HOSTED BY
ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2015, 22(5): 207−216

K-Domain Splicing Factor *OsMADS1* Regulates Open Hull Male Sterility in Rice

SUN Lian-ping[1, 2, #], ZHANG Ying-xin[1, #], ZHANG Pei-pei[1], YANG Zheng-fu[1], ZHAN Xiao-deng[1], SHEN Xi-hong[1], ZHANG Zhen-hua[1], HU Xia[1], XUAN Dan-dan[1], WU Wei-xun[1], LI Zi-he[1], CAO Li-yong[1], CHENG Shi-hua[1, 2]

([1]*National Center for Rice Improvement / Key Laboratory for Zhejiang Super Rice Research, China National Rice Research Institute, Hangzhou 310006, China;* [2]*College of Agronomy, Shenyang Agricultural University, Shenyang 110866, China;* [#]*These authors contribute equally to this work*)

Abstract: We identified the rice floral organ development mutant, termed as open hull and male sterile 1 (*ohms1*), from the progeny of the indica restorer line Zhonghui 8015 treated with ^{60}Co γ-ray irradiation. The *ohms1* mutant exhibited an open hull and lemma- and palea-like structure conversion between the anthers and stigma, which resulted in the *ohms1* mutant spikelet showing 'tridentate lemma'. The *ohms1* mutant was entirely sterile but had 60%–70% fertile pollen. Genetic analysis and gene mapping showed that *ohms1* was controlled by a single recessive gene, and the mutant gene was fine-mapped to a 42-kb interval on the short arm of chromosome 3 between markers KY2 and KY29. Sequence analysis of the four open reading frames in this region revealed that the mutant carried a single nucleotide transformation (A to G) at the last base of the fifth intron, which was likely corresponded to *ohms1* phynotype, in an MIKC type MADS-box gene *OsMADS1* (LOC_Os03g11614). Enzyme digestion and cDNA sequencing further indicated that the variable splicing was responsible for the deletion of the sixth exon in *ohms1*, but no structural changes in the MADS domain or amino acid frame shifts appeared. Additionally, real-time fluorescent quantitative PCR analysis showed that the *OsMADS1* expression level decreased significantly in the *ohms1* mutant. The expression levels of rice flowering factors and floral glume development-related genes also changed significantly. These results demonstrate that *OsMADS1* may play an important role in rice floral organ development, particularly in floral glume development and floret primordium differentiation.
Key words: rice; open hull; male sterile; *ohms1*; gene mapping; alternative splicing; floral organ

Floral organ development in rice, which signals the transformation of vegetative stage to reproductive stage, is a key process involved in rice reproduction and yield. The ABC model of dicotyledon floral organ development was first proposed to clarify the molecular regulatory mechanism on the basis of homeotic mutation and interaction relationships in *Arabidopsis thaliana* and snapdragon flowers (Coen and Meyerowitz, 1991), and gradually developed into the widely accepted ABCDE model with the later discoveries of D-class and E-class genes (Colombo et al, 1995; Mandel and Yanofsky, 1995; Ferrario et al, 2003; Ditta et al, 2004). The ABC model of floral organ development is conserved among different species in plants (Lohmann and Weigel, 2002). In recent years, many genes that regulate floral organ development have been reported in rice, and they can also be divided into the same five sub-groups according to the dicotyledon ABCDE model through homologous sequence alignments of MADS-box genes and studies on rice floral organ development mutants (Lamb and Irish, 2003).

A-class genes include *OsMADS14* (*RAP1A*),

Received: 19 May 2015; **Accepted:** 1 July 2015
Corresponding authors: CAO Li-yong (caoliyong@caas.cn); CHENG Shi-hua (shcheng@mail.hz.zj.cn)

Peer review under responsibility of China National Rice Research Institute
http://dx.doi.org/10.1016/j.rsci.2015.09.001

OsMADS15 (*DEP*), *OsMADS18* and *OsMADS2* (Jeon et al, 2000a; Fornara et al, 2004; Sentoku et al, 2005; Wang et al, 2010; Kobayashi et al, 2012; Lu et al, 2012), which are chiefly up-regulated in inferior palea, glumelle and lodicule at late spikelet development stages in rice, similar to the AP1-like genes in *Arabidopsis thaliana*. B-class genes in rice are relatively conserved. For example, *SUPERWOMEN1* (*OsMADS16*) specifies the lodicule and stamen identities in rice (Nagasawa et al, 2003; Xiao et al, 2003; Diao et al, 2013). *OsMADS2/4* is mainly expressed in the lodicule and stamen, whereas *OsMADS22* only controls lodicule formation without any influence on stamen development in rice (Arora et al, 2007; Yadav et al, 2007). The most representative C-class genes are *OsMADS3* and *OsMADS58* (Yamaguchi et al, 2006), which specify the stamen and carpel identities and are of great importance for the identification of floral meristem (Dreni et al, 2011). *DL* gene of *YABBY* gene family also belongs to the C-class genes controlling floral organ development. It regulates the formation and development of carpel and stamen in rice (Yamaguchi et al, 2004). D-class genes *OsMADS13* and *OsMADS29* mainly control ovule, carpel and seed development in rice (Dreni et al, 2007; Yin and Xue, 2012). The five SEP-like genes, *LEAFY HULL STERILE1* (*OsMADS1*), *OsMADS5*, *OsMADS7*, *OsMADS8* and *OsMADS34* (Jeon et al, 2000b; Prasad et al, 2005; Cui et al, 2010; Khanday et al, 2013), were reported to have E-class gene function in rice genome. Among these, *OsMADS1* is the most elucidated. Homeotic *OsMADS1* mutations lead to leafy hull sterility in rice. Moreover, it controls the identities and development of inferior palea and glumelle and accelerates early maturation in rice. Complete loss of *OsMADS1* function results in complete homeotic transformation of lodicule, stamen and carpel, and structures similar to inferior palea and glumelle form. All floral organs but inferior paleae homologously transform to foliaceous structures when *OsMADS1*, *OsMADS5*, *OsMADS7* and *OsMADS8* are simultaneously inhibited.

In addition, there are some non-MADS-box genes, such as *fon1* (Moon et al, 2006), *fon2* (Suzaki et al, 2009) and *fon4* (Chu et al, 2006), which control the number of floral organs; the floral organ regulators *STAMENLESS1* (Xiao et al, 2009); and *OsFOR1* (Jang et al, 2003), *MFS1* (Ren et al, 2013) and *G1* (Yoshida et al, 2009), which determine spikelet development and regulate rice floral organ development. Successful cloning of these genes associated with floral organ development has laid a theoretical foundation for research on rice reproductive development.

However, studies on monocotyledon floral organ development are thoroughly unclear for lacking of related mutant and phenotype analysis of relevant genes. There are few detailed studies on spikelet fertility caused by floral organ mutations as current studies have focused primarily on rice flower development and particularly on the homeotic transformation and development of different interior and exterior flower organs (Zhang et al, 2014). As a key organ of glumous flowers in rice, the size and shape of glume has a direct relationship with grain yield in rice. However, there is little research on glume development, and no reports on glume sterility genes cloned in the indica rice restorer line background. This study aimed to identify the molecular mechanism regulating glume development through the identification and phenotyping of *ohms1* mutant, an open hull sterile mutant from Zhonghui 8015 (Zh8015), and to map and clone the target gene.

MATERIALS AND METHODS

Rice materials

The floral organ development mutant *ohms1* was first identified from a ^{60}Co-γ-ray irradiated mutagenesis population of rice cultivar Zh8015 (an elite Chinese indica restorer line). The *ohms1* mutant, as the pollen acceptor, was crossed with Zhonghua 11 and 02428, respectively. The heterozygous F_1 plants were then self-pollinated to generate two F_2 populations for mapping of the *ohms1* gene. All plants were grown in paddy fields in Lingshui, Hainan Province of China in the spring of 2013 and in Hangzhou, Zhejiang Province of China in the summer of 2013. In the F_2 populations, homozygous lines displaying the *ohms1* phenotype were used for genetic mapping.

Pollen fertility and spikelet fertility investigation

At the heading stage, anthers at identical positions on the main panicles of wild type and mutant plants were selected to determine pollen fertility by 1% I_2-KI staining and observed with a 10 × 10 microscope to judge the fertility. According to pollen grain staining conditions and patterns, deep staining and incomplete staining (the pollen grain was round, but the staining was incomplete) were deemed as fertile, whereas light staining and irregular shape were sterile. Three visual fields that

contained at least 300 pollen grains for each glumous flower were observed randomly for pollen fertility.

When completely mature, all individual plants with mutant phenotypes were chosen, and five panicles were randomly selected from each individual plant for the investigation of fertility.

DNA extraction and genetic mapping

Total genomic DNA was extracted from young leaves following the modified cetyltriethyl ammonium bromide protocol described by Lu and Zheng (1992). Approximately 133 pairs of polymorphic microsatellite (SSR) primers that are evenly distributed on the 12 chromosomes were employed for the primary mapping of the *ohms1* locus. A high-density linkage map for fine-mapping in the target region was obtained by developing insertion-deletion (InDel) markers according to the publicly available rice genome sequence comparisons between Nipponbare and 9311 (http://www.gramene.org and http://blast.ncbi.nlm.nih.gov). A total of 1 496 mutant plants were selected from the F_2 population derived from the cross between the *ohms1* mutant and 02428 for fine-mapping of the *ohms1* locus. Linkage between molecular markers and the *ohms1* locus was determined following recessive-class approaches (Zhang et al, 1994). The molecular markers, including SSR and InDel markers, used for fine-mapping of *ohms1* are shown in Table 1. All of the primers were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd., China.

PCR was performed in a 12 μL reaction system containing 2 μL template DNA, 2 μL primer (20 μmol/L), 5 μL of 2 × *Taq* PCR Colorless Mix (Dingguo Changsheng Biotechnology Co., Ltd., Beijing, China), and 3 μL ddH_2O. The PCR amplification comprised an initial denaturation of 94 ºC for 5 min, followed by 35 cycles of 94 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 30 s. The reactions were completed with a final extension of 72 ºC for 8 min. The PCR products were separated by electrophoresis on 8% non-denaturing polyacrylamide gels and visualized by 0.1% $AgNO_3$ staining and formaldehyde and NaOH coloration.

Candidate gene analysis

All potential candidate genes were identified by referring to the Rice Genome Annotation Project Database (http://rapdb.dna.affrc.go.jp) for all open reading frames (ORFs) at the mapping interval and amplifying the genome segment of both *ohms1* mutant and wild type plants using high-fidelity DNA polymerase KOD-Plus-Neo. The first strand cDNA synthesis of both wild type and *ohms1* mutant was reverse transcribed using a TOYOBO First Strand cDNA Synthesis Kit with Oligo-dT(18) as reverse primer. Then, the full-length candidate gene cDNAs were amplified by high-fidelity DNA polymerase KOD-Plus-Neo. The PCR products were sent directly to Shanghai Sunny Biotechnology Co., Ltd. in China for gene sequencing. The results were spliced with Contig Express software and sequence alignments were made using the MegAlign software of DNASTAR to identify mutant sites.

Three-dimensional structure prediction of protein

The three-dimensional structures of target proteins in wild type and *ohms1* mutant were predicted by the homology modeling method. Protein models of wild type and *ohms1* mutant target genes were displayed by PyMOL Tutorial molecular 3D structure displaying software, and built in the same temple and coincided

Table 1. Primer sequences used in this study.

Primer	Forward primer (5′-3′)	Reverse primer (5′-3′)	Purpose
RM7576	CTGCCCTGCCTTTTGTACAC	GCGAGCATTCTTTCTTCCAC	Linkage analysis
RM7	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT	Linkage analysis
InD44	GGAATCCCTCCCTTCTTGTC	GGTCGGTAAAGACGGTGAAA	Fine mapping
InD45	CCAGGGATCTTCTCATCCAA	CCTGGCTAGCATACCACACA	Fine mapping
InD46	GCCATTGATCTTCTGCAGGT	TTTGTTGTCAATGCCCTGTT	Fine mapping
RD0304	GGCGTCACTGCTCGTA	GCCTGAAGCGTCCACA	Fine mapping
KY2	GTGGGAAGAAGAACATCAACTG	GCACACAAGATAAACCCAATCAGC	Fine mapping
KY12	ACCACGAGGGTGACCGTAGA	GCGAGGGTTGATGAGATAGCA	Fine mapping
KY17	CGAGAGGCGAAGGAAATAGAACG	CTCCTCCTCCTCCTGGTTCTCC	Fine mapping
KY25	CCATGGTCGCCATTGACACG	CCTGCTATAACACTCGCACAGATGC	Fine mapping
KY26	GGTGGTGAGCCAAGAACTGACC	CCTCAAGGAATCCTCGTAAGTCG	Fine mapping
KY29	CCAAGTGTGTCCGAGCTTAGTGC	TGAGTCAAAGCGAAAGTCAACAGG	Fine mapping
CAPS1	GCCATCGATCACCCTGAAAGTC	CTGATCAGCAAGAACAGTGC	*ohms1* site detection
CKY	AGCCAAACCACACCACCATAAAG	AGGACACTGTTTGCATTGGCT	cDNA sequencing

both of them.

Real time quantitative polymerase chain reaction (qRT-PCR)

Total RNA was extracted from young panicles (4–5 cm) of wild type and *ohms1* mutant plants using the TIANGEN RNAprep Pure Plant Kit. The first cDNA strand was synthesized from DNase I-treated RNA using Oligo-dT(18) primers in a 20-μL reaction based on a SuperScript III Reverse Transcriptase Kit (TOYOBO). The qRT-PCR analysis was performed on a Roche LightCycler 480 device using gene-specific primers, with rice *Actin* gene (*Os03g0234200*) as the reference gene (Khanday et al, 2013). Reactions containing SYBR Premix Ex *Taq* II (TaKaRa) were carried out in a final volume of 20 μL. qRT-PCR procedure was as follows: 95 ºC denaturation for 30 s, followed by 40 cycles of 95 ºC for 5 s, 60 ºC for 30 s, and 72 ºC for 15 s. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative levels of transcription (Schmittgen and Livak, 2008).

RESULTS

Main phenotypic characteristics of *ohms1* mutant

The *ohms1* mutant appeared basically normal in its main agronomic traits compared with the wild type Zh8015, except its 3–5 d delayed heading date and somewhat higher plant height under natural conditions in Hangzhou (Fig. 1-A and -B). The most striking morphological defects in *ohms1* mutant were observed in its florets (Fig. 1-C). The floral organ structure of Zh8015 exhibited normal palea, glumelle, stigmas and stamens, as well as a typical filaments structure that elongated at blossom time to make stamens out of the lemma for normal fertilization.

Obvious defects were observed in palea and glumelle of *ohms1* mutant. The *ohms1* spikelets consisted of three glume-like structures, including paleae and lemmas that exhibited open hull features. An extra glume-like structure formed between paleae and lemmas inside the stamens and stigmas, which looked like tridentate lemma. The mutant filament did not extend at blossom time, and only partial stamens extended out of the lemma (Fig. 1-C), likely resulting in abnormal blossom and fertilization. The pollen microscopic examination showed that the wild type Zh8015 pollen was almost completely fertile. In contrast, the *ohms1* mutant had completely sterile spikelets but 60%–70% fertile pollen (Fig. 1-D).

Genetic analysis of *ohms1* mutant

The seed-setting rate, lemma and palea of F_1 spikelets

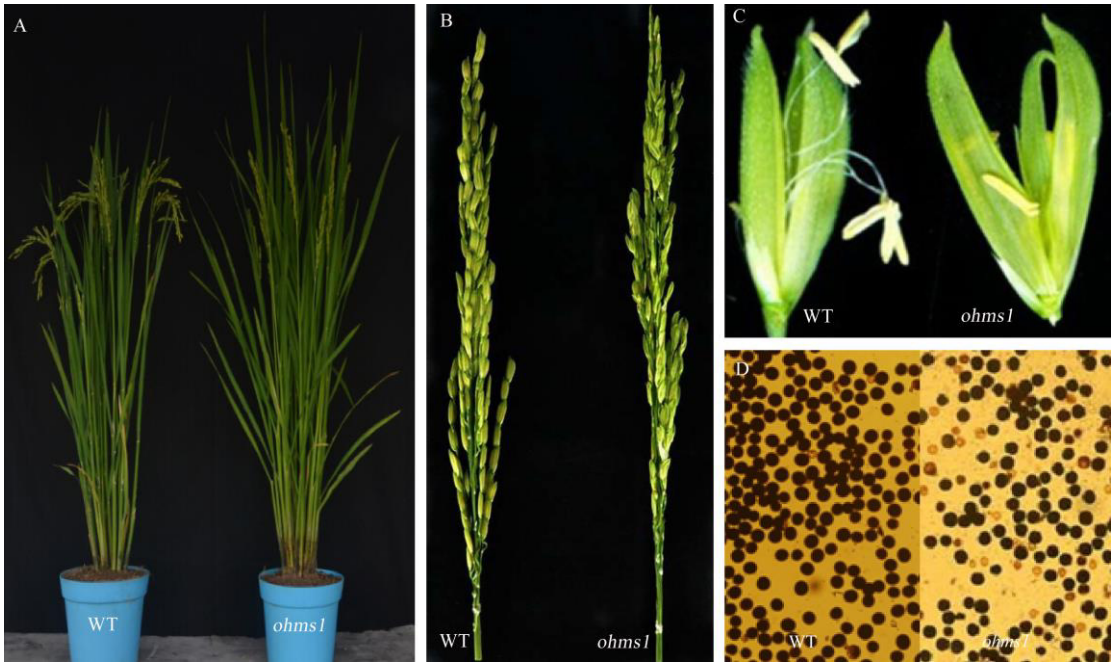

Fig. 1. Plant and floral organ developmental phenotypes of wild type (WT) Zhonghui 8015 and *ohms1* mutant at heading stage.

A, Wild type Zhonghui 8015 (Zh8015) and *ohms1* mutant plants; B, Panicles of wild type Zh8015 and *ohms1* mutant; C, Glumous flower of wild type Zh8015 and *ohms1* mutant; D, Pollen fertility of wild type Zh8015 and *ohms1* mutant.

Table 2. Segregation analysis of *ohms1* allele.

Combination	Seed-setting rate of F_1	F_2		$\chi^2(3:1)$	$\chi^2_{0.05}$
		No. of wild type plants	No. of mutant plants		
ohms1/ZH11	85.22	374	113	0.84	3.84
ohms1/02428	88.76	4658	1496	1.57	

ZH11, Zhonghua 11.

from two crossed combinations (*ohms1* × Zhonghua 11, *ohms1* × 02428) were similar to wild type. The number of wild type and mutant plants in both of two separate F_2 populations showed that the *ohms1* mutant trait was inherited as a monogenic recessive gene (Table 2).

Fine mapping of *ohms1* and candidate gene analysis

We selected a total of 1 496 mutant plants in the F_2 population of *ohms1* × 02428 for gene mapping. Linkage analysis showed that the *OHMS1* locus was mapped on the short arm of chromosome 3, flanked by the SSR loci RM7576 and InD45 and almost co-segregated with RM7576 (Fig. 2-A and -B). For fine-scale mapping, involving the use of six newly developed appropriate InDel markers (KY2, KY12, KY17, KY25, KY26 and KY29) and 1 496 F_2 segregants, we narrowed the location of *OHMS1* within a 42-kb segment bracketed by the markers KY2 and KY29 (Fig. 2-C).

Four ORFs were included at the 42 kb interval (Fig. 2-D), in which a MADS-box gene (LOC_Os03g11614) is involved in rice floral organ development, and have E-class floral homeotic genes function in regulating the identification and development of palea and glumelle (Jeon et al, 2000b). Therefore, LOC_Os03g11614 was favored as the *ohms1* candidate gene. Sequencing both the wild type Zh8015 and *ohms1* mutant target ORFs showed that the mutant had a single nucleotide transformation (A to G) at the intersection of the fifth intron and sixth exon (base 7269) of *OsMADS1* (Fig. 2-E).

cDNA sequence analysis and three-dimensional protein structure of the candidate gene

The *OsMADS1* gene consists of eight exons and seven introns (Fig. 2-E), with full length of 8 463 bp and 1

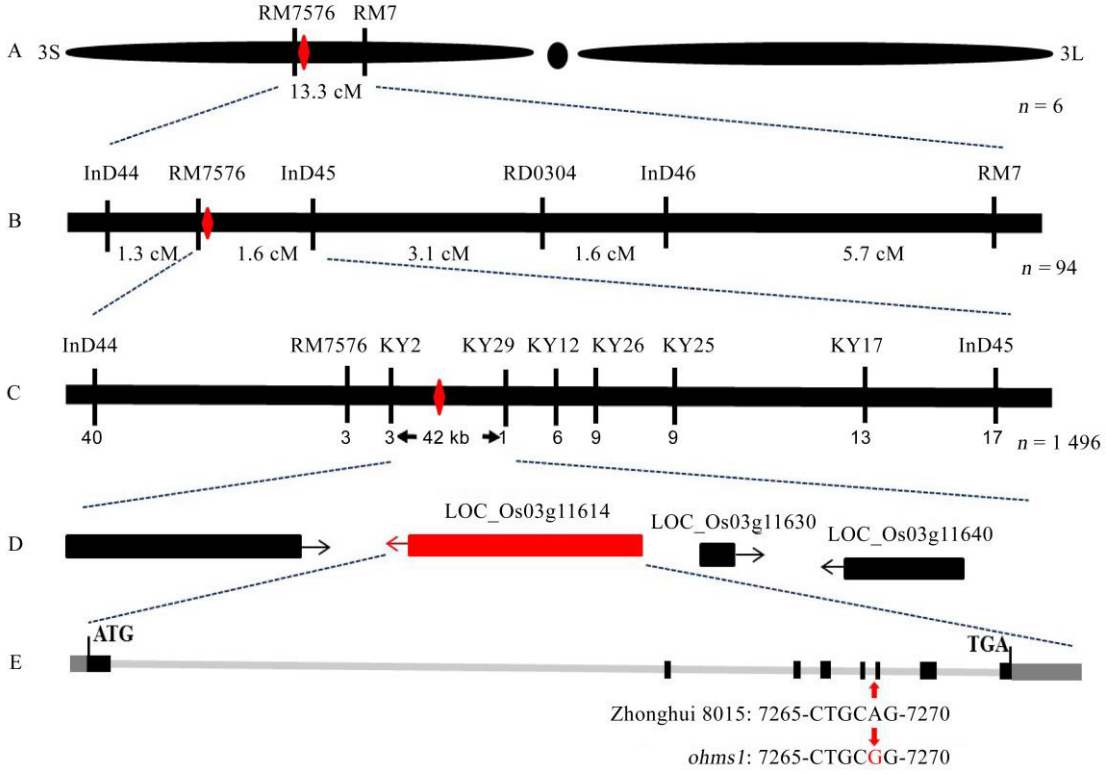

Fig. 2. Fine mapping and positional cloning of *ohms1* mutant gene.

A, The mutate gene was linked between RM7576 and RM7 on the short arm of chromosome 3; B, The mutant gene was preliminarily mapped between RM7576 and InD45; C, The *ohms1* mutant gene was fine-mapped to a 42-kb genomic region between markers KY2 and KY29; D, Four open reading frames existed in the region and the red one denote the target gene; E, Sequence analysis revealed that the mutant had a single nucleotide transformation (A to G) at the end of the fifth intron.

152 bp coding region. It encodes a protein that comprises 257 amino acids. Further cDNA sequencing showed the mutant had a 42-bp deletion that resulted in the sixth exon region (amino acids 155 to 168) in the *ohms1* mutant K-box domain to be completely lost compared with the wild type (Fig. 3-A). The alignment of the K-box domains of various MADS-box genes in rice showed that the amino acids in this region where the *ohms1* mutation occurred were conserved in all MADS genes, as previously observed. A pair of CAPS1 enzyme digestion primers were designed to test and verify this alternative splicing site for this mutation using endonuclease *Pst* I, and the enzyme digestion results confirmed our prediction (Fig. 3-B and -C). Hence, we hypothesized that the mutant site made the sixth exon spliced with the fifth intron of *OsMADS1*, thus changing the structure and function of the *OsMADS1* gene. The results strongly support the hypothesis that the *ohms1* mutant phenotype is caused by mutation in the *OsMADS1* gene.

The 3D structure model of *OsMADS1* amino acid sequences between wild type and *ohms1* mutant were made using the same template for protein structure comparison. No difference was found between the protein and MADS domain besides a deletion in the *OsMADS1* K-box domain of the mutant (Fig. 3-A to -C). The superposition of wild type and mutant 3D structure furtherly demonstrated this result (Fig. 3-D to -F).

Transcription of genes involved in lemma and palea development in *ohms1*

The K-box domain harboring the deletion in *ohms1* mutant is conserved in plant MADS genes, which is

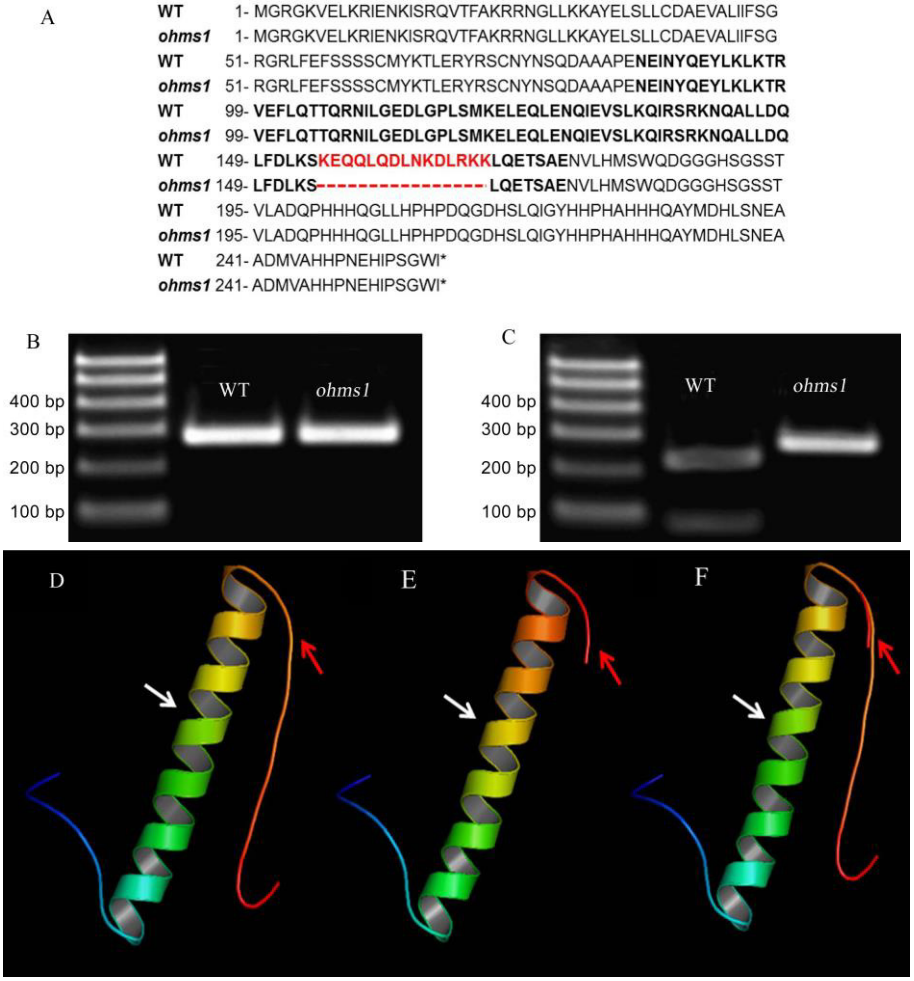

Fig. 3. Amino acid sequence analysis and protein structure of wild type (WT) and *ohms1* mutant.

A, WT and *ohms1* mutant protein sequences; The bold sequence are the *OsMADS1* K-box domain and the red sequences denote the deletion of *ohms1*; B and C, Electrophoresis before and after *Pst* I enzyme digestion, respectively; D to F, Comparison of the 3D protein structure models of wild type and the mutant *OsMADS1*; D, Wild type structure; E, *ohms1* mutant structure; F, Superposition of wild type and *ohms1*mutant sructures. The MADS domain and K-box domain of the *OsMADS1* protein were represented by white arrows and red arrows, respectively.

involved not only in development of lemma and palea, but also in patterning floral primordium formation and homeotic transformation in rice. Consequently, the expression levels of many other lemma/palea development-associated genes were greatly changed in the mutant compared to wild type. qRT-PCR analysis showed that the expression levels of *OsMADS1*, *OsMADS5*, *OsMADS7*, *OsMADS8*, *EG1* and *REP1* decreased in the mutant. However, the expression levels of *OsMADS14*, *OsMADS15*, *OsMADS32*, *OsMADS34*, *OsMADS50*, *OsMADS55* and *DP1* significantly increased. Particularly, *OsMADS55*, the downstream regulator of *OsMADS1* in floret primordia determination, appeared 6.99-fold higher expression level in the mutant (Fig. 4). We therefore demonstrated the great importance of *OsMADS1* in rice floral organ development.

Separation appeared in fertility of *ohms1* × 02428 progeny

The wild type Zh8015 showed normal spikelet fertility, in contrast to the *ohms1* mutant, which was perfectly sterile (Fig. 5-A and -B). However, some of open hull plants appeared partly fertile in F_2 population of *ohms1* × 02428 (Fig. 5-C). This may be due to there being no change or frame shift in core conserved domain of the *OsMADS1* protein caused by the *ohms1* mutation, and spikelet fertility was interactively restored by a possible epistatic K-box domain in 02428. However, further research on its molecular mechanism is still needed.

DISCUSSION

Many genes associated with lemma and palea development have been isolated in recent years. *OsMADS1*, which are more deeply researched, contributes not only to lemma and palea development, but also to the patterning of the inner whorl organs. The *OsMADS1* gene belongs to the plant type II of MADS-box genes (MIKC type), which are involved in rice floral organ development and exhibit E-class gene function (Jeon et al, 2000b). Moreover, *OsMADS1* is involved in the determination of floral organ

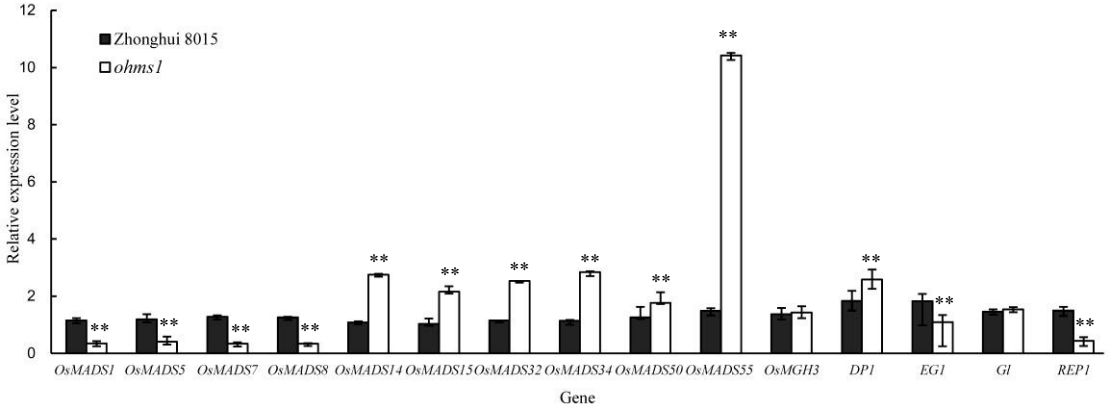

Fig. 4. qRT-PCR derived transcription profiles of a panel of genes associated with rice floral organ development.
** mean significant differences between wild type Zhonghui 8015 and *ohms1* mutant at the 0.01 level. Bars represent the standard error.

Fig. 5. Spikelet fertility of *ohms1* mutant and F_2 population.
A, Normal spikelet fertility of Zhobnghui 8015; B, Open hull and sterile spikelet of *ohms1* mutant; C, Open hull but fertile spikelets in F_2 population of *ohms1*/02428 (open hull but fertile spikelets are marked by red arrows).

identity, as well as in palea and glumelle development. As a result, the *ohms1* mutant exhibited an extra glume-like structure between the paleae and lemmas.

The *OsMADS1* gene contains eight exons and seven introns encoding 257 amino acids. Amino acids 85 to 175 constitute a semi-conserve K-box structural domain, homologous to the helical region of keratin. The most remarkable characteristic of this region is that it consists of hydrophobic amino acid residues. As a result, it can be folded to form continuous amphipathic helicesas as a region of interaction with protein molecule (Agrawal et al, 2005). In recent years, more in-depth studies have explored the molecular mechanism of *OsMADS1*. Jeon et al (2000b) reported its regulatory function in rice floral organ development by cloning an MADS domain mutation in *OsMADS1*, *lhs1*. Research on *afo*, an epigenetic mutation in *OsMADS1*, has shown the effects on the identification of floral meristems such as the inferior paleae, glumelle edge tissues and internal floral organs (Wang et al, 2010). Further studies revealed that *OsMADS1* affects the determination and development of palea and lemma using *Tos17* insertion or RNAi inhibition (Agrawal et al, 2005). Li et al (2008) have also proved the key function of *OsMADS1* in spikelet determinacy and development in gramineous plants using transposon insertion. However, the influence of mutations in the K-domain of *OsMADS1* gene in regulating rice floral organ development has not been elucidated. In this study, we successfully fine-mapped and cloned the open hull male sterile mutant *ohms1*, a novel mutant allele of *OsMADS1* caused by a single alternative splicing at the K-box domain of *OsMADS1* gene from the mutagenized population of Zh8015. A deletion of 14 amino acids of the sixth exon was located in the K-box domain of the *OsMADS1* gene, a sequence conserved among plant MADS-box genes. There were no changes in the MADS domain of the *OsMADS1* protein or any frame shift (Fig. 3).

Additionally, the *ohms1* mutant was entirely sterile but the pollen was not completely abortive (Fig. 1-D). Some *ohms1* plants appeared partly fertile in the F_2 separation population of *ohms1* × 02428, but the mechanism remains unclear. This may be due to another possible MIKC protein that is up- or down-streamed by an epistatic gene in 02428. This protein could independently interact with the *ohms1* K-box domain and fulfill its function, thereby partly restoring the spikelet fertility of open hull plant in the F_2 population of *ohms1* × 02428. Therefore, differences in the degree of conservation of the MADS domain and K domain in the *OsMADS1* gene and its interaction may facilitate the diversity of its contribution to controlling rice floral organ development and spikelet fertility. In addition, the wide compatibility site of 02428 may affect embryo sac fertility and ultimately restore the spikelet fertility of *ohms1* phenotype plants (Chen et al, 2008; Yang et al, 2012). Since the expression level of a large amount of genes associated with floral organ development was dependent on the presence of *ohms1* (Fig. 4), it is clear that *ohms1*, whatever its specific function is, is extremely important for normal reproductive in rice. However, further studies are imperative to explore the mechanism of floral organ development, spikelet fertility and the interaction in rice.

ACKNOWLEDGEMENTS

This study was supported by the National Key Technology R&D Program of China (Grant No. 2011BAD35B02), the National Key Transform Program of China (Grant No. 2011ZX08001-002), Zhejiang Provincial Natural Science Foundation of China (Grant No. LQ14C130003), and the Super Rice Breeding Innovation Team and Rice Heterosis Mechanism Research Innovation Team of the Chinese Academy of Agricultural Sciences Innovation Project, China (Grant No. CAAS-ASTIP-2013-CNRRI).

REFERENCES

Agrawal G K, Abe K, Yamazaki M, Miyao A, Hirochika H. 2005. Conservation of the E-function for floral organ identity in rice revealed by the analysis of tissue culture-induced loss-of-function mutants of the *OsMADS1* gene. *Plant Mol Biol*, **59**(1): 125–135.

Arora R, Agarwal P, Ray S, Singh A K, Singh V P, Tyagi A K, Kapoor S. 2007. MADS-box gene family in rice genome-wide identification, organization and expression profiling during reproductive development and stress. *BMC Genom*, **8**: 242–262.

Chen J J, Ding J H, Ouyang Y D, Du H Y, Yang J Y, Cheng K, Zhao J, Qiu S Q, Zhang X L, Yao J L, Liu K D, Wang L, Xu C G, Li X H, Xue Y B, Xia M, Ji Q, Lu J F, Xu M L, Zhang Q F. 2008. A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. *Proc Natl Acad Sci USA*, **105**(32): 11436–11441.

Chu H W, Qian Q, Liang W Q, Yin C S, Tan H X, Yao X, Yuan Z, Yang J, Huang H, Luo D, Ma H, Zhang D B. 2006. The *floral organ number4* gene encoding a putative ortholog of *Arabidopsis* CLAVATA3 regulates apical meristem size in rice. *Plant Physiol*, **142**(3): 1039–1052.

Coen E S, Meyerowitz E M. 1991. The war of the whorls: Genetic interactions controlling flower development. *Nature*, **353**: 31–37.

Colombo L, Franken J, Koetje E, van Went J, Dons H J, Angenent G C, van Tunen A J. 1995. The petunia MADS box gene *FBP11* determines ovule identity. *Plant Cell*, **7**(11): 1859–1868.

Cui R F, Han J K, Zhao S Z, Su K M, Wu F, Du X Q, Xu Q J, Chong K, Theissen G, Meng Z. 2010. Functional conservation and diversification of class E floral homeotic genes in rice (*Oryza sativa*). *Plant J*, **61**(5): 767–781.

Diao Z J, Li S P, Duan Y L, Zheng L L, Zhou Y C, Wu W R. 2013. Expression level of *DDF1* affects vegetative growth and reproductive development in rice. *Chin J Rice Sci*, **27**(4): 353–358. (in Chinese with English abstract)

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky M F. 2004. The *SEP4* gene of *Arahidopsis thaliana* functions in floral organ and meristem identity. *Curr Biol*, **14**(21): 1935–1940.

Dreni L, Jacchia S, Fornara F, Fornari M, Ouwerkerk P B, An G, Colombo L, Kater M M. 2007. The D-lineage MADS-box gene *OsMADS13* controls ovule identity in rice. *Plant J*, **52**(4): 690–699.

Dreni L, Pilatone A, Yun D P, Erreni S, Pajoro A, Caporali E, Zhang D B, Kater M M. 2011. Functional analysis of all agamous subfamily members in rice reveals their roles in reproductive organ identity determination and meristem determinacy. *Plant Cell*, **23**(8): 2850–2863.

Ferrario S, Immink R G H, Shchennikova A, Busscher-Lange J, Angenent G C. 2003. The MADS box gene *FBP2* is required for SEPALLATA function in petunia. *Plant Cell*, **15**(4): 914–925.

Fornara F, Parenicova L, Falasca G, Pelucchi N, Masiero S, Ciannamea S, Lopez-Dee Z, Altamura M M, Colombo L, Kater M M. 2004. Functional characterization of *OsMADS18*, a member of the AP1/SQUA subfamily of MADS box genes. *Plant Physiol*, **135**(4): 2207–2219.

Jang S, Lee B, Kim C, Kim S J, Yim J, Han J J, Lee S, Kim S R, An G. 2003. The *OsFOR1* gene encodes a polygalacturonase-inhibiting protein (PGIP) that regulates floral organ number in rice. *Plant Mol Biol*, **53**(3): 357–372.

Jeon J S, Lee S, Jung K H, Yang W S, Yi G H, Oh B G, An G. 2000a. Production of transgenic rice plants showing reduced heading date and plant height by ectopic expression of rice MADS-box genes. *Mol Breeding*, **6**(6): 581–592.

Jeon J S, Jang S, Lee S, Nam J, Kim C, Lee S H, Chung Y Y, Kim S R, Lee Y H, Cho Y G, An G. 2000b. *Leafy hull sterile1* is a homeotic mutation in a rice MADS box gene affecting rice flower development. *Plant Cell*, **12**(6): 871–884.

Khanday I, Yadav S R, Vijayraghavan U. 2013. Rice *LHS1/OsMADS1* controls floret meristem specification by coordinated regulation of transcription factors and hormone signaling pathways. *Plant Physiol*, **161**(4): 1970–1983.

Kobayashi K, Yasuno N, Sato Y, Yoda M, Yamazaki R, Kimizu M, Yoshida H, Nagamura Y, Kyozuka J. 2012. Inflorescence meristem identity in rice is specified by overlapping functions of three *AP1/FUL*-like MADS box genes and *PAP2*, a *SEPALLATA* MADS box gene. *Plant Cell*, **24**(5): 1848–1859.

Lamb R S, Irish V F. 2003. Functional divergence within the *APETALA3/PISTILLATA* floral homeotic gene lineages. *Proc Natl Acad Sci USA*, **100**(11): 6558–6563.

Li Y F. 2008. Map-based Cloning and Functional Analysis of *LEAFY HULL STERILE 1-3* (*lhs1-3*) and *PISTILLOID-STAMEN* (*PS*) Genes in Rice (*Oryza sativa* L. ssp. *indica*). Chongqing, China: Southwest University. (in Chinese with English abstract)

Lohmann J U, Weigel D. 2002. Building beauty: The genetic control of floral patterning. *Dev Cell*, **2**(2): 135–142.

Lu Y J, Zheng K L. 1992. A simple method for isolation of rice DNA. *Chin J Rice Sci*, **6**(1): 47–48. (in Chinese with English abstract)

Lu S J, Wei H, Wang Y, Wang H M, Yang R F, Zhang X B, Tu J M. 2012. Overexpression of a transcription factor *OsMADS15* modifies plant architecture and flowering time in rice (*Oryza sativa* L.). *Plant Mol Biol Rep*, **30**(6): 1461–1469.

Mandel M A, Yanofsky M F. 1995. The *Arabidopsis AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1*. *Plant Cell*, **7**(11): 1763–1771.

Moon S, Jung K H, Lee D E, Lee D Y, Lee J, An K, Kang H G, An G. 2006. The rice *FON1* gene controls vegetative and reproductive development by regulating shoot apical meristem size. *Mol Cells*, **21**(1): 147–152.

Nagasawa N, MiyOshi M, Sano Y, Satoh H, Hirano H, Sakai H, Nagato Y. 2003. *SUPERWOMAN1* and *DROOPING LEAF* genes control floral organ identity in rice. *Development*, **130**(4): 705–718.

Prasad K, Parameswaran S, Vijayraghavan U. 2005. *OsMADS1*, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. *Plant J*, **43**(6): 915–928.

Ren D Y, Li Y F, Zhao F M, Sang X C, Shi J Q, Wang N, Guo S, Ling Y H, Zhang C W, Yang Z L, He G H. 2013. *MULTI-FLORET SPIKELET1*, which encodes an AP2/ERF protein, determines spikelet meristem fate and sterile lemma identity in rice. *Plant Physiol*, **162**(2): 872–884.

Schmittgen T D, Livak K J. 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc*, **3**(6): 1101–1108.

Sentoku N, Kato H, Kitano H, Imai R. 2005. *OsMADS22*, an *STMADS11*-like MADS-box gene of rice, is expressed in non-vegetative tissues and its ectopic expression induces spikelet meristem indeterminacy. *Mol Genet Genom*, **273**(1): 1–9.

Suzaki T, Ohneda M, Toriba T, Yoshida A, Hirano H Y. 2009. *FON2 SPARE1* redundantly regulates floral meristem maintenance with *FLORAL ORGAN NUMBER2* in rice. *PLoS Genet*, **5**(10): 1–9.

Wang K J, Tang D, Hong L L, Xu W Y, Huang J, Li M, Gu M H, Xue Y B, Cheng Z K. 2010. *DEP* and *AFO* regulate reproductive habit in rice. *PLoS Genet*, **6**(1): 1–9.

Xiao H, Wang Y, Liu D F, Wang W M, Li X B, Zhao X F, Xu J C, Zhai W X, Zhu L H. 2003. Functional analysis of the rice AP3 homologue *OsMADS16* by RNA interference. *Plant Mol Biol*, **52**(5): 957–966.

Xiao H, Tang J F, Li Y F, Wang W M, Li X B, Jin L, Xie R, Luo H F, Zhao X F, Meng Z, He G H, Zhu L H. 2009. *STAMENLESS 1*, encoding a single C_2H_2 zinc finger protein, regulates floral organ identity in rice. *Plant J*, **59**(5): 789–801.

Yadav S R, Prasad K, Vijayraghavan U. 2007. Divergent regulatory *OsMADS2* functions control size, shape and differentiation of the highly derived rice floret second-whorl organ. *Genetics*, **176**(1): 283–294.

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka M, Nagato Y, Hirano H Y. 2004. The *YABBY* gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa*. *Plant Cell*, **16**(2): 500–509.

Yamaguchi T, Lee D Y, Miyao A, Hirochika H, An G, Hirano H Y. 2006. Functional diversification of the two C-class MADS box genes *OsMADS3* and *OsMADS58* in *Oryza sativa*. *Plant Cell*, **18**(1): 15–28.

Yang J Y, Zhao X B, Cheng K, Du H Y, Ouyang Y D, Chen J J, Qiu S Q, Huang J Y, Jiang Y H, Jiang L W, Ding J H, Wang J, Xu C G, Li X H, Zhang Q F. 2012. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. *Science*, **337**: 1336–1340.

Yin L L, Xue H W. 2012. The MADS29 transcription factor regulates the degradation of the nucellus and the nucellar projection during rice seed development. *Plant Cell*, **24**(3): 1049–1065.

Yoshida A, Suzaki T, Tanaka W, Hirano H Y. 2009. The homeotic gene long sterile lemma (G1) specifies sterile lemma identity in the rice spikelet. *Proc Natl Acad Sci USA*, **106**: 20103–20108.

Zhang G L, Zhang S T, Xiao L T, Tang W B, Xiao Y H, Chen L Y. 2014. Effect of high temperature stress on physiological characteristics of anther, pollen and stigma of rice during heading-flowering stage. *Chin J Rice Sci*, **28**(2): 155–166. (in Chinese with English abstract)

Zhang Q F, Shen B Z, Dai X K, Mei M H, Saghai-Marool M A, Li Z B. 1994. Using bulked extremes and ressessive classes to map genes for photoperiod-sensitive genic male sterility in rice. *Proc Natl Acad Sci USA*, **91**(18): 8675–8679.