Pak. J. Bot., 50(3): 1191-1196, 2018.

MAPPING THE *OSDMS-2* DOMINANT MALE STERILE RICE MUTANT

YANLING LIANG[2, 3*], HENGQI MIN[2, 3*], XIAOHUA XIAN[2, 3*], YUN CHEN[2, 3], MIN SHI[2, 3], SHUANGLIN DU[2, 3], KUN YANG[2, 3], YUANXIN HUANG[2, 3], JUN LV[2, 3#] AND YI ZHANG[1,2#]

[1]School of Agriculture, Yunnan University, Chenggong District, Kunming, Yunnan, 650500, China.
[2]Engineering Research Center of South Upland Agriculture, Southwest University, Tiansheng Road 2, Beibei, Chongqing 400715, China;
[3]College of Agronomy and Biotechnology, Southwest University, Tiansheng Road 2, Beibei, Chongqing 400715, China;
[*]Corresponding author's email: lvjun9898@163.com; *zy03392002@126.com*

Abstract

Dominant male sterility is one of the two most important types of male sterility. However, its underlying mechanism has not yet been characterized. In this study, the anthers of *OsDMS-2* dominant male sterile mutant were shorter and whiter than wild-type anthers. Moreover, pollen abundance was lower in mutant than wild-type anthers, with most mutant pollen grains being small and malformed. Inheritance analysis showed that male sterility was controlled by one dominant gene. However, gene mapping suggested that two loci, *OsDMS-2A* (on chromosome 2) and *OsDMS-2B* (on chromosome 8), might be responsible for the dominant sterility of the *OsDMS-2* mutant. The flanking markers of *OsDMS-2A* are C2D11 and C2D12, with genetic distances of 0.66 and 0.33 cM, respectively. The C8D3 and C8D7 markers flanking *OsDMS-2B* have genetic distances of 1.32 and 2.32 cM, respectively. Molecular mapping disagreeing with classical genetic analysis indicates *OsDMS-2* is a novel and valuable mutant for understanding male sterility genetic mechanism.

Key words: Dominant male sterility, Gene mapping, Rice.

Introduction

Genetic male sterility is common in higher plants. It can be classified as genic male sterility (i.e., controlled by nuclear genes) or genic-cytoplasmic male sterility (i.e., controlled by the complementary action of nuclear and cytoplasmic genes) (Kaul, 1988). According to whether the sterility phenotype is observed in the F_1 population, genic male sterility can be sub-classified as dominant or recessive genic male sterility.

Although dominant genic male sterility (DGMS) in plants was first observed in 1910 (in potato) (Salaman, 1910), there are only 24 confirmed cases, involving about 10 species (Li & Hong, 2009). DGMS is a gain-of-function phenotypic trait, and the mechanism regulating the mutation is complex. Dominant male sterile mutants reproduce through crosses between a heterozygous mutant and a fertile parent. Therefore, a homozygous dominant male sterile mutant is very difficult to generate. For these reasons, there has been relatively little research on dominant male sterility, in contrast to the abundant research on recessive male sterility.

To date, only one dominant male sterility gene, *MS5* in *Brassica napus* (Xin *et al.*, 2016), was cloned and functionally characterized. There are only six reported cases of dominant male sterile mutants in rice: Pingxiang dominant genic male sterile rice (*PDGMSR*) (Huang *et al.*, 2007; Yan *et al.*, 1989), low temperature thermo-sensitive dominant male sterile *TMS/8987* (Deng & Zhou, 1994; Li *et al.*, 1999), the dominant genic male sterile mutant of *Zhe9248* (Shu *et al.*, 2000), the dominant male sterile mutants of *1783* and *1789* (Zhu & Neil, 2000), and the Sanming dominant genic male sterile mutant (*SMS*) (Huang *et al.*, 2008; Yang *et al.*, 2012). Among these mutants, only three have been mapped. The *Ms-P* sterility gene in *PDGMSR* was mapped to a short interval of approximately 730 kb between the RM171 and RM6745 markers on chromosome 10 (Huang *et al.*, 2007), while the restorer gene of *Ms-P*, *Rfe*, was mapped between the RM6737 and RM6100 markers on chromosome 10 (Xue *et al.*, 2009). Although *Ms-P* and *Rfe* are located on chromosome 10, the intervals containing *Ms-P* and *Rfe* loci are situated in different regions (Huang *et al.*, 2007; Xue *et al.*, 2009). The *TMS* gene was mapped to chromosome 6 between the RM50 simple sequence repeat marker and the C235 restriction fragment length polymorphism marker, with genetic distances of 12.9 and 6.4 cM, respectively (Li *et al.*, 1999). Based on bulked segregant and linkage analyses, *SMS* was mapped to a 99-kb interval between the ZM30 and ZM9 InDel markers on chromosome 8 (Yang *et al.*, 2012).

With the current lack of research on dominant male sterility in rice, it is unlikely that the mechanism regulating rice male sterility will be fully characterized. Therefore, increasing the speed and volume of research on available dominant male sterile mutants, and identifying additional dominant male sterile mutants is crucial. We herein describe the mapping of a novel dominant male sterile rice mutant, *OsDMS-2*. Classical inheritance analysis indicated that one gene controlled the sterility phenotype in *OsDMS-2*. However, mapping results identified two loci on chromosomes 2 and 8 as responsible for the sterility phenotype.

Materials and Methods

Plant materials

Sterile parent: The *OsDMS-2* dominant male sterile rice mutant was detected in an *Oryza sativa* L. ssp. *indica* 8B1390 restorer line bred at Southwest University (Chongqing, China).

Fertile parents: Wild-type *O. sativa* L. ssp. *indica* 8B1390 and JH1 restorer lines were bred at Southwest University. Zhongjiu B is an *indica* maintainer line bred at the China National Rice Research Institute. Zhonghua 11 is an *O. sativa* L. ssp. *japonica* variety bred at the Institute of Crop Sciences of the Chinese Academy of Agricultural Sciences.

Characterization of the mutant phenotype: Rice fertility was investigated in different natural environments. Rice were grown under low temperature and long photoperiod conditions in Beibei, Chongqing, and under high temperature and short photoperiod conditions in Sanya, Hainan. Protruding anthers and pollen release were compared by visual inspection, and mature flowers at anthesis were photographed using a C-770 digital camera (Olympus, Tokyo, Japan). Glume appearance and anther size and color were examined using an SMZ1500 stereoscope (Nikon, Tokyo, Japan), and samples were photographed with a DS-5Mc digital camera (Nikon). Wild-type and *OsDMS-2* mutant anthers were crushed separately and treated with a 1% iodine-potassium iodide solution for 3–5 s to stain the starch of the pollen grains, which were photographed with an E600 microscope (Nikon).

Genetic analysis: Four combination crosses and four backcrosses were used to analyze the inheritance of male sterility in the *OsDMS-2* mutant. To generate the F_1 populations, the *OsDMS-2* mutant (female) was crossed with the 8B1390 (*indica*), JH1 (*indica*), Zhongjiu B (*indica*), and Zhonghua 11 (*japonica*) parents (male). To produce F_2 and BC_1F_1 populations, fertile F_1 plants were self-pollinated. Additionally, the sterile F_1 plants (as female parents) were backcrossed with 8B1390, JH1, Zhongjiu B, and Zhonghua 11. The spikelet fertility of the F_1s plants, and the segregation of spikelet fertility in BC_1F_1s and F_2 populations were measured during the flowering stage. The χ^2 test was used to assess the goodness-of-fit.

Molecular mapping

Mapping population: The BC_1F_1 mapping population was generated from the *OsDMS-2*/Zhonghua 11//Zhonghua 11 cross. Equal amounts of DNA (2 μg) from each of 15 male sterile and fertile plants were used to construct bulks of BC_1F_1 segregants. Bulked segregant analysis (Michelmore *et al.*, 1991) was used to identify linked markers. All fertile and sterile BC_1F_1 plants were genotyped for mapping. The plants were grown in the rice field of Southwest University.

Design of InDel primers: We developed 137 pairs of InDel markers for the entire genome and new linked markers as previously described (Zhang *et al.*, 2008).

DNA preparation: Fresh flag leaf samples (1.5 g) were harvested during the heading stage. Total DNA was extracted from the collected samples using cetyltrimethylammonium bromide according to an established procedure (Murray & Thompson, 1980). Additionally, DNA was extracted from each BC_1F_1 sterile and fertile plant as described by Wang *et al.*, (2002) with the following minor modifications: (1) tender leaf tissue (about 1 cm^2) was cut into small pieces and added to a 0.5-mL centrifuge tube; (2) 100 μL 0.125 M NaOH was added to the samples, which were then boiled for about 30 sec., and briefly held before proceeding to the next step; (3) 50 μL 1.0 M Tris-HCl (pH 8.0) and then 100 μL 0.125 M HCl were added to the samples, which were boiled for about 2 min, mixed, and kept at 4°C until needed.

Results

Isolation and phenotypic analyses of the *OsDMS-2* mutant: In 2008, one male sterile mutant was detected and named *OsDMS-2* (dominant male sterility-2). *OsDMS-2* plants were smaller and had a longer growth period than wild-type plants, but otherwise exhibited normal vegetative development. There were no obvious differences between the mutant and wild-type plants regarding agricultural traits, including tiller number, grains per panicle, and 1000-grain weight. Additionally, seed setting did not occur in self-crosses, and seed setting ratios were less than 20% when *OsDMS-2* was used as the female parent. The seed setting results indicated that *OsDMS-2* mainly exhibited a male sterile phenotype, despite its low female fertility level.

To clarify how the mutation influenced flower organs and pollen fertility, we compared the wild-type 8B1390 and mutant *OsDMS-2* plants in terms of the panicle, glume, anther, and pollen at anthesis. There were no apparent differences in the morphology of the panicle and glume. Additionally, the mutant anthers protruded normally (Fig. 1a–c). In wild-type plants, pollen was released from a dehiscent anther soon after the anther protruded from a glume (Fig. 1a). In contrast, pollen was not released from mutant anthers because of a lack of dehiscence among protruding anthers (Fig. 1b).

The stereoscopic and light microscopic results indicated that mutant anthers were shorter, thinner, and whiter than wild-type anthers (Fig. 2a and b). The wild-type plants also produced more pollen than the mutants. Additionally, *OsDMS-2* pollen grains were smaller and more malformed than normal, and they could not be stained by the iodine solution because of a failure to accumulate starch (Fig. 2c and d). The *OsDMS-2* plants were consistently sterile in both Chongqing (under low temperature and long photoperiod conditions) and Hainan (under high temperature and short photoperiod conditions), indicating that the male sterile phenotype of the *OsDMS-2* mutant was not influenced by variations in temperature or photoperiod.

Genetic analysis of the male sterile *OsDMS-2* mutant: To reveal the inheritance model of the mutant *OsDMS-2*, we crossed *OsDMS-2* with the male parents, 8B1390, JH1, Zhongjiu B, and Zhonghua 11. All of the resulting F_1 populations segregated into fertile and sterile plants with a 1:1 segregation ratio (Table 1). The sterile F_1s plants were backcrossed with their male parents to generate the BC_1F_1 populations, while the fertile plants were self-pollinated to produce F_2 plants. We then assessed the segregation of sterile and fertile plants in the BC_1F_1 and F_2 populations. All BC_1F_1 populations had fertility segregation ratios of 1:1 (Table 1), while all F_2 plants were fertile. The mutant phenotype was observed in F_1 plants, which indicated dominant inheritance of sterility. The 1:1 fertility segregation ratios in F_1 and BC_1F_1 populations suggested that the *OsDMS-2* sterility phenotype was likely controlled by a dominant gene.

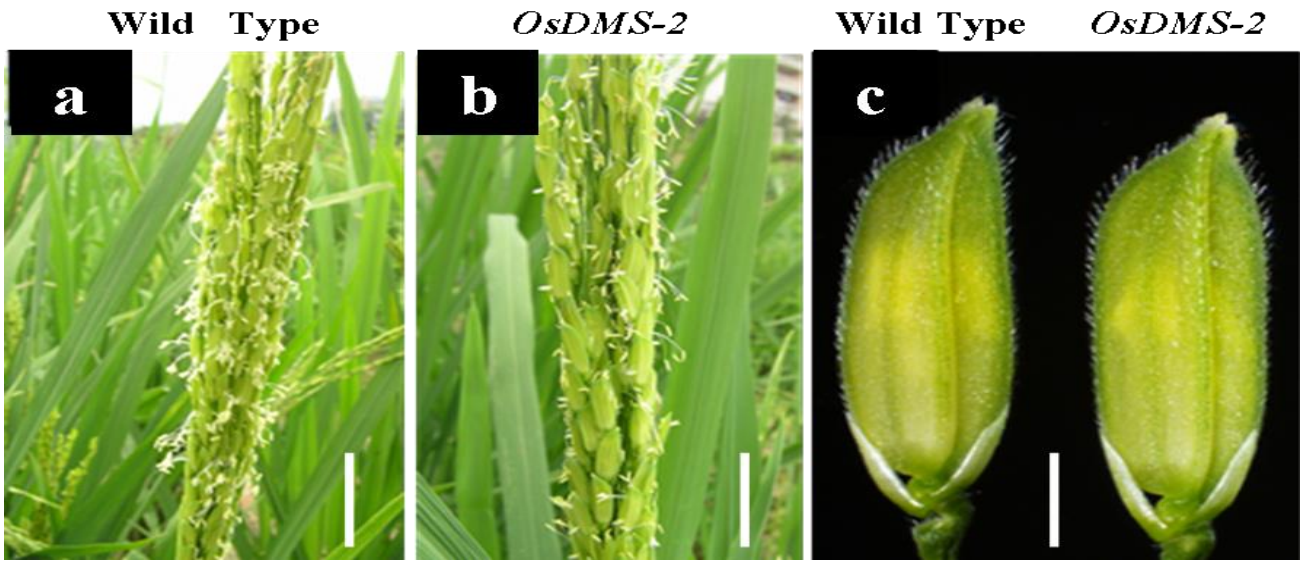

Fig. 1. Comparison of wild-type 8B1390 and mutant *OsDMS-2* panicles and glumes. Scale bars 2 cm in a and b, 0.25 cm in c.

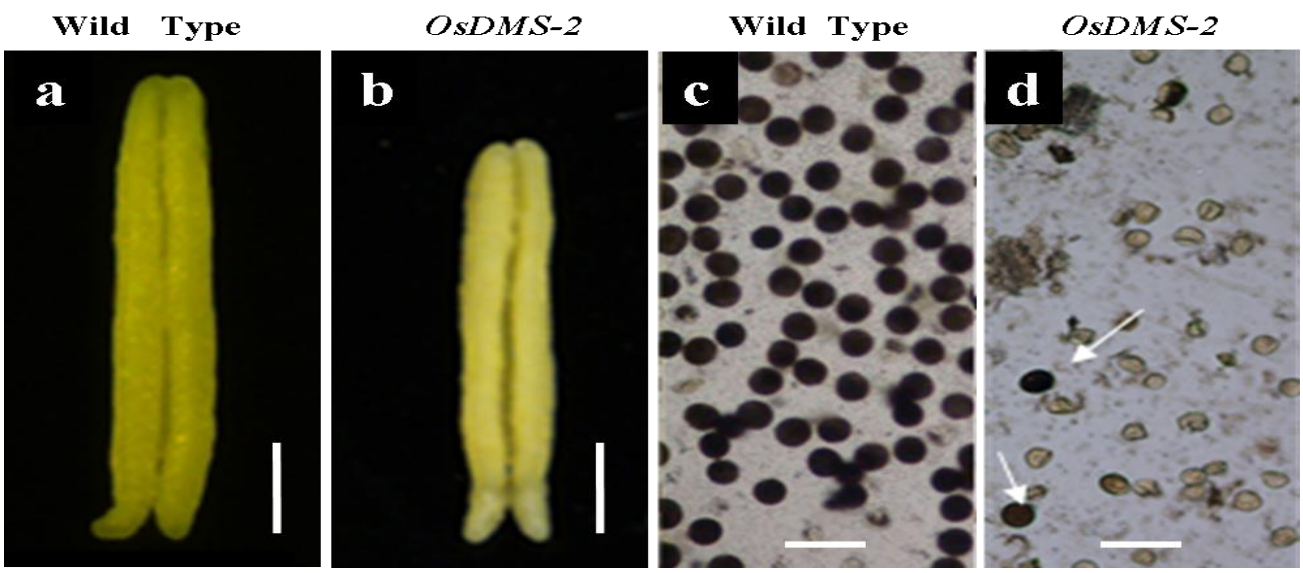

Fig. 2. Comparison of wild-type 8B1390 and mutant *OsDMS-2* anthers and iodine-stained pollen grains. Arrows indicate the few stained mutant pollen grains.
Scale bars 0.5 mm in a and b, 100 μm in c and d.

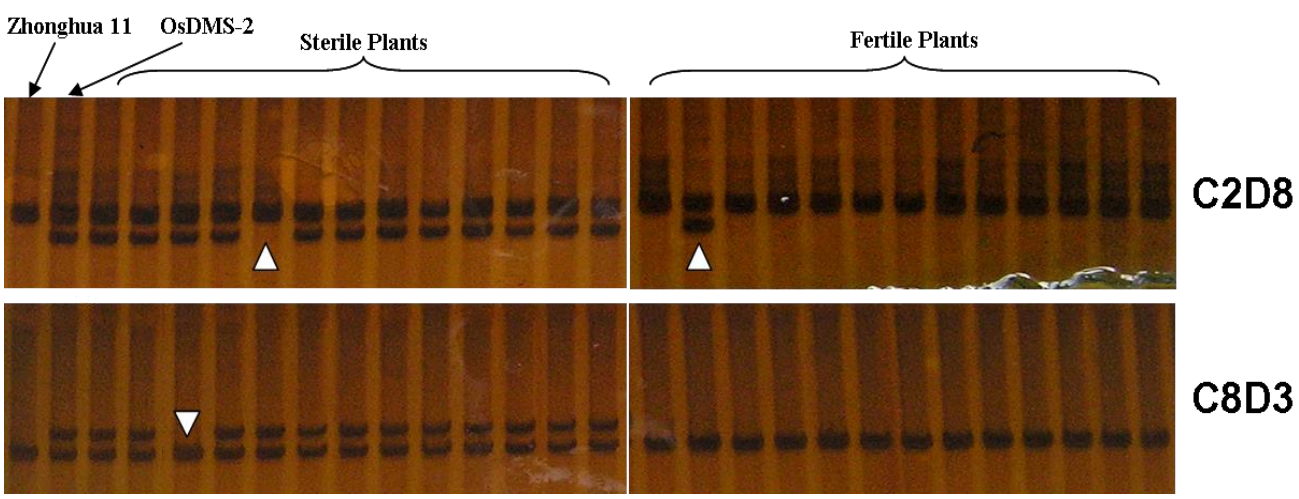

Fig. 3. Verification of selected linked InDel markers through the genotyping of individual plants. Arrowheads indicate recombinants.

Table 1. Fertility segregation ratios in F_1 and BC_1F_1 populations.

Combination	Observed		Expected		$\chi 2$ (1:1)
	No. of fertile plants	No. of sterile plants	No. of fertile plants	No. of sterile plants	
OsDMS-2/ 8b1390 (F_1)	25	22	23.5	23.5	0.085
OsDMS-2/8b1390// 8b1390 (BC_1F_1)	37	41	39	39	0.115
OsDMS-2/JH1 (F_1)	57	53	55	55	0.082
OsDMS-2/JH1// JH1 (BC_1F_1)	266	261	263.5	263.5	0.030
OsDMS-2/ZhongjiuB (F_1)	17	15	16	16	0.031
OsDMS-2/ZhongjiuB// ZhongjiuB (BC_1F_1)	43	48	45.5	45.5	0.176
OsDMS-2/Zhonghua11 (F_1)	83	93	88	88	0.461
OsDMS-2/Zhonghua11// Zhonghua11 (BC_1F_1)	147	155	151	151	0.162

Note: $\chi^2_{(0.05,1)} = 3.84$

Table 2. Primers used to detect markers linked to the *OsDMS-2* mutant gene.

Primer name	Forward primer sequence	Reverse primer sequence	Polymorphism	Chr. No.
C2D6	GTGATTGGCGGATTGATT	GTGCGTTCTCCTTGCTGT	no	2
C2D7	GGTAAGGAGCAAGGACAG	ACGATATTAGGTGGGATT	no	2
C2D8	ACCCAATCCTTACAATCAAC	CCTAGCCGAAACCTACCA	yes	2
C2D9	ATGTCCCAAATCTCCCTC	ACGCTCTGAATGCTGCTC	yes	2
C2D10	TAACGACCATCAACACCA	ATTCACAGAGCCGACTAA	no	2
C2D11	TCGGTGTCATAGTATGCTAATT	CATCTTTCCAGGTTGTCG	yes	2
C2D12	ACTCCCATCCTTCAATCT	CATCTAAGTAACCACGAAAT	yes	2
C2D13	CATCTCGGCTGCTCCATC	CTGCCCACAAGCTACCAC	yes	2
C2D14	TAGCCCGCTATTCTTCTC	TCCGCACTACAACTTCTTTA	no	2
C2D15	ACAGATTGGAGCTGTTTAT	ACCTATGTTGATGGCAGA	yes	2
C8D3	ACTAACCGAAACCCACAG	TATTGATGCCAATGCTTG	yes	8
C8D4	AAACATAGCCTGAATGCC	ACCACCTTAGTTCCCAAC	no	8
C8D5	GCATAATGGTAGCAAATT	AGAGGGTATGGGTGTAGT	no	8
C8D6	AGATTACGAGCGTGTTGA	CTTTATTTCCTTCGGTTC	no	8
C8D13	TGAATTATGAAGGACGACAG	GTGAAGAGGATGGTAGCG	yes	8
C8D14	TCGCTTCTTCTCCTGATT	TGGACCCTACCGAGTTTA	yes	8
C8D15	CCGATTGATTTACCGTCTG	CTGGTGGAGTACAACCCT	yes	8
C8D7	GACTTGTCTCAGTGTTTTGGAT	CGACTAATGGGGCTTTCC	yes	8
C8D10	TCGGATTGAGAAATGACC	AGGACTGAGAAGGGGAAA	yes	8

Screening and verifying molecular markers linked to the *OsDMS-2* mutant gene: To screen markers linked to the *OsDMS-2* mutant gene, 137 pairs of InDel primers were prepared. All primers were used to amplify *OsDMS-2* and Zhonghua 11 DNA. Polymorphic markers were subsequently used to amplify the fertile and sterile bulks, which consisted of DNA from 15 fertile or sterile plants from the *OsDMS-2*/Zhonghua 11//Zhonghua 11 BC_1F_1 population. We identified six primer pairs that detected polymorphisms on chromosomes 2 or 8 in the parents and progenies (Table 2).

To verify whether a marker was linked with the *OsDMS-2* mutant gene, all markers that detected polymorphisms in the bulks were further checked using 13 dominant sterile and 13 recessive fertile individuals from the BC_1F_1 *OsDMS-2*/Zhonghua 11//Zhonghua 11 population (Fig. 3: results for C2D8 and C8D3). Most of the sterile plants were heterozygous, while most of the fertile plants were homozygous recessive. The consistency between the observed phenotypes and genotypes indicated the 12 InDel markers on chromosomes 2 and 8 were linked. The results also suggested that two loci may be responsible for the dominant sterility phenotype of the *OsDMS-2* mutant, which is inconsistent with the classical genetics model of inheritance. We tentatively named these two loci *OsDMS-2A* and *OsDMS-2B*.

Gene mapping and the estimation of genetic distances: To map the *OsDMS-2A* locus, the C2D8 and C2D15 markers were used to analyze 147 fertile and 155 sterile individuals. Four and three recombinants were detected by C2D8 and C2D15, respectively (Table 3). There were no mutual interactions between the C2D8 and C2D15 recombinants, indicating that the *OsDMS-2A* locus was flanked by the two markers. The other polymorphic markers on chromosome 2 (i.e., C2D9, C2D11, C2D12, and C2D13) were subsequently used to assess all fertile and sterile individuals. We detected three, two, one, and two recombinants for C2D9, C2D11, C2D12, and C2D13, respectively (Table 3). According to the number of recombinants and the interactions among recombinant sets of each marker, the *OsDMS-2A* locus was suggested to be located between the C2D11 and C2D12 InDel markers, with genetic distances of 0.37 and 0.33 cM, respectively (Fig. 4). Based on the physical map produced from the Gramene data and the IRGSP-1.0 pseudomolecules of the rice genome (http://ensembl.gramene.org/Oryza_sativa/Info/Index), we determined that the *OsDMS-2A* locus was delimited to a physical distance of about 250 kb. We similarly determined that the *OsDMS-2B* locus was between the C8D3 and C8D7 InDel markers, with genetic distances of 1.32 and 2.32 cM, respectively (Fig. 4), and a physical distance of about 4.42 Mb. The linkage maps of the *OsDMS-2* loci and the linked markers are presented in Fig. 4.

Table 3. Recombinants detected using polymorphic markers.

Primer name	Serial no. of fertile recombinants	Serial no. of sterile recombinants	Total
C2D8	F31, F49, F86, F87	none	4
C2D9	F31, F86, F87	none	3
C2D11	F31, F87	none	2
C2D12	none	S35	1
C2D13	none	S35, S47	2
C2D15	none	S35, S47, S59	3
C8D3	F37, F51	S35, S59	4
C8D13	none	none	0
C8D14	none	none	0
C8D15	none	none	0
C8D7	F31, F34, F46, F56, F83, F84, F105	none	7
C8D10	F21, F31, F34, F37, F49, F50, F59, F86, F87, F105	S20, S36, S56	13

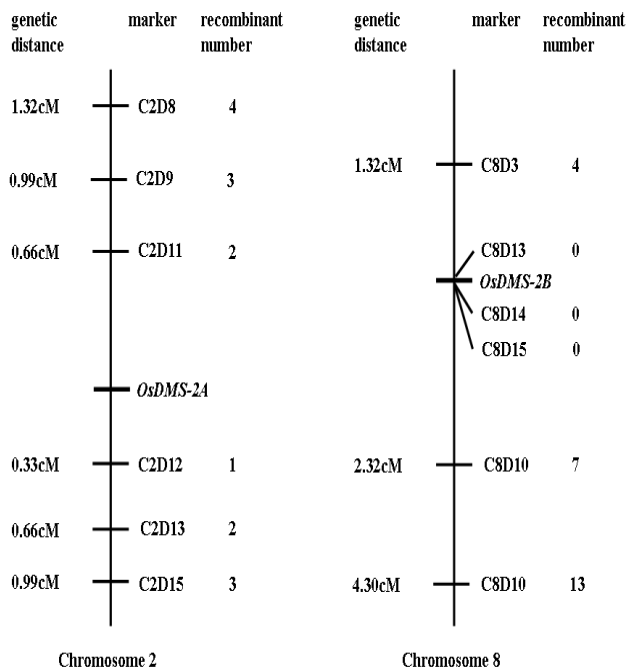

Fig. 4. Linkage maps of the *OsDMS-2* loci

Discussion

Research on dominant male sterile mutants should ideally lead to the comprehensive characterization of the mechanism regulating male sterility. However, the generation of dominant male sterile mutants including the generation of homozygous dominant male sterile mutants through sexual reproduction is very difficult. Prior to this study, only six dominant male sterile mutants had been detected in rice, and genes of only three of these had been mapped. (Deng & Zhou, 1994; Huang *et al.*, 2007; Huang *et al.*, 2008; Li *et al.*, 1999; Shu *et al.*, 2000; Yan *et al.*, 1989; Yang *et al.*, 2012; Zhu & Neil, 2000). To ensure that the mechanism regulating male sterility is fully characterized, more dominant male sterile mutants are needed.

OsDMS-2 is a new dominant male sterile rice mutant. It exhibits some general traits associated with sterility, including abnormal anther color and size, smaller and malformed pollen, and a lack of seeds. Additionally, when *OsDMS-2* served as a female parent, the seed setting ratio was lower than 20%, indicating the female sterility of this mutant.

Based on classical genetics, the sterility of *OsDMS-2* should be controlled by a single dominant gene because the segregation ratios were 1:1 in the F_1 and BC_1F_1 populations. However, our *OsDMS-2* gene mapping results were inconsistent with the expected results regarding the number of loci controlling the sterility phenotype (i.e., two loci instead of the expected single locus). Further studies are required to explain the discrepancy between the expected and observed results. Nevertheless, considering that the *OsDMS-2* mutant was derived from 8B1390 line, the *osdms-2a* and *osdms-2b* loci in the 8B1390 must have mutated to *OsDMS-2A* and *OsDMS-2B* in the *OsDMS-2* mutant. Additionally, only gametes with *osdms-2a*/*osdms-2b* or *OsDMS-2A*/*OsDMS-2B* are produced (i.e., no gametes with *OsDMS-2A*/*osdms-2b* or *osdns-2a*/*OsDMS-2B*). The mutated dominant loci may be inherited by sterile plants, while the recessive loci may be inherited by fertile plants during the production of segregated populations. Another hypothesis that explains the difference in the number of loci is that gametes with *OsDMS-2A*/*osdms-2b* or *osdns-2a*/*OsDMS-2B* fail to develop or cannot fuse with other gametes during fertilization. Alternatively, embryos resulting from the recombinant gametes are not viable. These hypotheses should be tested in future studies, which should focus on fine mapping these loci and analyzing gene functions. Future investigations involving the *OsDMS-2* mutant will likely be helpful to clarify the mechanism regulating male sterility.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (31370349; http://www.nsfc.gov.cn), the Natural Science Foundation of Chongqing Science and Technology Commission (CSTC, 2013jjB80003; http://www.ctin.ac.cn), and the Fundamental Research Funds for the Central Universities (XDJK2014C054, XDJK2015A014; http://kjc.swu.edu.cn/ kxjsc/index.php).

References

Deng, X.J. and K.D. Zhou. 1994. Study on fertility transition and inheritance of the lower temperature thermo-sensitive dominant male-sterile rice 8987. *J. Sichuan Agric. Univ.*, 12(3): 376-382.

Huang, T.Y., Y.P. Wang, B.T. Ma, Y.Q. Ma and S.G. Li. 2007. Genetic analysis and mapping of genes involved in fertility of pingxiang dominant genic male sterile rice. *J. genet. & genom.* = Yi chuan xue bao 34(7): 616-622. doi: 10.1016/S1673-8527(07)60070-8.

Huang, X.B., Z.H. Tian, Z.Q. Deng, J.T. Zheng, C.B. Lin and J.X. Tang. 2008. Preliminary identification of a novel sanming dominant male sterile gene in rice (*Oryza sativa* L.). *Acta agronomica sinica*, 34(10): 1865-1868. doi: 10.3321/j.issn:0496-3490. 2008. 10.026.

Kaul, M.L.H. 1988. Male sterility in higher plants. Springer-Verlag, Berlin ; New York.

Li, S.G., K.D. Zhou and L.H. Zhu. 1999. Inheritance analysis and molecular mapping of a rice thermo-sensitive dominant genic male sterility gene. *Chinese Science Bulletin*, 44(9): 965-968. doi: 10.3321/j.issn:0023-074X.1999.09.013.

Li, W.J. and T.Z. Hong. 2009. Research overview of dominant genic male sterile gene in rice. *Anhui Agri. Sci. Bull.*, 15(11): 76-79. doi: 10.3969/j.issn.1007-7731.2009.11.039.

Michelmore, R.W., I. Paran and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis - a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci.* USA 88(21): 9828-9832. doi: DOI 10.1073/pnas.88.21.9828.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, 8(19): 4321-4325. doi: 10.1093/nar/8.19.4321.

Salaman, R.N. 1910. Male sterility in potatoes, a dominant mendelian character; with remarks on the shape of the Pollen in wild and domestic varieties. *J. Linn. Soci. Lond. Bot.*, 39(272): 301-312. doi: 10.1111/j.1095-8339.1910.tb02481.x.

Shu, Q.Y., D.X. Wu, Y.W. Xia and M.W. Gao. 2000. Induction of dominant male sterility by using ^{60}Co-γ rays iradiation in rice oryza sativa L. *Acta Agri. Nucl. Sin.*, 14(5): 274-278. doi: 10.3969/j.issn.1000-8551.2000.05.004.

Wang, X.F., J.B. Yang, T.H. Xiang, L. Li and D.H. Ni. 2002. A new method for pcr reaction from alkali-treated rice leaf tissues. chin. *J. Rice Sci*., 16(1): 67-70. doi: 10.3321/j.issn:1001-7216.2002.01.013.

Xin, Q., Y. Shen, X. Li, W. Lu, X. Wang, X. Han, F.M. Dong, L.L. Wan, G.S. Yang, D.F. Hong and Z.K. Cheng. 2016. MS5 mediates early meiotic progression and its natural variants may have applications for hybrid production in brassica napus. *Plant Cell*, 28(6): 1263-1278. doi: 10.1105/tpc.15.01018.

Xue, K., X.S. Peng and H.H. He. 2009. Mapping the genes involved in fertility of pingxiang dominant genic male sterile rice by using fluorescent-labeled SSR makers. *Acta Agri. Universit. Jiangxie.*, 31(5): 841-845. doi: 10.3969/j.issn.1000-2286.2009.05.013.

Yan, L.A., J.C. Zhang, C. Zhu, Y.H. Ou, J.N. Li, and Y.H. Cai. 1989. The preliminary evaluation of a dominant male-sterile gene in rice. *Acta Agrono. Sin.*, 15(2): 174-181.

Yang, Z.M., X.F. Xie, X.B. Huang, F.Q. Wang, Z.J. Tong, Y.L. Duan, T. Lan and W.R. Wu. 2012. Mapping of sanming dominant genic male sterility gene in rice. *Heredi.*, 34(5): 615-620. doi: 10.3724/SP.J.1005.2012.00615.

Zhang, Y., J.X. Mao, K. Yang, Y.F. Li, J. Zhang, Y.X. Huang, F.C. Shen and C.D. Zhang. 2008. Characterization and mapping of a male-sterility mutant, tapetum desquamation (t), in rice. *Geno.*, 51(5): 368-374. doi: 10.1139/G08-013.

Zhu, X.D. and R.J. Neil. 2000. Identification of dominant male sterile mutants in rice (*Oryza sativa* L.). *Acta Agri. Nucl. Sin.*, 14(5): 279-283. doi: 10.3969/j.issn. 1000-8551.2000.05.005.

(Received for publication 15 February 2017)