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Identification and Cloning of Tillering-Related Genes *OsMAX1* in Rice

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Abstract: Tillering is an important agronomic trait which has a direct impact on plant type and grain yield. Strigolactones are a class of important phytohormones regulating rice tillering. *ATMAX1* is an important gene involved in strigolactone biosynthesis through encoding the protein P450 in *Arabidopsis*. Based on sequence BLASTp, we identified five homologous genes of *ATMAX1* in rice, i.e., *OsMAX1a*, *OsMAX1b*, *OsMAX1c*, *OsMAX1d* and *OsMAX1e*. Among them, *OsMAX1a* and *OsMAX1e* showed stable and high expression in rice tissues. In addition, we observed that *OsMAX1a* and *OsMAX1e* can rescue the branched phenotype and the influences caused by *MAX1* mutation in *Arabidopsis*. Moreover, the expression of *OsMAX1a* and *OsMAX1e* can respond to phosphate deficiency and different phytohormones, especially GR24, a strigolactone analogue. Therefore, it is concluded that *OsMAX1a* and *OsMAX1e* are involved in the biosynthesis of strigolactones and regulated rice tillering.
Key words: rice; strigolactone; *OsMAX1*; gene cloning; tillering; phytohormone

Tillering is an important agronomic trait which can influence plant type and grain yield. Besides, as a multigenic trait, tillering is affected by a lot of factors, including fine adjustment, comprehensive expression of many genes, environments and plant hormones. (Domagalska and Leyser, 2011; Wang and Li, 2011; Ruyter-Spira et al, 2013). Strigolactones (SLs) are a class of important plant hormones regulating plant branching. Plant can adjust their phenotypes through regulating the biosynthesis of SLs under different environments.

As a class of carotenoid derivatives, SLs are demonstrated to be signaling molecules in rhizosphere (Cardoso et al, 2011). In recent years, with the rapid development of molecular biology, it is found that SLs also function as plant hormones to inhibit shoot branching and modulate root architecture (Dun et al, 2009). Besides, SLs can control the plant root, root hair and lateral branch growths, stem elongation, leaf senescence, flower development and the responses to drought and salt stress (Stirnberg et al, 2002; Snowden et al, 2005; Gomez-Roldan et al, 2008; Umehara et al, 2008; Ruyter-Spira et al, 2013). Moreover, SLs can also promote the establishment of symbiosis between terrestrial plants and arbuscular mycorrhizal fungi that help plants to improve nutrient uptake. Under low phosphate (P) conditions, the exudation of SLs into rhizosphere is strongly enhanced, which promotes the symbiosis of arbuscular mycorrhizal fungi and the response to phosphate deficiency (Akiyama et al, 2005; Kohlen et al, 2011). Furthermore, the exudation of SLs in root can stimulate seed germination in both *Striga* and *Orobanche* (Cook et al, 1966).

Lots of genes, including *DWARF3* (*D3*), *D10*, *D53*, *D14* (*HIGH-TILLERING DWARF2* (*HTD2*), *D88*), *D17* (*HTD1*), *D27* and *OsMADS57*, are involved in the biosynthetic pathway of SLs in rice (Zou et al, 2006; Arite et al, 2009; Hamiaux et al, 2012; Zhou et al, 2013). Among them, in a D14 and D3-dependent manner, SLs induce the degradation of D53 by proteasome and thus promote axillary bud outgrowth (Jiang et al, 2013; Zhou et al, 2013). In addition, *OsMADS57* interacts

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with *OsTB1* and targets *D14* to control the outgrowth of axillary buds in rice (Guo et al, 2013). Many studies showed that *MORE AXILLARY GROWTH 1* (*MAX1*), *MAX2*, *MAX3*, *MAX4*, *RAMOSUS 1* (*RMS1*), *RMS4*, *RMS5*, *DECREASED APICAL DOMINANCE 1* (*DAD1*), *DAD2*, *DAD3* and *DAD4* also participate in SL biosynthesis in some other species (Beveridge, 2000; Stirnberg et al, 2002; Sorefan et al, 2003; Wang et al, 2013).

Previous research showed that the biosynthesis of SLs is started with the isomerization of β-carotene by β-carotene isomerase D27, followed by the cleavage of β-carotene by carotenoid cleavage dioxygenase 7 (CCD7) and CCD8, which results in the formation of carlactone (Booker et al, 2004; Lin et al, 2009; Wang et al, 2013). Some genes responsible for the conversion of carlactone to SLs have been identified. *MAX1*, encoding a cytochrome P450 (CYP) in *Arabidopsis*, has been suggested to be a candidate P450 protein capable of transforming SL precursors to bioactive SL intermediates in the downstream of D27, CCD7 and CCD8. Then SL signaling is mediated by an F-box protein (MAX2 in *Arabidopsis*; D3 in rice) and an α/β-hydrolase D14. It is clear that *MAX1* play an essential role in SL biosynthesis. In addition, the mutation of the above genes appears to result in branched and dwarf phenotypes.

In this study, using molecular genetics, we identified and cloned two candidate genes, *OsMAX1a* and *OsMAX1e*, homologous to *AtMAX1*. It is presented that *OsMAX1a* and *OsMAX1e* had similar functions with *AtMAX1* and were involved in the biosynthesis of SLs to regulate rice tillering. Therefore, this study will be useful for elucidating the molecular mechanisms of rice tillering regulated by SLs and SL biosynthetic pathway. In addition, it can explain how monocotyledons control plant type under different nutritional conditions through adjusting SL biosynthesis and activity.

MATERIALS AND METHODS

Gene sequence BLASTp and evolution analysis

In this study, the gene and protein sequences of *MAX1* were downloaded from *Arabidopsis thaliana* database. *ATMAX1* amino acid sequence served as the probe. We obtained more than 100 homologous sequences in NCBI by BLASTp. Then 34 plant protein sequences were selected for multiple sequence alignment through the software MEGA5. The map of phylogenetic tree was created by the neighbor-joining method (Supplemental Fig. 1).

Sequence analysis of *OsMAX1* genes in rice

Using the tool of homologous sequence alignment in NCBI, we predicted the *AtMAX1* homologous genes in rice genome database (http://rice.plantbiology.msu.edu/index.shtml) and identified five *OsMAX1* candidate genes with high homology to *AtMAX1*, *OsMAX1a* (LOC_Os01g50530 and LOC_Os01g50520, and cDNA sequencing showed that these two annotated loci are in the same ORF), *OsMAX1b* (LOC_Os06g36920), *OsMAX1c* (LOC_Os01g50590), *OsMAX1d* (LOC_Os01g50580) and *OsMAX1e* (LOC_Os02g12890) (Table 1). These candidate genes shared 50%–63% sequence homology with *AtMAX1*.

These five candidate genes were searched in Gramene (http://www.gramene.org/Multi/blastview) to determine their chromosomal distributions. Using genome sequence and cDNA sequence, we analyzed the structural characteristics of their introns and exons (Fig. 1). The results showed that these *OsMAX1* candidate genes

Table 1. *OsMAX1* candidate genes in rice.

Gene	ID	Chromosome	Amino acid size
OsMAX1a	LOC_Os01g50530	1	412
	LOC_Os01g50520	1	
OsMAX1b	LOC_Os06g36920	6	549
OsMAX1c	LOC_Os01g50590	1	517
OsMAX1d	LOC_Os01g50580	1	385
OsMAX1e	LOC_Os02g12890	2	548

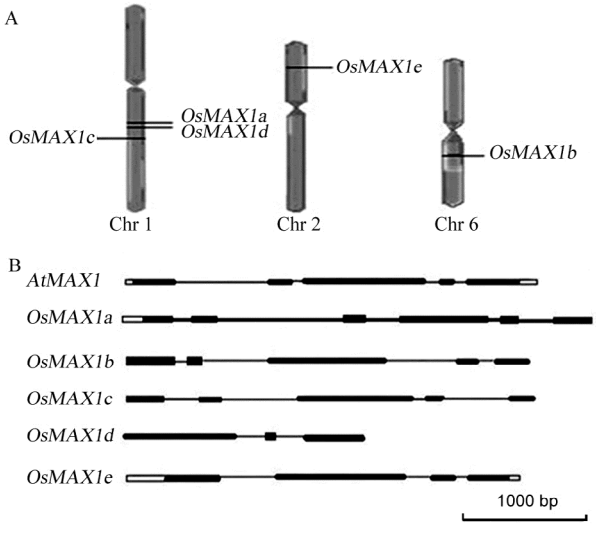

Fig. 1. Chromosomal location and gene structure of rice *OsMAX1* candidate genes.

Chr, Chromosome. Black boxes indicate exon and the connecting lines indicate intron in part B.