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ORIGINAL PAPER

Fine mapping and candidate gene analysis of *ptgms2-1*, the photoperiod-thermo-sensitive genic male sterile gene in rice (*Oryza sativa* L.)

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Abstract Photoperiod-thermo-sensitive genic male sterile (PTGMS) rice exhibits a number of desirable traits for hybrid rice production. The cloning genes responsible for PTGMS and those elucidating male sterility mechanisms and reversibility to fertility would be of great significance to provide a foundation to develop new male sterile lines. Guangzhan63S, a PTGMS line, is one of the most widely used indica two-line hybrid rice breeding systems in China. In this study, genetic analysis based on F_2 and BC_1F_2 populations derived from a cross between Guangzhan63S and 1587, determined a single recessive gene controls male sterility in Guangzhan63S. Molecular marker techniques combined with bulked-segregant analysis (BSA) were used and located the target gene (named *ptgms2-1*) between two SSR markers RM12521 and RM12823. Fine mapping of the *ptgms2-1* locus was conducted with 45 new Insertion–Deletion (InDel) markers developed between the RM12521 and RM12823 region, using 634 sterile individuals from F_2 and BC_1F_2 populations. *Ptgms2-1* was further mapped to a 50.4 kb DNA fragment between two InDel markers, S2-40 and S2-44, with genetic distances of 0.08 and 0.16 cM, respectively, which cosegregated with S2-43 located on the AP004039 BAC clone. Ten genes were identified in this region based on annotation results from the RiceGAAS system. A nuclear ribonuclease Z gene was identified as the candidate for the *ptgms2-1* gene. This result will facilitate cloning the *ptgms2-1* gene. The tightly linked markers for the *ptgms2-1* gene locus will further provide a useful tool for marker-assisted selection of this gene in rice breeding programs.

Abbreviations

BSA	Bulked-segregant analysis
CMS	Cytoplasmic-genetic male sterility
InDel	Insertion–Deletion
MAS	Marker-assisted selection
PCR	Polymerase chain reaction
PGMS	Photo-sensitive genic male sterile
PTGMS	Photoperiod-thermo-sensitive genic male sterile
SSR	Simple sequence repeats
TGMS	Thermo-sensitive genic male sterile

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Introduction

Rice (*Oryza sativa* L.) is one of the most important grain crops worldwide and provides a staple food for almost half of the world's population (Delseny et al. 2001). As the world population continues to grow and arable land is lost, world food security depends heavily on increasing rice grain yield (Gupta et al. 2006). Hybrid rice has made a tremendous contribution to food security both in China and

many other countries. It has been demonstrated that hybrid rice technology increased yields by 20–30% under unchanged irrigated conditions in China and other countries during the past two decades (Yuan 1998; Virmani 2003). Presently in China, the area occupied by hybrid rice comprises more than 50% of the total region under rice cultivation (You et al. 2006).

Hybrid rice seed production involves the use of male sterility systems. Two well-known male sterility systems have been used to produce hybrid seeds, CMS (cytoplasmic-genetic male sterility) and PTGMS. Compared with the three-line system, the two-line system, or PTGMS, has many advantages for hybrid seed production (Yuan 1990). First, in the PTGMS line, male sterility is induced by environmental factors (such as temperature and photoperiod) of a particular range, duration or concentration at a sensitive stage of plant development (Tamerlane et al. 2005). Therefore, the line can be used to produce hybrid seed for male sterile lines under long-day conditions or when the environmental temperature is above a critical point. Subsequently, the plants self-propagate. The plants can also be used as maintainer lines under short-day conditions or below a critical point during sensitive growth stages. Second, there is an increased probability of finding heterotic hybrid combinations, because any normal fertile variety can be used as a parental line, which can be applied to restore fertility in the F_1. In short, these advantages make the two-line system widely employed in hybrid rice seed production.

Two-line hybrid rice development has been met with widespread success. It has increased yields by 5–10% over those of the equivalent three-line hybrid rice and the planting area in China has continued to rise each year from the early 1990s. In 2007, the area planted in two-line hybrid rice was over 3.1 million hm^2 in China, accounting for 11% of the total rice acreage (Yang et al. 2009).

However, the PTGMS system exhibits shortcomings. A sudden drop in temperature can be disastrous due to a reversion to fertility, which results in selfed seeds of the female parent, during hybrid seed production at high temperatures (Nas et al. 2005). Consequently, development of a new PTGMS line is vital to overcome the instability in the expression of this trait. One proposed solution is to combine available PTGMS genes into a single background via MAS. Recombination of PTGMS genes will not only solve the problem of incomplete sterility of PTGMS lines, but also provide a very effective way to develop various types of elite PTGMS lines. Li et al. (2009) recombined the photo-sensitive genic male sterile (PGMS) gene from Nongken58S and the thermo-sensitive genic male sterile (TGMS) genes from AnnongS and found that male sterility in rPTGMS (recombinogenic PGMS and TGMS genes) plants with low critical sterility-inducing temperature was more complete and stable.

To date, 13 PTGMS genes have been identified in rice: *pms1* (Liu et al. 2001), *pms2* (Zhang et al. 1994), *pms3* (Lu et al. 2005), *rpms1* (Peng et al. 2008), *rpms2* (Peng et al. 2008), *tms1* (Wang et al. 1995), *tms2* (Yamaguchi et al. 1997), *tms3* (Subudhi et al. 1997), *tms4* (Dong et al. 2000), *tms5* (Yang et al. 2007), *tms6* (Lee et al. 2005), *rtms1* (Jia et al. 2001) and *Ms-h* (Koh et al. 1999), which have been mapped to chromosomes 7, 3, 12, 8, 9, 8, 7, 6, 2, 2, 5, 10 and 9. *Pms1* was mapped to an 85 kb DNA fragment (Liu et al. 2001); *pms2* was mapped to the interval between markers RG348 and RG191 (Zhang et al. 1994); *pms3* was mapped to a 28.4 kb DNA fragment containing five ORFs (Lu et al. 2005); *rpms1* and *rpms2* controlled male sterility of YiD1S, a reverse PGMS line; *rpms1* was mapped to the interval between SSR markers RM22980 and RM23017 and *rpms2* was mapped to the interval between SSR markers RM23898 and YDS926 (Peng et al. 2007); *tms1* was mapped to the interval between markers RZ562 and RG978 (Wang et al. 1995); *tms2* was located between R643A and R1440 (Yamaguchi et al. 1997); *tms3* was mapped to the interval between RFLP markers OPAC3640 and OPAA7550 (Subudhi et al. 1997); *tms4* was mapped on chromosome 2 from a mutant rice line, TGMS-VN1, and linked to an AFLP marker E5/M12-600 at a distance of 3.3 cM (Dong et al. 2000); *tms5* gene was first mapped to the interval between STS marker C365-1 and CAPs marker G227-1 (Wang et al. 2003) and was more recently physically mapped to a 19 kb DNA fragment located on BAC AP004039 (Yang et al. 2007); *tms6* was fine mapped to the interval between markers RM3351 and E60663 (Lee et al. 2005); *rtms1* was mapped between RM239 and RG257 with genetic distance of 3.6 and 4.0 cM, respectively (Jia et al. 2001); *ms-h* was mapped between RG451 and RZ404 with genetic distance of 2.5 and 3.3 cM, respectively (Koh et al. 1999).

In the present study, we mapped a new PTGMS gene, *ptgms2-1*, in rice variety Guangzhan63S, one of the most widely used PTGMS lines in the indica two-line hybrid rice breeding systems in China. This achievement is a major step toward the ultimate goal of cloning the *ptgms2-1* gene to elucidate the mechanisms involved in male sterility. Fine mapping of this gene will serve as the point of departure to select new PTGMS lines with high yield, quality, resistance and general combining ability (GCA) by MAS for hybrid rice breeding.

Materials and methods

Plant materials and mapping population

Guangzhan63S, a rice variety with stable male sterility, was cooperatively selected by the North Japonica Hybrid

Rice Research Center and Hefei Fengle Seed Corporation in China (Yang et al. 2002). Jiangxi Academy of Agricultural Sciences selected the upland variety 1587. The F_1 plants were generated from Guangzhan63S 'female' and 1587 'male' parents, which were planted in a paddy field in Sanya (18°N, 109°E), Hainan Province, China, in the winter of 2007. A small F_2 population for primary mapping consisted of 398 plants and was obtained by selfed F_1 plants grown in the experimental farm at Lixiahe Region Agricultural Research Institute of Jiangsu, Yangzhou (33°N, 119°E), China, in the summer of 2008. The sterile individuals from the F_2 were selected to cross to 1587 to produce a BC_1F_1 population. The BC_1F_1 plants were planted in Sanya in the winter of 2008. A large BC_1F_2 population for fine mapping consisted of 2,200 plants, which were grown in Yangzhou in the summer of 2009. From 1 July to 14 August 2008 and 2009, the daily average temperature in Yangzou was 24.6–32.8°C and 24.7–32.9°C, respectively (provided by Yangzhou Weather Bureau), which were higher than the critical temperature of 24°C to induce sterility in Guangzhan63S (Yang et al. 2002).

Fertility characterization

Pollen and spikelet fertility was evaluated using the protocol of Shan et al. (2009). Pre-flowering spikelets were collected and dipped into 1% (v/v) I_2 in 3% (v/v) KI. The anthers were then removed from the spikelet, placed on glass slides, crushed into powder and used to assess fertility using a light microscope. Plants were considered completely male sterile if the pollen did not stain and plants exhibiting more than 90% darkly stained pollen were considered fertile.

DNA extraction and molecular marker analysis

Genomic DNA was extracted from fresh-frozen leaves of each individual using the CTAB method according to Rogers and Bendich (1988). The extracted DNA was dissolved in ddH_2O. A total of 445 SSR markers were used in this study, of which information for 400 markers was downloaded from http://www.gramene.org. A high-density linkage map for fine mapping in the target region was obtained by developing 45 new InDel (Insertion–Deletion) markers according to the publicly available rice genome sequence comparisons between Nipponbare and 9311 (http://rgp.dna.affrc.go.jp) using Primer Premier 5.0 software. Sangon Co, Ltd. (Shanghai, China) synthesized the SSR and InDel primers. DNA amplification was performed by PCR with the following parameters: an initial cycle of 5 min at 95°C; 33 cycles of 30 s at 94°C, 30 s at 55°C, 40 s at 72°C; and a final cycle of 5 min at 72°C. Reactions were carried out in 96-well PCR plates of 25 μL containing 1 μmol/L of each primer, 200 μmol/L of dNTPs, 5 ng of DNA template, 2 mmol/L $MgCl_2$, 2.5 μL 10× buffer (supplied by Sheng-gong Inc. with Tag polymerase) and 1 U of Tag polymerase. Amplification products were analyzed on 3.5% agarose gels stained with ethidium bromide, and photographed with a UVP system.

Linkage and gene mapping

Bulked segregant analysis (BSA) (Michelmore et al. 2004) was used to screen the polymorphic markers between two DNA pools. The pools were constructed by combining 10 DNA samples selected from sterile and fertile plants from the F_2 population. The polymorphic markers were considered putatively linked to the target gene, and subsequently confirmed by the F_2 segregation population. Based on the results of primary mapping of the target gene, new InDel markers were developed in the anchored region using the method described above, and the sterile plants of F_2 and BC_1F_2 were used for fine mapping the target gene. Linkage analysis was conducted using MAPMAKER/EXP 3.0 (Lander et al. 1987). The Kosambi mapping function was applied to transform the recombination frequency to mapping distance (cM).

Gene annotation

The delimited gene region was annotated using the online system RiceGAAS (http://www.ricegaas.rgp.dna.affrc.go.jp) and the AP004039 sequence (http://www.ncbi.nlm.nih.gov). Based on gene annotation results and the AP004039 sequence (http://www.ncbi.nlm.nih.gov), we designed PCR primers and amplified the putative gene of the delimited region in Guangzhan63S and 1587. The PCR products were purified and sequenced by TaKaRa Co, Ltd. (Dalian, China). The sequence was analyzed by DNAMAN software.

RT-PCR analyses

Total RNAs were extracted from roots, stems, leaves and panicle of Guangzhan63S and 1587 during the heading stage. RNA extraction followed the Trizol reagent protocol provided by the manufacturer (Invitrogen) with subsequent *DNase* I (TaKaRa) treatment. RT-PCR experiments were performed with cDNA synthesis kit (TaKaRa) and approximately 1 μg of total RNA from each sample was used for first-strand cDNA synthesis. RT-PCR was conducted to amplify gene 1 transcript using first-strand cDNA and *OsActin* was also amplified as a control. The primer pair (GeneF: 5′-AGGAGACCTGCGTCATCTTCC-3′ and GeneR: 5′-GAATGGCGTGGTAGGTCTTGA-3′) was used in RT-PCR analysis. PCR mixture (25 μL) contained

1 μmol/L of each primer, 200 μmol/L of dNTPs, 5 ng of cDNA template, 2 mmol/L $MgCl_2$, 2.5 μL 10× buffer and 1 U Taq DNA polymerase. The amplification reaction was carried out using the following profile: 5 min at 95°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, 40 s at 72°C, and a final cycle of 5 min at 72°C. The PCR products were electrophoresed on a 1.5% (w/v) agarose gels stained with ethidium bromide, and photographed with a UVP system.

Results

Genetic analysis of the photoperiod-thermo-sensitive genic male sterility in Guangzhan63S

The pattern of inheritance in the photoperiod-thermo-sensitive genic male sterile gene derived from Guangzhan63S was analyzed using F_1, F_2, BC_1F_1 and BC_1F_2 populations. F_1, BC_1F_1 and 1587 exhibited normal fertility. These results indicated that sterility is a recessive trait in Guangzhan63S. The F_2 and BC_1F_2 populations exhibited trait segregation as observed in the fertile and sterile plant types. The fertile plants possessed normal anthers and more than 90% darkly stained pollen, and the sterile plants had thin anthers and the complete absence of stained pollen (Fig. 1). The segregation pattern of fertile to sterile plants in the F_2 and BC_1F_2 followed a 3:1 ($\chi_c^2 = 0.97 < \chi^2_{0.05} = 3.84$ and $\chi_c^2 = 0.12 < \chi^2_{0.05} = 3.84$, respectively) phenotypic ratio (Table 1). All the above results clearly indicated that a single recessive gene controls male sterility in Guangzhan63S.

Primary mapping of *ptgms2-1*

In order to determine which chromosome the target gene resides on, approximately 400 SSR markers distributed on 12 chromosomes were used to investigate polymorphisms between the two gene pools which were constructed by combining 10 DNA samples selected from sterile and fertile plants from the F_2 population. The results showed that four SSR markers on chromosome 2 were polymorphic between the two pools, suggesting that the target gene might be located on rice chromosome 2. The four polymorphic markers RM12345, RM12521, RM12823 and

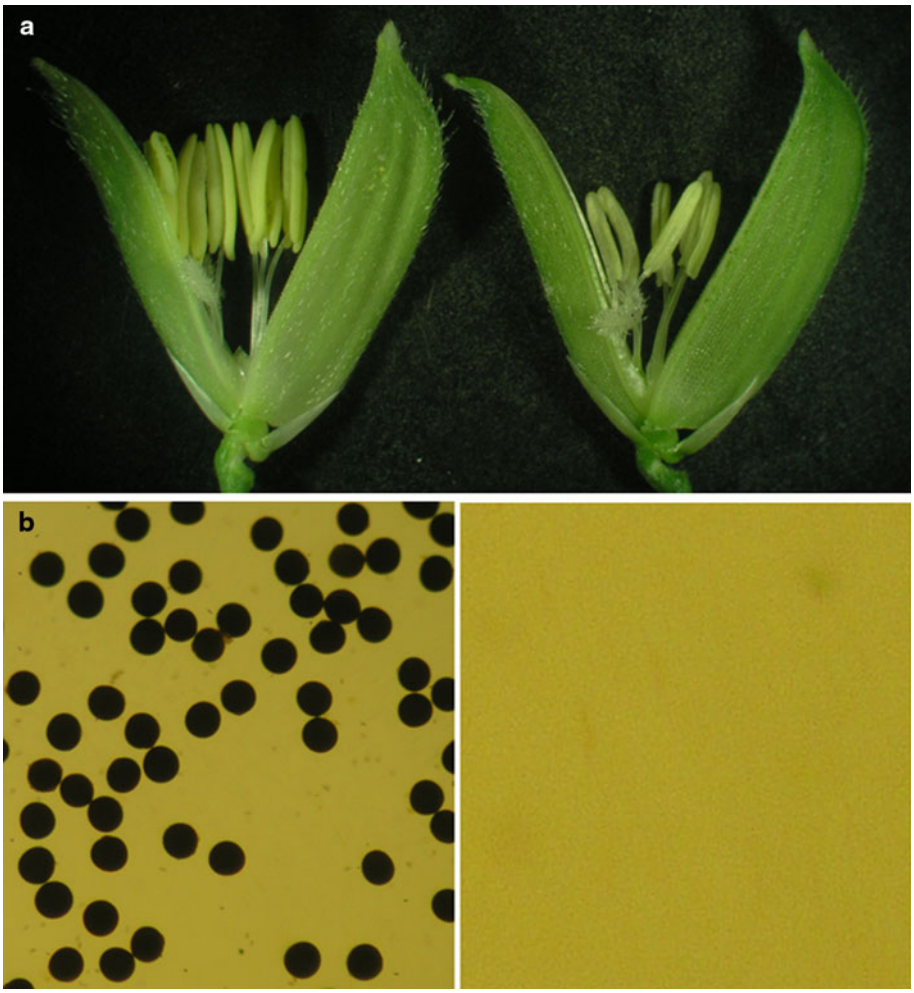

Fig. 1 Comparison of the anthers and pollens of the fertile and sterile plant in F_2 and BC_1F_2 population. **a** *left* a spikelet of fertile plant with normal anthers as 1587; *right* a spikelet of sterile plant with thin anthers as Guangzhan63S; **b** *left* a mature anther of fertile plant was squashed and stained with 1% (v/v) of I_2 in 3% (v/v) KI, and the pollens were round and darkly stained; *right* a mature anther of sterile plant was squashed and stained with 1% (v/v) of I_2 in 3% (v/v) KI, no pollen was found

Table 1 Fertility of the F_2 and BC_1F_2 derived from Guangzhan63S/Han1587

Population	Total	Fertile individuals	Sterile individuals	Fertile individuals/sterile individuals	$\chi^2_{0.05}$ (3:1) = 3.84
F_2	398	307	91	3.37	0.97
BC_1F_2	2,200	1,657	543	3.05	0.12

RM13034 were further employed to construct the primary map of the target gene using 91 F_2 sterile individuals. The target gene named *ptgms2-1* was located between RM12521 and RM12823, with respective genetic distances of 17 and 10.4 cM (Fig. 2a).

Fine mapping of *ptgms2-1*

Fine mapping a gene of interest is the key step in a map-based cloning approach. The *ptgms2-1* locus was finely mapped using a large BC_1F_2 population comprised of 2,200 plants. In addition, 45 new InDel markers were synthesized between the two SSR markers RM12521 and RM12823. Eight polymorphisms were detected in Guangzhan63S and 1587 (Table 2). These polymorphic markers were used to analyze the sterile plants in the F_2 and BC_1F_2 progeny. Finally, the location of the *ptgms2-1* gene was narrowed down to a 50.4 kb region defined by markers S2-40 and S2-44, with genetic distances of 0.08 and 0.16 cM, respectively, and cosegregated with S2-43 located on the AP004039 BAC clone (Fig. 2b).

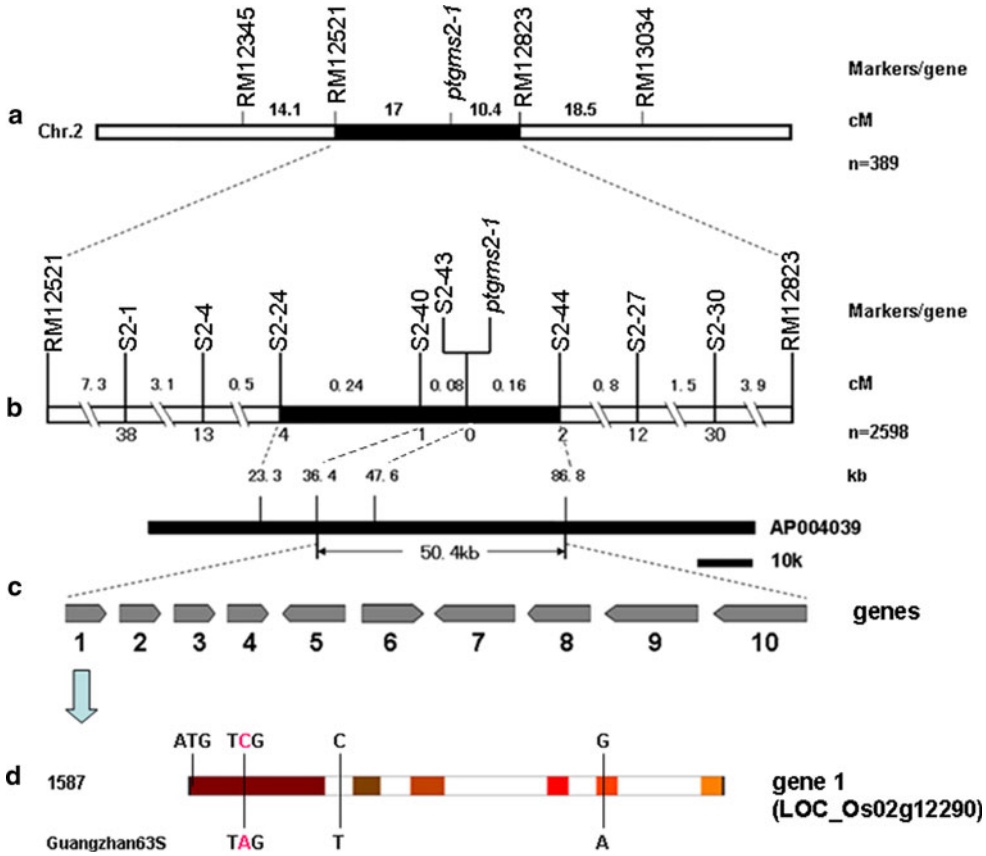

Fig. 2 Genetic and physical maps of the *ptgms2-1* gene and candidate gene analysis. **a** Linkage map of chromosome 2 constructed using 91 sterile plants from 398 F_2 individuals. The *ptgms2-1* gene was mapped to the region between markers RM12521 and RM12823. Numbers show genetic distance between adjacent markers. **b** Fine mapping of the *ptgms2-1* gene. The *ptgms2-1* gene was restricted to the region between markers S2-40 and S2-44 using 634 sterile plants from a total of 2,598 F_2 and BC_1F_2 individuals. The number of recombinants between the markers and *ptgms2-1* is indicated under the linkage map. Numbers show genetic distance between adjacent markers. **c** Candidate region of the *ptgms2-1* locus and the annotated gene in japonica Nipponbare from http://www.ricegaas.rgp.dna.affrc.go.jp. The *ptgms2-1* gene was narrowed down to a 50.4 kb region in the BAC clone AP004039. There were 10 annotated genes in this region. **d** Sequence comparison between 1587 and Guangzhan63S on the gene 1 coding region. The structure of gene 1 is based on the annotation results from the RiceGAAS system (http://www.ricegaas.rgp.dna.affrc.go.jp). SNP1 at site 71 created a premature stop codon in Guangzhan63S allele. SNP2 at site 508 was delimited in non-coding regions. SNP3 at site 1469 was a nonsense mutation

Table 2 Information of the polymorphic InDel markers

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	Size of PCR products (bp)	T_m (°C)
S2-1	CTGATCATCTCATGCCTCCTACG	TCCATCTCCCAATATGACCAACC	167	54
S2-4	CTTGAGTGGGGACACCAAGT	CAATCCCCTTCCCATCTCTT	102	55
S2-24	ATGGCCTCGTCGAGTTCTAA	CGGTCAGCTGAATTTCTCTGT	110	55
S2-27	AGGGAGCGCAAATTTTATCA	CAATACTCCCTCCGGTTTCA	222	55
S2-30	CGGCTAGGGCTAGGGTTC	CCTCCTTCTCTCTCCTCGCT	131	55
S2-40	AGCTATTAGAGAGCCCCTG	CTGAATCAATCTCCGTCTGTTA	150	55
S2-43	GAAGTCAAAAGATAGTAGTTCCCTC	GACTTATGTGATGGACAAAACTGA	331	56
S2-44	CCCTTGCTTTTGTTGGTGTT	CTCGTTTCCCACCTCACATT	198	55

Candidate gene analysis

The available sequence annotation databases (http://www.ricegaas.rgp.dna.affrc.go.jp) indicated ten annotated genes (Gene 1: LOC_Os02g12290, Gene 2: LOC_Os02g12300, Gene 3: LOC_Os02g12310, Gene 4: LOC_Os02g12320, Gene 5: LOC_Os02g12330, Gene 6: LOC_Os02g 12340, Gene 7: LOC_Os02g12350, Gene 8: LOC_Os02g 12360, Gene 9: LOC_Os02g12370 and Gene 10: LOC_ Os02g12380) are present in the 50.4 kb target region (Fig. 2c). Gene 1 is a nuclear ribonuclease Z (RNase Z), Gene 2 is a pectate lyase, which is responsible for cell wall degradation and soft-rotting of plant tissue (Rodríguez et al. 2002); gene 3 is a hypothetical protein; gene 4 an unknown protein similar to Os02g0214500, which is a no apical meristem (NAM) protein; gene 5 is a putative polyprotein; gene 6, 7 and 9 are unknown proteins; gene 8 codes a histone deacetylase 2; and gene 10 codes a YLP motif-containing protein 1.

Recently, Perwez and Kushner (2006) demonstrated that *E. coli* RNase Z played a significant role in mRNA decay. Smith (2004) found that when the expression of RNase Z using RNAi was reduced, *Caenorhabditis elegans* were slow-growing and sterile. Sterility resulted from a drastic reduction in germline proliferation and the generation of germline nuclei in the cell-cycle was arrested. These results suggested a potentially important new role for the RNase Z family of proteins in organisms.

Results demonstrated that *ptgms2-1* controlled male sterility in Guangzhan63S, indicated by the absence of stained pollen in the Guangzhan63S line. Therefore, we speculated that gene 1 was a viable candidate for the underlying *ptgms2-1* locus. We tested this hypothesis by designing PCR primers (G1-1F: 5′-TGTTGAAGAAGC ATCCTGGGC-3′, G1-1R: 5′-CATTTGGACGACATAG AAGGGTG-3′; position in AP004039: 38,271–40,724; fragment length: 2,454 bp) based on the AP004039 sequence and amplified the gene 1 coding region in Guangzhan63S and 1587. The sequencing results indicated that there were three single nucleotide polymorphisms (SNP1, SNP2 and SNP3) between Guangzhan63S and 1587 (Fig. 2d). SNP1 at site 71 created a premature stop codon in the Guangzhan63S allele. SNP2 at site 508 delimited non-coding regions. SNP3 at site 1469 was a nonsense mutation. The gene 1 coding region was subsequently sequenced for two indica and nine japonica rice varieties (Table 3). Comparison of the predicted coding sequences showed that gene 1 was normal exclusive of Guangzhan63S. We examined the expression of gene 1 in different rice organs by RT-PCR analysis and the expression pattern was shown in Fig. 3. Gene 1 was expressed in various organs, including roots, stems, leaves and panicle in 1587. However, it was only normally expressed in the roots, and the expression was significantly reduced in the stems, leaves and panicle in Guangzhan63S. Therefore, we ascertained that gene 1 is the *ptgms2-1* candidate.

Discussion

Following pedigree analysis of Guangzhan63S it was determined that the sterile gene was derived from Nongken58S, a japonica photoperiod-sensitive genic male sterile (PGMS) line. The sterile gene of Nongken58S was initially transferred to 7001S (China Yearbook on High Technology Industry 080120 1996), a japonica PGMS line, then to N422S (Li et al. 1995), another japonica PGMS line, via 7001S. Finally, the sterile gene was bred into Guangzhan63S by N422S (Yang et al. 2002). However, a fertility alteration of Guangzhan63S was detected in response to temperature, which differed from the three progenitor lines, Nongken58S, 7001S and N422S. The results indicated that the sterility gene responded differently to environmental conditions, which showed photoperiod-sensitivity when transferred into japonica backgrounds, while temperature-sensitivity when into indica background.

It was previously reported that the original Nongken58S mutation was located on chromosome 12 (Lu et al. 2005).

Table 3 Plant materials used for sequence analysis

Name	Species	Origin	SNP1	SNP2	SNP3	Phenotype
Balilla	*O. japonica*	Italy	G**C**G	T	A	fertile
Koshihikari	*O. japonica*	Japan	G**C**G	T	A	fertile
Huaidao15	*O. japonica*	China	G**C**G	T	A	fertile
Nipponbare	*O. japonica*	Japan	G**C**G	T	A	fertile
Wuxiangjing	*O. japonica*	China	G**C**G	T	A	fertile
Wuyujing3	*O. japonica*	China	G**C**G	T	A	fertile
Xudao3	*O. japonica*	China	G**C**G	T	A	fertile
Zaofeng9	*O. japonica*	China	G**C**G	T	A	fertile
9516	*O. rufipogen*	China	G**C**G	T	A	fertile
Guangluai4	*O. indica*	China	G**C**G	T	A	fertile
9311	*O. indica*	China	G**C**G	T	A	fertile
1587	*O. indica* (up-land rice)	China	T**C**G	C	G	fertile
Guangzhan63S	*O. indica* (PTGMS line)	China	T**A**G	T	A	sterile

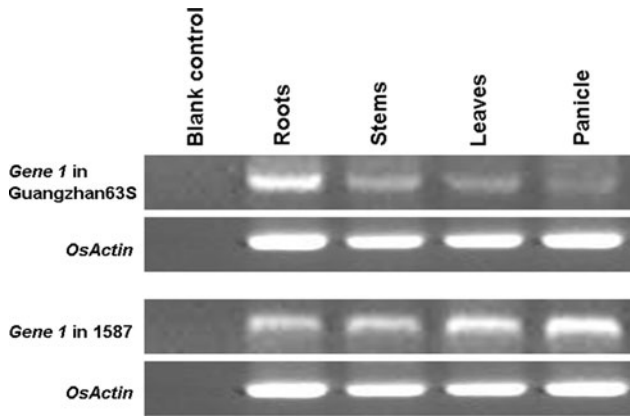

Fig. 3 RT-PCR analysis of the candidate gene expression in specific tissues of Guangzhan63S (sterile) and 1587 (fertile) rice plants. The amplification of the *OsActin* was used as a control to show that approximately equal amounts of total RNA had been used in the RT-PCR analysis

Zhang et al. (1994) demonstrated the 32001S PGMS line fertility trait, derived its PGMS genes from Nongken58S, and was controlled by two sterile genes *pms1* and *pms2*. The two genes were located on chromosome 7 and chromosome 3, respectively. In this study, the Guangzhan63S sterile gene was also derived from Nongken58S, but mapped to the short arm of chromosome 2. All the above results suggest that the sterile gene locus of PTGMS lines derive the PTGMS gene from Nongken 58S, but the gene has the capacity to diverge under certain conditions.

Currently, the sterile genes of the PTGMS lines that are used in indica two-line hybrid rice breeding systems in China has two main sources, Nongken58S or AnnongS. The sterile gene of AnnongS *tms5* was physically mapped to a 19 kb DNA fragment (Yang et al. 2007). The most significant result of this work is fine physical mapping the *ptgms2-1* locus which was derived from Nongken58S to a DNA fragment of 50.4 kb. Additionally, we identified a nuclear ribonuclease Z gene, which has not been reported in plants, as the candidate gene for the *ptgms2-1* locus in the target region. This is an important step toward map-based cloning this gene and aids in understanding the PTGMS mechanisms at the molecular level. Guangzhan63S is a non-pollen type and widely used PTGMS line with stable male sterility. It is highly necessary to identify the corresponding mutated gene in Guangzhan63S for transferring the gene for the development of new rPTGMS lines with more complete and stable sterility. The tightly linked markers for the *ptgms2-1* gene locus characterized in this study will further provide a useful tool for MAS of this gene in rice breeding programs.

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