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Molecular Markers and Candidate Genes for Thermo-Sensitive Genic Male Sterile in Rice

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Abstract: The discovery of thermo-sensitive genic male sterility (TGMS) has led to development of a simple and highly efficient two-line breeding system. In this study, genetic analysis was conducted using three F_2 populations derived from crosses between IR68301S, an *indica* TGMS rice line, and IR14632 (*tropical japonica*), Supanburi 91062 (*indica*) and IR67966-188-2-2-1 (*tropical japonica*), respectively. Approximately 1:3 ratio between sterile and normal pollen of F_2 plants from the three populations revealed that TGMS is controlled by a single recessive gene. Bulked segregant analysis using simple sequence repeat (SSR) and insertion-deletion (InDel) markers were used to identify markers linked to the *tms* gene. The linkage analysis based on the three populations indicated that the *tms* locus was located on chromosome 2 covering the same area. Using IR68301S × IR14632 F_2 population, the results showed that the *tms* locus was located between SSR marker RM12676 and InDel marker 2gAP0050058. The genetic distance from the *tms* gene to these two flanking markers were 1.10 and 0.82 cM, respectively. InDel marker 2gAP004045 located between these two markers showed complete co-segregation with the TGMS phenotype. In addition, InDel marker vf0206114052 showed 2.94 cM linked to the *tms* gene using F_2 populations of IR68301S × Supanburi 91062. These markers are useful tool for developing new TGMS lines by marker-assisted selection. There were ten genes located between the two flanking markers RM12676 and 2gAP0050058. Using quantitative real-time PCR for expression analysis, 7 of the 10 genes showed expression in panicles, and response to temperatures. These genes could be the candidate gene controlling TGMS in IR68301S.

Key words: hybrid rice; thermo-sensitive genic male sterility; insertion/deletion; simple sequence repeat; marker-assisted selection

Rice (*Oryza sativa* L.) is one of the most important global food crops. The world's population has doubled since the early 1960s, therefore, existing yields of the major cereal crops will be insufficient to meet the food needs of the future (FAO, 2018). In addition, arable lands have declined worldwide, thus an increase in yield will be necessary to meet this demand (Bruinsma, 2003). Rice production in Thailand signifies a portion of the Thai economy and is a major export product. However, yield of Thai rice is less than that of the neighboring countries such as Indonesia, Vietnam, Malaysia, Laos and the Philippines (USDA, 2016). Yields have increased with the development of hybrid rice currently planted in many countries. The trend of hybrid rice development in both commercialization and research of private companies has been increasing.

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Hybrid rice technology has increased yields by 20%–30% under unchanged irrigation conditions (Yuan, 1998; Virmani, 2003). Hybrid rice seeds of large scale commercial production have been released to the market in China since 1976. The Ministry of Agriculture of China develops super rice breeding programs to increase rice yield per planting area, resulting that average rice yields in China rose from 1.89 t/hm^2 in 1949 to 6.71 t/hm^2 in 2012 (Cao and Zhan, 2014). In Thailand, average rice yield is only 2.89 t/hm^2 in 2018 (Thai Rice Exporter Association, 2018). Many countries which adopt hybrid rice technology are able to achieve higher yields.

Hybrid rice seed production involves the use of male sterility systems. Two well established male sterility systems in rice are cytoplasmic genetic male sterility (CMS), a three-line system, and environmentally sensitive genic male sterility (EGMS), a two-line system including photoperiod-sensitive genic male sterility (PGMS) and thermo-sensitive genic male sterility (TGMS). The discovery of PGMS and TGMS has led to development of a simple and highly efficient two-line breeding system in hybrid rice seed production. Several EGMS-related genes have been mapped such as *tms1* (Wang et al, 1995), *tms2* (Yamaguchi et al, 1997; Pitnjam et al, 2008), *tms3* (Subudhi et al, 1997; Lang et al, 1999), *tms4* (Dong et al, 2000), *tms5* (Wang et al, 2003; Nas et al, 2005; Jiang et al, 2006; Yang et al, 2007), *tms6* (Lee et al, 2005), *tms7*(t) (Li et al, 2005), *tms8* (Hussain et al, 2011), *tms9* (Sheng et al, 2013), *tms9-1* (Qi et al, 2014), *tmsX* (Peng et al, 2010), *pms1* (Liu et al, 2001; Zhou et al, 2011), *pms2* (Zhang et al, 1994), *pms3* (Ding et al, 2012a, b), *ptgms2-1* (Xu et al, 2011), *p/tms12-1* (Zhou et al, 2012), *rpms1* (Peng et al, 2008) and *rtms1* (Jia et al, 2001). Candidate genes for EGMS have been reported, Myb-like DNA-binding domain containing protein (Zhou et al, 2011), a nuclear ribonuclease Z gene (Xu et al, 2011; Zhou et al, 2014), ORMDL (Pitnjam et al, 2008; Chueasiri et al, 2014) and the putative pollen specific protein encoded by *SF21* (Nas et al, 2005). In addition, several genes controlling male sterility phenotype have been reported in rice such as undeveloped *tapetum 1* (*udt1*) (Jung et al, 2005), tapetum degeneration retardation (*tdr*) (Li et al, 2006), CER-like protein causing wax-deficient *anther 1* (*wda 1*) (Jung et al, 2006), and LRR receptor kinase affecting *msp1* (Nonomura et al, 2003).

IR68301S is a TGMS rice line obtained from International Rice Research Institute (IRRI), the Philippines. Male sterility in IR68301S is stable in a high temperature (≥ 28 ºC), and it has been used for the development of two-line hybrid in Thailand. In this study, we mapped a gene controlling TGMS in IR68301S, and identified candidate genes using expression analysis. The knowledge in this study will be useful for rice breeding programs.

MATERIALS AND METHODS

Rice materials and phenotypic screening

An F_2 population, generated from a cross between IR68301S (B2), an *indica* TGMS mutant rice line, and IR14632 (B30), a *tropical japonica* rice line, was used for identification of linked markers to the *tms* gene. These plants were obtained from IRRI. In addition, the other F_2 populations generated from crosses between B2 and Supanburi 91062 (B11), a Thai *indica* rice line having high yield, and between B2 and IR67966-188-2-2-1 (KM9), a *tropical japonica* rice line, were also used. The *indica* and *japonica* crosses were used to increase chances for polymorphic marker identification, and then these markers were used for linkage analysis. The F_2 populations were planted in paddy fields for observation on pollen sterility/fertility in summer of 2011, 2012 and 2013, in which the maximum temperatures were 33 ºC–37 ºC. Pollen sterility and fertility of the F_2 plants were evaluated on flowering day by observing anthers with the naked eyes and under a microscope after staining with 1% I_2-KI solution. The χ^2 test was applied to determine the F_2 segregation ratio of sterility and fertility.

Genotyping and linkage analysis

Genomic DNA was extracted from fresh leaves of individual F_2 sterile plants generated from all the three crosses using the cethy trimethylammonium bromide (CTAB) method (Murray and Thomson, 1980). Polymorphic markers between parents and bulked segregant analysis (BSA) using pooled DNA from 10 individuals each for fertile and sterile plants, were applied to identify linked markers to *tms* gene. The resulting linked markers were used to determine the genotype of the individual F_2 plants. Primers for single nucleotide polymorphism (SNP), and insertion-deletion (InDel) markers were designed from sequences obtained from GRAMENE database (http://www.gramene.org) and RiceVarMap (http://ricevarmap.ncpgr.cn/). Amplification of DNA fragments using simple

sequence repeat (SSR) and InDels was performed as previously described by McCouch et al (2002) and Pitnjam et al (2008). The resulting PCR products were electrophoretically separated on 3% agarose gel or 6% acrylamide gel, and DNA patterns were observed by ethidium bromide or silver staining. The recombination frequency (c) was calculated with the formula:

$c = (N_1 + N_2) / 2N$

where N is the total number of sterile plants surveyed, N_1 is the number of sterile individuals with the homozygous band of the fertile parent, and N_2 is the number of individuals with heterozygous bands (Peng et al, 2010). Recombination frequency was converted into genetic distance (cM) for linkage analysis.

Expression analysis

Segregating wild type male fertile and TGMS male sterile BC_3F_5 plants generated from Pathum Thani 1, a Thai elite line, crossed with B2 were used for expression analysis, These plants were grown under natural condition at National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (14º04′50.6″ N and 100º36′09.0″ E), Pathum Thani Province, Thailand. Pre-germinated grains were seeded in a tray and then the seedlings were transplanted into plastic pots (without hole at the bottom, diameter of 20.5 cm and height of 16.0 cm) with one seedling per pot. About one month before flowering, each of at least five plants/line were moved to controlled growth rooms at temperatures (24 ± 2) ºC and (32 ± 2) ºC under 80% relative humidity, 12 h light/12 h darkness until flowering. For expression analysis, young panicles about 13−14 cm in length (dyad stage) were harvested from BC_3F_5 wild type and TGMS mutant plants at the same day. Total RNA was extracted using TRIZOL™ reagent (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) experiments were performed with cDNA synthesis kit (Fermentus, Lithuania) using cDNAs transcribed from total RNA. Based on information in GRAMENE database, genes located between two flanking markers tightly linked to *tms* gene were used for expression analysis by qRT-PCR. The qRT-PCR was performed using Bio-RAD iCycler iQ5 Machine (BioRAD, USA) and all reactions were conducted in 96-well plates (BioRad, USA). The qPCRBIO SyGreen Mix Lo-ROX (PCR Biosystems) assay was used in a total volume of 10 μL per reaction. Each reaction mixture contained 1 μL cDNA, 2 μL distilled water, 1 μL (10 μmol/L) forward primer, 1 μL (10 μmol/L) reverse primer and 5 μL of 2× qPCR BIO SyGreen Mix. All qRT-PCR plates were carried out with following cycling condition: 95 ºC for 3 min, following 35 cycles of 95 ºC for 30 s, 57 ºC to 63 ºC for 30 s and 72 ºC for 30 s, then 95 ºC for 1 min and a melting curve from 60 ºC to 95 ºC in 0.5 ºC increments. *Actin* was used as an internal control. Three replications were performed for all reactions. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative gene expression (Livak and Schmittgen, 2001). The significantly of qRT-PCR were calculated by *R* program (http://cran.r-project.org/) with ANOVA and Duncan's new multiple range test.

RESULTS

Pollen staining

Mature anthers of the TGMS mutant and wild type grown in controlled growth rooms under fertile and sterile conditions were observed. There was no pollen produced in anthers of TGMS mutant plants under sterile condition (32 ± 2) ºC (Fig. 1-A), while under the fertile condition of (24 ± 2) ºC, pollen production was similar to that of wild type plants (Fig. 1-B). At these two conditions, wild type plants produced normal pollen (Fig. 1-C and -D).

Genetic analysis of *tms* gene controlling TGMS in IR68301S

By growing the F_2 populations under sterile condition of (32 ± 2) ºC, the segregation patterns of fertile to sterile plants in the F_2 populations were near to 3:1 ratio in all the three populations (Table 1). The results indicated that the TGMS of IR68301S was controlled by a single recessive gene.

Mapping of *tms* gene controlling TGMS in IR68301S

A total of 119 SSR, 10 SNP and 283 InDel markers were used to identify polymorphism between parents from the three crosses. The results showed that 53, 3 and 7 markers were polymorphic between the parents from B2 × B30, B2 × B11 and B2 × KM9, respectively.

The resulting polymorphic markers were applied to identify linked markers to the *tms* gene using BSA. And 24 out of the 53 markers showed linkage to the *tms* gene in F_2 male sterile plants from B2 × B30. In addition, three and four markers showed linkage to the *tms* gene in F_2 male sterile plants from B2 × B11 and B2 × KM9, respectively (Supplemental Table 1).

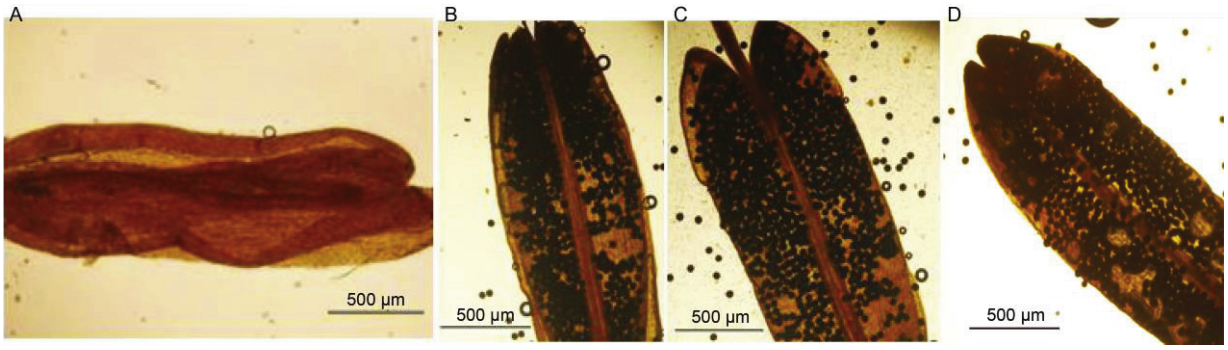

Fig. 1. Pollen staining of thermo-sensitive genic male sterility (TGMS) mutant and wild type.

A, Abortive anther of the TGMS mutant grown under high temperature (32 ± 2) ºC. B, Normal anther of the TGMS mutant grown under low temperature (24 ± 2) ºC. C, Normal anther of wild type grown under high temperature (32 ± 2) ºC. D, Normal anther of wild type grown under low temperature (24 ± 2) ºC.

According to GRAMENE and RiceVarMap databases, these markers are on chromosome 2 at the position of 4.19 to 7.44 Mb.

The linked markers identified from each cross were used to genotype the F_2 male sterile. These F_2 male sterile plants selected from flowering plants in summers (33 ºC–37 ºC) were completely sterile. A total of 217, 259 and 450 F_2 male sterile plants from B2 × B30, B2 × B11 and B2 × KM9 were used for genotyping, respectively. Segregation patterns were shown in Fig. 2. A linkage analysis based on the three F_2 populations showed that the *tms* gene in IR68301S was located on chromosome 2 (Fig. 3). Using F_2 sterile individuals of B2 × B30, the results showed that the *tms* gene was located between SSR marker RM12676 at 5.74 Mb and InDel marker 2gAP0050058 at 5.81 Mb. The genetic distance of the *tms* gene from the two markers was 1.10 and 0.82 cM, respectively. In addition, InDel marker 2gAP004045 showed complete co-segregation with the TGMS phenotype (Fig. 3-A). Using F_2 sterile individuals of B2 × B11, the results showed that *tms* gene was located between RM126016 at 4.69 Mb and InDel marker vf0206114052 at 6.11 Mb. The genetic distance of the *tms* gene from the two markers was 14.97 and 2.94 cM, respectively (Fig. 3-B). Using F_2 population of B2 × KM9, the results showed that four SSR markers, RM126016, RM12649, RM6378 and RM12674 were linked to the *tms* gene. The genetic distance of the *tms* gene from these markers were 12.50, 8.75, 8.25 and 6.25 cM, respectively (Fig. 3-C).

Table 1. Segregation patterns of F_2 populations for fertility and sterility.

F_2 population	Total No. of lines	No. of fertile individuals	No. of sterile individuals	Segregation rate	χ^2
B2 × B30	917	700	217	3.22	0.910
B2 × B11	981	722	259	2.79	0.980
B2 × KM9	1 784	1 334	450	2.96	0.042

B2, IR68301S; B30, IR14632; B11, Supanburi 91062; KM9, IR67966-188-2-2-1.

The value of $\chi^2_{0.05}$ is 3.84.

Expression analysis of candidate genes located inside the flanking markers

Based on information in GRAMENE database, there are ten genes (Table 2) located between the two flanking markers, RM12676 and 2gAP0050058. Nine out of the ten genes were annotated as expressed proteins, and one was annotated as a conserved hypothetical protein. These genes were tested for expression in panicles of wild type and TGMS mutant plants under fertile and sterile conditions. The results showed that the expressions of seven genes were detected in the tested tissues, but those of *Os02g0202950*, *Os02g0203000* and *Os02g0203300* could not be detected (data not shown). All the seven expressed genes showed different expression levels under fertile and sterile conditions or between the TGMS mutant and the wild type. Genes *Os02g0202600*, *Os02g0202800* and *Os02g0202900*

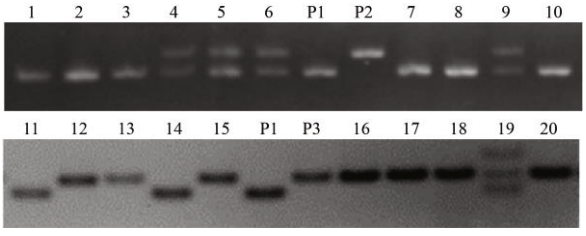

Fig. 2. Samples of genotyping F_2 male sterile plants.

P1, Female parent (IR68301S, B2); P2, Male parent (IR14632, B30); 1–10, F_2 male sterile individuals of B2 × B30 (*indica* × *japonica*) using Os02g12370 marker; P3, Male parent (Supanburi 91062, B11); 11–20, F_2 male sterile individuals of B2 × B11 (*indica* × *indica*) using vf0206114052 marker.

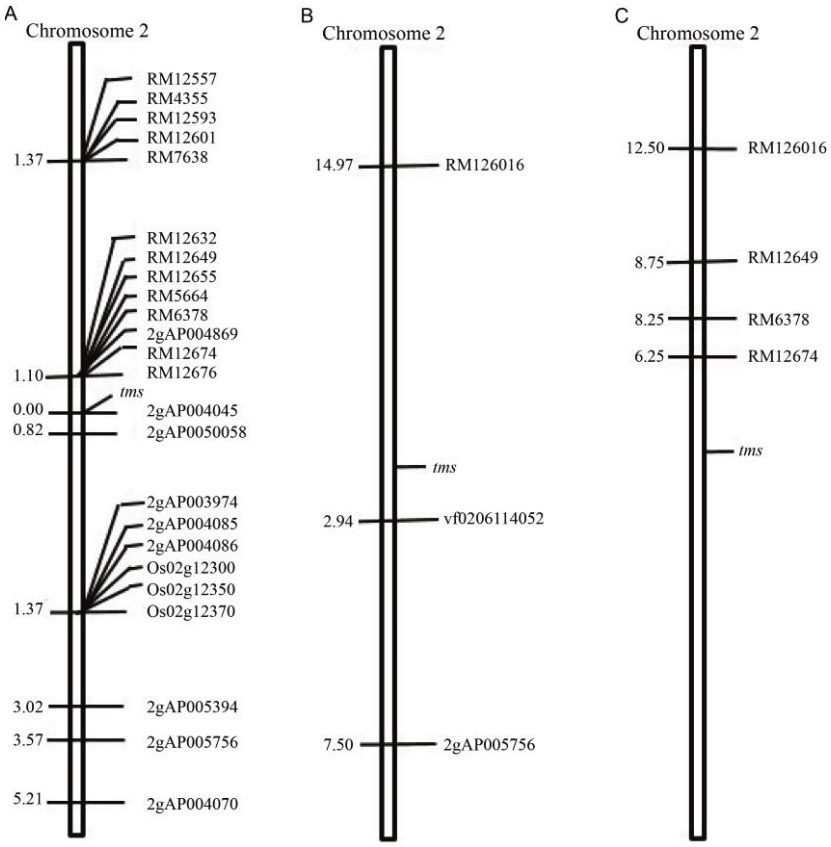

Fig. 3. Genetic linkage map of thermo-sensitive genic male sterility gene on chromosome 2.

The linkage maps were analyzed based on male sterile F_2 populations of IR68301S × IR14632 (A), IR68301S × Supanburi 91062 (B) and IR68301S × IR67966-188-2-2-1 (C). Distances of each marker in centiMorgans (cM) from *tms* gene were given on the left side of the genetic map.

showed significantly different expression levels between the two conditions in the TGMS rice plants but no significantly different expression levels were detected between the two conditions in the wild-type rice plants (Fig. 4). *Os02g0202800* and *Os02g0202900* showed higher expression levels under fertile condition than sterile condition, while *Os02g0202600* showed higher expression levels under sterile condition than the fertile conditions. These three genes showed higher expression levels in the TGMS mutants than in the wild type under both conditions. These two genes *Os02g0203401* and *Os02g0203500* showed a similar pattern of expression both in the mutant and wild-type rice plants, by showing higher expression under lower temperature condition. These genes *Os02g0202500* and *Os02g0203700* showed a similar pattern of expression by showing higher expression under lower temperature condition in the TGMS mutants, but higher expression under higher temperature condition in wild-type plants (Fig. 4).

Table 2. Genes located between the two flanking markers RM12676 and 2gAP0050058.

Gene	Description
Os02g0202500	Conserved hypothetical protein
Os02g0202600	Tetratricopeptide-like helical domain containing protein
Os02g0202800	Zinc ion binding protein
Os02g0202900	OsFBK3-F-box domain and kelch repeat containing protein
Os02g0202950	Concanavalin A-like lectin/glucanases superfamily and Legume lectin domain
Os02g0203000	Carbohydrate binding, sequence-specific DNA binding, sequence-specific DNA binding transcription factor activity
Os02g0203300	UDP-glucuronosyl/UDP-glucosyltransferase
Os02g0203401	hypothetical gene, protein coding
Os02g0203500	Putative uncharacterized protein OJ1135_F06.25; Putative uncharacterized protein P0544H11.7
Os02g0203700	Zinc finger protein; Zinc finger transcription factor ZFP30

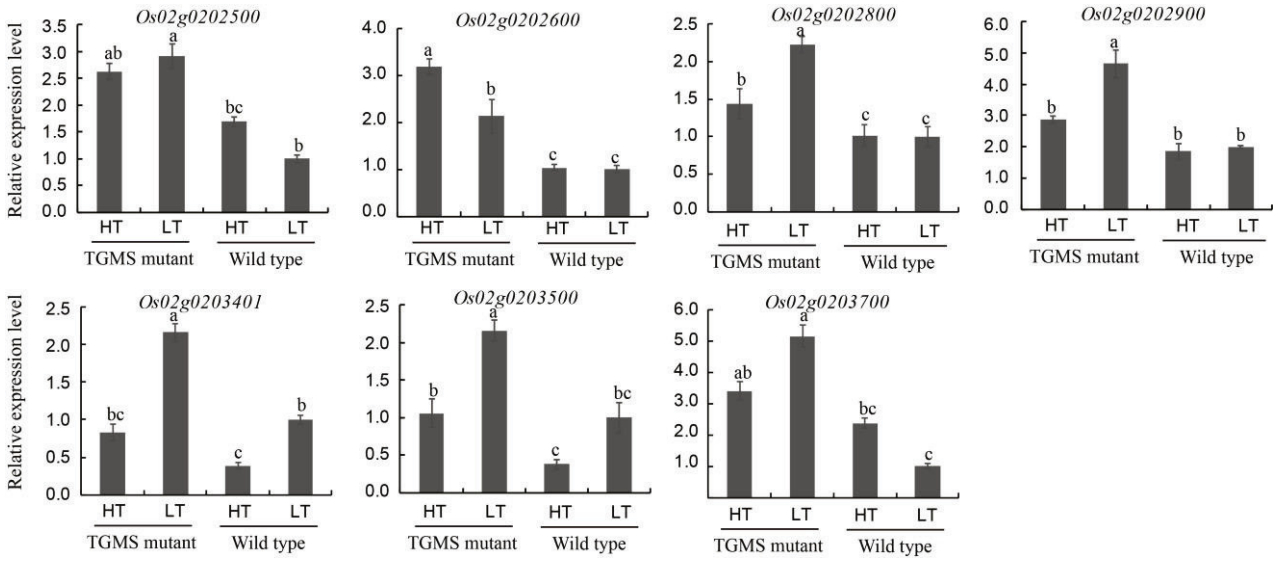

Fig. 4. Relative expression levels of genes in panicles by quantitative real-time PCR.

HT, High temperature condition (32 ºC); LT, Low temperature condition (24 ºC); TGMS, Thermo-sensitive genic male sterility.

Data represent Mean ± SE, and the same lowercase letter(s) indicate no significant difference at $P < 0.05$ by Duncan's new multiple range test.

DISCUSSION

The segregation patterns of fertile to sterile plants in the three F_2 populations followed the 3:1 ratio, which is typical of Mendelian low, indicating that the TGMS of an *indica* IR68301S is controlled by a single recessive gene, which is in agreement with other studies (Borkakati and Virmani, 1996; Lopez et al, 2003; Wang et al, 2003; Hussain et al, 2011; Qi et al, 2014).

Using the three mapping populations, the *tms* gene in IR68301S was mapped on chromosome 2. Using B2 × B30 F_2 population, the *tms* gene was located between SSR marker RM12676 and InDel marker 2gAP0050058. In addition, InDel marker 2gAP004045, located between these two markers, showed complete co-segregation with the TGMS phenotype. These markers are useful tool for developing new TGMS lines by marker-assisted selection (MAS) and identifying the TGMS individuals at earlier stages of line development in rice breeding program. Using F_2 population from *indica* parents (B2 and B11), only two markers were linked to the *tms* gene. The genetic distance of the *tms* gene from the two markers was 14.97 and 7.50 cM. Since major subspecies of rice in Thailand is *indica*, the polymorphic and tightly linked markers generated from *indica* parents will be more practical than the linked markers generated from *indica* × *japonica* crosses. Therefore, several markers nearby the flanking region were designed and test for polymorphism between the two *indica* parents (B2 and B11). The 30 InDel markers covering flanking region from 5.50 to 6.28 Mb were designed to test polymorphism between these two parents (Supplemental Table 2). The results showed that only vf0206114052 at 6.11 Mb showed polymorphism and linked (2.94 cM) to the *tms* gene. Therefore, this marker will be useful in *indica* breeding programs. The two tightly linked InDel markers, vf0206114052 and 2gAP004045, will be useful for *indica* × *indica* and *indica* × *japonica* rice breeding programs, respectively.

Candidate genes for EGMS have been reported. The locus of *pms3* (photo period-sensitive male sterility) and *P/TMS12-1* (photo- or thermo-sensitive genic male sterility) represent the same locus on chromosome 12 conferring PGMS and TGMS traits (Zhou et al, 2012). This report revealed that a SNP C-to-G between the fertile lines and sterile lines led to increasing of methylation in putative promoter region of this non-coding gene, resulting in premature programmed cell death (PCD) in developing anthers, thus causing PGMS in *japonica* and TGMS in *indica* subspecies.

The *Os02g0214300* is a candidate gene of *tms5* which locates on chromosome 2 at the position of 6.39 Mb (htpp://www.gramene.org) and this gene encodes RNase Z^{S1}. Experimental results revealed that at high temperature RNase Z^{S1} loses its function and causes defective pollen production (Zhou et al, 2014). This enzyme maintained mRNAs of Ub_{L40} at normal level and led to male fertility. LOC_Os02g12290, a nuclear

ribonuclease Z gene was identified as the candidate for the *ptgms2-1* gene, located on chromosome 2 in Guangzhan 63S, a *japonica* PTGMS line (Xu et al, 2011).

The gene controlling TGMS in IR68301S was located between the two flanking markers at the position of 5.74 to 5.81 Mb. There were no previously reported EGMS genes at this position. There were 10 genes located between the flanking markers, however, we detected the expressions level of only 7 genes in the TGMS mutant and the wild type under both conditions. All these genes responded to temperatures by showing different expression levels under fertile and sterile conditions in the TGMS rice plants. Three genes *Os02g0202600*, *Os02g0202800* and *Os02g0202900* showed similar expression levels under fertile and sterile conditions in wild type rice plants but they showed significantly different levels of expression in the mutant plants under sterile condition. Interestingly, *Os02g0202600* showed significantly higher expression level under sterile condition than under fertile condition in the TGMS rice plants, while it showed similar expression levels under both conditions in the wild type rice plants. The *Os02g0202600* encodes a tetratricopeptide-like helical domain protein (TPR). Shin et al (2014) reported that the N-terminal tetratricopeptide repeat1 (TPR1) domain is essential for its interaction with pectate lyase-like proteins (PLLs) in petunia, maize and *Arabidopsis*. Lacking the TPR1 domain, no interaction resulting in pollen that failed to germinate. *Oryza sativa* pollen calmodulin-binding protein (OsPCBP) is a Ca^{2+}-dependent calmodulin-binding (CaMBP) protein and it contains six TPR motifs that can interact with protein in pollen to regulate germination and also involve in starch accumulation. The absence or reduction of starch can cause abortive pollen (Zhang et al, 2012). This information indicated that the TPR domain is associated with pollen development. *Os02g0202800* encodes a zinc ion binding protein and *Os02g0203700* encodes a zinc finger protein. The zinc finger family proteins are transcription factors (TF) involved in abiotic stress. Bai et al (2015) reported that three zinc finger TF genes (LOC_Os06g14180, LOC_Os12g37800, LOC_Os11g14000) are up-regulated and two zinc finger TF genes (LOC_Os01g50750 and LOC_Os05g44550) are down-regulated following cold stress treatment in rice male sterile lines. In *Chrysanthemum morifolium*, the result of RNA sequencing showed that *BBX24*, a zinc finger TF protein gene associated with flowering time and tolerance to abiotic stress (Yang et al, 2014). Yang et al (2015) reported that zinc finger CCCH domain-containing proteins are two (Unigene43080_Zhong-531 and Unigene43085_Zhong-531) of differential expression genes (DEGs) which respond to temperature interacting with nitrogen at meiosis stage of rice spikelet. In our study, *Os02g0202800* and *Os02g0203700* showed higher expression levels under lower temperature in the TGMS plants, while in wild type rice plants, *Os02g0203700* showed lower expression levels under lower temperature and *Os02g0202800* showed similar expression levels under both conditions. *Os02g0202900* encodes an F-box domain and kelch repeat containing protein. F-box proteins are critical for degradation of cellular proteins. The F-box protein-encoding genes have specific and/or overlapping expression during floral transition as well as panicle and seed development. The F-box protein encoding genes are involved in different abiotic stress conditions (Jain et al, 2007; Chunthong et al, 2017). The TPR domains were predicted in rice F-box proteins responsible for processing and/or translation of mRNAs. DNA-binding domains such as zinc finger, ring finger and helix loop helix are also found in rice F-box proteins, which may be directly or indirectly involved in transcriptional regulation (Small and Peeters, 2000).

Os02g0202500 encodes a conserved hypothetical protein. It showed higher level of expression under the higher temperature condition than under the lower temperature condition in the wild type rice plants, but in the TGMS rice plants the expression of this gene was similar under both conditions. *Os02g0203500* encodes a putative uncharacterized protein. It showed similar expression patterns in the TGMS and wild type rice plants but the expression levels were higher in the TGMS than in wild type rice plants under both conditions. Although the functions of *Os02g0202500*, *Os02g0203500* and *Os02g0203401* are not clear but their expressions in our study suggested that they could be involved in TGMS. Therefore, further study is needed to identify the candidate gene controlling TGMS in IR68301S rice line.

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SUPPLEMENTAL DATA

The following materials are available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Table 1. Markers on chromosome 2 linked to the *tms* gene using F_2 male sterile plants from IR68301S × IR14632, IR68301S × Supanburi 91062 and IR68301S × IR67966-188-2-2-1.

Supplemental Table 2. InDel markers on chromosome 2 covering flanking region from 5.50 to 6.28 Mb.

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