 87.5% of the recurrent parent genetic background, respectively. In order to increase the recurrent parent genetic background, MAS was applied at BC_2F_1 generation by using more number of loci for providing opportunity to find individual plants with the highest genetic background of the recurrent parent. As a result, two plants from BC_2F_1 generation were found to carry 97.22% genetic background. These plants had genetic background higher than BC_2F_1 generation. Selfed seeds from selected BC_2F_1 plants were planted at a temperature higher than 30°C and phenotypic selection was employed at flowering stage for selection of the sterile plants.

CONCLUSION

The advantage of genetic background selection at the early stage of selection was able to speed up the breeding program. It could reduce the generation of backcross to meet the genetic similarity to recurrent parent. The high genetic background of recurrent parent plant which was identified by SSR marker was selected for backcrossing in each step. The new TGMS lines showed stable male sterility under high temperature conditions (>30°C). However, it is necessary to study a critical temperature of the new TGMS lines. The development of stable TGMS lines is an important step of two-line hybrids rice system in Thailand environment.

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REFERENCES

Benbouza, H., J.M. Jacquemin, J.P. Baudoin and G. Mergeai, 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. Biotechnol. Agron. Soc. Environ., 10: 77-81.

Borkakati, R.R. and S.S. Virmani, 1996. Genetics of thermosensitive genic male sterility in rice. Euphytica, 88: 1-7.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer and E.C.K. Pang, 2005. An introduction to markers, Quantitative Trait Loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142: 169-196.

Gupta, P.K. and K.R. Varshney, 2004. Cereal Genomics: An Overview. In: Cereal Genomics, Gupta, P.K. and K.R. Varshney (Eds.). Kkuwer Academic Publishers, Dordrecht, Netherlands, pp: 19-34.

Hwa, C.M. and X.C. Yang, 2008. Fixation of hybrid vigor in rice: Opportunities and challenges. Euphytica, 160: 287-293.

Jain, S.M., S.D. Brar and S.B. Ahloowalia, 2002. Molecular Techniques in Crop Improvement. 2nd Edn., Kkuwer Academic Publishers, Dordrecht, The Netherlands, pp: 257-280.

Jena, K.K. and D.J. Mackill, 2008. Molecular markers and their use in marker-assisted selection in rice. Crop Sci., 48: 1266-1276.

Maruyama, K., H. Araki and H. Kato, 1990. Thermo-sensitive genetic male sterility induced by irradiation. Proceeding of the 2nd International Rice Genetic Symposium, April 21-25, 1990, Manila, Philippines, pp: 227-232.

Matthayatthaworn, W., P. Sripichitt, C. Phumichai, S. Rungmekarat, S. Uckarach and T. Sreewongchai, 2011. Development of Specific Simple Sequence Repeat (SSR) markers for non-pollen type thermo-sensitive genic male sterile gene in rice (*Oryza sativa* L.). Afr. J. Biotechnol., 10: 16437-16442.

Peng, H.F., X.H. Chen, Y.P. Lu, Y.F. Peng and B.H. Wan *et al*., 2010. Fine mapping of a gene for non-pollen type thermosensitive genic male sterility in rice (*Oryza sativa* L.). Theoret. Applied Genet., 120: 1013-1020.

Phillips, R.L. and K.I. Vasil, 2001. DNA-Based Markers in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, ISBN: 9780792368656, pp: 49-58.

Sreewongchai, T., T. Toojinda, N. Thanintorn, C. Kosawang, A. Vanavichit, D. Tharreau and P. Sirithunya, 2010. Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. Plant Breed., 129: 176-180.

Tanee, S., M. Weerachai, P. Chalermpol and S. Prapa, 2014. Introgression of gene for non-pollen type thermo-sensitive genic male sterility to thai rice cultivars. Rice Sci., 21: 123-126.

Virmani, S.S. and P.C. Voc, 1991. Induction of photo- and thermo-sensitive male sterility in indica rice. Agronomy Abstract, Vol. 119, American Society of Agronomy, USA.

Virmani, S.S., R.C. Aquino and G.S. Khush, 1982. Heterosis breeding in rice (*Oryza sativa* L.). Theoret. Applied Genet., 63: 373-380.

Wang, Y.G., Q.H. Xing, Q.Y. Deng, F.S. Liang, L.P. Yuan, M.L. Weng and B. Wang, 2003. Fine mapping of the rice thermo-sensitive genic male-sterile gene *tms5*. Theoret. Applied Genet., 107: 917-921.

Yuan, L.P., 1987. [Strategy conception of hybrid rice breeding]. Hybrid Rice, 1: 1-4, (In Chinese).

Yuan, L.P., 1998. Hybrid Rice in China. In: Advance Hybrid Rice Technology, Virmani, S.S., E.A. Siddiq and K. Muralidharan (Eds.). International Rice Research Institute, Philippines, pp: 27-33