Theor Appl Genet (2007) 114:939–946
DOI 10.1007/s00122-006-0491-z

ORIGINAL PAPER

Fine mapping of *pss1*, a pollen semi-sterile gene in rice (*Oryza sativa* L.)

Wanchang Li · Ling Jiang · Shirong Zhou · Chunming Wang · Linglong Liu · Liangming Chen · Hiroshi Ikehashi · Jianmin Wan

Received: 27 May 2006 / Accepted: 21 December 2006 / Published online: 6 February 2007

Abstract During routine seed increase procedures in rice, semi-sterile plants are common; however, such semi-sterility mutants in rice varieties have been only rarely analyzed genetically. W207-2 is a semi-sterile selection from the *japonica* rice variety Nipponbare. In this report, we found the female gamete of W207-2 was normal, and its semi-sterility was unaffected by growth duration but was conditioned by a recessive nuclear gene whose action leads to pollen semi-sterility and anther indehiscence, and the gene was named as *pss1* (pollen semi-sterile). Using an F_2 population derived from the two parents W207-2 and Dular and a pooled DNA strategy, *pss1* was mapped to an interval on chromosome 8 defined by the two SSR loci RM6356 and RS41. The position of *pss1* was confirmed in another F_2 population derived from the cross W207-2 × Nipponbare. Over 2,000 homozygous *pss1* segregants from the large W207-2 × Dular F_2 population were used to fine map *pss1* to a 0.04 cM segment flanked by a CAPs marker L2 and a dCAPs L3 marker. Sequences for both markers are present on a single PAC clone, and the physical distance between them is about 28 kb. Analysis of the PAC sequence predicts the presence of five open reading frames, they are as follows: putative ribonuclease PH, putative avr9 elicitor response protein, kinesin1-like protein, putative protein RNP-D precursor and putative 40S ribosomal protein S13. This result would be helpful in cloning the *pss1* gene.

Communicated by T. Sasaki.

W. Li · L. Jiang · S. Zhou · C. Wang · L. Liu · L. Chen · J. Wan (✉)
State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China
e-mail: wanjm@njau.edu.cn

J. Wan
Crop Science Research Institute, Chinese Academy of Agricultural Sciences, Beijing 10081, China

H. Ikehashi
Department of Plant and Resources College of Bioresources, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 2528510, Japan

W. Li
College of Life Sciences, Henan Normal University, Xinxiang 453002, China

Introduction

Male-sterile mutants are valuable for understanding the mechanisms of pollen development, and for the production of F_1 hybrid seeds as well. The sporophytic male sterile phenotype has been identified in rice and a number of other higher plant species. Nuclear genes involved in the determination of male fertility are involved in a range of biological processes, including the determination of reproductive cell fate, meiosis, maturation of microspores, and dehiscence of anthers (Chaudhury 1993). In rice, male sterility is frequently inherited in a monogenic recessive fashion, such as the *ms/ms* genotype is sterile, and both the *Ms/Ms* and *Ms/ms* types are fully fertile. This type of sterility has been widely exploited in rice breeding (Singh and Ikehashi 1981). Semi-sterile mutants are less common, but have also been documented in the literature. In one of the earliest such reports, Terao (1921) described a

spontaneous semi-sterile mutant in the rice variety Sekiyama, showing that the phenotype was associated with pollen abortion, and wild type and semi-sterility followed the segregation ratio 1:1. Thus this semi-sterility was considered to be under the control of a gene with gametophytic action. During routine seed increase procedures in rice, semi-sterile plants are common, and since these represent off-types, their removal by roguing becomes a time-consuming activity. The frequency of semi-sterile plants was estimated to at a rate of 1.68×10^{-4} for Nakate-Shinsenbon, and 8.52×10^{-5} for Nipponbare at the Kyoto Prefecture Seed Increase Farm (Ikehashi and Yamaguchi 2000). Such semi-sterility mutants in rice varieties have been only rarely analyzed genetically, because the estimation of segregation ratio is complicated by loss through sterility of certain genotypes. This has largely prevented the undertaking of formal analyses of the molecular mechanism(s) associated with the male partial-sterile character.

In 1991, a spontaneous semi-sterile selection was obtained from the *japonica* rice variety Nipponbare in Japan. The mutant line W207-2 was genetically stable, showing 50% pollen fertility and 40% spikelet fertility. Yu et al. (2005) roughly mapped a semi-sterile gene, *ss-1(t)*, to a region of chromosome 8 defined by the SSR loci RM152 and RM6863 using an F_2 population from the cross W207-2 × CPSLO17. This genetic window is, however, too wide (15.1 cM) to allow for a candidate gene approach, based on the full rice genome sequence, to be taken. The major objective of this study was to analyses the causes of this semi-sterile phenomenon and fine-map the *pss1* (pollen semi-sterile) gene in a narrow interval, which should lay the basis for its map-based cloning, leading to an eventual understanding of the genetic mechanism(s) underlying pollen semi-sterility in molecular level in rice.

Materials and methods

Plant materials

The semi-sterile mutant W207-2 was crossed with both Nipponbare, and the *indica* variety Dular. In order to exclude the possibility of confounding effects arising from the use of a wide *indica* × *japonica* cross, the widely compatible *indica* variety Dular was chosen as the parent of the mapping population, since it carries a suite of neutral alleles *S-7*^{n} (Yanagihara et al. 1992), *S-5*^{n}, *S-8*^{n}, *S-9*^{n}, *S-16*^{n} and *S-17*^{n} (Wan et al. 1993, 1996; Wan and Ikehashi 1995, 1996). The two F_2 populations consisting of 291 and 1,730 individual plants, respectively were grown in the summer season of 2004; while in 2005, an larger W207-2 × Dular F_2 population with 9,600 plants was grown. Parental plants and F_1s were included as checks, and all experiments were grown in the field at the Jiangsu Academy of Agricultural Sciences, Nanjing, China.

Investigation of pollen and spikelet fertility

To estimate the level of pollen fertility, three spikelets were collected from each plant shortly before anthesis, and fixed in 70% ethanol. Three anthers were sampled at random from each spikelet. Between 100 and 200 stained pollen grains (1% I_2–KI solution) were inspected by light microscopy. Using the classification system of Chaudhary et al. (1981), round, dark brown pollen grains were assessed as fertile, while irregularly shaped, yellow or clear ones were classed as sterile. Spikelet fertility was calculated as the proportion of fertile spikelets to all spikelets on three panicles for each plant at maturity. Hand saturated pollination was employed to study the biology of semi-sterility in W207-2. Twenty panicles of semi-sterile W207-2 were bagged before flowering, of which ten were fertilized with W207-2 and ten with Nipponbare pollen every day for 4 days. One month later, seed set in the bagged spikelets was measured.

Molecular marker analysis

Genomic DNA was isolated from fresh leaves of field-grown plants using the method Dellaporta et al. (1983) with minor modifications. The extracted DNA was dissolved in TE buffer (10 mM Tris base, 0.1 mM EDTA, pH 8.0), and used to make DNA pools from either 20 fertile or 20 semi-sterile individuals. SSR genotyping was following the procedures of Chen et al. (1997) with minor modifications. PCRs were carried out in a volume of 10 µl containing 10 mM Tris–HCl pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 50 µM of each dNTP, 0.2 µM of each primer, 0.5 U *Taq* polymerase (TaKaRa, Dalian) and 20 ng of template. The amplification regime was 94°C for 5 min, followed by 35 cycles of 94°C/30 s, 55°C/30 s and 72°C/60 s, with a final extension step of 7 min at 72°C. Amplicons were separated by 8% non-denaturing PAGE, and silver stained (Sanguinetti et al. 1994). Details of all the markers used are published at the Gramene website (http://www.gramene.org/microsat). Linkage maps were constructed according to Temnykh et al. (2000) and McCouch et al. (2002).

Development of new PCR-based markers flanking *pss1*

New SSR markers were produced from the Nipponbare genome sequence (http://www.rgp.dna.affrc.go.jp), using the Primerprimer5.0 and SSRIT routines (http://www.gramene.org/db/searches/ssrtool). Predictions of polymorphism were made by comparisons of the in silico amplicons from Nipponbare and the *indica* cultivar 93-11 (http://www.rice.genomics.org.cn/index.jsp). To develop additional PCR-based markers flanking *pss1*, genomic sequences of W207-2 and Dular were contrasted. Primers were designed to generate CAPS (cleaved amplified polymorphic sequence, Konieczny and Ausubel 1993) or dCAPS (derived CAPS; Michaels and Amasino 1998; Neff et al. 1998) markers.

Linkage analysis and gene mapping

Linkage groups were determined using MAPMAKER/EXP 3.0 (Lander et al. 1987). The Kosambi mapping function was used to transform the recombination frequency to mapping distance (cM). To normalize the variance, % pollen sterility was transformed by the arcsine_square root function. Interval QTL mapping was applied through MAPMAKER/QTL 1.1 (Lander et al. 1987) with a LOD threshold of 3.0 and a probability level of 0.01 for declaring the presence of a putative QTL. For the high resolution mapping of *pss1* among the 2,100 semi-sterile segregants from the W207-2 × Dular F_2 population, the recombination frequency c between a marker and *pss1* was calculated using the method of Zhang et al. (1994), where $c = (N_1 + N_2/2)/2{,}100$, in which N_1 is the number of individuals homozygous for the fertile parent marker allele, and N_2 the number of heterozygotes.

Results

Analysis of semi-sterility

Experiments over successive years indicated that the semi-sterility character is stably expressed, showing about 50% pollen fertility and 40% spikelet fertility. The major agricultural traits of W207-2 were no significant difference with Nipponbare exception for low fertility. In 2004, the pollen and spikelet fertility of W207-2, the semi-sterile mutant line, and other parents as well as reciprocal F_1 hybrids was shown in Table 1. The results indicated that the semi-sterility was controlled by recessive nucleus genes with no cytoplasmic effect.

When W207-2 was hand-pollinated with W207-2, its spikelet fertility was about 51%, while when hand-pollinated with Nipponbare, it reached about 86%. This was taken to indicate that the female fertility of W207-2 is normal, and that its spikelet semi-sterility is due to lesions in microsporogenesis. Despite the semi-sterility of the pollen, there should, in principle, be a sufficient number of fertile pollen grains present to successfully fertilize each spikelet. An additional feature of the W207-2 phenotype is that its anthers are thin and almost indehiscent, while those of Nipponbare are plump and dehiscent (Fig. 1). Thus, anther indehiscence acts to further reduce the number of effective pollen grains shed onto the stigma, resulting in its marked spikelet semi-sterility.

Correlation analysis in the W207-2/Nipponbare F_2 population showed that heading date is not correlated with either spikelet fertility ($r = 0.1962$) or pollen fertility ($r = 0.0825$). Thus, semi-sterility is unaffected by growth duration. A highly significant positive correlation ($r = 0.9119$) obtains between pollen and spikelet fertility, as predicted if the spikelet semi-sterility in W207-2 is caused by pollen semi-sterility. Since spikelet fertility is jointly affected by anther dehiscence and

Table 1 Pollen and spikelet fertilities of the parents and reciprocal F_1

Varieties and F_1s	Pollen fertility (%)	Spikelet fertility (%)
Nipponbare	97.0 ± 0.5	94.4 ± 1.1
W207-2	51.0 ± 1.2	40.1 ± 1.1
Dular	98.6 ± 0.7	91.7 ± 1.7
W207-2/Nipponbare	91.1 ± 1.7	87.5 ± 1.4
Nipponbare/W207-2	93.2 ± 1.9	88.1 ± 1.6
W207-2/Dular	90.9 ± 1.6	87.1 ± 1.3
Dular/W207-2	94.6 ± 1.9	89.5 ± 1.2
Nipponbare/Dular	91.6 ± 0.9	91.4 ± 1.2
Dular/Nipponbare	97.7 ± 0.8	89.9 ± 1.7

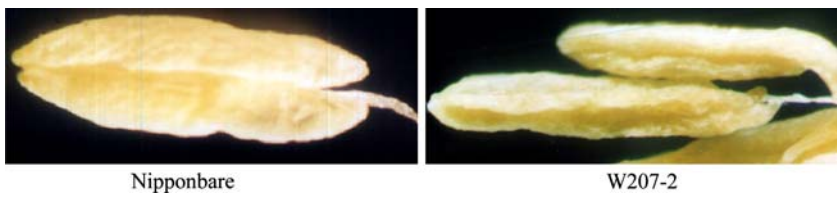

Fig. 1 Anthers of Nipponbare and W207-2

pollen fertility, we chose pollen fertility as the measure of fertility.

Frequency distribution of pollen fertility in the W207-2/Nipponbare F_2 population was continuous and bimodal, with two distinct peaks around 55 and 95%. One of the peaks fertility was similar to the semi-sterile parent (W207-2), and the other was similar to the F_1. According to the apparent low valley (70%), the W207-2/Nipponbare F_2 population could be divided into two groups, the fertile plants group (70–97.6%) including 221 individuals and the semi-sterile plants group (14.3–70%) including 70 individuals. χ^2 showed that fertile plants and semi-sterile plants fit to 3:1 ratio ($\chi^2_c = 0.0928 < \chi^2_{0.05,1} = 3.84$). These results suggested that the pollen semi-sterility of W207-2 was a single recessive nucleus gene controlled sporophytic inheritance.

Preliminary mapping of *pss1*

A collection of 565 SSR markers, dispersed across the rice genome, was surveyed for marker informativeness between the fertile and semi-sterile bulks, and between the two parents W207-2 and Dular. Five SSR loci—RM178 on chromosome 5, RM118 on chromosome 7, and RM506, RM152 and RM6863 on chromosome 8, emerged as candidates for linkage from this exercise. Based on the linkage maps of chromosomes 5, 7 and 8 constructed using 182 randomly chosen individuals from the W207-2 × Dular F_2 population, only one major locus controlling pollen semi-sterility could be identified with a high LOD score (48.3) and a substantial PVE (phenotypic variation explained) (70.5%). The locus was located in the region on chromosome 8 between SSR loci RS41 and RM6356 (Fig. 2).

To confirm that this region includes *pss1*, 97 chromosome 8 SSR markers (including 32 newly developed ones), covering the whole chromosome, were tested for polymorphism between W207-2 and Nipponbare. Of these, only two—RM3702 and RS41—were informative. The location of *pss1* was refined by a mapping analysis involving 291 W207-2 × Nipponbare F_2 individuals. An ANOVA showed that the pollen fertility of homozygotes carrying the W207-2 allele at RM3702 and RS41 was significantly lower than those carrying the alternative allele, either as homozygotes or heterozygotes, both of which express a level of pollen fertility indistinguishable from wild type (Table 2). According to the rice molecular linkage map published by McCouch et al. (2002), the marker RM3702 located on chromosome 8 between two markers RS41 and RM6356, thus the recessive allele at *pss1* present in W207-2 is located in the region of RM3702 and RS41

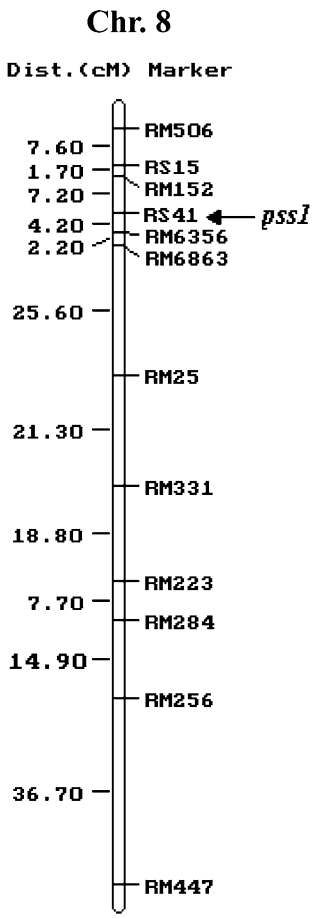

Fig. 2 Location of the pollen semi-sterility gene (*pss1*) on chromosome 8 using W207-2 × Dular F_2 population ($n = 182$)

Table 2 Genetic effect of pollen fertility in each group classified by genotypes of markers in W207-2 × Nipponbare F_2 population

Marker	Genotype[a]	Total	Mean (%)	t Test	
				0.05	0.01
RM3702	1/1	71	48.63	a	A
	1/2	160	90.09	b	B
	2/2	60	88.46	b	B
RS41	1/1	72	48.90	a	A
	1/2	161	89.39	b	B
	2/2	58	88.23	b	B

[a] 1/1 denoted genotype of W207-2, 2/2 denoted genotype of Nipponbare, 1/2 denoted genotype of F_1 hybrid

on chromosome 8, and the Nipponbare allele acts in a completely dominant fashion over that of W207-2.

Fine mapping of *pss1*

To further define the *pss1* map position, another 70 markers, including 32 developed SSR markers and 8 CAPs (or dCAPs) markers mapping in the vicinity of

RS41 and RM6356 were screened against W207-2 and Dular. Of these, seven SSRs and three CAPs (dCAPs) markers were informative (Table 3). Then, these ten informative markers and RS41 and RM6356 were used to genotype the entire fine-mapping population of 2,100 *pss1* segregants. CAPs marker L2 uncovered one recombinant event with respect to *pss1* on one side, and the dCAPs marker L3 also defined one recombinant event on the other side. Thus, *pss1* was restricted to a 0.04 cM region flanked by L2 and L3 on the short arm of chromosome 8 (Fig. 3). These two markers are present on a single Nipponbare PAC (P0470F10), and the physical distance between them is about 28 kb. The sequence of this clone (http://www.ncbi.nlm.nih.gov) displays a putative ribonuclease PH, a putative avr9 elicitor response protein, a kinesin1-like protein, a putative RNA-binding protein RNP-D precursor, a putative 40S ribosomal protein S13 and a part of another putative 40S ribosomal protein S13.

Discussion

Male-sterile mutations provide an ideal source material for a range of genetic and molecular biological studies of reproductive biology. For example, to test whether the asymmetric division that resulted in the formation of the vegetative cell and the generative cell, two mutants *sidecar pollen* (Chen and McCormick 1996) and *gemini pollen* (Park et al. 1998) were screened in Arabidopsis. At the mature pollen stage in *sidecar pollen* heterozygotes, ~50% of the pollen was normal, ~43% was aborted, and ~7% showed the sidecar phenotype. *Gemini pollen* encodes a microtubule-associated protein (MAP215 family), and the *gemini pollen* phenotype is now believed to be attributable to a defect in the correct positioning of cytokinetic phragmoplast at pollen mitosis I (Twell et al. 2002). Therefore, this male semi-sterile mutant, W207-2, would also provide a useful material for studying the pollen development in rice.

Many factors affect spikelet fertility, including pollen and embryo sac sterility (Song et al. 2005), anther indehiscence (Maekawa et al. 1997) and non-synchronization of male and female gametogenesis in the same spikelet (Liu et al. 1997). In this research, we found that the pollen fertility of W207-2 was semi-sterile and the anther dehiscence was poor. Though the female gamete of W207-2 was normal, the spikelet fertility was low for the two reasons. Liu et al. (2004) showed that a reduced affinity (or compatibility) between gametes could be an important cause of sterility in *indica* × *japonica* hybrids in addition to pollen and

Table 3 All markers newly developed in this study which had polymorphisms between W207-2 and Dular

Marker	Marker type	Forward primer (5′-3′)	Reverse primer (5′-3′)	PAC/BAC location	Restriction enzyme
RS15	SSR	TAGAACTACCACCGCAAGG	AAGCAGCAACCAAAACACT	P0007D08	
RS22	SSR	AGTTCTTCGCCCAGTG	TGCTCCTAGTCGTCCC	P0498H04	
RS24	SSR	AGGACGCTTGAAGAGGAAA	ATGACAGGCTGGGTGAGTG	P0498H04	
RS41	SSR	GAGGACCTGAACCTGTCTAAAT	TGCCATGATGATAACGTGAAT	P0470F10	
RS43	SSR	ACCATGTCGGTCTTAT	TACACCAGGAGCAAAT	P0470F10	
RS413	SSR	CGCTTATGTGGCAGTA	AAAGGCAAAGAGGAGA	P0470F10	
RS73	SSR	AAAAAGCAGAGGAGGAGAT	CCACACAAGACTGAAATTG	OSJNBa0073J19	
L1	dCAPS	CTTGATTGATCTCGCAGAGTTCGTTGAT	ACCAGGGTGGCGATCTTCTCAGACT	P0470F10	Bcl I
L2	CAPs	AAGCCAATGCAGTAACTATAACC	CTTTAGCAGCGAGATGTCCAA	P0470F10	Sca I
L3	dCAPs	AAGAACTAAGAAAGGAAATCAC	ATATATCAGAATCAACAAGTTTAA	P0470F10	Dra I

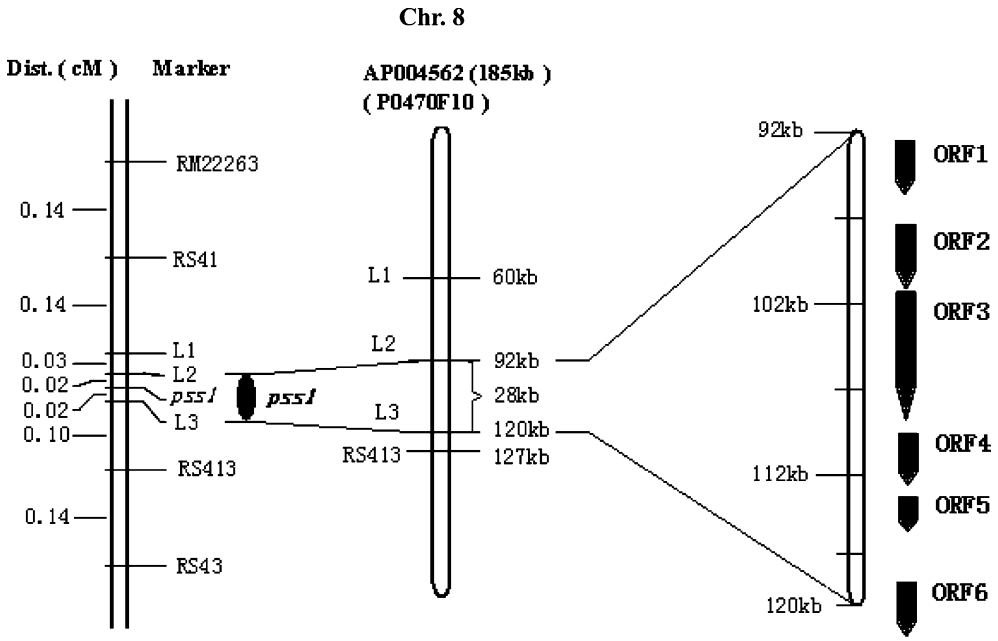

Fig. 3 High-resolution genetic and physical maps of the *pss1* locus on rice chromosome 8 using W207-2 × Dular F_2 population (n = 2,100), ORF1–ORF6, respectively, denotes putative ribonuclease PH, putative avr9 elicitor response protein, kinesin1-like protein, putative protein RNP-D precursor, putative 40S ribosomal protein S13 and putative 40S ribosomal protein S13

embryo sac fertility. In principle, semi-sterile pollen types should produce sufficient viable pollen grains to effect full fertilization of the spikelet. However, as shown in some intraspecific hybrids, 50% pollen stainability can still lead to low seed-set, and this has been ascribed to a poor level of pollen germination (Lin et al. 1992). Thus pollen germination needs to be tested to determine whether it contributes to spikelet semi-sterility or other factors, such as compatibility, etc.

There were many reports on hybrid semi-sterility of *japonica*/*indica*, and several hypotheses had been proposed to explain the genetic mechanism of such inter-subspecific hybrid semi-sterility, including allelic interaction at a single locus (Ikehashi and Araki 1986), duplicate gametophytic lethal model (Oka 1974) or epistatic interaction between loci (Li et al. 1997), and recombination within putative differentiated "supergenes" (Li et al. 1997). However, the semi-sterility of W207-2 could not be explained by these hypotheses, and it was a homozygous semi-sterility (*pss1*/*pss1*) but not allelic interaction. When W207-2 was crossed with other *japonica* varieties or wide-compatibility varieties, the F_1 fertility was normal and the segregated fertile plants: semi-sterile plants fitted to 3:1 in the F_2 population, thus we thought the semi-sterility of W207-2 was one recessive gene controlled sporophytic inheritance.

In a related study, Sobrizal et al. (2001) mapped the pollen semi-sterility gene *S27(t)* to a region between the loci G2132 and L128 on chromosome 8, using a BC_4F_2 population derived from the hybrid Taichung 65 × *Oryza glumaepatula* (IRGC105668). Two spikelet fertility QTL *spf8* and *f8* appear to co-locate to a region on chromosome 8 (Wang et al. 1998; Song et al. 2005), but linkage relationships suggest that these three loci are not identical with *pss1*. The fine mapping of *pss1* refined the gene within a 28 kbp PAC, the full sequence of which is represented in the Nipponbare genome sequence. Of the predicted gene sequences present in this clone, there are five open reading frames.

RNase PH was one of the exoribonucleases that catalyzed the 3′ end processing of tRNA, and it removed nucleotides following the CCA sequence of tRNA precursors by phosphorolysis and generated mature tRNAs with amino acid acceptor activity (Ishii et al. 2003). RNase PH also played the major role in mRNA and rRNA degradation/processing (Butler 2002).

The *Avr9* gene encoded secreted cysteine-rich peptides, and their elicitor response proteins elicited a set of defense responses for plant disease resistance (Blatt et al.1999).

Kinesins performed functions including transport of vesicles, organelles, or chromosomes along MTs (microtubules), crosslinking and antiparallel sliding of MT arrays, and, possibly, mediation of MT polymerization and depolymerization (Yang et al. 2003). Several kinesins had been found to be associated with mitosis and/or meiosis (Nishihama et al. 2002; Yang et al.

2003). For example, in *Arabidopsis*, *TES* encoded a plant-specific kinesin required for meiotic cytokinesis (Yang et al. 2003). Its mutant, *tes*, had the following features: (1) reduced fertility following self-pollination, (2) production of relatively small numbers of pollen grains, and (3) unimpaired female fertility (Spielman et al. 1997). Another *ATK1* gene in *Arabidopsis*, encoding a kinesin, was important for spindle function in male meiosis. Its mutant *atk1-1*, loss-of-function by T-DNA, had reduced number of viable pollen grains, abnormal male meiosis and normal female gamete, and lastly resulted in male semi-sterility. The abnormal spindle was the likely cause for the abnormal chromosome segregation and the subsequent spore and pollen defects (Chen et al. 2002). The features of W207-2 are more likely similar to these two mutants, *tes* and *atk1-1*.

RNP D was a kind of RNA-binding proteins. It regulated gene post-transcriptional events including pre-mRNA splicing, polyadenylation, mRNA transport, translation and stability/decay, either directly or indirectly (Burd and Dreyfuss 1994). For example, hnRNP D, depending on promoter context and cell type, could function as activators or repressors. In human, hnRNP D binding to the CR2 promoter region reduced promoter activity (Tolnay et al. 2002). In *Arabidopsis*, two RNA-binding proteins were involved in floral development (Macknight et al. 1997; Schomburg et al. 2001). The ribosomal proteins (RPs) were major components of the biosynthetic machinery, and it represented up to 15% of cellular protein (Mager 1988).

Knocking out of *AtRPS13A* (a gene homologous to cytoplasmic ribosomal protein S13) caused *Arabidopsis* aberrant growth and development. However, no phenotypic changes were observed during reproductive growth (Ito et al. 2000).

We assume that the candidate gene of *pss1* is most possibly one of the ORF1, ORF3 and ORF4 among the five ORFs functions. Furthermore, using RT-PCR, we find no differences of these three genes transcripts between the mutant and wild type in anther development. This suggests post-transcriptional regulation might involve in *pss1* lesion. Now, we are attempting to identify the *pss1* gene via sequencing the whole 28 kb fragment and gene transformation.

Acknowledgments We are grateful to Dr. F.L. Gu in Jiangsu Academy of Agricultural Sciences, for kindly managing the field experiments. We thank Dr. C.L. Wang, S.W. Guo and the graduate students Sh. D. Huang, L. Zhao, T. Guo and L.N. Zheng for their help in the experiments. This research is supported by the grants from the National High Technology Research and Development Program of China (2006AA100101, 2006AA10Z1A5), the National Natural Science Foundation of China, Jiangsu Science and Technology Development Program (BG2004303, BG2006301) and PCSIRT.

References

Blatt MR, Grabov A, Brearley J, Hammond-Kosack K, Jones JDG (1999) K^+ channels of *Cf-9* transgenic tobacco guard cells as targets for *Cladosporium fulvum Avr9* elicitor-dependent signal transduction. Plant J 19:453–462

Burd CG, Dreyfuss G (1994) Conserved structures and diversity of functions of RNA-binding proteins. Science 265:615–621

Butler JS (2002) The yin and yang of the exosome. Trends Cell Biol 12:90–96

Chaudhury AM (1993) Nuclear genes controlling male fertility. Plant Cell 5:1277–1283

Chaudhary RC, Virmani SS, Khush GS (1981) Patterns of pollen abortion in some cytoplasmic-genetic male sterile lines of rice. Oryza 18:140–142

Chen YCS, McCormick S (1996) *Sidecar pollen*, an *Arabidopsis thaliana* male gametophytic mutant with aberrant cell divisions during pollen development. Development 122:3243–3253

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Chen CB, Marcus A, Li WX, Hu Y, Calzada JV, Grossniklaus U, Cyr RJ, Ma H (2002) The Arabidopsis *ATK1* gene is required for spindle morphogenesis in male meiosis. Development 129:2401–2409

Dellaporta SL, Wood T, Hicks TB (1983) A plant DNA mini preparation: version II. Plant Mol Biol Rep 1:19–21

Ikehashi H, Araki H (1986) Genetics of F1 sterility in remote crosses of rice. In: IRRI (eds) Rice genetics. IRRI, Manila, pp 119–130

Ikehashi H, Yamaguchi (2000) Semi-sterile mutant in seed increase of rice (in Japanese). Nougyou Oyobiengei 75:700–704

Ishii R, Nureki O, Yokoyama S (2003) Crystal structure of the tRNA processing enzyme RNase PH from *Aquifex aeolicus*. J Biol Chem 278:32397–32404

Ito T, Kim GT, Shinozaki K (2000) Disruption of an *Arabidopsis* cytoplasmic ribosomal protein S13-homologous gene by transposon-mediated mutagenesis causes aberrant growth and development. Plant J 22:257–264

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Lander ES, Green P, Abrahamson J (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage map of experimental and natural populations. Genomics 1:174–181

Li Z, Pinson SRM, Paterson AH, Park WD, Stancel JW (1997) Genetics of hybrid sterility and hybrid breakdown in an inter-subspecific rice (*Oryza sativa* L.) population. Genetics 145:1139–1148

Lin SY, Ikehashi H, Yanagihara S, Kawashima A (1992) Segregation distortion via male gametes in hybrids between *Indica* and *Japonica* or wide-compatibility varieties in rice (*Oryza sativa* L). Theor Appl Genet 84:812–818

Liu YS, Zhu LH, Sun JS, He P, Liu YS, Zhu LH, Sun JS (1997) Mapping quantitative trait loci for reproductive barriers occurring in hybrid between *indica* and *japonica* rice. Acta Bot Sin 39:1099–1104

Liu HY, Xu CG, Zhang Q (2004) Male and female gamete abortions, and reduced affinity between the uniting gametes as the causes for sterility in an *indica/japonica* hybrid in rice. Sex Plant Reprod 17:55–62

Macknight R, Bancroft I, Page T, Lister C, Schmidt R, Love K, Westphal L, Murphy G, Sherson S, Cobbett C, Dean C (1997) *FCA*, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. Cell 89:737–745

Maekawa M, Inukai T, Shinbashi N (1997) Genic analysis of hybrid sterility caused by anther indehiscence between distantly related rice varieties. Euphytica 94:311–318

Mager WH (1988) Control of ribosomal protein gene expression. Biochim Biophys Acta 949:1–15

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). DNA Res 9:199–207

Michaels SD, Amasino RM (1998) A robust method for detecting single-nucleotide changes as polymorphic markers by PCR. Plant J 14:381–385

Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J 14:387–392

Nishihama R, Soyano T, Ishikawa M, Araki S, Tanaka H, Asada T, Irie K, Ito M, Terada M, Banno H, Yamazaki Y, Machida Y (2002) Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/mapkkk complex. Cell 109:87–99

Oka HI (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77:521–534

Park SK, Howden R, Twell D (1998) The *Arabidopsis thaliana* gametophytic mutation *gemini pollen 1* disrupts microspore polarity, division asymmetry and pollen cell fate. Development 125:3789–3799

Sanguinetti CJ, Dias Neto E, Simpson AJG (1994) Rapid silver staining and recover of PCR products separated on polyacrylamide gels. Biotechniques 17:915–919

Schomburg FM, Patton DA, Meinke DW, Amasino RM (2001) *FPA*, a gene involved in floral induction in *Arabidopsis*, encodes a protein containing RNA-recognition motifs. Plant Cell 13:1427–1436

Singh RJ, Ikehashi H (1981) Monogenic male sterility in rice: Induction and identification. Crop Sci 21:286–289

Sobrizal Y, Matsuzaki Y, Yoshimura A (2001) Mapping of a gene for pollen semi-sterility on chromosome 8 of rice. Rice Genet Newsl 18:59–61

Song X, Qiu SQ, Xu CG, Li XH, Zhang QF (2005) Genetic dissection of embryo sac fertility, pollen fertility, and their contributions to spikelet fertility of intersubspecific hybrids in rice. Theor Appl Genet 110:205–211

Spielman M, Preuss D, Li FL, Browne WE, Scott RJ, Dickinson HG (1997) *TETRASPORE* is required for male meiotic cytokinesis in *Arabidopsis thaliana*. Development 124:2645–2657

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequence in rice (*Oryza sativa* L.). Theor Appl Genet 100:697–712

Terao H (1921) Mutation of semi-sterility and its inheritance in rice (in Japanese). Jpn J Genet 1:45–54

Tolnay M, Vereshchagina LA, Tsokos GC (2002) NF-κB regulates the expression of the human complement receptor 2 gene. J Immunol 169:6236–6243

Twell D, Park SK, Hawkins TJ, Schubert D, Schmidt R, Smertenko A, Hussey PJ (2002) Mor1/Gem 1 has an essential role in the plant-specific cytokinetic phragmoplast. Nat Cell Biol 4:711–714

Wan J, Ikehashi H (1995) Identification of a new locus *S-16* causing hybrid sterility in native rice varieties (*Oryza sativa* L.) from Tai-hu lake region and Yannan province, China. Breed Sci 45:461–470

Wan J, Ikehashi H (1996) List of hybrid sterility gene loci (HSG-Li) incultivated rice (*Oryza sativa* L.). Rice Genet Newsl 13:110–113

Wan J, Yanagihara S, Kato K, Ikehashi H (1993) Multiple alleles at a new locus causing hybri sterility between a Korean *indica* variety and *Javanica* variety in rice (*Oryza sativa* L.) (in Japanese). Jpn J Breed 43:507–516

Wan J, Yamaguchi Y, Kato H, Ikehashi H (1996) Two new loci for hybrid sterility in rice (*Oryza sativa* L.). Theor Appl Genet 92:188–190

Wang J, Liu KD, Xu CG, Li XH, Zhang Q (1998) The high level of wide compatibility of variety Dular has a complex genetic basis. Theor Appl Genet 97:407–412

Yanagihara S, Kato H, Ikehashi H (1992) A new locus for multiple alleles causing hybrid sterility between an Aus varieties and *Javaica* varieties in rice (*Oryza sativa* L.) (in Japanese). Jpn J Breed 42:793–801

Yang CY, Spielman M, Coles JP, Li Y, Ghelani S, Bourdon V, Lemmon BE, Scott RJ, Dickinson HG (2003) TETRASPORE encodes a kinesin required for male meiotic cytokinesis in Arabidopsis. Plant J 34:229–240

Yu WW, Wang CM, Ikehashi H, Wan JM (2005) Mapping of a novel gene for semi-sterility in rice (*Oryza sativa* L.). Breed Sci 55:15–20

Zhang Q, Shen BZ, Dai XK, Mei MH, Maroof MAS, Li ZB (1994) Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. Proc Natl Acad Sci USA 91:8675–8679