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The gene *OsIAA9* encoding auxin/indole-3-acetic acid proteins is a negative regulator of auxin-regulated root growth in rice

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Abstract

Auxin signal transduction in plants depends on regulation by short-lived nuclear plant proteins called auxin/indole-3-acetic acid (Aux/IAA) proteins. The *OsIAA9*, which is highly and rapidly induced by auxin, encodes the entire Aux/IAA domain characteristic of the Aux/IAA family in rice. The promoter region of *OsIAA9* contains several *cis*-elements. Analysis of *P_{OsIAA9}::GUS* transgenic plants indicates a specific expression of *OsIAA9* in roots and seedling shoots, especially lateral roots and root tips. Based on real-time PCR, the expression of *OsIAA9* was induced by multiple hormones and abiotic stresses. Furthermore, ectopic overexpression of *OsIAA9* in rice led to fewer crown and lateral roots and reduced the inhibition of root elongation by auxin. These observations indicate that *OsIAA9* was a negative regulator of auxin-regulated root growth.

Additional key words: abscisic acid, crown and lateral roots, gibberellic acid, jasmonates, kinetin, *Oryza sativa*, salicylic acid, transgenic plants.

Introduction

Auxin, the first plant hormone discovered, regulates multiple plant developmental processes including, but not limited to, apical dominance, lateral root formation, vascular differentiation, embryo patterning, and shoot elongation (Muday and DeLong 2001, Jenik and Barton 2005, Leyser 2005). Auxin/indole-3-acetic acid (Aux/IAA) proteins are plant-specific transcriptional regulators, which share a four-domain structure. They act as co-receptors with auxin receptor transport inhibitor response 1 (TIR1) and function as transcriptional repressors by interacting with auxin response factor (ARF) proteins through conserved domains III and IV. Rice has 31 potential non-redundant Aux/IAA transcription factor genes (Jain *et al.* 2006, Song *et al.* 2009a), and the function of some has been identified. Some of them function in root development, such as *OsIAA3* in crown root formation, *OsIAA11* and *OsIAA13* in lateral root development (Kitomi *et al.* 2012, Zhu *et al.* 2012), and *OsIAA23* in postembryonic maintenance of the quiescent center in

roots (Ni *et al.* 2011). Understanding the regulation of root architecture in crop plants is of great agronomic importance because root architecture is a key determinant of plant anchorage and efficiency of water and nutrient uptake and adjusts plant growth to handle abiotic stresses. Unlike *Arabidopsis*, which has a primary root that iteratively branches to generate several orders of lateral roots, rice has predominantly shoot-borne crown roots that branch sequentially to form a herringbone-like structure (Rebouillat *et al.* 2009). Thus, unravelling genes which are involved in root development is key for improving agronomic characteristics of rice. We previously found that *OsIAA9* and *OsIAA20* are sister genes, which are highly and rapidly induced by auxin. They are tissue- or organ-specific, and they are up-regulated in plumules grown in the dark (Song *et al.* 2009a). Constitutive expression of *OsIAA9* in *Arabidopsis* leads to a reduced gravitropic response and alters the number and distribution of starch granules (Luo *et al.* 2015).

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Abbreviations: ABA - abscisic acid; Aux/IAA - auxin/indole-3-acetic acid; ARF - auxin response factor; 2,4-D - 2,4-dichlorophenoxyacetic acid; GUS - β -glucuronidase; IAA - indole-3-acetic acid; JA - jasmonic acid; MS - Murashige and Skoog; SA - salicylic acid; TIR1 - transport inhibitor response 1.

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The aim of this research was to identify the function and localization of *OsIAA9* in rice and to follow *OsIAA9* expression induced by multiple hormones and abiotic

stresses. We also used *OsIAA9*-overexpressing plants, which had fewer crown roots, were defective in lateral root formation, and were resistant to exogenous auxin.

Materials and methods

Constructs and rice transformation: To make an overexpression construct, the full-length cDNA of *OsIAA9* was amplified from Minghui 63 (*Oryza sativa* L. ssp. *indica*) by reverse transcription (RT)-PCR using primers 5'-ATAGGTACCAGAAATCCAGCC AAGAAG-3' and 5'-TATGGATCCTGAGATGTG CCCAACTACA-3'. The sequencing-confirmed PCR fragment was digested by *KpnI* and *BamHI* and inserted into pCAMBIA1301U. A 1.7 kb fragment containing the *OsIAA9* promoter region was amplified from genomic DNA of an upland rice (*Oryza sativa* L. ssp. *japonica*) cv. IRAT109 with primers 5'-TATAAGCTTAAT CTTTGGTTCCGAGTTA-3' and 5'-TATGGATCCCTT CTTGGCTGGATTCTA-3', and the sequencing-confirmed PCR fragment was digested by *HindIII* and *BamHI* and placed into pCAMBIA1391Z-GUS (www.cambia.org, AF234312). Both constructs were transformed into *japonica* rice cv. Zhonghua11, a genotype supporting a highly efficient transformation using the *Agrobacterium*-mediated transformation method. Transgenic calli and plantlets were selected based on hygromycin (50 mg dm⁻³) resistance.

Plants and treatments: Zhonghua11 seedlings were germinated and grown at a temperature of 28 °C, a 14-h photoperiod, an irradiance of 50 µmol m⁻² s⁻¹, and an air humidity of 60 % for about three weeks before treatment with phytohormones. The phytohormones used were: 100 µM indole-3-acetic acid (IAA), 100 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 100 µM jasmonic acid (JA), 100 µM salicylic acid (SA), 100 µM abscisic acid (ABA), 100 µM gibberellic acid (GA), and 100 µM kinetin (KT). Treatments lasted for 6 or 12 h. Detailed information on drought (Dro) and salinity (Salt) treatments is in Song *et al.* (2009a).

Transgenic seeds were germinated on a Murashige and Skoog (MS) medium. Plantlets were transferred onto an MS medium containing different concentration of hormones and grown at 28 °C, a 14-h photoperiod, and an irradiance of 50 µmol m⁻² s⁻¹. Detailed information on plant treatments is in Song *et al.* (2009a). Methods used for auxin treatment of transgenic plants are described in Song *et al.* (2009b), and Song and Xu (2013).

Blotting RNA and real-time PCR: Total RNA was isolated from T0 rice leaves using a *TRIzol* reagent

(Invitrogen, Carlsbad, CA, USA). The total RNA (15 µg) from 24 samples was resolved on a 1.2 % (m/v) agarose gel containing 2 % (m/v) formaldehyde and blotted onto *Hybond* (Invitrogen) nylon membranes. The RNA gel blots were hybridized with an α-³²P-labeled *OsIAA9* sequence-specific probe at 65 °C overnight. Blots were washed with a *Hybond* washing buffer, and the signal was detected by radiography. Real-time PCR methods are described in Song *et al.* (2009b); they used specific primers listed in Table 1 Suppl.

Yeast two-hybrid analysis: Full-length *OsIAA9*, *OsIAA1*, and *OsIAA4* were inserted into yeast expression vectors PAD502 and PDBLeu (Invitrogen, Carlsbad, CA, USA). Yeast (*Saccharomyces cerevisiae* L.) strain MAV502 was used to determine the interaction affinity. A colony-lift filter assay (β-galactosidase assay) was performed as described by the manufacturer (Invitrogen), and the results confirmed by growing the transformants on synthetic complete medium without leucine, tryptophan, and histidine (SC-3) and containing 10 mM 3-amino-triazole. Primers used are listed in Table 2 Suppl.

β-Glucuronidase (GUS) staining: Histochemical GUS analysis was performed as described by Song *et al.* (2009a). Transgenic plant samples were incubated with a GUS staining solution containing 100 mM NaH₂PO₄ buffer (pH 7.0), 0.5 % (m/v) *Triton X-100*, 0.5 mg cm⁻³ 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, and 20 % (v/v) methanol. After staining, the tissue samples were mounted on slides and observed and photographed under a microscope. Seedlings, roots, leaves, and flower organs were incubated in a solution of 1 mM 5-bromo-4-chloro-3-indolyl glucuronide and in a 50 mM sodium phosphate buffer (pH 7.0) at 37 °C for 13 - 20 min.

Genotyping T-DNA mutant of *OsIAA9*: A T-DNA mutant of *OsIAA9* was obtained from the *Rice Mutant* database (<http://RMD.ncpgr.cn>). A PCR was conducted to identify the genotype of the progeny from a T-DNA insertion line for the *OsIAA9* gene using three primers: a forward primer (5'-CGATCGATCACAACTACTAG-3'), reverse primer (5'-GAGATGTGCCCACTACAGT-3'), and T-DNA-specific left border primer (NTBL5, 5'-AATCCAGATCCCCCGAATTA-3').

Results

In previous analysis of *OsIAA* gene expression, I noticed that *OsIAA9* (AK073365, LOC_Os02g56120) is highly and rapidly induced by auxin and is specifically expressed in some tissues (Song *et al.* 2009a). Therefore, in this study, I was interested in identifying the function of *OsIAA9*. Based on phylogenetic analysis, *OsIAA9* and *OsIAA20* form one subclade (Song *et al.* 2013). *OsIAA9* contains all the domains conserved by Aux/IAA proteins including domain I, represented as LRLGLT; domain II, represented as VVGWPPV; and domains III and IV (Jain *et al.* 2006). Domains III and IV of Aux/IAA can form homo- or hetero-dimers with other Aux/IAA proteins. As

OsIAA9 is apparently a canonical Aux/IAA protein that harbors domains III and IV, I believed it can form homo- or hetero-dimers. To test this hypothesis, full-length *OsIAA9*, *OsIAA1*, and *OsIAA4* were cloned into PAD502 and PDBLeu. Yeast colony filter assays (Fig. 1A) and growth of transformants (Fig. 1B) demonstrated that *OsIAA9* can form a homodimer or heterodimer with *OsIAA1* (a canonical Aux/IAA protein) and *OsIAA4* (a dominant mutation-type domain II Aux/IAA protein). Similar results were reported in identifying the function of *OsIAA1* (Song *et al.* 2009b). These observations indicate that *OsIAA9* was a canonical Aux/IAA gene.

Fig. 1. *A* - Yeast two-hybrid assay of *OsIAA1*-*OsIAA9*, *OsIAA4*-*OsIAA9*, and *OsIAA9*-*OsIAA9* in β -galactosidase colony filter assay (yeast transformants were grown on YPAD medium and colony filter-lift assays were performed). 1 - pBD-*OsIAAx* (X = 1, 4, and 9, as indicated in the figure) + pAD-*OsIAA9*; 2 - pBD-*OsIAAx* (X = 1, 4, and 9, as indicated); 3 - pAD-*OsIAA9*; 4 - positive control; 5 - positive controls (positive 4 is a little bit stronger than 5); 6 - negative control. *B* - Growth of yeast transformants on synthetic complete medium without leucine, tryptophan, and histidine containing 10 mM 3-aminotriazole. 1 - negative control; 2 - positive control; 3 - pAD-*OsIAA9*; 4 - pBD-*OsIAAx* (X = 1, 4, and 9); 5 - negative control; 6 - pBD-*OsIAAx* (X = 1, 4, and 9) + pAD-*OsIAA9*. (1 and 5 are the same transformants). (X stand for different *OsIAA* genes, *OsIAA1* on left, *OsIAA4* on middle and *OsIAA9* on right)

Tissue and organ expression patterns from collection of rice expression profiles *CREB* (<http://crep.ncpgr.cn>; Wang *et al.* 2010) showed that more *OsIAA9* transcript accumulates in the callus, endosperm, radicle, and seed after imbibition (Song *et al.* 2009a). To further investigate the spatial and developmental regulation of *OsIAA9*, I cloned the *OsIAA9* promoter to drive GUS expression and introduced the construct into rice for histochemical analysis. Around 10 independent T2 transgenic lines containing the *OsIAA9* promoter constructs were analyzed throughout the life cycle of rice. The *OsIAA9* was expressed in roots and seedling shoots (Fig. 2) but not in any other tissues or organs (data not shown). Detailed histochemical analyses of the GUS activity of *P_{OsIAA9}:GUS* lines show that *OsIAA9* was expressed in the coleoptiles and roots at the seedling stage (Fig. 2A,B), including the root tip (Fig. 2C, lateral root stele (Fig. 2D), and lateral roots (Fig. 2E,F) suggesting a role of *OsIAA9* in root development, especially of lateral roots. The *OsIAA9* promoter activity was visible during early lateral root formation at the sites of primordia initiation and was very

high in the vasculature of the emerging lateral roots (Fig. 2E,F).

To find putative *cis*-elements of *OsIAA9*, I analyzed the promoter of 2000-bp regions upstream from ATG using the *PLACE 30.0* database (Higo *et al.* 1999). The search results suggest that the sequence of this fragment contained numerous DNA elements predicted to be responsive to auxin, drought, abscisic acid (ABA), salicylic acid (SA), and high salinity. The DNA elements included three copies of TGTCTC (AuxREs for ARF binding); two copies of ACGTG (a drought-inducible ABRE-like element); a copy of CACGTG (ABA-inducible); two copies of TGACG (IAA/SA-inducible); a copy of TTGAC (SA-inducible); several copies of GNGGTG, GTGGNG, and GAAAAA (salt-inducible); and two copies of TGACG and GCCGCC (JA-related element) (data not shown). These predictions suggest that the functions of *OsIAA9* might be associated with various phytohormone signals and abiotic stresses. To further explore the possible involvement of *OsIAA9* in the regulation of hormone- and abiotic stress-related genes,

the expressions of *OsIAA9* in response to exogenous hormones (IAA, 2,4-D, JA, SA, gibberellic acid, ABA, and kinetin) and abiotic stresses (drought and salt) were examined by real-time PCR. The *OsIAA9* was strongly induced by IAA (7-fold up-regulated), 2,4-D (10-fold up-regulated), JA (12-fold up-regulated), SA (5-fold up-

regulated), and salinity (10-fold up-regulated) and slightly stimulated by drought (3-fold up-regulated), but no significant changes were detected following treatment with ABA, kinetin, or gibberellic acid (Fig. 3). These results suggest that *OsIAA9* had multiple functions in rice.

Fig. 2. An expression pattern of *OsIAA9* as indicated by the β -glucuronidase (*GUS*) reporter gene driven by the *OsIAA9* promoter. The *GUS* expression in seedling (A, B), the seminal root tip and root stele (the elongation zone) (C), the root tip of lateral root (D), lateral roots (E), at the base of lateral root (F).

Fig. 3. Relative expressions (normalized to time point 0 in each treatment) of *OsIAA9* in response to hormones and abiotic stresses (see materials and methods for details and abbreviations) as determined by real-time PCR.

To further test the function of *OsIAA9*, I cloned full-length *OsIAA9* driven by the maize ubiquitin promoter and transformed it into rice (Fig. 4A). Leaves of all 22 independent lines of transgenic plants were collected to detect the expression of *OsIAA9*. Analyses of the RNA-blot indicate 13 overexpression lines relative to the wild type (Fig. 4B). The *OsIAA1*-overexpressing plants exhibited semi-dwarfism and increased leaf angles (Song *et al.*

2009b) and *OsIAA4*-overexpressing plants exhibited semi-dwarfism and increased tiller angles (Song *et al.* 2013). When I observed *OsIAA9*-overexpressing transgenic plants in the field, no obvious morphological changes in plant height, tiller number, leaf angle, or tiller angle (data not shown) were found.

Auxins play crucial roles in root development including regulation of growth of the main root in

Arabidopsis and the seminal root in rice. Because *OsIAA9* responds rapidly and highly to auxins, all *OsIAA9* protein has the typical Aux/IAA conserved domains including domain I, II, III and IV. I next focused on the root morphology of *OsIAA9*-overexpressing plants. Root elongation assays are a typical way to investigate the responses of genes to hormone application. To investigate the function of *OsIAA9* in the auxin signalling pathway, I tested the responsiveness of *OsIAA9*-overexpressing transgenic plants to application of 2,4-D, a synthetic auxin (Song *et al.* 2014). Five independent positive lines (O2, O11, O12, O17, and O19) and one non-overexpressing line (O21) were chosen for a root elongation assay. I first tested the responsiveness of *OsIAA9*-overexpressing plants in an MS medium containing 0.1 μM 2,4-D. The *OsIAA9*-overexpressing plants grew better than WT plants in medium containing 0.1 μM 2,4-D (Fig. 4D-I), whereas no differences were observed for the wild type or non-

overexpressing line O21 (Fig. 4C) suggesting that overexpressing *OsIAA9* in rice led to resistance to exogenous auxin. Then, dose responsiveness of *OsIAA9*-overexpressing plants to 2,4-D was tested. The *OsIAA9*-overexpressing plants were grown in an MS medium containing different concentrations of 2,4-D, and data were collected after two weeks of growth. The roots of transgenic lines were significantly longer than the roots of the wild type (Fig. 4J), further confirming *OsIAA9*-overexpressing plants were less sensitive to the inhibitory effect of auxin than the wild type. Finally, the responsiveness of *OsIAA9*-overexpressing plants to the natural auxin IAA were tested. Transgenic plants exhibited resistance to IAA compared to the wild type (data not shown).

To characterize the root phenotype of *OsIAA9*-overexpressing plants under normal growth conditions, germinated seeds of *OsIAA9*-overexpressing plants and

Fig. 4. Root elongation assay of *OsIAA9*-overexpressing plants treated with 2,4-D. The overexpression construct for rice transformation (A). The RNA gel-blot analysis of transgenic and wild-type (WT) plants (B). Transgenic overexpressing plants O-2 (D), O-11 (E), O-12 (F), O-17 (G), and O-19 (H), non-overexpressing transgenic plant O-21, and WT plants were grown in an Murashige and Skoog medium with 0.1 μM 2,4-D. Quantification of seminal root length in D-F (I). The germinated seeds of *OsIAA9*-overexpressing (O-12, O-17, and O-19) and WT plants were transferred to MS medium containing 0 (1), 0.01 (2), 0.05 (3), 0.1 (4), and 0.5 (5) μM 2,4-D and root lengths was measured after two weeks (J). Error bars indicate SE, $n = 20$.

wild-type plants were transferred to an MS medium to observe differences in their growth (Fig. 5A). One of the most striking characteristics of ectopic overexpression of *OsIAA9* was a decrease in the number of crown roots (Fig. 5B,C). In two-week-old wild-type seedlings grown in tubes, the crown root number was about eight per plant (Fig. 5D). However, *OsIAA9*-overexpressing transgenic plants had only about four crown roots per plant (Fig. 5D). The second strong feature of the phenotype was a decrease in the number of lateral roots (Fig. 5E,F). The lateral root density was about 18 roots cm^{-1} in the wild type but about

10 roots cm^{-1} in *OsIAA9*-overexpressing plants (Fig. 5G). To determine whether exogenous auxin affects this reduction in number of crown roots in *OsIAA9*-overexpressing plants, the wild-type and *OsIAA9*-overexpressing plants were grown in an MS medium both with and without 2,4-D. Taken together, these observations suggest that ectopic overexpression of *OsIAA9* resulted in a decreased crown root number, caused defective lateral root formation, and impaired auxin sensitivity in roots.

Fig. 5. Root phenotypes of *OsIAA9*-overexpressing plants. Appearance of *OsIAA9*-overexpressing plants and wild-type plants under normal growth conditions (A). The crown root number of *OsIAA9*-overexpressing plants (B) and wild-type plants under normal growth conditions (C). Quantification of the number of crown roots in *OsIAA9*-overexpressing and wild-type plants (D). Root morphology of *OsIAA9*-overexpressing plants (E) and wild-type plants under normal growth conditions (F). Lateral root density of *OsIAA9*-overexpressing and wild-type plants (G). Error bars indicate SE, $n = 20$.

To further characterize the function of *OsIAA9*, I crossed a mutant of *OsIAA9* with T-DNA inserted in the first intron (Fig. 6A) to the wild type for homozygosity testing. Out of 12 T1 generation plants, 3 were homozygous (M1, M6, and M12), 5 were heterozygous (M4, M5, M7, M8, and M9), and 4 corresponded to the wild type (M2, M3, M10, and M11) (Fig. 6B). Seminal root length was checked under normal growth conditions or after treatment with auxin; no significant difference was

observed between homozygous (M12) and wild-type plants (M10) (Fig. 6C) suggesting functional redundancy of Aux/IAA proteins.

Auxin/IAA proteins possess a potent transcriptional co-repression activity through domain I, which contains an EAR [ethylene response factor (ERF)-associated amphiphilic repression (EAR), defined by the consensus sequence patterns of either LxLxL or DLNxxP] motif for interaction with the co-repressor protein TOPLESS

(Tiwari *et al.* 2004). Protein sequence analysis showed that *OsIAA9* harbors domain I. To determine whether *OsIAA9* functions as a co-repressor of transcriptional regulation of auxin responses, as do other *Aux/IAA* proteins, I examined the effects of *OsIAA9* overexpression on the induction of early auxin responsive genes, including *OsIAA1*, *OsIAA4*, *OsIAA9*, and *OsIAA20*, in the whole plant. The induction

of three canonical *Aux/IAA* genes, *OsIAA1*, *OsIAA9* itself, and *OsIAA20*, was suppressed by exogenous auxin application in *OsIAA9*-overexpressing plants, whereas the induction of one non-canonical *Aux/IAA* gene, *OsIAA4*, was not significantly influenced by exogenous auxin (Fig. 2 Suppl.) suggesting that ectopic overexpression of *OsIAA9* repressed auxin-induced gene expression.

Fig. 6. *A* - Characterization of *osiaa9* mutants. The T-DNA insertion site in the *OsIAA9* gene. Boxes and lines (not drawn to scale) represent exons and introns, respectively. *B* - The insertion site and genotype of the mutant as confirmed by PCR using a pair of gene-specific primers (GL+GR) and a T-DNA border primer (NTBL5). *C* - Seminal root length with and without 2,4-D treatment. Homozygous mutant (M12) and wild-type (M10) seeds were germinated on an Murashige and Skoog medium and transferred to an MS medium containing 0 or 0.05 μM 2,4-D. Means ± SE, $n = 20$.

To further check the regulation of auxin signaling by *OsIAA9*, the expression of *OsIAA9* in *OsIAA1*- and *OsIAA4*-overexpressing transgenic plants was tested. The induction of *OsIAA9* in *OsIAA1*-overexpressing plants was

significantly suppressed (Fig. 3A Suppl.); however, there was no clear change in *OsIAA4*-overexpressing plants (Fig. 3B Suppl.). Taken together, these results suggest that *OsIAA9* was a major auxin negative regulator in rice.

Discussion

Auxin is an important phytohormone that plays a crucial role in plant growth and development, acting either alone or interplaying with other phytohormones. One of the best known functions of auxin is its regulation of root development. It facilitates root growth and elongation (Peret *et al.* 2009, Peer *et al.* 2011) and is required for the initial cell division during development of lateral root primordia (De Smet *et al.* 2006, 2007). In this study, I focused on identifying a canonical *Aux/IAA* gene, *OsIAA9*, in rice; promoter sequence analysis of *OsIAA9* identified several *cis*-elements related to auxin, SA, drought, and salinity. Real-time PCR shows that the expression of *OsIAA9* was highly induced by IAA, 2,4-D, SA, and JA implying multiple functions of *OsIAA9* in hormone

mediated responses. The *OsIAA9* was also highly induced by salinity and slightly induced by drought but no significant change was found after ABA treatment indicating that *OsIAA9* may mediate plant abiotic stress responses independent of the ABA pathway. The β-glucuronidase staining shows *OsIAA9* mainly as a root specifically expressed gene. Ectopic overexpressions of *OsIAA1* and *OsIAA4* in rice cause multiple phenotypic changes including a decreased plant height, increased leaf/tiller angle, and insensitivity to auxin in roots (Song *et al.* 2009b, Song and Xu 2013). Unlike *OsIAA1* and *OsIAA4*, however, ectopic overexpression of *OsIAA9* did not cause any visible phenotypic change in aboveground parts, but did reduce inhibition of root elongation in

response to exogenous auxin. One explanation for the difference between *OsIAA9* and *OsIAA1/OsIAA4* ectopic overexpression in aboveground parts may be due to the different expression pattern of each gene. The *OsIAA1* is highly expressed throughout the entire life cycle (Song *et al.* 2009b), and *OsIAA4* shows a very low expression throughout the entire life cycle (Song and Xu 2013), whereas *OsIAA9* was specifically expressed in seedling roots and shoots.

Interactions between phytohormones play important roles in the regulation of plant growth and development. There is an increasing evidence of extensive interaction between phytohormones also in the adaptation to external stimuli (Wolters and Jurgens 2009). These interactions are in some cases governed by shared components of signal transduction pathways, but in other cases reflect the modulation that a given hormone exerts on the synthesis or action of another (Hoffmann *et al.* 2011). The *OsIAA9* is highly induced by auxin and JA implying that *OsIAA9* may act through *OsARFs* to influence crosstalk between auxin and JA. Further work is need to determine the phenotypic characterization of *OsIAA9* in JA response and to identify how *OsARF* mediates the interplay between JA and auxin responses in rice.

Salinity is one of the most common environmental stresses with detrimental effects on plant growth and development (Munns and Tester 2008). When plants are exposed to stresses, they often respond with a partial or complete arrest of growth. This response is considered an adaptive strategy for survival enabling plants to reallocate multiple resources towards combating stress (Xiong and Zhu 2001). Plants use two different strategies to deal with a high soil salinity. One strategy is activation of pathways

that allow the plant to export or compartmentalize salt. Relying on their phenotypic plasticity, the second strategy is the adjustment of their root system architecture and the direction of root growth to avoid locally high salt concentrations (Galvan-Ampudia and Testerink 2011). Modulation of root system architecture by salt stress involves differential regulation of auxin distribution in the root, which determines both direction of growth and development of lateral roots (Galvan-Ampudia and Testerink 2011). In this study, I observed that *OsIAA9* was significantly accumulated under salt treatment and slightly under drought stress. The *OsIAA9* may function at the intersection between auxin and abiotic stress signalling. Furthermore, *OsIAA9*-overexpressing *Arabidopsis* shows an enhanced tolerance to osmotic stress and salt stress (http://www.saltscience.or.jp/g_summary/2009/0905-E.pdf), and *OsIAA9* is mainly a root-expressed gene with an ectopic overexpression of *OsIAA9* in rice causing an abnormal root phenotype. All these results imply that *OsIAA9* may be involved in adjustment of root system architecture and the direction of root growth to avoid locally high salt concentrations.

Auxin signalling transduction depends on the rapid degradation of Aux/IAA proteins. The Aux/IAA proteins function as negative feedback regulators to modulate the exact regulation of auxin related physiological process. The high auxin responsiveness of *OsIAA9* shows that it may be an important auxin negative feedback regulator, and the inhibition of expression of *OsIAA9* in *OsIAA1*-overexpressing plants, and the suppression of several auxin response *OsIAAs* in *OsIAA9*-overexpressing plants suggest that *OsIAA9* is an important auxin negative feedback regulator in rice.

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