Constitutive Expression of OsIAA9 Affects Starch Granules Accumulation and Root Gravitropic Response in *Arabidopsis*

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Auxin/Indole-3-Acetic Acid (Aux/IAA) genes are early auxin response genes encoding short-lived transcriptional repressors, which regulate auxin signaling in plants by interplay with Auxin Response Factors (ARFs). Most of the Aux/IAA proteins contain four different domains, namely Domain I, Domain II, Domain III, and Domain IV. So far, all Aux/IAA mutants with auxin-related phenotypes identified in both *Arabidopsis* and rice (*Oryza sativa*) are dominant gain-of-function mutants with mutations in Domain II of the corresponding Aux/IAA proteins, suggesting that Aux/IAA proteins in both *Arabidopsis* and rice are largely functional redundantly, and they may have conserved functions. We report here the functional characterization of a rice Aux/IAA gene, OsIAA9. RT-PCR results showed that expression of OsIAA9 was induced by exogenously applied auxin, suggesting that OsIAA9 is an auxin response gene. Bioinformatic analysis showed that OsIAA9 has a repressor motif in Domain I, a degron in Domain II, and the conserved amino acid signatures for protein-protein interactions in Domain III and Domain IV. By generating transgenic plants expressing GFP-OsIAA9 and examining florescence in the transgenic plants, we found that OsIAA9 is localized in the nucleus. When transfected into protoplasts isolated from rosette leaves of *Arabidopsis*, OsIAA9 repressed reporter gene expression, and the repression was partially released by exogenously IAA. These results suggest that OsIAA9 is a canonical Aux/IAA protein. Protoplast transfection assays showed that OsIAA9 interacted ARF5, but not ARF6, 7, 8 and 19. Transgenic *Arabidopsis* plants expressing OsIAA9 have increased number of lateral roots, and reduced gravitropic response. Further analysis showed that OsIAA9 transgenic *Arabidopsis* plants accumulated fewer granules in their root tips and the distribution of granules was also affected. Taken together, our study showed that OsIAA9 is a transcriptional repressor, and it regulates gravitropic response when expressed in *Arabidopsis* by regulating granules accumulation and distribution in root tips.

**Keywords:** OsIAA9, auxin signaling, gravitropism, lateral root formation, *Arabidopsis thaliana, Oryza sativa*
INTRODUCTION

In Arabidopsis, Aux/IAA genes are one of the early auxin response gene families (Hagen and Guilfoyle, 2002). Aux/IAA proteins are short-lived transcription repressors that involve in the regulation of auxin signaling (Guilfoyle and Hagen, 2007; Guilfoyle, 2015). Most of the Aux/IAA proteins contain four conserved domains, namely, Domain I, Domain II, Domain III, and Domain IV. Domain I is an active repression domain containing a conserved LxLxL motif. Domain II contains a conserved degron and is responsible for the stability of Aux/IAA proteins. Domains III and IV are similar to the conserved C-terminal dimerization domain of Auxin Response Factors (ARFs), and are required for homo dimerization among Aux/IAA proteins, and hetero dimerization between Aux/IAA proteins and ARFs (Ulmasov et al., 1997, 1999; Ramos et al., 2001; Tiwari et al., 2001, 2003, 2004; Dreher et al., 2006; Nanao et al., 2014).

Aux/IAA proteins regulate auxin signaling by interplay with ARFs (Guilfoyle and Hagen, 2007; Guilfoyle, 2015). When cellular auxin levels are low, Aux/IAA proteins are stable, and they can form dimers with ARF activators that bound on the TGTCTC Auxin Response Elements (AuxREs) in the promoter regions of the auxin response genes, thus inhibiting the expression of auxin response genes. Elevated cellular auxin levels will result in the activation of auxin receptor TIR1 (Dharmasiri et al., 2005; Kepinski and Leyser, 2005), leading to the ubiquitylation and then degradation of Aux/IAA proteins via the 26S proteasome (Tan et al., 2007), thus allowing the ARF activators to activate auxin response genes (Ulmasov et al., 1999; Tiwari et al., 2001, 2004; Guilfoyle and Hagen, 2007).

So far all the aux/iaa mutants identified in Arabidopsis, including single T-DNA insertion mutants, double, and triple mutants of closely related Aux/IAA genes, showed no visible developmental defects (Overvoorde et al., 2005), suggesting that Aux/IAA genes are largely redundant functionally. On the other hand, several different types of phenotypes were observed in the dominant aux/iaa mutants. For example, the iaa18-1 mutant has aberrant cotyledon placement in embryos (Ploense et al., 2009), the axr2-1/iaa7 mutant has agravitropic root and shoot growth, and a short hypocotyl and stem (Naggal et al., 2000), the iaa16-1 mutant has restricted adult plant growth and ablished fertility in homozygous (Rinaldi et al., 2012), the iaa18 and iaa28 mutants are severely defective in lateral root formation (Rogg et al., 2001; Uehara et al., 2008), and the sbr-1/iaa14 mutant completely lacks lateral roots (Fukaki et al., 2002). Some of the dominant gain-of-function iaa mutants even have opposite phenotypes. For example, the axr3/iaa17 mutant has defects in root hair development, while shy2/iaa3 mutant has longer root hairs (Knox et al., 2003). The shy2/iaa3 mutant also has other phenotypes including enlarged cotyledons, short hypocotyls, and altered auxin-regulated root development (Tian and Reed, 1999; Tian et al., 2002). These results suggest that stability of Aux/IAA proteins is crucial for their functions in regulating plant growth and development.

Consistent with the fact that Aux/IAA protein are unstable proteins, Arabidopsis transgenic plants expressing wild type Aux/IAA genes from Arabidopsis and grape (Vitis vinifera) are also morphological indistinguishable from wild type plants (Park et al., 2002; Fujita et al., 2012; Kohno et al., 2012). However, auxin-related phenotypes were observed in Arabidopsis transgenic plants expressing mutated Aux/IAA gene with a mutation in Domain II, or Aux/IAA gene lacking Domain II (Park et al., 2002; Fukaki et al., 2005; Sato and Yamamoto, 2008).

Phenotypic changes were observed in knock-down mutants of Aux/IAA genes in tomato (Solanum lycopersicum) (Wang et al., 2005a; Bassa et al., 2012; Deng et al., 2012; Su et al., 2014), and expressing wild type PtrlIAA14.1, a poplar Aux/IAA gene in Arabidopsis resulted in phenotypic changes (Liu et al., 2015), suggesting the functions of Aux/IAA from at least some plant species may different from that in Arabidopsis.

In rice (Oryza sativa), however, all the identified Aux/IAA mutants with auxin-related phenotypes are dominant gain-of-function mutants with mutations in the Domain II of corresponding Aux/IAA proteins (Jun et al., 2011; Zhu et al., 2011; Kitomi et al., 2012), and auxin-related phenotypes were also observed in transgenic rice plants expressing mutated or dominant mutation-type rice Aux/IAA genes (Nakamura et al., 2006; Song and XU, 2013), suggesting that Aux/IAA proteins in rice may regulate auxin signaling in a way similar to those in Arabidopsis.

We report here the characterization of OsIAA9, a rice Aux/IAA gene. Expression of OsIAA9 was greatly induced by exogenously supplied IAA. Bioinformatics and protoplast transfection assay results showed that OsIAA9 is a canonical Aux/IAA protein. When expressed in Arabidopsis in a wild type form, however, OsIAA9 affected lateral root formation and root gravitropic response.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The Japonica rice (Oryza sativa) variety Nipponbare was used for Auxin treatment and OsIAA9 gene cloning. The Arabidopsis thaliana (Arabidopsis) ecotype Columbia-0 (Col-0) was used for plant transformation and protoplasts isolation. DR5:GUS transgenic plants were in Col background (Wang et al., 2005b).

For auxin treatment and RNA isolation from rice seedlings, rice seeds were germinated and grown on water for 10 days, and treated with 10 μM IAA for 4 h before RNA was isolated. For plant transformation and protoplasts isolation, Arabidopsis seeds were sown directly into soil pots and kept in a growth room. For phenotypic analysis, Arabidopsis seeds were sterilized and sown on 0.8% (w/v) phytoagar solidified ½ MS (Murashige & Skoog) (Murashige and Skoog, 1962) plates with vitamins (PlantMedia) and 1% (w/v) sucrose, unless indicated otherwise. The plates were kept at 4°C in darkness for 2 days, and then moved into a growth room. Rice plants were grown at 28°C, and Arabidopsis plants at 20°C, with a 16 h/8 h (light/darkness) photoperiod.

Phylogenetic Analysis

Closely related Arabidopsis and rice Aux/IAA proteins to OsIAA9 were identified by BLAST searching Arabidopsis and
rice proteome database\(^1\) using the entire amino acid sequence of OsIAA9. Full-length amino acid sequences of OsIAA9 and closely related Arabidopsis and rice Aux/IAA proteins were subjected to phylogenetic analysis using “One Click” mode with default settings on Phylogeny\(^2\).

**Auxin Treatment**

To examine the expression of OsIAA9 in response to auxin, 10-day-old rice seedlings were treated with 10 \(\mu\)M IAA for 4 h in darkness on a shaker at 40 rpm. Samples were frozen in liquid N\(_2\) and kept at \(-80^\circ\)C for RNA isolation.

**RNA Isolation and RT-PCR**

Total RNA from rice and Arabidopsis seedlings was isolated using the procedures described previously (Wang et al., 2014; Guo et al., 2015; Liu et al., 2015). cDNA was synthesized by Oligo(DT)-primed reverse transcription using the EazyScript First-Strand DNA Synthesis Super Mix (TransGen Biotech) according to the manufacturer’s instructions.

RT-PCR was used to examine the expression of OsIAA9, and Arabidopsis gene ACTIN2 (ACT2) or rice gene OsACT2 were used as controls for RT-PCR.

** Constructs**

The reporters constructs LexA-Gal4:GUS and Gal4:GUS, and the effector constructs GD, LD-VP, CAT, and ARFs used for protoplast transfection were as described previously (Tiwari et al., 2003; Wang et al., 2005b, 2007; Liu et al., 2015).

To generate HA or GD tagged OsIAA9 constructs for protoplast transfection assays, the full-length open-reading frame (ORF) of OsIAA9 was amplified by RT-PCR using RNA isolated from rice seedlings, and cloned in frame with an N-terminal HA or GD tag into the \(p\)UC19 vector under the control of the 35S promoter (Tiwari et al., 2003; Wang et al., 2005b).

The 35S:OsIAA9 construct in \(p\)UC19 was digested with proper enzymes, and subcloned into the binary vector \(p\)PZP211 (Hajdukiewicz et al., 1994) for plant transformation.

To generate GD tagged OsIAA9CTD for protoplast transfection, ORF sequence of OsIAA9CTD (corresponding to the amino acid residues 84–182) was amplified by PCR using 35S:OsIAA9 plasmids as template, and cloned in frame with an N-terminal GD tag into the \(p\)UC19 vector under the control of the 35S promoter.

To generate GFP tagged OsIAA9 for subcellular localization analysis of OsIAA9, GD tag in 35S:GD-OsIAA9 construct was replaced with a GFP tag, subcloned into the binary vector \(p\)PZP211, and used for plant transformation.

The primers used for gene cloning and gene expression analysis of OsIAA9 are OsIAA9-F, 5′-CAACATATGGAGGCT GGACCTTGGGCT-3′, OsIAA9-R, 5′-CAACTTAAAGTATTACC CAGTATCTTCAGGC -3′, and OsIAA9CTD-F, 5′-CAACATAT GTCGGGCGGGGCGGT-3′. The primers used to amplify ACT2 and OsACT2 were described previously (Guo et al., 2015).

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\(^1\)http://phytozome.jgi.doe.gov/pz/portal.html

\(^2\)www.phylogeny.fr

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**Plant Transformation and Transgenic Plants Selection**

About 5-week-old Arabidopsis plants with several mature flowers on the main inflorescence were transformed using the floral dip method (Clough and Bent, 1998). T1 seeds were sterilized and grown on 1/2 MS plates containing 50 \(\mu\)g/ml kanamycin to select transgenic plants. At least five transgenic lines with similar phenotypes were obtained. Phenotypes of transgenic plants were examined in the T1 generation, and confirmed in following several generations. Represent homozygous T3 or T4 transgenic plants were used for further analysis.

**Plasmid DNA Isolation, Protoplasts Isolation, Transfection, and GUS Activity Assays**

The procedures for plasmids preparation, protoplast isolation, transfection, and GUS activity assay have been described previously (Wang et al., 2005b, 2007, 2014, 2015; Zhou et al., 2014; Guo et al., 2015; Liu et al., 2015). Briefly, plasmids of the reporter and effector genes were prepared using the GoldHi EndoFree Plasmid Maxi Kit (Kangwei), and co-transfected into protoplasts isolated from rosette leaves of \(~4\)-week-old Col wild type Arabidopsis plants. The transfected protoplasts were incubated under darkness at room temperature for 20–22 h before GUS activities were measured using a SynergyTM HT microplate reader (BioTEK).

**Gravitropic Response Assays**

Gravitropic response was measured as described by Li et al. (2011). Briefly, sterilized seeds were grown vertically on 1/5 MS plates for 4 days. The plates were photographed and the plates were then turned \(90^\circ\). The plates were photographed again 1 day later. Angles of the angle between gravity and the root were measured using NIH Image J\(^3\).

**Starch Granule Staining**

Starch granule was stained by following the procedures described by Li et al. (2011).

**Microscopy**

Photographs of Arabidopsis seedlings were taken under a Motic K dissection microscope equipped with an EOS 1100D camera. Root length was then measured using NIH Image J. GFP florescence of OsIAA9-GFP transgenic Arabidopsis seedlings was examined under an Olympus FV1000 confocal microscope.

**RESULTS**

OsIAA9 is an Auxin Response Gene That Encodes a Canonical Aux/IAA Protein

Available experimental evidences suggest that functional mechanism of Aux/IAA may be conserved in rice and Arabidopsis.

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\(^3\)http://rsweb.nih.gov/ij
Arabidopsis. To test if this is the case, we decided to identify a canonical rice Aux/IAA gene, and study its functions in the regulation of auxin signaling and plant growth and development in Arabidopsis.

OsIAA9 was chosen because it is one of the Aux/IAA genes whose expression was highly induced by 2,4-D, a synthesized auxin (Jain et al., 2006). As shown in Figure 1A, expression of OsIAA9 was also highly induced by IAA, a natural occurred auxin. By using the entire amino acid sequence of OsIAA9 to BLAST rice and Arabidopsis protein database⁴, we identified and selected several Aux/IAA proteins that have relative higher amino acid sequence similarity to OsIAA9. Phylogenetic analysis showed that OsIAA9 and OsIAA20 are paralogs. The most closely related Arabidopsis Aux/IAA to OsIAA9 is IAA31 (Figure 1B), an Aux/IAA protein with mutated Domain II (Figure 1C). These results are largely consistent with that reported by Jain et al. (2006).

Previously experiment showed that canonical Aux/IAA proteins contain an LxLxL repressor motif in Domain I, a GWPPV degron core sequence in Domain II, conserved KR residues between Domain I and Domain II that are crucial for the degradation of Arabidopsis Aux/IAA proteins, some conserved amino acid residues in Domain III and Domain IV that are require for protein–protein interaction among Aux/IAA proteins or between Aux/IAA proteins and ARFs (Tiwari et al., 2001, 2003, 2004; Dreher et al., 2006; Nanao et al., 2014). As shown in Figure 1C, OsIAA9 has all the features for a canonical Aux/IAA protein. In addition, OsIAA9 also has the conserved W residue found in OsIAAs and OsARFs that is crucial for protein-protein interaction (Ni et al., 2014).

⁴http://phytozome.jgi.doe.gov/pz/portal.html
OsIAA9 is a Transcription Repressor and its Stability is Affected by Auxin

In Arabidopsis, canonical Aux/IAA proteins are short-lived proteins whose stability is affected by auxin, and they function as transcription repressors. To examine if OsIAA9 is a transcription repressor and its stability is affected by auxin, we first examine it subcellular localization by examining transgenic Arabidopsis plants expressing GFP-OsIAA9 under the control of the 35S promoter. As shown in Figure 2, OsIAA9 is predominantly localized in nucleus.

We then examined if OsIAA9 functions as transcriptional repressor by using protoplast transfection assays. Plasmids of effector gene GD-OsIAA9 or control gene GD, activator gene LD-VP, and the reporter gene LexA-GAL4:GUS were contransfected into protoplasts, and GUS activities were measured after the transfected protoplasts were incubated in the presence and absent of 1.0 µM IAA. As shown in Figure 3A, in the absence of IAA, co-transfection of control gene GD and activator gene LD-VP activated the reporter gene, while co-transfection of effector gene GD-OsIAA9 and activator gene LD-VP resulted in repression of the reporter gene. In the presence of IAA, the repression on the expression of the reporter gene by co-transfected OsIAA9 gene was partially released (Figure 3A), indicating that OsIAA9 is a transcription repressor, and it is unstable in the presence of auxin. When transfected into protoplasts with an integrated auxin response reporter gene DR5:GUS, OsIAA9 repressed the expression of the reporter gene (Figure 3B), suggesting that OsIAA9 regulates auxin response gene expression.

OsIAA9 Interacts With ARF5 in Plant Cells

In Arabidopsis, Aux/IAA proteins regulate auxin signaling through interacting with ARF activators. So far only five ARF...
activators in *Arabidopsis* have been shown to be transcriptional activators (Wang et al., 2005b). Having shown that OsIAA9 is a transcriptional repressor and it regulates auxin reporter gene expression (Figure 3), we wanted to further examine if OsIAA9 interacts with any of the ARF activators by using protoplast transfection assays.

Because Aux/IAA proteins have been shown to be active transcription repressor, and Domain III and IV are the domains that required for the interaction among Aux/IAA proteins and between Aux/IAA protein and ARFs, we made a GD-OsIAA9CTD construct by fusing OsIAA9 C-terminal Domain (Domain III and IV) with GD, and cotransfected it with ARF activator effector genes and the reporter gene Gal4:GUS into protoplasts. GD gene was cotransfected as a control. GUS activity was measured after incubation. As shown in Figure 4, cotransfection of GD gene with all the ARF activator genes does not have any effects on the expression of the reporter gene, while cotransfection of GD-OsIAA9CTD gene with ARF5 gene, but not other ARF activator genes activated Gal4:GUS reporter, suggesting that OsIAA9 specifically interacts with ARF5.

**Expression of OsIAA9 in Arabidopsis Affects Lateral Root Formation and Root Gravitropic Response**

The results described above indicate that OsIAA9 is a canonical Aux/IAA protein. Arabidopsis transgenic plants overexpressing wild type Arabidopsis and grape Aux/IAA genes are morphologically similar to wild type plants (Park et al., 2002; Fujita et al., 2012; Kohno et al., 2012). However, expression of PtrIAA14.1, a canonical Aux/IAA protein encoding wild type poplar Aux/IAA gene in Arabidopsis resulted in auxin-related phenotypes including semi-draft with increased number...
of branches, down-curling leaves, and greatly reduced fertility (Liu et al., 2015). To further explore if OsIAA9 functions similar to canonical Aux/IAA proteins in Arabidopsis, we generated transgenic plant expressing OsIAA9 under the control of the 35S promoter (35S:OsIAA9). As shown in Figure 5A, transgenic Arabidopsis plants have reduced root gravitropic response as indicated by the root orientations. Expression of OsIAA9 in the transgenic plants was confirmed by RT-PCR (Figure 5B). Quantitative analysis showed that root elongation is largely unaffected in the Arabidopsis transgenic plants expressing OsIAA9 (Figure 5C). However, the transgenic plants produced more lateral roots (Figure 5D).

Transgenic plants expressing GFP-OsIAA9 also resulted in abnormal root gravitropic response (Figure 5A), indicating that GFP-OsIAA9 protein is functional, thus the plants were used to examine subcellular localization of OsIAA9 (Figure 2).

To further examine the roles of OsIAA9 in regulating root gravitropic response, we tested the gravitropic response of Col wild type and OsIAA9 transgenic Arabidopsis seedlings to changed gravity. Seedlings were grown on vertical plates for 4 days, and pictured to measure the root directions. The plates were then turned 90° to change the gravity, photographs were taken again after 24 h. As shown in Figure 6A, wild type seedlings grew straight down toward the gravity direction before and after the plates were turned, whereas roots of transgenic seedling were in random directions before and after the plates were turned. Quantitative results of the root direction further confirmed our observation (Figure 6B).

Starch Granules Accumulation is Affected in the OsIAA9 Transgenic Arabidopsis Seedlings

Starch granules in root tips play an important role during the sensing of gravity stimulus. Because the root gravitropic response was altered in the OsIAA9 transgenic plants, we suspected that starch granule formation in the transgenic plants may be disrupted. To test if this is the case, we compared starch granule formation in wild type and the transgenic Arabidopsis seedlings by staining the roots with Lugol’s solution, which contains iodine that can react with starch granules to generate a visible bright blue color. We found that in wild-type root tips, strong staining was observed in several different layers of columella cells, while in root tips of the OsIAA9 transgenic Arabidopsis seedlings, only slight staining was observed, and there is no clear stained cell layers could be observed (Figure 7).

DISCUSSION

Interplay of Aux/IAA proteins and ARFs regulates auxin signaling in Arabidopsis (Hagen and Guilfoyle, 2002). Characterization of gain-of-function mutants in Arabidopsis revolved that Aux/IAA genes regulated many aspects of plant
growth and development (Nagpal et al., 2000; Rogg et al., 2001; Fukaki et al., 2002; Uehara et al., 2008; Ploense et al., 2009; Rinaldi et al., 2012). So far all experimental evidences suggest that Aux/IAA proteins in Arabidopsis and rice may regulate auxin signaling and plant growth and development in a similar manner (Jain et al., 2006; Nakamura et al., 2006; Jun et al., 2011; Zhu et al., 2011; Kitomi et al., 2012; Song and Xu, 2013). Our results in this report show that OsIAA9 is a canonical Aux/IAA protein, it regulates auxin signaling in a way similar to that of the canonical Aux/IAA proteins in Arabidopsis. However, unlike the Arabidopsis canonical Aux/IAA proteins, OsIAA9 has been studied so far, OsIAA9 regulates lateral root formation and root gravitropic response when expressed in Arabidopsis in an unmutated form.

**OsIAA9 is a Canonical Aux/IAA Protein**

All canonical Aux/IAA proteins contain four conserved domains. An LxLxL motif-containing domain, a degron-containing domain, and two domains required for protein–protein interaction (Ulmasov et al., 1997, 1999; Ramos et al., 2001; Tiwari et al., 2001, 2003, 2004; Dreher et al., 2006; Jain et al., 2006; Nanao et al., 2014).

Bioinformatics analysis showed that OsIAA9 is closely related to IAA31, a Domain II mutated type Arabidopsis Aux/IAA protein (Figure 1B). However, it contains all the features of a canonical Aux/IAA protein has, including the conserved KR residues crucial for 26 proteasome degradation of Aux/IAA proteins (Dreher et al., 2006), and the conserved W residue crucial for protein–protein interactions of OsIAAs and OsARFs (Ni et al., 2014) (Figure 1B). In consist with the canonical Aux/IAA protein features it has, OsIAA9 functions as a transcription repressor, its stability is affected by Auxin, it regulates auxin response repoter gene expression (Figure 3), and it interacts with ARF5 in transfected protoplasts (Figure 4). These results suggest that OsIAA9 is a canonical Aux/IAA protein, it regulates auxin signaling in a way similar to all other canonical Aux/IAA proteins.

**Expression of Unmutated OsIAA9 in Arabidopsis Resulted in Auxin Related Phenotypes**

So far all the aux/iaa mutants identified in Arabidopsis and rice with phenotypes are gain-of-function mutants with mutations occurred within Domain II of corresponding Aux/IAA proteins (Tian and Reed, 1999; Nagpal et al., 2000; Rogg et al., 2001; Fukaki et al., 2002; Tian et al., 2002; Knox et al., 2003; Uehara et al., 2008; Ploense et al., 2009; Jun et al., 2011; Zhu et al., 2011; Kitomi et al., 2012; Rinaldi et al., 2012). On the other hand, expression of mutated type, but not wild type Arabidopsis Aux/IAA genes in Arabidopsis resulted in auxin-related phenotypes (Park et al., 2002; Fukaki et al., 2005; Sato and Yamamoto, 2008). Expression of mutated or dominant mutation-type rice Aux/IAA genes in rice also resulted in auxin-related phenotypes (Nakamura et al., 2006; Song and Xu, 2013), suggest that stability of Aux/IAA proteins are crucial for their functions in regulating plant growth and development, and that function mechanisms of Arabidopsis and rice Aux/IAA proteins may be conserved. Similar to other canonical Aux/IAA proteins, OsIAA9 functions as a transcription repressor, and its stability is affected by auxin as mentioned above. However, transgenic Arabidopsis plants expressing OsIAA9 showed several auxin-related phenotypes, including increased lateral root formation and reduced root gravitropic response (Figures 5 and 6). Previously we have showed that expression of wild type PtrIAA14.1, a canonical Aux/IAA gene from poplar, resulted in phenotypes changes in Arabidopsis (Liu et al., 2015), but the phenotypes are different from that of Arabidopsis transgenic plants expressing OsIAA9. On the other hand, among all the five ARF activators, both PtrIAA14.1 and OsIAA9 interacted only with ARF5 in transfected protoplasts (Liu et al., 2015; Figure 4), indicating that interaction between ARF5 and OsIAA9 may contribute little, if any to the resulted phenotypes in the transgenic plants. In summary, we showed that OsIAA9 is a canonical Aux/IAA protein, and it causes auxin-related phenotypes including lateral root formation and gravitropic response when expressed in Arabidopsis.

**AUTHOR CONTRIBUTIONS**

SW conceived the study. SL and SW designed the experiments. SL, QL, SL, NMP, and HT performed the experiments. SL and SW analyzed the data. SW drafted the manuscript. All authors read and approved the final manuscript.

**ACKNOWLEDGMENTS**

This work was supported by the Key Laboratory of Molecular Epigenetics of MOE (130014542), the Department of Human Resources and Social Security of Jilin Province (http://hrss.ji.gov.cn) and the Programme for Introducing Talents to Universities (B07017). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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December 2015 | Volume 6 | Article 1156

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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