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Loss of function of *OsMADS34* leads to large sterile lemma and low grain yield in rice (*Oryza sativa* L.)

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Abstract Rice (*Oryza sativa* L.) is one of the most important food crops, especially in Asia. The spikelet is a characteristic structure of grass inflorescences that determines crop output. However, the molecular mechanism that controls spikelet development and grain yield in rice remains unclear. In this study, we isolated a new *osmads34* allelic mutant (i.e., *osmads34-t*). The *osmads34-t* mutant showed more primary branch numbers, short panicles, and long sterile lemmas. The sterile lemmas were transformed into the lemmas and had the lemma identity in the *osmads34-t* mutant, suggesting that the sterile lemma and lemma are homologous organs. Additionally, *osmads34-t* displayed smaller grains on its secondary branches of panicles and a lower seed-setting rate. These results suggest that *OsMADS34* plays an important role in determination of grain size and yield in rice. *OsMADS34* was expressed in tested organs and tissues, and its green fluorescent protein (GFP) signal was located in the nucleus. The result of this study will be used to understand the identity of unique organs in grass spikelets and may improve grain yield in breeding practice.

Keywords Rice (*Oryza sativa* L.) · *OsMADS34* · Spikelet · Sterile lemma · Grain yield

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Introduction

A typical eudicot flower has four whorls: sepals, petals, stamens, and carpels. The ABC model for floral organ specialization was established in 1991 based on model eudicot species, *Arabidopsis thaliana* and *Antirrhinum majus*, which facilitates the better understanding of flower development (Coen and Meyerowitz 1991). In recent advanced studies, this ABC model was developed to an ABCDE model. Most of the genes involved in this model, which have been identified as members of the MADS-box gene family, have been used to explain the genetic mechanism and regulatory network underlying flower development in eudicot and monocot species (Hong et al. 2010; Li et al. 2011; Dreni et al. 2011; Khanday et al. 2013).

Grass species, comprising one of the largest monocot families, are quite different from eudicots in terms of

floral architecture, which have spikelets and florets (Yoshida and Nagato 2011). In rice, each ordinary spikelet consists of two rudimentary glumes, two sterile lemmas and a floret. From the periphery to the center, the floret contains a pair of interlocked lemma and palea, two lodicules, six stamens and a pistil (Yoshida et al. 2009). Sterile lemmas, which are highly derived grass-specific organs, are larger than rudimentary glumes, but smaller than lemmas (Ikeda et al. 2004). Rudimentary glumes are regarded as severely reduced bract organs, and sterile lemmas represent vestigial glume of two lateral florets (Hong et al. 2010; Gao et al. 2010; Kobayashi et al. 2010; Ren et al. 2013). Although these unique organs, including rudimentary glumes, sterile lemmas, lemmas, and paleas, share some homology, their identities are still controversial (Zhang and Yuan 2014; Ren et al. 2015).

Rice is a major food crop for half of the global population (Abacar et al. 2016; Guo et al. 2014). Grain yield is directly determined by four components: the number of panicles, the number of grains per panicle, grain weight, and the ratio of filled grains (Zuo and Li 2014). Grain weight is mainly affected by grain size including grain length, width, and thickness (Xing and Zhang 2010). To date, there are many quantitative trait loci (QTLs) and genes involved in regulation of grain size that have been cloned in rice mainly included *GRAIN WEIGHT 2* (*GW2*), *GRAIN SIZE ON CHROMOSOME 2* (*GS2*), *GRAIN SIZE 5* (*GS3*), *GRAIN LENGTH 3.1* (*GL3.1*), *GRAIN SIZE 5* (*GS5*), *GRAIN WEIGHT 5* (*GW5*), *GRAIN WEIGHT 7* (*GW7*)/*GRAIN LENGTH ON CHROMOSOME 7* (*GL7*), *DRAWF 61* (*D61*), *BRASSINOSTEROID–DEPENDENT 1* (*BRD1*), and *SHORT GRAIN1* (*SG1*) (Song et al. 2007; Hu et al. 2015; Fan et al. 2006; Qi et al. 2012; Xu et al. 2015; Weng et al. 2008; Wang et al. 2015a; Wang et al. 2015b; Yamamuro et al. 2000; Mori et al. 2002; Nakagawa et al. 2011). *GW2* encodes a RING-type protein with E3 ubiquitin ligase activity that regulates grain width and weight (Song et al. 2007). *GS2* encodes growth-regulating factor 4 (OsGRF4), which regulates cell size and cell numbers (Hu et al. 2015). *GS5* encodes a putative serine carboxypeptidase and functions as a positive regulator of grain size (Xu et al. 2015). *GW5* encodes a nuclear polyubiquitin-binding protein which controls seed width and weight (Weng et al. 2008). *GL7* encodes a protein homologous to *A. thaliana* LONGIFOLIA proteins, which regulates longitudinal cell elongation (Wang et al. 2015b). These findings suggest that these genes determined grain weight and size largely by regulating cell proliferation and expansion of the hull, and these genes' further application in breeding program will facilitate to improve rice supply.

In this study, we isolated a new *osmads34* allelic mutant (*osmads34-t*) with variable defect. More primary branch numbers per panicle, short panicles, and long sterile lemmas were found in the *osmads34-t* mutant, which was similar to phenotypic defects of the reported *osmads34* mutant (Gao et al. 2010; Kobayashi et al. 2010). Differently, the *osmads34-t* exhibited small grains and low seed-setting rate. Sequencing analysis displayed that the mutated *LOC_Os03g54170* (i.e., *osmads34-t*) gene contained four base pair (bp) deletions, resulting in premature translational termination. Complementation test and bioinformatics analysis revealed that *LOC_Os03g54170* is indeed *OsMADS34*.

Material and method

Plant materials

The *osmads34-t* mutant was derived from Zhonghua11 (ZH11) (*O. sativa L. japonica*) undergoing ethyl methyl sulfate (EMS) mutagenesis. ZH11 was used as the wild-type strain for phenotypic observation. The *osmads34-t* mutant was further crossed with an *Indica* rice variety, Zhefu802 (ZF802) (*O. sativa L. indica*), to construct the F_2 population. All plants were cultivated in paddies in Sanya, China.

Agronomic traits and genetic analysis

All plants were grown under normal cultural conditions. At maturity stage, the agronomic traits of grains, including grain width, grain length, and kilo-grain weight, and the primary and secondary branch numbers were investigated and compared between the wild type and *osmads34-t* mutant. The segregation of normal and abnormal plants was determined, and the ratio was analyzed by χ^2 test.

Microscopy observation

At heading stage, the spikelets were fixed in FAA solution (50% ethanol, 10% formaldehyde, and 5% glacial acetic acid) over night at 4 °C, then dehydrated with ethanol series (50, 70, 85, 95, 100, 100, 100%) and

infiltrated with xylene series (33, 50, 67, 100, 100, 100% solubility in 100% ethanol) (Ren et al. 2013). Finally, the samples were embedded in paraffin, sectioned into 8-μm thick sections, and stained with 1% safranine (solubility in 95% ethanol) and 1% fast green (solubility in 95% ethanol) for histological analysis. Scanning electron microscopy (SEM) analysis was conducted using a NIKON ECLIPSE 90i microscope (Yu et al. 2015).

Map-based cloning

For fine mapping of the *OsMADS34*, six simple sequence repeat (SSR) markers were developed on comparisons between *Indica* 93-11 and *Japonica* Nipponbare according to the web data which include the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), Rice Data (http://www.ricedata.cn/gene/), and Gramene (http://www.gramene.org). The primers used in this study are listed in Supplementary Table 1.

Gene expression analysis

Using TRIzol reagent (Invitrogen, USA), total RNA was extracted from inflorescences at booting stage and floral organs at heading stage in the *osmads34-t* and wild type. Then, using a ReverTra qPCR RT kit (Toyobo Corporation, China), 2 mg RNA sample was reverse-transcripted into single-strand cDNA. And three biological repeats of qPCR were conducted with an ABI Prism 7000 Sequence Detection System and SYBR Green PCR Master Mix kit (Applied Biosystems, USA). Quantitative real-time PCR experiments of MADS-box genes (*OsMADS1*, *OsMADS6*, *OsMADS14*, and *OsMADS15*), *DL* gene, and grain regulation genes (*SMG1*, *GW2*, *GS3*, *GS5*, and *GL7*) were performed using a Power SYBR Green PCR Master Mix kit (Applied Biosystems). And the rice actin gene was used as an internal control (primers listed in Supplementary Table1). For the β-glucuronidase (GUS) staining assay, a 2.18-kb sequence of the *OsMADS34* promoter was amplified by PCR (primers listed in Supplementary Table 1) and the PCR products were digested with *HindIII* and *BamHI.* Then, the fragment was cloned into the pCAMBIA1391Z vector.

Tissue for GUS staining was gently fixed in 90% acetone on ice for 20 min and then rinsed in 50 mM $NaPO_4$, pH 7.2, 0.5 mM $K_3Fe(CN)_6$, and 0.5 mM $K_4Fe(CN)_6$. Then, the tissue was placed in staining solution (50 mM NaPO4, pH 7.2, 2 mM X-gluc, 0.5 mM $K_3Fe[CN]_6$, and 0.5 mM $K_4Fe[CN]_6$), which was vacuum infiltrated and incubated at 37 °C overnight (Sieburth and Meyerowitz 1997).

Subcellular localization

The coding sequence (CDS) of *OsMADS34* was amplified without stop codon, and this contained restriction enzyme sites *PstI* and *XbaI* (primers listed in Supplementary Table1). The *OsMADS34*-GFP fusion vector was constructed by the insertion of *OsMADS34*-CDS into 35S-EGFP-(pCAMBIA1300) (Wu et al. 2016). The green fluorescent protein (GFP) and *OsMADS34*-GFP plasmids were then transformed into rice protoplasts (Bart et al. 2006). GFP fluorescence was observed under a confocal laser-scanning microscope (ZEISS LSM 700, Germany).

Result

The *osmads34* mutant shows elongated sterile lemmas

No obvious differences were observed between the wild type and *osmads34-t* during the vegetative phase. At the heading stage, the sterile lemmas of the wild type were small, triangular structures with an average length approximately one fourth of the lemmas and paleas. By contrast, the *osmads34-t* mutant showed two elongated sterile lemmas which resembled the wild-type lemmas or paleae in size (Fig. 1a, f). We also investigated the morphology and the number of components of the four whorls of floral organs, finding that these organs were normal (Fig. 1b, g).

Paraffin section exhibited that wild-type lemmas and paleas had four cell layers, as well as five and three vascular bundles, respectively. The sterile lemmas of the wild type had only one vascular bundle (Fig. 1c, d). By contrast, in the *osmads34-t* mutant, the sterile lemmas had 46 vascular bundles (Fig. 1h, i). SEM analysis revealed that in the wild type, the epidermis of the lemma and palea had numerous protrusions and trichomes, whereas the epidermis of sterile lemmas had few protrusions and trichomes (Fig. 1e). However, in the *osmads34-t* mutant, the epidermis of sterile lemmas possessed numerous protrusions and trichomes, which

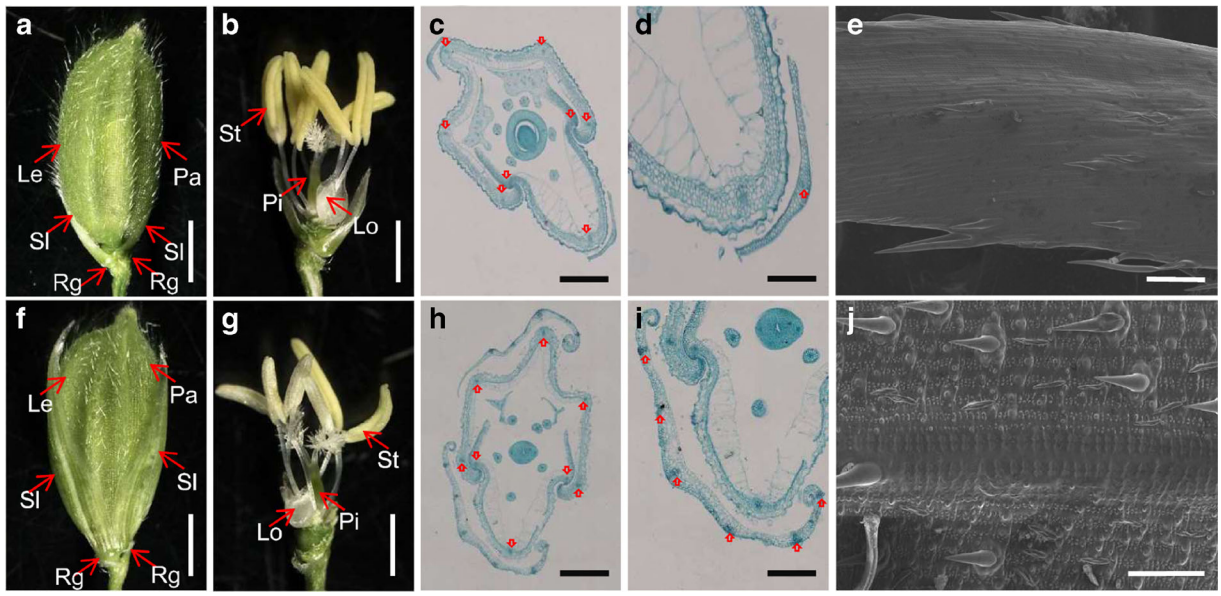

Fig. 1 Phenotypes of spikelets in the wild type and osmads34-t mutant. **a**, **b** Spikelet of wild type. **c**, **d** Histological analysis of wild-type spikelet. **e** Epidermal surface of wild-type sterile lemma. **f**, **g** Spikelet of *osmads34-t*. **h**, **i** Histological analysis of *osmads34-t* spikelet. **j** Epidermal surface of *osmads34*. *Le* lemma, *Pa* palea, *Sl* sterile lemma, *Rg* rudimentary glume, *Pi* pistil, *Lo* lodicule. *Red arrows* represent vascular bundles in **a**, **d**, **h**, **i**. *Bars* = 2 mm in **a**, **b**, **f**, **h**, 100 μm in **c**, **h**, 30 μm in **d**, **i**, and 100 μm in **e**, **j**

was similar to the epidermis of wild-type lemmas (Fig. 1j).

In order to understand the identity of abnormal sterile lemmas in *osmads34-t*, we examined the transcript levels of the hull (i.e., lemma and palea) identity genes *OsMADS1*, *OsMADS14*, and *OsMADS15*, the lemma identity gene *DROOPING LEAF* (*DL*), and the palea identity gene *OsMADS6* in the *osmads34-t*. Abundant levels of *OsMADS1*, *OsMADS14*, *OsMADS15*, and *DL* transcripts were detected in the long sterile lemmas of *osmads34-t*, but no *OsMADS6* transcripts were found, suggesting that the sterile lemmas were transformed into lemma-like organs in the *osmads34-t* mutant (Fig. 2).

Early spikelet development in the *osmads34-t* mutant

Spikelet development is divided into eight stages based on the identity of later organs according to Ikeda et al. (2004). In the current study, we examined young spikelets at different developmental stages from the wild type to *osmads34-t* by SEM. During Sp4, the palea primordium formed at the opposite side to the lemma and sterile lemma was growing and had no significant difference between the wild type and *osmads34-t* mutant (Fig. 3a, e). However, in the *osmads34-t* mutant, lodicule primordia and stamen primordia formed and the sterile lemma was much larger than that of the wild type, i.e., nearly the size of the palea during Sp5 and Sp6 (Fig. 3b, f). During Sp7 and Sp8, the carpel primordium was formed and the length of sterile lemma was close to that of the palea and lemma in the *osmads34-t* mutant (Fig. 3c, d, g, h). However, at Sp4–Sp8, neither the morphology nor the number was altered in the *osmads34-t* mutant compared to the wild type.

OsMADS34 affects grain yield

The *osmads34-t* grains, on secondary branches, were obviously different from the wild type. Compared with the wild type, the *osmads34-t* mutant had smaller grains on its secondary branches, but the grains on primary branches were not affected (Fig. 4a, b, h, i). Additionally, both the kilo-grain weight and the weight of kilo-brown rice were remarkably reduced in the *osmads34-t* mutant (Fig. 4g). The *osmads34-t* mutant also had a lower seed-setting rate than the wild type (Fig. 4j).

Further tests showed that the pollen viability of spikelets on secondary branches was greatly reduced in the *osmads34-t* mutant compared to the wild type,

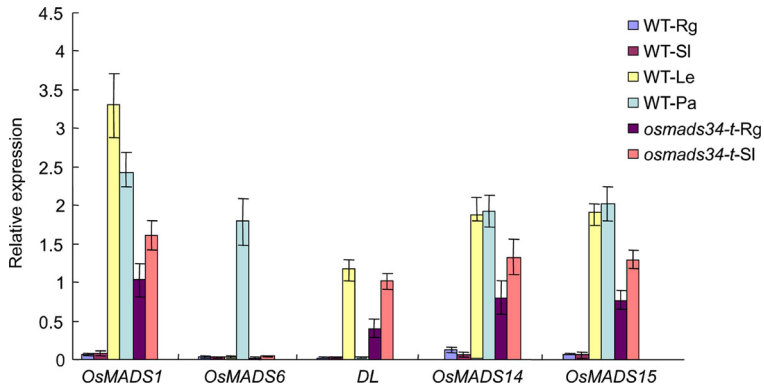

Fig. 2 Relative expression levels of floral organ identity genes in the wild type and *osmads34-t* floral organs. *WT-Rg* rudimentary glume of wild type, *WT-Sl* sterile lemma of wild type, *WT-Le* lemma of wild type, *WT-Pa* palea of wild type, *osmads34-t-Rg* rudimentary glume of *osmads34-t*, *osmads34-t-Sl* sterile lemma of *osmads34-t*. *Error bars* indicate SD

whereas the pollen viability of spikelets on primary branches was not altered (Fig. 4c–f). Moreover, the number of primary branches increased, and the number of secondary branches decreased in the mutant compared to the wild type (Fig. 4k). Most importantly, *osmads34-t* exhibited shorter panicles and fewer grains than wild type.

Next, we examined the expressions of several genes involved in the regulation of grain weight and size. The expressions of *SMG1*, *GW2*, *GS3*, and *GL7* were higher in the mutant than in the wild type, while the expression of *GS5* was unaffected (Supplementary Figure 1). These results may indicate that *OsMADS34* functions in the regulation of grain yield and size.

Isolation of *OsMADS34*

When the *osmads34-t* mutants were crossed with ZF802, an indica variety, all F1 individuals exhibited normal phenotype as the wild type. In F2 population, the segregation rate of wild type and abnormal plants fits the ratio of 3:1, which indicates that the mutated traits are controlled by a single recessive nuclear gene. The F_2 population was used to map the *OsMADS34* gene locus. More than 1300 plants in this population exhibited the mutant phenotypes. We used 160 SSR markers that are evenly distributed on rice chromosome1–12 to screen the target gene by bulked segregant analysis. Markers RM227 and RM15948 on

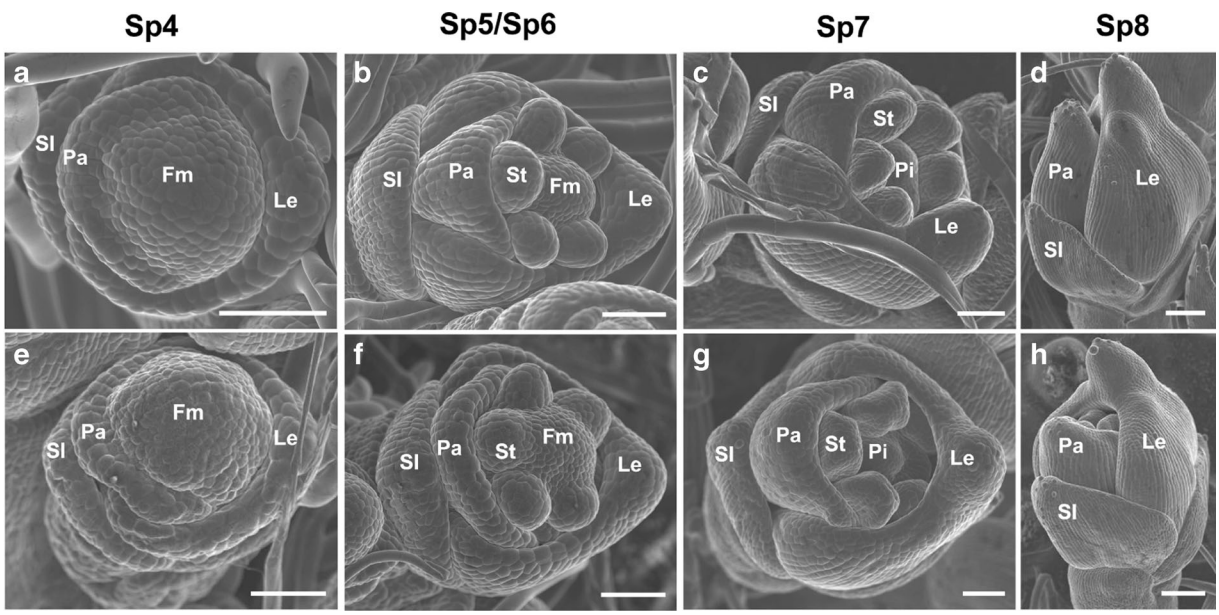

Fig. 3 Spikelets at early developmental stages in the wild type and *osmads34-t*. **a**–**d** Development of the spikelet in the wild type. **a**Sp4. **b** Sp5–6. **c** Sp7. **d** Sp8. **e**–**h** Development of the spikelet in *osmads34-t*. **e** Sp4. **f** Sp5–6. **g** Sp7. **h** Sp8. *Sl* sterile lemma, *Le* lemma, *Pa* palea, *Fm* floral meristem, *Pi* pistil. *Bars* =50 μm in **a**–**h**

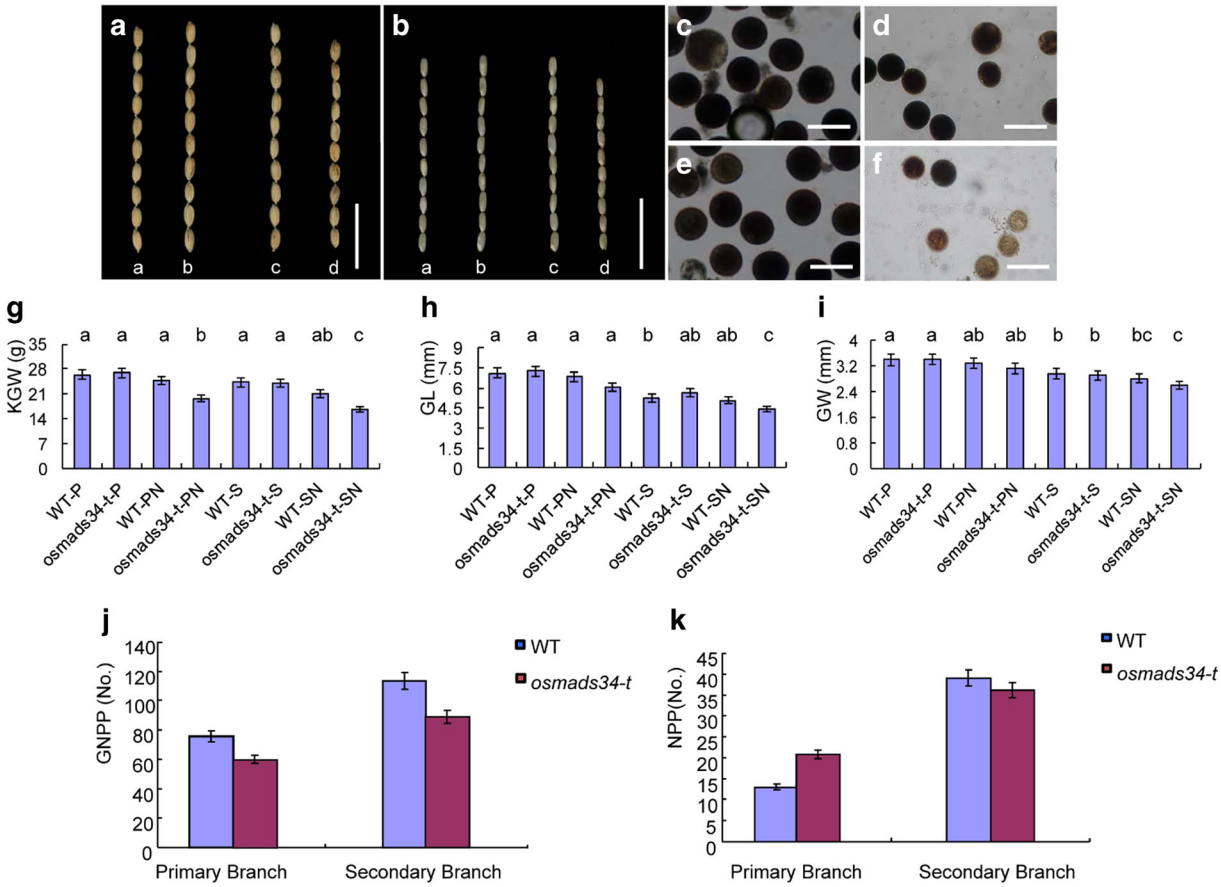

Fig. 4 Phenotype and grain yield in the wild type and *osmads34-t* mutant. **a**, **b** Grains of wild type and *osmads34-t*. **a**-*a* Grains of wild type on primary branch. **a**-*b* Grains of *osmads34-t* on primary branch. **a**-*c* Grains of wild type on secondary branch. **a**-*d* Grains of *osmads34-t* on secondary branch. **b**-*a* Brown rice grains of wild type on primary branch. **b**-*b* Brown rice grains of *osmads34-t* on primary branch. **b**-*c* Brown rice grains of wild type on secondary branch. **b**-*d*) Brown rice grains of *osmads34-t* on secondary branch. **c** Pollen grains in the wild-type stamen on primary branch. **d** Pollen grains in the *osmads34-t* stamen on primary branch. **e** Pollen grains in the wild type stamen on secondary branch. **f** Pollen grains in the *osmads34-t* stamen on secondary branch. **g** Kilo-grain weight of wild type and *osmads34-t*, including grains and brown rice on primary and secondary branches. **h** Grain length of wild type and *osmads34-t*, including grains and brown rice on primary and secondary branches. **i** Grain length of wild type and *osmads34-t*, including grains and brown rice on primary and secondary branches. **j** Grain numbers per panicle on primary branch and secondary branch of wild type and *osmads34-t*. **k** Branch numbers per panicle of wild type and *osmads34-t*. *KGW* kilo-grain weight, *GL* grain length, *GW* grain width, *WT-P* grains on primary branch in wild type, *osmads34-t-P* grains on primary branch in the *osmads34-t*, *WT-PN* brown rice on primary branch in the wild type, *osmads34-t-PN* brown rice on primary branch in the *osmads34-t*, *WT-S* grains on secondary branch in wild type, *osmads34-t-S* grains on secondary branch in the *osmads34-t*, *WT-SN* brown rice on secondary branch in the wild type, *osmads34-t-SN* brown rice on secondary branch in the *osmads34-t*, *WT* wild type, *GNPP* grain numbers per panicle, *NPP* numbers per panicle. *Bars* = 2 cm in **a**, **b** and 50 μm in **c**–**f**. *Error bars* indicate SD. The presence of the same lowercase letter denotes a non-significant difference between the means **g**–**i**

chromosome 3 had polymorphisms between the wild-type DNA pools and *osmads34-t* DNA pools. Using these markers, we examined 212 F_2 recessive individuals finding that the target gene was located between RM227 and RM15948.

For fine mapping of the locus, 24 pairs of SSR markers were developed, four of which had polymorphism. Using these four markers, 1326 screened F_2 recessive individuals narrowed down this gene to 143 kilobase (Kb) regions (primers listed in Supplementary Table 1). In this region, *LOC_Os03g54170* encoded a predicted OsMADS34 protein with the conserved MADS-box domain (Supplementary Figure 3; http://www.ricedata.cn; http://rice.plantbiology.msu.edu) may be involved in the regulation of spikelet or floral development.

Next, sequencing suggested that *LOC_Os03g54170* gene has four nucleotide (GGAT) deletions in the genomic sequence of *osmads34-t*, which leads to a frameshift and premature translational termination (Fig. 5a, b). To further test the linkage between the mutant phenotype and *osmads34-t*, *LOC_Os03g54170*, a wild-type genomic fragment containing a 2021-bp region upstream of the start codon and a 1008-bp region downstream of the stop codon, was transformed into the *osmads34-t* mutant. In this complementation test, all mutant phenotypes were rescued in the *osmads34-t* mutant (Fig. 5c–h; Supplementary Figure 2).

Meantime, we performed the protein sequence alignment. The result showed that the LOC_Os03g54170 protein contains a highly conserved domain with the proteins of other species such as rice, sorghum, maize, wheat, and *Arabidopsis* (Supplementary Figure 4). The results also suggest that LOC_Os03g54170 shared high amino acid sequence similarity with the known E-function genes *OsMADS1* and *OsMADS5*, which possessed a conserved MADS-box domain (Supplementary Figure 3). Further, *LOC_Os03g54170* is also reported and is an allele of the *OsMADS34* gene (Gao et al. 2010; Kobayashi et al. 2010). Therefore, all findings suggested that *LOC_Os03g54170* is a member of E-class

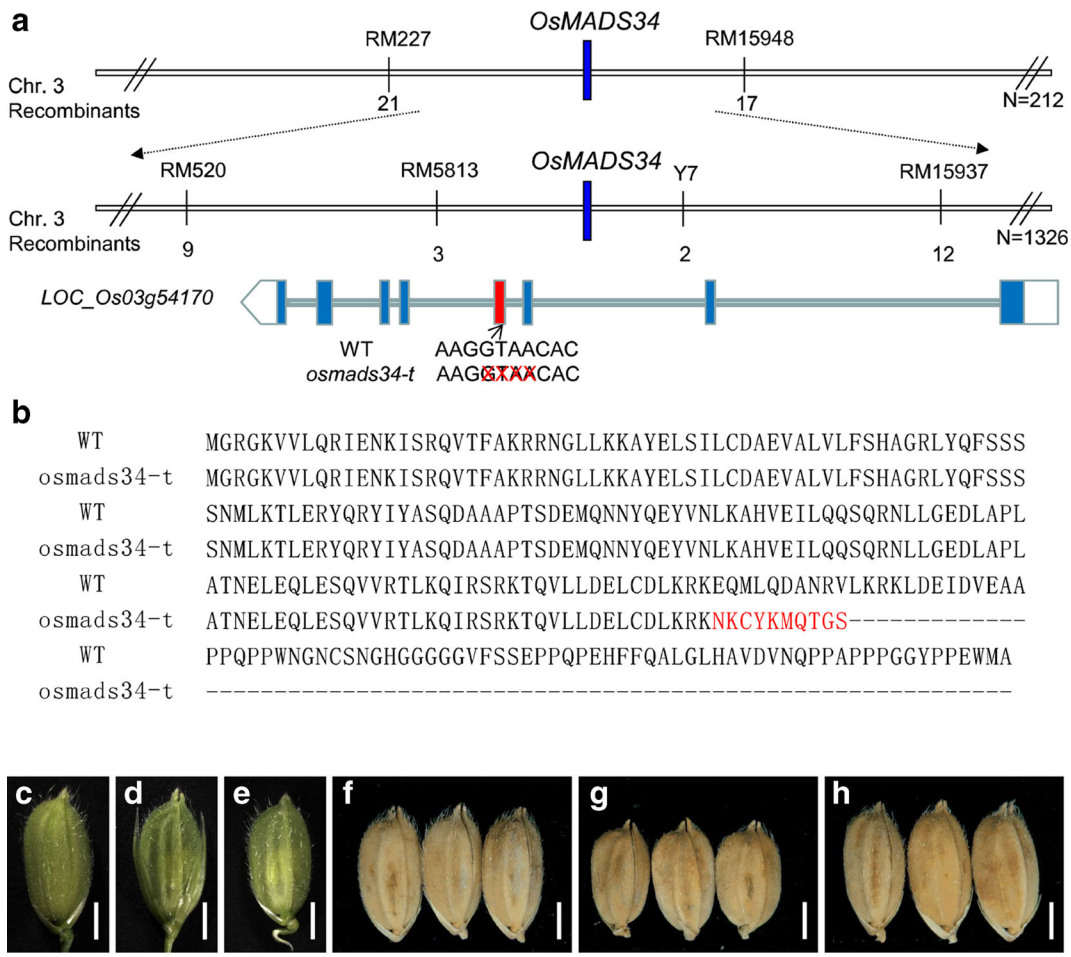

Fig. 5 Isolation of the *OsMADS34* gene. **a** Map position of the *OsMADS34* locus. The mutation sites, deletion of 4 bp, are shown. **b** An AFD1 gene encodes 239 amino acid expression proteins, while *osmads34-t* encodes 176 amino acid expression proteins due to frame shift. **c** Spikelet of wild type. **d** Spikelet of *osmads34-t* mutant. **e** Spikelet of transgenic plants. **f** Grains of wild type on secondary branch. **g** Grains of *osmads34-t* on secondary branch. **h** Grains of transgenic plants on secondary branch. *Bars* = 2 mm in **c**–**h**

gene family and confirmed that *LOC_Os03g54170* is an *OsMADS34* gene.

Expression pattern of *OsMADS34* and protein localization

To examine the expression pattern of *OsMADS34*, we performed qRT-PCR in the wild type. *OsMADS34* was expressed in all tissues and organs examined, including roots, culms, leaves, sheaths, panicles, rudimentary glumes, sterile lemmas, lemmas, and paleae, with strong expressions detected in young panicles and reproductive organs (Fig. 6a).

To monitor the tissue-specific expression of *OsMADS34*, we constructed an *OsMADS34* promoter: a GUS expression vector. GUS signals were observed in all of the tissues examined, with the strongest signals detected in young panicles (Fig. 6b–f). We also examined the subcellular localization of OsMADS34 in rice protoplasts, finding that fluorescent signals of OsMADS34 were detected in the nucleus. Taken together, these results suggested that OsMADS34 is a nuclear protein that may function as a transcription factor (Fig. 6g–l).

Discussion

Grain size and number are important determinants of grain yield (Qian et al. 2016). To date, numerous genes controlling rice grain size have been identified, such as *GW2*, *GS2*, *GS3*, *DWARF 1* (*D1*), *DWARF 2* (*D2*),

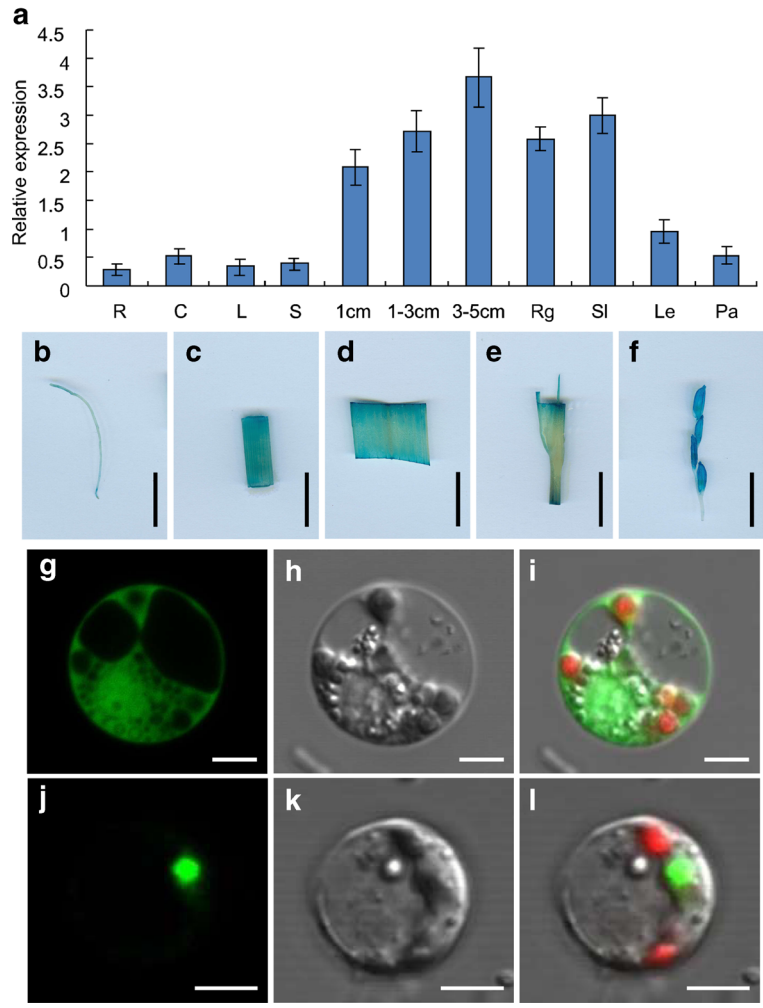

Fig. 6 Expression pattern of *OsMADS34*. **a** Relative expression of *OsMADS34* in different tissues was detected by qPCR. **b**–**f** Gus signal were observed in different tissues. **b** Root. **c** Culm. **d** Leaf. **e** Sheath. **f** Young panicles. **g**–**l** GFP fusion protein. **g** Digital Image Control (DIC) image. **h** Bright-field image. **i** Merged GFP fusion protein. **j**–**l** *OsMADS34*-GFP. **j** DIC image. **k** Bright-field image. **l** Merged image of OsMADS34-GFP fusion protein. *R* root, *C* culm, *L leaf*, *S* sheath, *1 cm* young panicles (≤1 cm), *1–3 cm* young panicles (1–3 cm), *3–5 cm* young panicles (3–5 cm), *Rg* rudimentary glume, *Sl* sterile lemma, *Le* lemma, *Pa* palea. *Bars* =1 cm in **b**–**f** and 2 μm in **g**–**l**. *Error bars* indicate SD

SMALL GRAIN 1 (*SMG1*), *DENSE AND ERECT PANICLE 1* (*DEP1*)/*ERECTPOSE PANICLE* (*EP*), and *DENSE AND ERECT PANICLE 2* (*DEP2*) (Song et al. 2007; Hu et al. 2015; Qi et al. 2012; Miura et al. 2009; Hong et al. 2003; Duan et al. 2014; Huang et al. 2009; Wang et al. 2009; Li et al. 2010). However, the molecular mechanisms that affect final rice supply are complicated and remain to be described. Although *OsMADS34* was reported to control spikelet development (Gao et al. 2010; Kobayashi et al. 2010), its involvement in the regulation of grain yield has not previously been described. In this study, we found that the *osmads34-t* mutant bore small grains on secondary branches, but not on primary branches. Compared with the wild type, *osmads34-t* grains on secondary branches were shorter in length, but are equal in width, which resulted that the grain weight was markedly decreased. The *osmads34-t* mutant also displayed shorter panicles and a lower seed-setting rate which was caused by the defective pollen grains of spikelets on secondary branches. Using qRT-PCR and GUS analyses, we found that *OsMADS34* was highly expressed in spikelets, which is consistent with the phenotype observations of the *osmads34-t* mutant. In previous studies, most of MADS-box genes are primarily involved in regulating flower development, but how they regulate seed development is currently unclear. *OsMADS29*, a rice MADS-box family member, is expressed in the ovule. Knockdown of *OsMADS29* causes shrunken seeds and a reduced grain-filling rate (Yang et al. 2012; Yin and Xue 2012). Through these findings, together with the current results, we suggest that MADS-box genes including *OsMADS34* and *OsMADS29* play important roles in determining grain yield and size. Further, functional analysis and appalment of *OsMADS34* and other MADS-box genes will provide a new way to improve grain yield in rice breeding.

In this study, the *osmads34-t* sterile lemma exhibited a similar histological structure, resembling the wild-type lemma. In wild type flowers, *DL* and *OsMADS6* were mainly expressed in the lemma and palea, respectively (Li et al. 2011). But *DL* was ectopically expressed, and no *OsMADS6* expression was detected in the sterile lemma of the *osmads34-t* mutant (Fig. 2). Meantime, our results also displayed that the transcripts of *OsMADS1*, *OsMADS14*, and *OsMADS15* were primarily in the lemma and palea of wild-type flowers (Prasad et al. 2005; Kobayashi et al. 2012; Lu et al. 2012), but their expressions were also observed in the elongated sterile lemma of the *osmads34-t* mutant (Fig. 2). These information suggested that the sterile lemma was transformed into the hull-like organ and acquired the lemma identity in part in the *osmads34-t* mutant. Similarly, in the reported *g1*/*ele* and *eg1* mutants, the sterile lemma is also transformed homeotically into a lemma-like organ (Li et al. 2009; Yoshida et al. 2009; Hong et al. 2010). These findings imply that *OsMADS34*, *G1*/*ELE*, and *EG1* maintain the identity of sterile lemmas and restrain the elongation of sterile lemmas in the development of rice spikelet. A current hypothesis suggests that the spikelet originally contained three florets, in which two lateral florets degenerated into lemmas, which subsequently degenerated into the sterile lemmas during evolution (Kellogg 2009; Ren et al. 2013). In the *osmads34-t*, *g1*/*ele* and *eg1* mutants, the sterile lemmas are enlarged and transformed into lemmas, which supports the hypothesis that the sterile lemma and lemma may be homologous structures. However, more studies are needed to identify more related mutants and to clone the corresponding genes involved in the regulation of the sterile lemma and lemma in rice.

In summary, our findings about the *osmads34-t* mutant will not only facilitate further study of the genetic mechanism of flower development, but they also provide a new way to improve rice yield in breeding practice.

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