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SHORT COMMUNICATION

OsPrMC3 is Involved in Seed Development and in Determining Seed Yield as a Branching Inhibitor

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Abstract We here show that OsPrMC3 affects seed yields by regulating tillering. *OsPrMC3* is highly expressed in leaves and mature seeds, although its expression is detected in all tissues, and its mutant *osprmc3* has more tillers and less grain, indicating its crucial role in determining grain yield as a tillering inhibitor.

Keywords grain · OsPrMC3 · rice · tillering · yield

Tillering, an important agronomic trait, enhances grain production. Rice tiller is a specialized grain-bearing branch that forms on the unelongated basal internode and grows independently of the mother stem (culm) by means of its own adventitious roots. Rice tillering occurs as a two-stage process: the formation of an axillary bud at each leaf axil and subsequent outgrowth of the bud. So far, several genes controlling rice tillering have been isolated, including MONOCULM1 (MOC1), which participates in axillary meristem initiation in rice (Li et al., 2003), and OsTB1, a rice orthologue of maize TB1 (Teosinte Branched1), regulates axillary bud outgrowth (Doebley et al., 1997; Lukens and Doebley, 2001; Hubbard et al, 2002). Recent results also demonstrated that overexpression of OsTB1 causes less tillering in rice (Takeda et al., 2003).

Another group of branching/tillering includes dwarf (d) mutants D3, D17/HTD1, D10, D27, and D14, which show increased branches and reduced plant height (Ishikawa et al., 2005; Zou et al., 2006; Arite et al., 2007; Lin et al., 2009; Arite et al., 2009; Gao et al., 2009; Liu et al., 2009). This is suggested to be caused by a deficiency in synthesizing strigolactones and in their signaling. Strigolactones are phytohormones that inhibit plant shoot branching (Umehara et al., 2008).

Gibberellin (GA) and brassinosteroid (BR) are the two major factors responsible for dwarfism although it arises from various types of defects. Thus, their biosynthesis- or perception-deficient mutants show the dwarf phenotype (Mandava, 1988; Clouse and Sasse, 1998; Fujioka and Yokota, 2003). A soluble GA receptor, GIBBERELLIN-INSENSITIVE DWARF1 (GID1) (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006) belongs to a large family of lipases that are related to various carboxylesterases (Marshall et al., 2003). Structural analyses of GID1 proteins from rice and Arabidopsis revealed that they have a GA-binding pocket (Murase et al., 2008). The rice *gid1-1* mutant has a severe dwarf phenotype that is typical of rice GA-related mutants (Itoh et al., 2001; Sasak et al., 2003; Sakamoto et al., 2004), and transgenic rice overexpressing GID1 display a GA-hypersensitive phenotype. The GA receptors were first implemented as a part of the carboxylesterase family (Vandenbussche et al., 2007). The rice Dwarf88 (D88) mutant for the gene encoding a putative esterase has excessive numbers of shorter tillers and smaller panicles and seeds compared to the wild-type (Gao et al., 2009).

Interestingly, OsPrMC3 (Accession number: NP_001057131) displays homology to GA receptors at the amino acid level and contains esterase and lipase domains. Therefore, OsPrMC3 is expected to be affected in development including tillering and leaf growth.

Thus, the morphological and physiological roles of OsPrMC3 during rice tillering and seed development were analyzed using loss-of-function mutants.

A T-DNA insertion mutant of OsPrMC3 was first isolated by performing polymerase chain reaction (PCR) with *OsPrMC3*

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Table 1 Primer sequences used for PCR

Primer sequences for real time RT-PCR (PCR product: 200 bp (F/R))
Forward primer (F) 5'-TCATCCGCGTCTACGTGAGCGGCC-3' Reverse primer (R) 5'-CGCCGCCGTGGAAGTACACGAGGA-3'
Primer sequences for selection of *osprmc3* mutant (PCR Product: 1108 bp (LP/RP), 700 bp (BP/RP))
Left primer (LP) 5'-GGTACTCGACGGACACCG-3' Right primer (RP) 5'-GTTATCAACCGGTTGTTCGG-3' Border primer (BP) 5'-CCACAGTTTTCGCGATCCAGACTGAATG-3'

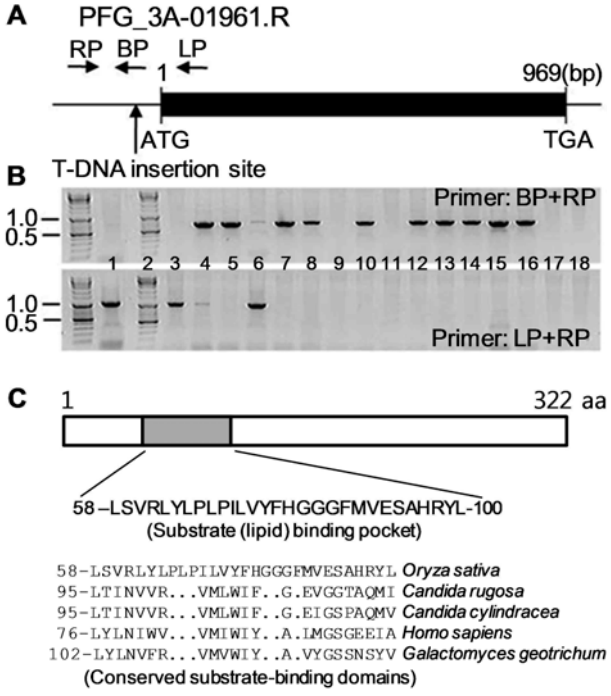

Fig. 1 Isolation of the *osprmc3* mutant. (A) Schematic diagram of the T-DNA insertion mutant, PFG_3A-01961.R provided by Dr. Gynheung An, POSTECH. T-DNA insertion and primer-binding sites are indicated by arrows. (B) Independent transgenic lines were analyzed by PCR using two sets of primers, as described in Table 1. In non-transgenic lines, 0.7 and 1 kb fragments were amplified by PCR with BP and RP, and LP and RP, respectively, whereas were not amplified by these primer sets in transgenic homozygote lines. Number nine among the homozygous lines was chosen and used for further study. (C) OsPrMC3 has a conserved domain for binding to lipid substrates. The substrate binding region was indicated as an amino acid sequence. Amino acid sequences of five lipases from different species [OsPrMC3 (Accession number: NP_001057131), *Oryza sativa*; Triacylglycerol lipase (Accession number: 1LPP), *Candida rugosa*; Cholesterol esterase (Accession number: 1CLEA), *Candida cylindracea*; Bile salt-activated lipase (Accession number: 1F6W_A), *Homo sapiens*; Triacylglycerol hydrolase (Accession number: 1THG), *Galactomyces geotrichum*] were aligned.

gene-specific and T-DNA-specific primers (Table 1), and confirmed that the T-DNA was inserted into the 5'UTR of the *OsPrMC3* gene (Fig. 1A and B). However, only one homozygous line, number nine, was chosen among the homozygous lines for

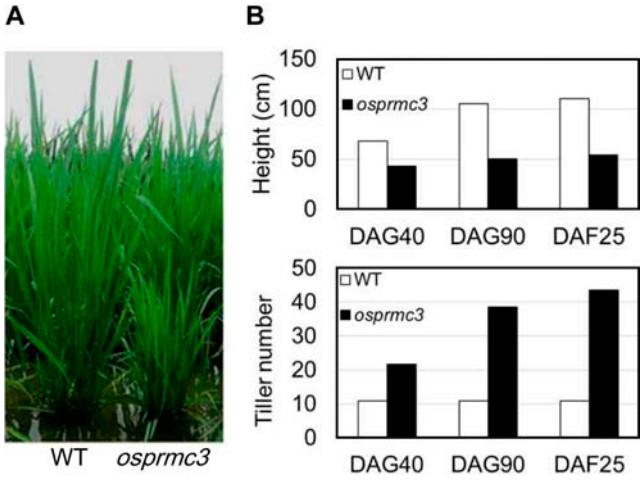

Fig. 2 Phenotypic analysis of the *osprmc3* mutant. (A) Wild-type and *osprmc3* mutant were grown in the field and then photographed. The mutants show dwarfism with a higher number of tillers than the wild-type. (B) The heights and number of tillers of the field-grown *osprmc3* mutant were examined at three different developmental stages. The mutant had much more tillers than the wild-type, but was much smaller than that of the wild-type. DAG, Day after germination. DAF, Day after flowering.

further study because all of them show the same phenotypic features. A comparison of the amino acid sequences showed that it has a binding site for lipids such as triacylglycerol and cholesterol in its N-terminal region (Fig. 1C), indicating that it can act as a lipase or esterase.

The phenotype of number nine was then examined with the naked eye and by microscopy. The results showed that *osprmc3* is much smaller and has more tillers than the wild-type plant (Fig. 2A). For example, the heights of the wild type and *osprmc3* mutant plants at maximum tiller stage were 68.1 and 43 cm, respectively (Fig. 2B). After flowering, the heights reached 110.3 and 53.7 cm, respectively, at the mature stage. At DAG40, the number of tillers was 10.9 for the wild type and 21.6 for the *osprmc3* mutant (Fig. 2B). After flowering, the number of tillers remained the same in the wild-type but increased continuously up to 43.5 in the *osprmc3* mutant (Fig. 2B), suggesting that OsPrMC3 is an esterase or lipase that regulates height and tiller development before and after flowering.

In addition, we investigated leaf length and width of the *osprmc3* mutant at DAG40 and found that leaf length was 42 cm for the wild-type and 24 cm for *osprmc3*. Interestingly, the leaf width of the wild-type was much narrower than that of the *osprmc3* mutant (Fig. 3A). In addition, the number and width of veins in the *osprmc3* mutant was lower than in the wild type (Fig. 3A), indicating that OsPrMC3 activity is necessary for leaf development.

Further investigation was made on the possible role of OsPrMC3 in seed set and development using *osprmc3* mutant lines. To this end, the size and number of panicles, number of seeds per panicle, the color and dry weight of the seeds, and the

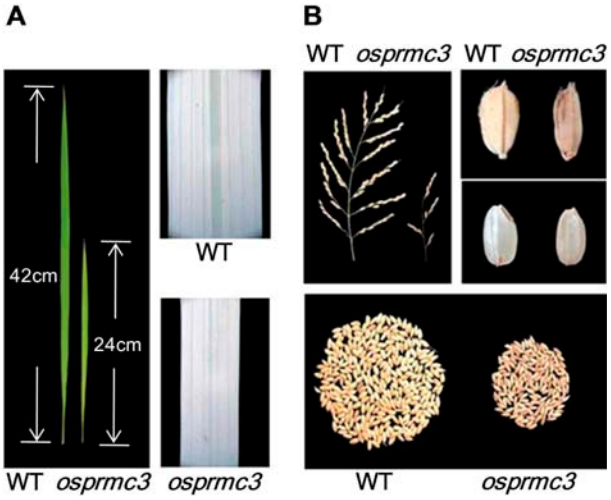

Fig. 3 Morphological features of the leaf and seed of wild-type and *osprmc3* mutant rice. Wild-type and *osprmc3* mutant were grown in the field, and leaf and seed morphologies were examined. (A) Leaf length of the mutant is shorter than that of the wild-type, and leaf width and vein greatly decreased in the mutant. (B) The number of panicle branches and seeds per panicle in *osprmc3* mutant are significantly low in the mutant, leading to fewer seeds. Seed size is also smaller in the mutant than in the wild-type.

Table 2 Numbers and weights of the seeds in *osprmc3* mutants

	No. of seed per panicle	No. of panicle branch	% of mature seed	Dry weight per seed (mg)
WT	101	13	97.5	27.1
osprmc3	7.8	4	57.4	15.4

abortion ratio in the *osprmc3* mutant were examined. The panicle size of the *osprmc3* mutant was much shorter than that of the wild type (Fig. 3B), and the number of branches in each panicle was 13 and 4 in the wild type and *osprmc3* mutant (Table 2), respectively, suggesting that OsPrMC3 can control panicle development. The number of seeds per panicle was 101 and 7.8 in the wild type and *osprmc3* mutant (Fig. 3B, Table 2), respectively, and the dry weights of the seeds were 27.1 and 15.4 mg for the wild type and *osprmc3* mutant, respectively (Table 2). Finally, upon examination of the seed abortion, approximately 50% of the seeds of the *osprmc3* mutant were abortive (Table 2). Taken together, the results strongly suggest that OsPrMC3 plays an important role in seed set and rice maturation.

On the basis of the phenotypic analyses, we speculated that the phenotype would be correlated with the expression pattern during development. To check the relationship between the phenotypes of the *osprmc3* mutant and the expression of the *OsPrMC3* gene, the expression pattern of the *OsPrMC3* gene in various rice organs during whole development was examined by quantitative real time real-time reverse transcription (RT)-PCR. Result showed that the transcript level of OsPrMC3 was the highest in the young leaf during vegetative growth (Fig. 4). Interestingly, morphological analysis of the mutant revealed that the *osprmc3* mutant had much

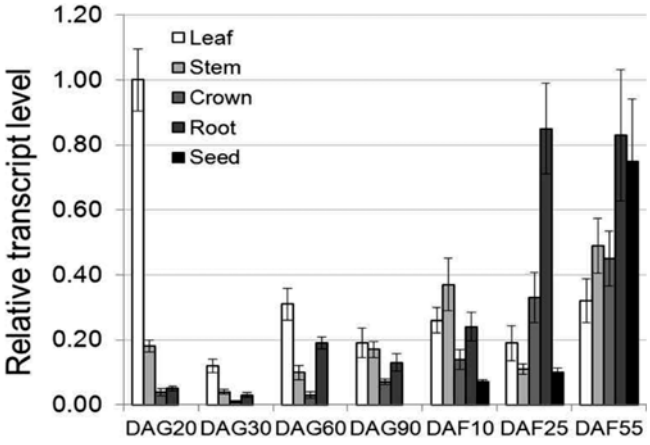

Fig. 4 Expression profile of the *OsPrMC3* gene. Total RNA was isolated from various tissues of field-grown wild-type plants at the indicated stages, and the transcript level of *OsPrMC3* was analyzed by quantitative real-time RT-PCR using the gene-specific primers described in Table 1. *OsPrMC3* expression was the highest in young leaves during vegetative development, but increased significantly in roots and seeds after flowering with a slight change in crowns and stems. DAG, Day after germination. DAF, Day after flowering.

narrower leaves and veins (Fig. 3A). These results suggest that OsPrMC3 is absolutely required for leaf development. Notably, its expression level is dramatically increased in seed during maturation (Fig. 4), implying that OsPrMC3 plays an important role in not only leaf development during the vegetative stage but also in seed development during the ripening stage. These results indicate that the morphological features and abnormal seed yields of the *osprmc3* mutant are strictly correlated with the stage- and organ-specific expression of the *OsPrMC3* gene.

It is well known that tillers are lateral organs that develop during the vegetative stage in rice. However, panicle branches are also lateral organs but at the reproductive stages in rice (Wang and Li, 2011). In addition, in several cases, these two kinds of lateral organs are regulated by common mechanisms and change coordinately, although the number of tillers or panicle branches is not always undergoing a similar change in most cases (Wang and Li, 2011). The *osprmc3* mutant had an increased number of tillers, but showed reduced panicle size, panicle branching, and grain size compared to the wild type, indicating that two organs are not coordinately regulated by OsPrMC3.

In conclusion, the results of the present study demonstrated that the dwarfism, abnormal leaf growth, and panicle branching caused by the OsPrMC3 mutation results in abnormal seed development, which in turn results in abnormal seeds and low grain yield. Further expression analysis of OsPrMC3 using reporter genes such as β-glucuronidase and green fluorescent protein could provide helpful information as to how OsPrMC3 regulates tillering and panicle development. Moreover, identification of OsPrMC3 substrates may provide crucial clues for understanding the role of OsPrMC3 in the control of rice architecture by acyl compound or other lipid modification.

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