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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

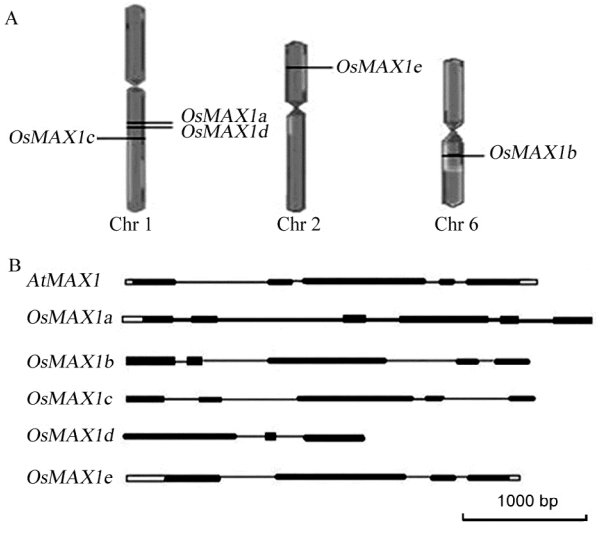

Fig. 1. Chromosomal location and gene structure of rice *OsMAX1* candidate genes.

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

had different exon and intron structures. The proteins encoded by these *OsMAX1* genes belonged to the CYP711 protein family. In addition, there is a very high homology in the amino acid sequences of *MAX1* (Supplemental Fig. 2). Through protein cluster analysis, we discovered that the P450 proteins encoded by *MAX1* genes widely existed in monocot and dicotyledonous plants, and there were lots of conserved branches in monocot plants.

Transcription analysis of *OsMAX1* genes in rice

Nipponbare was grown on full nutrition for three weeks. A total of 50 mg homogenized ground roots and stems were used to extract RNA by using 10 mL Trizol (Invitrogen) and RNA was further purified with chloroform. After precipitation with 70% ethanol, RNA was recovered with RNAEasy Mini Kit column (Qiagen) and DNA was removed using the DNAase I Kit (Qiagen) according to the manufacturer's instructions.

Semi-quantitative primers were designed by the software Primer Premier 5 (PREMIER Biosoft). The primer sequences were RTOsMAX1a (F/R), TCATC TGGCCAGGCTCC/CCTGGGGTTCCCTTCATTGA GC and RTOsMAX1e (F/R), ACCCCCTGGGGAG ACTGCAT/CTCGATGCCGAGACGCCTCT.

cDNA was synthetized from 1 μg total RNA per sample using the Script cDNA Synthesis Kit (BioRad) following the manufacturer's instructions. Finally, the products were analyzed with 1.5% agarose gel electrophoresis.

Construction of over-expression and RNA interference vectors

The full-length sequence of *OsMAX1e* was acquired through the amplification of Nipponbare DNA. The coding regions of *OsMAX1a* were also acquired through the amplification of Nipponbare cDNA. Then the DNA fragments of *OsMAX1e* and *OsMAX1a* were connected to pEASYTM-T1 Simple-Cloning Vector (TransGene), respectively. The correct recombinant plasmids T1-OsMAX1a and T1-OsMAX1e were confirmed by sequencing. T1-OsMAX1a and pHB were connected after being digested by *Xba* I and *Sac* I restriction endonucleases. Meanwhile, T1-OsMAX1e and pHB were digested by *Xba* I and *Hind* III restriction endonucleases before connecting. Ultimately, we constructed the vectors pHB-OsMAX1a and pHB-OsMAX1e driven by the 35S promoter.

The 400 bp mRNA sequences were used to construct the RNA interference vectors RNAi-OsMAX1a and RNAi-OsMAX1e. Because *OsMAX1* has multiple copies, the conservative sequence of *OsMAX1a* was also selected to design another RNAi fragment, i.e., RNAi-OsMAX1CON. All RNAi-OsMAX1-sense sequences were acquired through the amplification of Nipponbare cDNA (the primer sequences are shown in Table 2). RNAi-OsMAX1-sense was digested by *Sal* I and *Hind* III restriction endonucleases and then connected with the vector pSK-int to generate intermediate vector pSK-Sense. RNAi-antisense was digested by *CoR* I/*Sac* I restriction endonucleases and then connected with the pSK-Sense vector to construct pSK-RNAi. Two pSK-RNAi vectors pSK-OsMAX1a -RNAi and pSK-OsMAX1e-RNAi were generated. The reconstructed plasmid pSK-RNAi was digested with *Sal* I and *Sac* I restriction endonucleases and the 1 kb segments were recycled for RNAi. Connecting the purified RNAi clips with pOsAct2-1-nos, we got the recombinant plasmids OsMAX1a-RNAi and OsMAX1e-RNAi, and further validated with Sal I and Sac I restriction endonucleases. The construction of interference vector and the structure of pHB-OsMAX1 are showed in Supplemental Fig. 3.

Genetic transformation in rice

Callus induction and transgeneration were conducted on rice mature embryos (Hiei et al, 1994; Huang et al, 2001). Transgenic T_1 plants were selected with hygromycin and transgene expression was verified. Then, we planted transgenic seedlings in a field under consistent condition.

According to the method of CTAB extraction, the

Table 2. Sequence of RNAi markers.

Marker	Sence primer	Anti-sence primer
RNAi-OsMAX1CON-F	5′-GCGTCGACGTTCTCAAGAGGCTTCGCTG-3′	5′-CGAGCTCGTTCTCAAGAGGCTTCGCTG-3′
RNAi-OsMAX1CON-R	5′-CCCAAGCTTCAAGGTAGGGGAATTTGGTCT-3′	5′-CGGAATTCCAAGGTAGGGGAATTTGGTCT-3′
RNAi-OsMAX1a-F	5′-GCGTCGACTCCTGAAGGAAGCGATGAGA-3′	5′-CGAGCTCTCCTGAAGGAAGCGATGAGA-3′
RNAi-OsMAX1a-R	5′-CCCAAGCTTCACCTCTGGCTCTGGGAAAT-3′	5′-CGGAATTCCACCTCTGGCTCTGGGAAAT-3′
RNAi-OsMAX1e-F	5′-GCGTCGACGTCCAGCTCGCTGTCCACCA-3′	5′-TCCCCCGGGGTCCAGCTCGCTGTCCACCA-3′
RNAi-OsMAX1e-R	5′-CCCAAGCTTGAGACGAGGTGGAAGCGCATG-3′	5′-CGGAATTCGAGACGAGGTGGAAGCGCATG-3′

Underlined part represents enzyme locus.

DNA of transgenic plants was extracted for PCR detection. The primer sequences were as follows: cyt108(F), GCACTGGGCGCACTTGGTCT; cyt108(R), TTCGCCTCTTCTTCCTCCTCTCG. Statistical analysis was performed by the SPSS software. Further, we analyzed the tiller number and plant height of positive transgenic plants in T_2 generation (significant *t*-test).

Inflorescence infection of *Arabidopsis*

The cultivation of *Arabidopsis* seedlings and inflorescence infection were based on the method of Xu et al (2010). Transgenic T_1 plants were selected on nutrition soil medium containing Basta. After getting the transgenic seeds and seedlings, we sprayed the seedlings evenly with 1:5000 Basta solution twice a week. Finally, the normal green seedlings were positive transgenic lines.

Gene expression analysis

Rice hydroponic fluid (Cock et al, 1976) was used for planting rice seedlings which were treated with different kinds of phytohormones after four weeks. The concentrations of cytokinins, kinetin (KT), NPA (auxin inhibitor, N-1-naphthylphalamic acid) and indole-3-acetic acid (IAA) solutions were 100 μmol/L. The concentration of GR24 (stigolactones) solution was 5 μmol/L. P-deficiency was achieved by treating plants without phosphate in hydroponic fluid. Then, we sampled the rice seedlings at 1, 4 and 18 h after treatment, respectively. The seedlings were used to extract RNA and perform quantitative real-time PCR (RT-qPCR) for analyzing expression levels of the candidate genes *OsMAX1a* and *OsMAX1e*. The RT-qPCR reactions were prepared using iQ SYBR Green Supermix (BioRad). For each reaction, 0.3 μmol/L of each primer and 1 μL of 10-fold diluted template cDNA were used. The amplification was detected using BioRad RT-qPCR detection system and thermocycler. The expression data were the average of three replicates.

RESULTS

***OsMAX1a* and *OsMAX1e* have stable expression in rice**

Previous studies showed that *AtMAX1* is expressed in roots and stems of *Arabidopsis* (Turnbull et al, 2002; Booker et al, 2005). Therefore, the candidate genes expressed in roots and stems were speculated to have similar function with *AtMAX1*.

We analyzed the expression of five candidate genes in the roots and stems of rice, and the results showed that *OsMAX1a* and *OsMAX1e* expressed stably in the roots and stems of rice. Besides, the expression of *OsMAX1a* was higher in roots than in stems, and the expression of *OsMAX1e* was very stable in both roots and stems (Fig. 2). However, *OsMAX1b*, *OsMAX1c* and *OsMAX1d* only had weak or unstable expression in the roots and stems. Thus, it is concluded that the expression patterns of *OsMAX1a* and *OsMAX1e* are closer to *AtMAX1*. Therefore, *OsMAX1a* and *OsMAX1e* were target objects in subsequent studies.

Over-expression of *OsMAX1a* and *OsMAX1e* can rescue branched phenotype of *Arabidopsis max1* mutant

Arabidopsis max1 mutants have branched phenotype. In order to verify the functions of *OsMAX1a* and *OsMAX1e* in *Arabidopsis*, the over-expression vectors of these two genes were transferred into *max1* mutants. Finally, the positive transgenic T_2 plants were obtained using Basta selection, which had normal phenotype (Fig. 3). It suggests that over-expressing *OsMAX1e* and *OsMAX1a* can rescue the branched phenotype of *Arabidopsis MAX1*. It is concluded that these genes can inhibit the growth of lateral branches in *Arabidopsis*. Thus, *OsMAX1a* and *OsMAX1e* have similar function with *AtMAX1*, suggesting that these two rice cytochrome P450 genes are *AtMAX1* orthologs.

Interference of *OsMAX1a* and *OsMAX1e* can affect rice tillering phenotype

Interference vectors containing the characteristic fragment cyt108 were transferred into rice through the mediation of *Agrobacterium*. Then, the identification of positive transgenetic offspring was conducted through amplification of the cyt108 fragment. Because

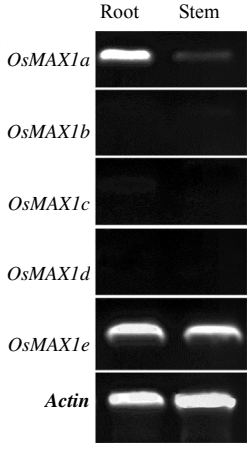

Fig. 2. Transcriptional analysis of *OsMAX1*.

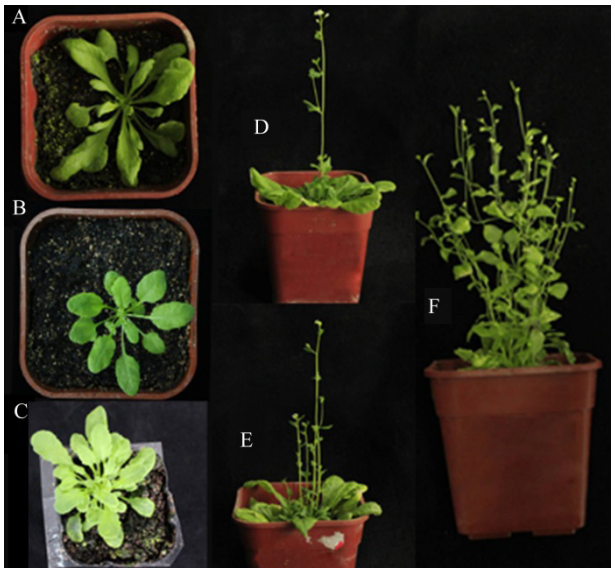

Fig. 3. Over-expression of *OsMAX1a* and *OsMAX1e* in *Arabidopsis max1* mutant.

A and D, Over-expression of *OsMAX1a* in *Arabidopsis max1*; B and E, Over-expression of *OsMAX1e* in *Arabidopsis max1*; C and F, *Arabidopsis max1*.

cyt108 is a shortened cytochrome C in rice, positive transgenic plants should show dual-bands in electrophoresis after PCR (Fig. 4). Compared to Nipponbare, the tillering numbers of *OsMAX1a*-RNAi and *OsMAX1e*-RNAi plants were increased by nearly 40%, although their height changes were not obvious (Fig. 5).

In addition, it was found that the tiller number of *OsMAX1CON*-RNAi plants was twice as much as that of Nipponbare, although they had a bit lower height than Niponbbare (Fig. 5). These results suggest that the interference of conserved sequence can suppress the expression of multiple *OsMAX1* genes, so that the change of phenotype is more obvious. The interference of *OsMAX1a* and *OsMAX1e* can increase rice tillering number. Therefore, these two genes function as inhibiting rice tillering. Besides, the phenotype of transgenic plants did not change intensely. On this basis, it can be concluded that multiple similar genes have the similar function on controlling rice tillering. The functional redundancy of *OsMAX1* genes can maintain the stability of rice phenotype.

Decrease of *OsMAX1a* and *OsMAX1e* expression in interfered offspring of rice

In rice lines *OsMAX1a*-RNAi, *OsMAX1e*-RNAi and *OsMAX1CON*-RNAi, the expression of *OsMAX1a* and *OsMAX1e* decreased significantly compared with Nipponbare, and the *OsMAX1a* expression declined more obviously (Fig. 6).

P-deficiency can improve expressions of *OsMAX1a* and *OsMAX1e* in rice

P-deficiency can improve the biosynthesis of SLs (Umehara et al, 2008; Kohlen et al, 2011). In order to study the relationship between *OsMAX1* expression and P-deficiency, we analyzed the expression levels of *OsMAX1a* and *OsMAX1e* treated with P-deficiency using RT-qPCR. Our results showed that P-deficiency can significantly improve the expression of these candidate genes (Fig. 7) to improve the synthesis of SLs in rice. Therefore, *OsMAX1a* and *OsMAX1e* are involved in the biochemical pathway of SL synthesis.

Expression of *OsMAX1a* and *OsMAX1e* induced by different phytohormones

Using RT-qPCR, we measured the expression of *OsMAX1a* and *OsMAX1e* in rice seedlings treated with different plant hormones. By applying GR24, it was observed that the expression of *OsMAX1a* and *OsMAX1e* decreased significantly after only 1 h of GR24 treatment, but their expression rebounded slightly after 18 h of GR24 treatment (Fig. 8). The above results might be caused by exogenous GR24, which inhibited the synthesis of SLs in rice, and thus inhibited the expression of *OsMAX1a* and *OsMAX1e*. In addition, rice reduced the biosynthesis of phytohormones to maintain normal growth. It is confirmed that *OsMAX1a* and *OsMAX1e* can participate in the biosynthesis of phytohormones.

Then we detected that the expression of *OsMAX1a*

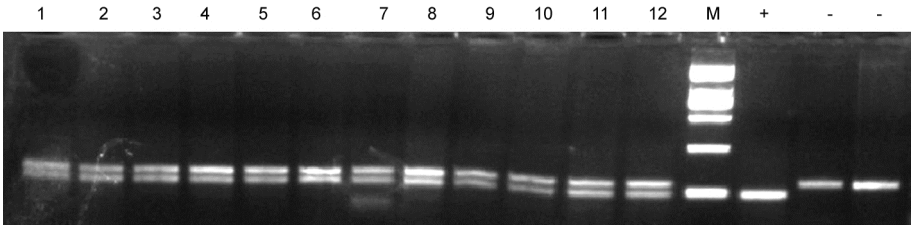

Fig. 4. PCR analysis of transgenic rice plants.

M, Marker; +, Positive control; -, Negative control; Lanes 1 to 12, Transgenic rice plants.

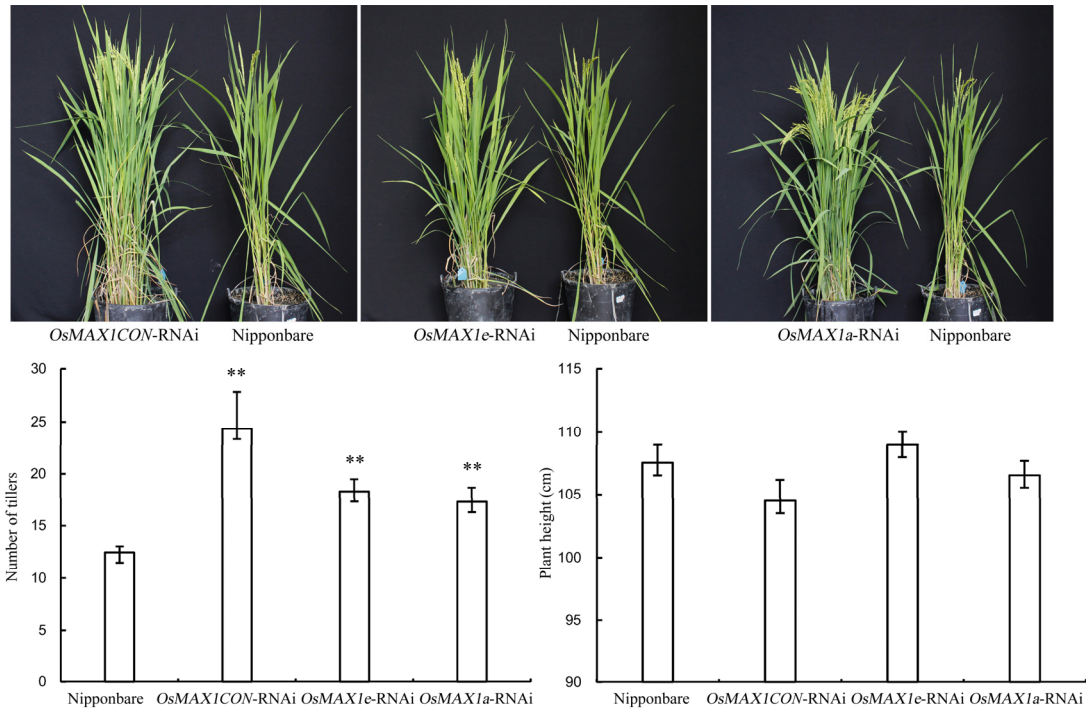

Fig. 5. Tillering numbers and plant heights of Nipponbare, *OsMAX1CON*, *OsMAX1a* and *OsMAX1e* interference in rice.
OsMAX1CON-RNAi, Conserved region interference of *OsMAX1* gene.
** means significant difference at the 0.01 level. Bar represents the standard error.

and *OsMAX1e* decreased obviously after the treatment of IAA. The expression of these genes significantly decreased after 1 h of IAA treatment but gradually restored after being treated for 4 or 18 h (Fig. 8). On the contrary, the impact of NPA treatment was opposite to that of IAA treatment. The expression of these two genes increased significantly after 1 h of NPA treatment, gradually returned to normal level after 4 h of NPA treatment, and showed a trend of reduction after 18 h of NPA treatment (Fig. 8). In addition, the expression of these two genes significantly decreased after treated with KT, and the decreasing extent was more obvious with the prolongation of KT treatment (Fig. 8).

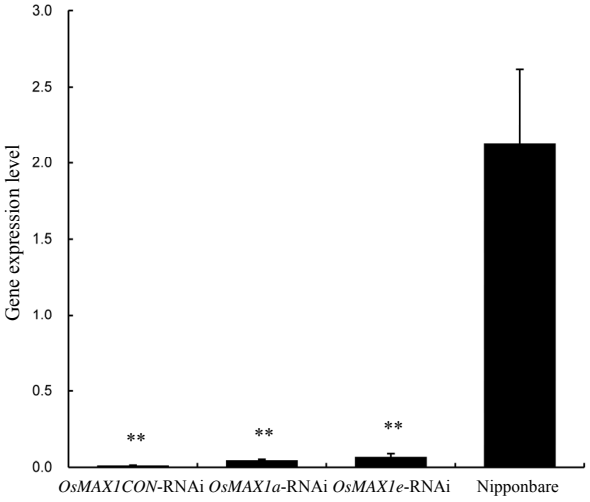

Fig. 6. Expression levels of *OsMAX1CON*, *OsMAX1a* and *OsMAX1e* interference offspring in rice.
** means significant difference at the 0.01 level. Bar represents the standard error.

DISCUSSION

AtMAX1 is a single copy gene in *Arabidopsis* genome.

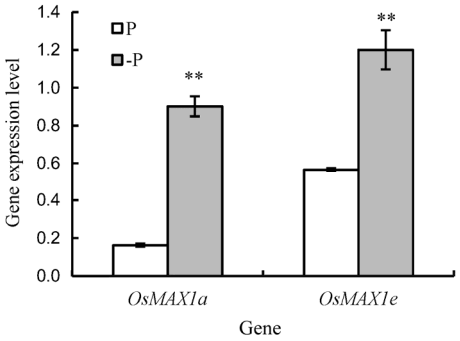

Fig. 7. Expression levels of *OsMAX1a* and *OsMAX1e* as affected by phosphate (P)-deficiency.
** means significant difference at the 0.01 level. Bar represents the standard error.

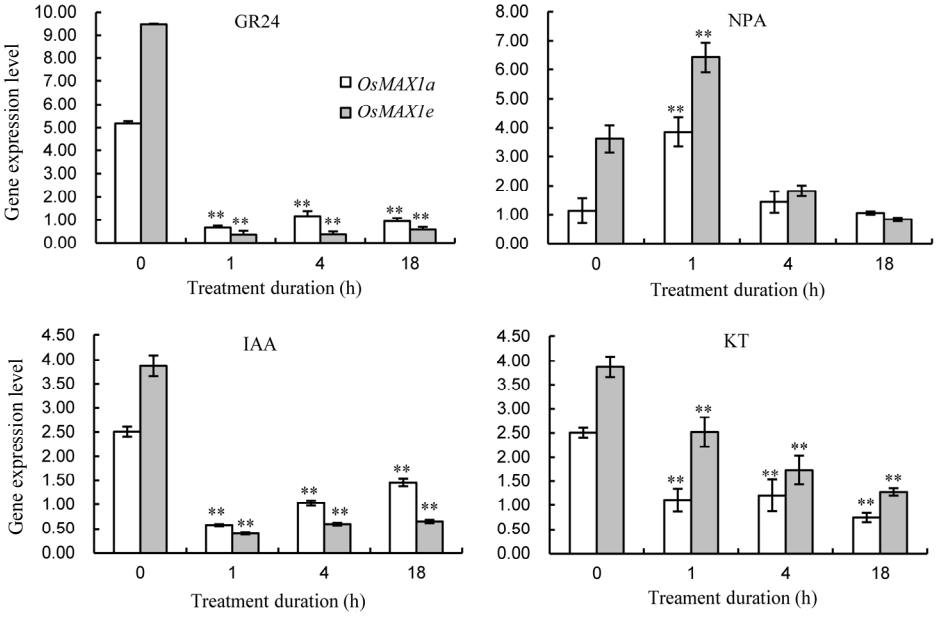

Fig. 8. Expression levels of *OsMAX1a* and *OsMAX1e* after GR24, N-1-naphthylphalamic acid (NPA), indole-3-acetic acid (IAA) and kinetin (KT) treatments.

** means significant difference at the 0.01 level. Bar represents the standard error.

Its mutation can increase branches (Drummond et al, 2011). In this study, the over-expression of either *OsMAX1a* or *OsMAX1e* can rescue the branched phenotype in *MAX1* mutants. It suggests that *OsMAX1a* and *OsMAX1e* have similar function with *AtMAX1* which can inhibit the growth of lateral branches. In addition, it illustrates that the proteins encoded by *MAX1* are conserved in both monocot and dicotyledonous plants.

RNA interference was adopted to verify the function of *OsMAX1a* and *OsMAX1e* in rice. Because the five candidate *OsMAX1* genes share high homology, the specific sequences of *OsMAX1a* and *OsMAX1e* were chosen as interference fragments to construct *OsMAX1a*-RNAi and *OsMAX1e*-RNAi vectors, respectively. Besides, the conserved sequences of these five genes were used to construct the interference fragment *OsMAX1CON*-RNAi. Compared to Nipponbare, the tillering number of *OsMAX1CON*-RNAi plants increased by 94.64% and the plant height decreased by 2.84%. These results suggest that the conserved domain being interfered has the greatest impact on rice growth. In addition, the interference of specific sequences of these two genes also had significant influences on rice growth.

The above phenotypes are probably due to the hairpin structure formed by the common sequence of these five *OsMAX1* genes. This hairpin structure can interfere the expression of multiple genes, thus rice showed increased tillers and decreased height. In interfered rice T_2 offspring, the expression of *OsMAX1a* and *OsMAX1e* declined significantly compared with Nipponbare. Thus, the above results also demonstrate that these two candidate genes have important functions in rice growth. Moreover, the phenotype of transgenetic plants is not as significant as mutants (e.g., *d3*, *d14*, *d10* and *d27*), which indicates that *OsMAX1* genes have overlapped function. Multiple *MAX1* genes can maintain the stability of *MAX1* functions in different species. It can explain why we cannot explore the phenotype of single *MAX1* gene mutation in rice.

A large number of studies have shown that the biosynthesis of SLs is increased during P-deficiency (Yoneyama et al, 2007). We simulated P-deficiency in hydroponic nutrient solution. Compared to normal condition, the expression of *OsMAX1a* and *OsMAX1e* increased significantly. Thus, P-deficiency may induce the expression of *OsMAX1a* and *OsMAX1e* by promoting the biosynthesis of SLs. In addition, we treated rice seedlings with GR24, and our results indicate that rice may suppress the expression of *OsMAX1a* and *OsMAX1e* through feedback regulation, which results in the reduction of SL biosynthesis.

Thus, the expression of *OsMAX1a* and *OsMAX1e* may respond to the change of SLs level. In addition,

the expression of *OsMAX1a* and *OsMAX1e* are also regulated by IAA and KT. In recent years, a lot of experimental data show that SLs, IAA and cytokinin synergistically regulate plant branching (Alder et al, 2008, 2012). It is speculated that *OsMAX1a* and *OsMAX1e* affect the formation of rice tillering through the synergistic regulation of SL biosynthesis by IAA, cytokinin and SLs. The sensitivity of *OsMAX1a* and *OsMAX1e* expression to external hormones indicates that various kinds of phytohormones can affect SL biosynthesis in rice. Therefore, the expression of these two genes can be adjusted to promote the adaption ability of rice to external environments.

In summary, *OsMAX1a* and *OsMAX1e* play an important role in controlling rice tillering through regulating the biosynthesis of SLs in rice. Remarkably, the functions of *MAX1* in rice are performed by a number of different genes, which is different with the single-gene mechanism in dicotyledonous plants.

Previously, the correlation between the function of CYP711 proteins encoded by *OsMAX1* and their characteristics is also not clear. Therefore, this study is important for revealing the underlying mechanisms of how SLs influence rice tillering and elucidating the relationship among plant type, the change of composition and the biosynthesis of SLs in rice.

SUPPLEMENTAL DATA

The following materials are available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Fig. 1. Phylogenetic tree of P450 proteins.

Supplemental Fig. 2. Alignment of amino acid sequences of MAX1 proteins.

Supplemental Fig. 3. Construction of interference vector (A) and the structure of pHB-OsMAX1 (B).

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