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ORIGINAL ARTICLE

IDD10 is Involved in the Interaction between NH_4^+ and Auxin Signaling in Rice Roots

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Abstract NH_4^+ is an important nitrogen resource for rice plants in paddy soil. Therefore, it is likely that NH_4^+-triggered plant growth interacts with phytohormone-mediated developmental mechanisms. Our previous transcriptomic analysis revealed that many genes involved in auxin signaling and efflux are sensitive to NH_4^+. In the current study, we found that NH_4^+ treatment causes a delayed gravity response in rice roots. To further elucidate the interlocking relationship between NH_4^+ and auxin signaling during root development, we utilized mutants and overexpressors of a key NH_4^+ signaling transcription factor *INDETERMINATE DOMAIN 10* (*IDD10*), encoding a transcription factor that regulates the expression of NH_4^+ uptake and N-assimilation genes. We obtained several lines of evidence that auxin affects NH_4^+-mediated gene expression and root development in rice plants via IDD10. First, the gravity response was delayed in *idd10* roots and accelerated in *IDD10* overexpressor (*IDD10 OX*) roots in the absence and (especially) presence of NH_4^+. Second, *idd10* plants showed strong root coiling only in the presence of NH_4^+. However, treatment of 1-N-naphthylphthalamic acid (NPA), a polar auxin transport inhibitor suppressed the NH_4^+-specific root phenotype of *idd10*. Third, the expression of NH_4^+-responsive auxin-related genes was affected in *idd10* and *IDD10* overexpressors. Finally, *IDD10* expression was induced by IAA and suppressed by NPA. These findings suggest that the gene expression patterns and phenotypes triggered by NH_4^+ are influenced by the actions of auxin during root development, pointing to a regulatory circuit between NH_4^+ and auxin signaling that functions in root development in rice.

Keywords: Ammonium, Auxin, IDD10, Microarray, Root growth

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Introduction

NO_3^- and NH_4^+ are the major forms of nitrogen resources for higher plants. Nitrogen (N) is an important macroelement required for the biosynthesis of cellular molecules such as amino acids and nucleotides. The reduction of NO_3^- to NH_4^+ consumes 12–26% of the available photosynthetically generated reductant in a plant (Patterson et al. 2010). Therefore, NH_4^+ is an energetically favorable N source. However, NH_4^+ is toxic to many plant species at high concentrations (Britto DT 2002), although rice utilizes NH_4^+ as the major N source. Early genomic responses of rice to exogenous NH_4^+ have been surveyed under various levels of N supply (Lian et al. 2006; Cai et al.2012; Xuan et al. 2013; Yang et al. 2015a; 2015b; Chandran et al. 2016). Many genes have been identified that respond to changes in cellular N status in rice. These genes are involved in diverse aspects of metabolism and signaling, including nitrogen and carbon metabolism, stress responses, and hormonal signaling. These findings are similar to those obtained from studies investigating the effects of nitrate on gene expression in *Arabidopsis thaliana* (Wang et al. 2000; Britto DT 2002; Wang et al. 2003; Scheible et al. 2004; Bi et al. 2007; Gifford et al. 2008).

Many studies have shown that in Arabidopsis, hormonal signaling pathways are tightly connected with NH_4^+-related plant growth and stress responses. The auxin-resistant *aux1*, *axr1*, and *axr2* mutants are insensitive to the NH_4^+-mediated inhibition of root growth (Cao et al. 1993). The application of NH_4^+ to shoots causes the auxin influx carrier AUX1 to inhibit lateral root emergence (Li et al. 2011). *ARG1* (*ALTERED RESPONSE TO GRAVITY1*) is required for normal *AUX1* expression and basipetal auxin transport in the root apex, and *arg1* mutants are sensitive to NH_4^+ (Zou et al. 2013). Ethylene production in shoots is associated with NH_4^+-mediated lateral root inhibition (Li et al. 2013). The activation of ABA signaling reduces NH_4^+-induced stress in a mutant of *AMOS1* (*AMMONIUM OVERLY SENSITIVE1*)/*EGY1* (*ETHYLENE-DEPENDENT, GRAVITROPISM-DEFICIENT, AND YELLOW-GREEN-LIKE PROTEIN1*) (Li et al. 2012). In rice, RAVL1 (*RELATED TO ABI3/VP1-LIKE1*), a key brassinosteroid (BR) signaling transcription factor, was recently found to regulate BR-mediated induction of *AMT1;2* and NH_4^+ uptake (Xuan et al. 2016).

In rice, high concentrations of NH_4^+ induce primary root coiling in the light, which is rescued by the inhibition of NH_4^+ assimilation (Hirano et al. 2008; Shimizu et al. 2009). However, few studies have explored the relationship between hormones and NH_4^+ signaling during root development in rice. We previously showed that INDETERMINATE DOMAIN10 (IDD10) regulates NH_4^+-mediated gene expression and root growth in rice (Xuan et al. 2013). However, how IDD10 regulates root growth under NH_4^+ conditions is currently unclear.

In this study, we analyzed the effects of NH_4^+ on the expression of auxin-related genes and the response of roots to gravity in rice plants. We utilized *IDD10* mutant and overexpressor plants with altered expression of NH_4^+ uptake and N-assimilation genes to analyze NH_4^+-mediated gravity responses in root tips and auxin-related gene expression. The results shed light on the relationship between NH_4^+ and hormonal signaling/action during root growth.

Results

Auxin-related Gene Expression and Gravity Response are Influenced by NH_4^+ in Rice Roots

We previously obtained the NH_4^+-responsive transcriptomic profiles of roots from 17-day-old rice seedlings at the autotrophic stage that had been grown without N supply after 3 h of NH_4^+ treatment (Xuan et al. 2013; Chandran et al. 2016). Analysis of the microarray data revealed that many phytohormone genes (especially those related to auxin) are highly sensitive to NH_4^+ treatment. In the current study, we further analyzed the expression of auxin-related genes that responded to NH_4^+ within 3 h of treatment. Table 1 and Fig. 1A show the list of auxin-signaling and efflux-related genes and their expression patterns that were previously identified by microarray analysis whose expression levels differed more than 2-fold before and after NH_4^+ treatment. We performed qRT-PCR analysis to verify the NH_4^+-triggered expression of auxin-signaling genes, including *OsIAA24* (*LOC_Os07g08460*), *OsIAA19* (*LOC_Os05g48590*), *OsGH3-13* (*LOC_Os11g32510*), and *OsIAA10* (*LOC_Os02g57250)* (Fig. 1B). The qRT-PCR data confirmed the NH_4^+-responsive expression patterns of most genes identified from the microarray data.

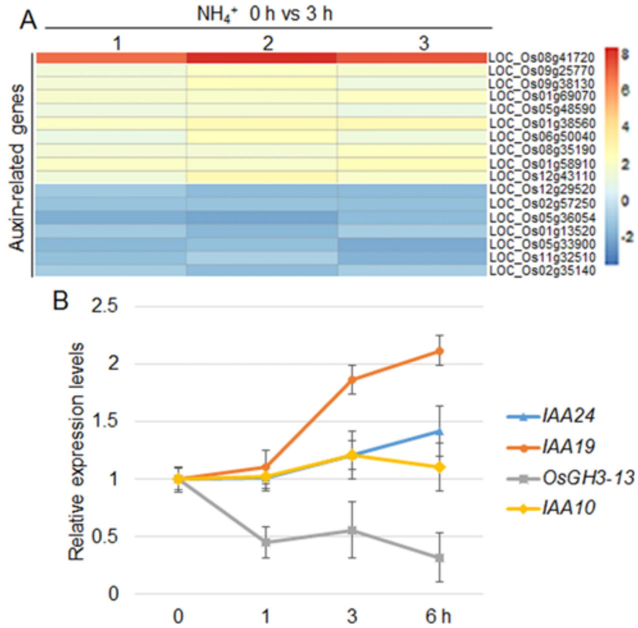

Fig. 1. NH_4^+-dependent auxin-related gene expression. (A) Heat map showing the auxin related genes whose expression levels were altered at least 2-fold after 3 h of NH_4^+ treatment in rice roots. Gene expression is shown with a pseudocolor scale, with blue denoting low expression levels and red denoting high expression levels ($P<0.05$). '1', '2', and '3' on the map indicate the repeat number of the microarray experiments. (B) Seventeen-day-old seedlings were transferred to nutrient solution containing 0.5 mM $(NH_4)_2SO_4$. Total cellular RNA was extracted from whole roots 0, 1, 3, and 6 h after transfer. qRT-PCR was performed to measure the expression levels of auxin-signaling genes (*OsIAA24* (*LOC_Os07g08460)*, *OsIAA19* (*LOC_Os05g48590)*, *OsGH3-13* (*LOC_Os11g32510)*, and *OsIAA10* (*LOC_Os02g57250)*. Sample mRNA levels were normalized with respect to those of *UBIQUITIN* mRNA. Data represent means ± SE (n=3).

Since a few *AUX/IAA* and auxin efflux carrier/transporter genes are regulated by NH_4^+ treatment, we explored the

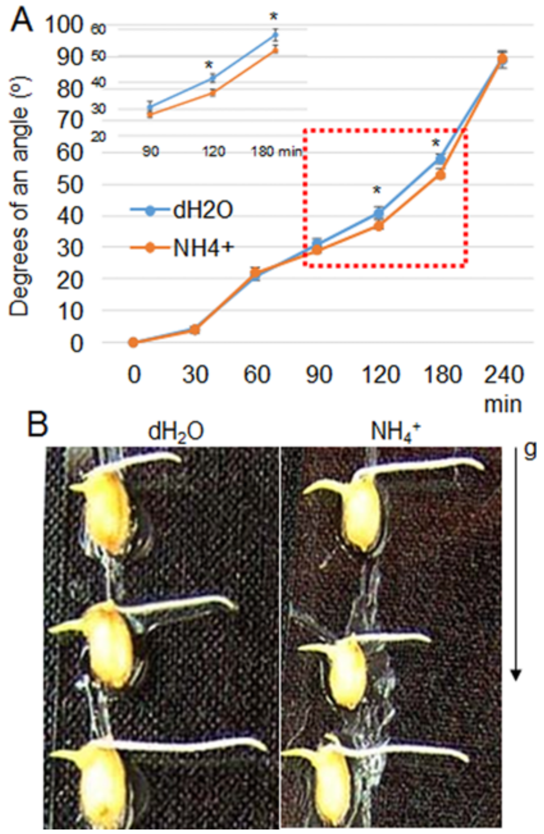

Fig. 2. The effects of NH_4^+ on gravity responses in wild-type roots. (A) Two-day-old seedlings grown in water were transferred to dH_2O or 0.5 mM $(NH_4)_2SO_4$ solution, and the gravity direction was changed 90°. Bending angles of root tips in water and NH_4^+ solution were measured at various time points. The experiments were repeated at least three times, and data represent means ± SE ($n > 10$). The region in the red dotted box in above is shown at a larger scale. Significant differences in gravity responses in dH_2O and 0.5 mM $(NH_4)_2SO_4$ solution are shown (*$P<0.05$). (B) Roots tips of plants grown in dH_2O or 0.5 mM $(NH_4)_2SO_4$ 120 min after the gravity direction was changed 90° are shown. "g" indicates gravity, and the black arrow indicates the direction of gravity.

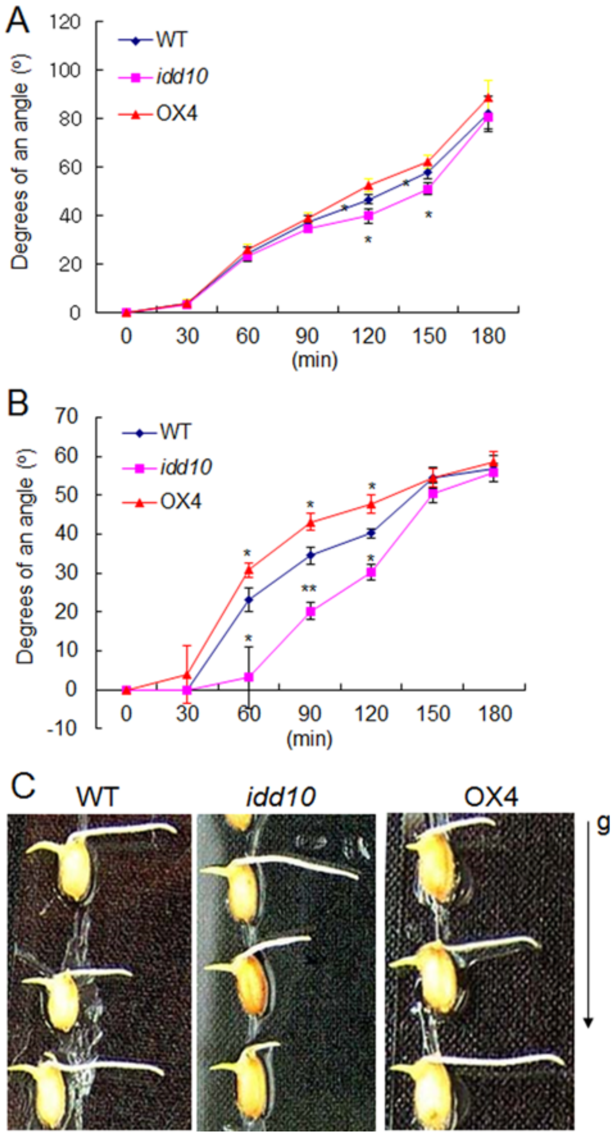

Fig. 3. The effects of NH_4^+ on gravity responses in root tips of wild-type, *idd10*, and *IDD10 overexpression* (OX4) plants. Two-day-old seedlings grown in water were transferred to dH_2O (A) and 0.5 mM $(NH_4)_2SO_4$ solution (B), and the gravity direction was changed 90°. Bending angles of root tips in water (A) and NH_4^+ solution (B) were measured at various time points. (C) Photographs showing root tips of plants grown in 0.5 mM $(NH_4)_2SO_4$ solution 90 minutes after the gravity direction was changed. "g" indicates gravity, and the black arrow indicates the direction of gravity. The experiments were repeated at least three times, and data represent means ± SE ($n > 10$). Significant differences in gravity responses between wild type, *idd10*, and OX4 are shown (*$P<0.05$, **$P<0.01$).

phenotypic effect of NH_4^+ on auxin signaling by examining the gravity response of roots grown in the presence of NH_4^+. Two-day-old wild-type plants grown in water were transferred to buffer (1 mM MES buffer, pH 5.8) containing 0 or 0.5 mM $(NH_4)_2SO_4$. The primary root direction was set to 90° against the direction of gravity. We measured the root tip angles at various time points (Fig. 2A). The gravity response of root tips occurred more rapidly in control buffer than in NH_4^+ solution, especially after 120 and 180 min of treatment (Fig. 2). These results indicate that NH_4^+ treatment delays the gravity response of root tips. To examine whether 1 mM NH_4^+ is toxic to root tip growth, we added 0.1 and 0.05 mM $(NH_4)_2SO_4$ to the same solutions. However, we detected little difference among NH_4^+ concentrations. Moreover, by adding NO_3^- to the same buffer, we examined whether the type of N resource affects the gravity response in rice root tips, finding that NO_3^- did not affect the gravity response of root tips (Fig. S1).

NH_4^+ Enhances Sensitivity to Gravity Mediated by IDD10

IDD10 is a transcriptional activator that induces many NH_4^+-uptake and N-assimilation genes (Xuan et al. 2013). To investigate the possibility that IDD10 mediates the interaction between NH_4^+ and auxin signaling, we examined the gravity response in the roots of *idd10* mutant and *IDD10* overexpressor plants. Two-day-old seedlings grown in dH_2O were subjected to a 90 degree change in orientation with respect to gravity. We compared the gravity responses in root tips every 30 min among 2-day-old wild-type, *idd10*, and *IDD10* overexpressor (OX4) plants. The gravity response was slower in *idd10* compared to wild-type plants but significantly faster in OX4 versus wild type at 120 and 150 min after the change in gravity direction (Fig. 3A). To examine whether NH_4^+ affects the IDD10-mediated sensitivity of these plants to gravity, 2-day-old plants were transferred to the same solution containing 0.5 mM $(NH_4)_2SO_4$. OX4 exhibited a faster and stronger gravity response in NH_4^+ solution than in the same buffer without NH_4^+. Root tips of OX4 began to bend 30 min after stimulation (Fig. 3B, C). Wild-type roots did not show any response to gravity at up to 30 min of treatment. By contrast, *idd10* responded to gravity more slowly than wild-type plants in the presence of NH_4^+. These results suggest that both NH_4^+ and IDD10 have stimulatory effects on the gravity sensitivity of roots. We also investigated whether supplying NH_4^+ to roots would affect the gravity responses of coleoptiles and shoots, finding that NH_4^+ treatment did not alter the responses of coleoptiles or shoots to gravity in *idd10* or OX4 plants (Fig. S2).

NPA Treatment Rescues the NH_4^+-triggered Root Coiling of *idd10*

The *idd10* mutant exhibits distinct coiling phenotypes in roots grown in NH_4^+-containing solution (Xuan et al. 2013). As shown in Fig. 4, primary root tips were strongly coiled in the presence of modified 0.5X MS medium containing 10 mM NH_4^+. However, the phenotype was not detected in plants grown in 10 mM nitrate and dH_2O. To investigate whether NH_4^+-induced root coiling is related to aberrations in auxin signaling and homeostasis, we added the auxin efflux inhibitor NPA to 0.5X MS growth medium containing 10 mM NH_4^+ (Fig. 5A). The addition of 50 nM NPA to the medium suppressed the root-coiling phenotype of *idd10* (Fig. 5). The primary root length was similar in 3-day-old wild-type and *idd10* mutant plants, even though NPA treatment slightly inhibited primary root growth in both wild-type and *idd10* plants. These results strongly suggest that the action of NH_4^+ is closely associated with auxin signaling during root development.

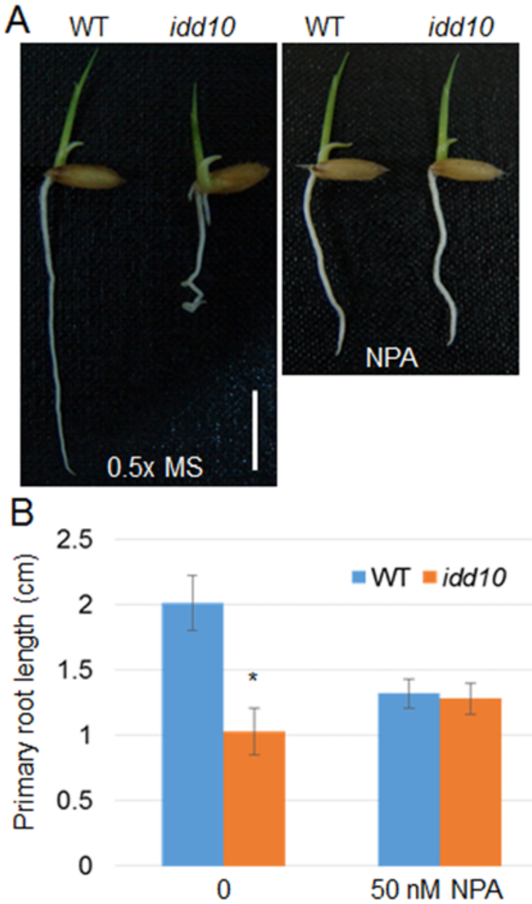

Fig. 5. The effects of the polar auxin transport inhibitor NPA on NH_4^+-dependent *idd10* root growth. (A) Wild-type and *idd10* plants were grown in 0.5x MS medium or 0.5x MS medium containing 50 nM 1-N-naphthylphthalamic acid (NPA). Three-day-old seedlings were photographed. Bar=0.5 cm. (B) Primary root length in wild-type and *idd10* plants shown in (A). Significant differences between wild-type and *idd10* plants are shown (*$P<0.05$).

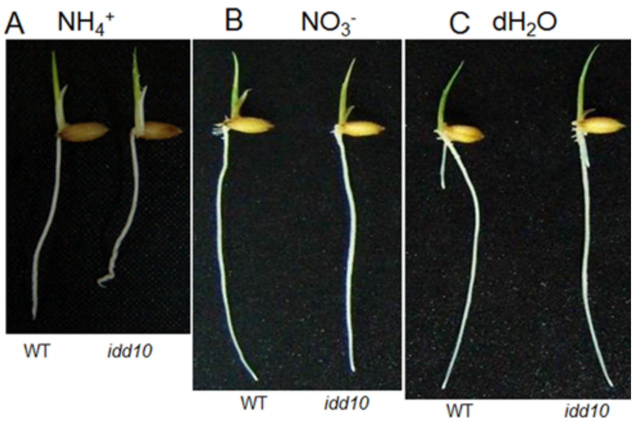

Fig. 4. Root phenotypes of 3-day-old wild-type (WT) and *idd10* mutant seedlings grown in 0.5X MS containing 10 mM NH_4NO_3 (A) and a modified 0.5X MS containing only 10 mM of KNO_3 (B) as the sole nitrogen source, or dH_2O (C).

Analysis of NH_4^+-triggered Expression of Auxin-related Genes in *idd10* and OX4 Roots

To further explore the role of IDD10 in the interaction between auxin and NH_4^+, we analyzed the expression of 18

Table 1. Auxin-related genes regulated by NH_4^+

Locus no.	Gene description	Fold (Log2)	STDEV
Auxin-related genes			
LOC_Os12g29520	auxin response factor, putative, expressed	-1.54	0.052
LOC_Os02g57250	OsIAA10 - Auxin-responsive Aux/IAA gene family member	-2.06	0.221
LOC_Os04g36054	auxin response factor 9	-1.46	0.328
LOC_Os01g13520	auxin response factor 1	-2.02	0.319
LOC_Os05g33900	auxin-induced protein 5NG4	-1.63	0.434
LOC_Os11g32510	OsGH3-13 - Auxin-responsive GH3 gene family member	-1.29	0.283
LOC_Os02g35140	auxin response factor 7	1.67	0.234
LOC_Os09g25770	auxin-induced protein 5NG4	1.59	0.524
LOC_Os09g38130	auxin efflux carrier component	1.79	0.226
LOC_Os01g69070	auxin efflux carrier component	1.14	0.192
LOC_Os05g48590	OsIAA19 - Auxin-responsive Aux/IAA gene family member	2.52	0.384
LOC_Os01g36560	auxin-induced protein 5NG4	1.39	0.538
LOC_Os06g50040	OsSAUR29 - Auxin-responsive SAUR gene family member	1.61	0.333
LOC_Os08g35190	auxin-repressed protein	1.89	0.467
LOC_Os01g58910	auxin-induced protein 5NG4	1.81	0.631
LOC_Os12g43110	OsSAUR58 - Auxin-responsive SAUR gene family member	7.34	0.606
LOC_Os08g41720	auxin efflux carrier component	1.21	0.068
LOC_Os07g08460	OsIAA24 - Auxin-responsive Aux/IAA gene family member	1.22	0.035

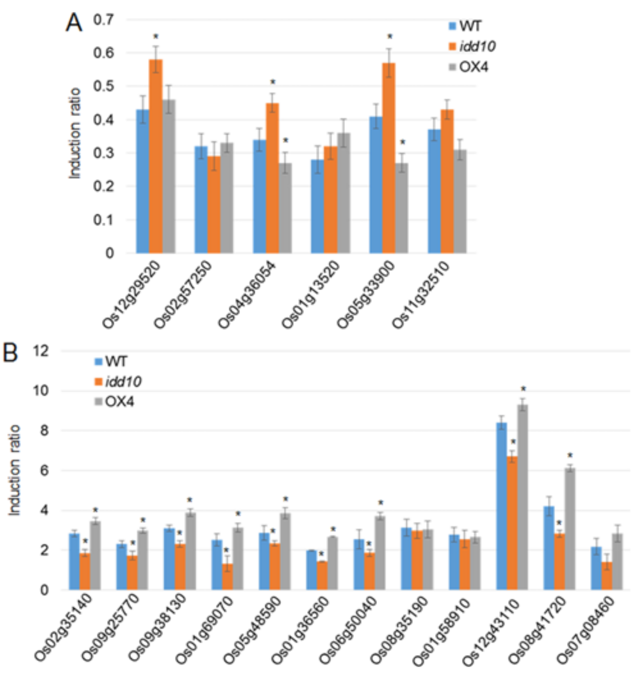

Fig. 6. NH_4^+-dependent expression of auxin-related genes in wild-type, *idd10*, and *IDD10* overexpressor (OX4) plants. (A) The expression levels of six auxin-related genes suppressed by NH_4^+ in wild-type roots (Table 1) were examined in *idd10* and OX4 roots 3 h after NH_4^+ treatment. (B) The expression levels of 12 auxin-related genes induced by NH_4^+ (Table 1) were examined in *idd10* and OX4 roots 3 h after NH_4^+ treatment. Significant differences in expression levels among wild type, *idd10*, and OX4 are shown (*$P<0.05$).

auxin-related genes whose expression levels were altered by at least 2-fold by NH_4^+ treatment (Table 1) in wild-type, *idd10*, and OX4 roots before and 3 h after NH_4^+ treatment. Among these 18 genes, six genes were suppressed by NH_4^+ treatment while the 12 remaining genes were induced by this treatment. Among the six NH_4^+-suppressed genes (*LOC_Os12g29520*, *LOC_Os02g57250*, *LOC_Os04g36054*, *LOC_Os01g13520*, *LOC_Os05g33900*, and *LOC_Os11g32510*), three (*LOC_Os12g29520*, *LOC_Os04g36054*, and *LOC_Os05g33900*) were suppressed less strongly by NH_4^+ in *idd10* than in wild type. Meanwhile, two genes (*LOC_Os04g36054* and *LOC_Os05g33900*) were suppressed more strongly in OX4 than in wild type (Fig. 6A). We also examined the expression levels of 12 NH_4^+-induced genes by qRT-PCR, including *LOC_Os02g35140*, *LOC_Os09g25770*, *LOC_Os09g38130*, *LOC_Os01g69070*, *LOC_Os05g48590*, *LOC_Os01g36560*, *LOC_Os06g50040*, *LOC_Os08g35190*, *LOC_Os01g58910*, *LOC_Os12g43110*, *LOC_Os08g41720*, and *LOC_Os07g08460*. Nine of these genes (*LOC_Os02g35140*, *LOC_Os09g25770*, *LOC_Os09g38130*, *LOC_Os01g69070*, *LOC_Os05g48590*, *LOC_Os01g36560*, *LOC_Os06g50040*, *LOC_Os12g43110*, and *LOC_Os08g41720*) were induced by NH_4^+ at lower levels in *idd10* than in wild type. Conversely, the same nine genes were induced by NH_4^+ at higher levels in OX4 than in wild-type plants (Fig. 6B). These results suggest that many auxin genes might function in the responses to NH_4^+ via a regulatory mechanism mediated by IDD10.

Auxin-dependent Expression of *IDD10*

Since our results indicated that auxin-related genes can be

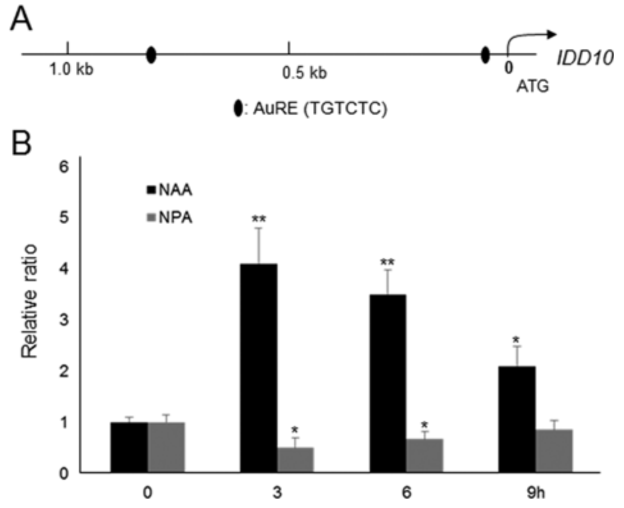

Fig. 7. Auxin-dependent *IDD10* expression. (A) Schematic diagram showing the locations of AuREs (auxin-responsive elements) within the 1.0 kb *IDD10* promoter. Back ovals indicate AuREs. (B) qRT-PCR was performed to examine the effects of auxin and the auxin polar transport inhibitor NPA on *IDD10* expression. Seven-day-old seedlings were treated with 1 μM NAA or 1 μM NPA. The expression levels of *IDD10* were measured 0, 3, 6, and 9 h after treatment. Expression levels were normalized against that of *UBIQUITIN* mRNA. Significant induction by NAA and suppression by NPA were detected (*$P<0.05$, **$P<0.01$).

induced by both NH_4^+ and IDD10, we next investigated whether auxin signaling influences *IDD10* expression. Sequence analysis of the *IDD10* promoter showed that two putative auxin responsive elements (AuRE; TGTCTC) are located within 1 kb of this promoter (Fig. 7A). To investigate whether *IDD10* functions in response to auxin signaling, we treated 7-day-old plants grown in dH_2O with 1 μM NPA or NAA and monitored *IDD10* expression at 0, 3, 6, and 9 h of treatment. NAA treatment induced *IDD10* expression at 3, 6, and 9 h, with the highest expression detected at 3 h. By contrast, NPA treatment repressed the expression of *IDD10* at 3 and 6 h, and the expression levels recovered at 9 h (Fig. 7B). These results suggest that the expression of *IDD10* is influenced by auxin signaling and homeostasis.

Discussion

NH_4^+ is important for plant growth, development, and yield in rice. Our previous efforts exploring the early genomic response to NH_4^+ in rice roots led to the identification of approximately 2000 genes whose expression levels changed by at least 2-fold within 3 h after NH_4^+ treatment (Xuan et al. 2013). These genes are involved in diverse metabolic processes, molecule transport, stress responses, and hormone signaling. In the current study, we verified that the expression of auxin-related *AUX/IAA* and putative auxin efflux carrier genes is regulated by NH_4^+ (Table 1). Furthermore, a root gravity test showed that supplying the plants with NH_4^+ delayed the gravity response in root tips (Fig. 2). These observations suggest that NH_4^+ affects auxin signaling or transport, which helps control the gravity response in roots. Interestingly, nitrate treatment did not affect gravity responses in the root tips of young rice seedlings (Fig. S1). Moreover, the application of NH_4^+ did not interfere with gravity responses in shoots and coleoptiles (Fig. S2). Therefore, it is likely that the specific effect of NH_4^+ on gravity responses is cell-type dependent. In Arabidopsis, foliar application of NH_4^+ increases ethylene production, which further affects AUX1 activity, thereby inhibiting lateral root formation (Li et al. 2011; Li et al. 2013). The nitrate transporter NRT1.1 transports auxin under low nitrogen conditions in lateral roots (Krouk et al. 2010), suggesting that there are diverse relationships between auxin and nitrogen signaling.

IDD10 is a transcription factor that regulates N-linked gene expression in rice roots (Xuan et al. 2013). Phenotypically, mutant roots exhibit root tip coiling in an NH_4^+ dosage-dependent manner. Typically, wild-type roots also exhibit tip coiling when exposed to a high concentration of NH_4^+ (Hirano et al. 2008). Root coiling and growth in *idd10* plants are much more sensitive to NH_4^+ compared to wild-type plants, even though *idd10* plants accumulate less NH_4^+ than wild-type plants (Xuan et al. 2013). The current results show that IDD10 influences the NH_4^+-induced change in the magnitude of expression of many auxin-related genes (13 out of 18) (Fig. 6). These genes include putative auxin efflux carrier genes and *OsIAA19* (Fig. 6; Table 1). Furthermore, *idd10* and *IDD10 OX* root tips exhibited slower and more rapid responses, respectively, to changes in the direction of gravity compared to wild type. The application of NH_4^+ further affected gravity sensitivity in the root tips of *idd10* and overexpressor plants (Fig. 3). Additional evidence that IDD10 is involved in the interaction between NH_4^+ and auxin was obtained by the observation that the application of NPA, a polar auxin transport inhibitor, rescued the coiling defects of *idd10* roots in 0.5x MS medium (Fig. 4). These data strongly suggest that the altered gravity response of *idd10* roots might be related to a dysfunction of the auxin transport process. The AuRE motifs located within 1 kb of the promoter (Fig. 7A) might mediate the induced expression of *IDD10* by NAA and its suppression by NPA treatment in roots. This observation further supports the possibility that *IDD10* is involved in an auxin signaling regulatory circuit. Regulatory circuits between hormones and NH_4^+ signaling have been reported in Arabidopsis. Indeed, some rice lines overexpressing the Arabidopsis *AtEIN3* homolog, *OsEIL1*, showed a root-coiling phenotype (Mao et al. 2006). Furthermore, foliar NH_4^+ supply inhibited lateral root formation caused by the inhibition of ethylene overproduction-mediated AUX1 activity (Li et al. 2011; Li et al. 2013).

In summary, we showed that NH_4^+ treatment regulates hormonal gene expression and the gravity response in rice root tips. Furthermore, IDD10 mediates the interaction between NH_4^+ and auxin signaling in terms of both gene expression and the response of root tips to gravity. These results provide useful information for understanding the molecular mechanisms underlying NH_4^+-dependent root growth and development in rice plants.

Materials and Methods

Plant Materials and Growth Conditions

After germination, wild-type, *idd10*, and *IDD10* overexpression plants were grown in dH_2O in a greenhouse for 14 days. The seedlings were grown for an additional 3 days in N-free nutrient solution (Abiko et al. 2005), followed by transfer to the same nutrient solution containing 0.5 mM $(NH_4)_2SO_4$, pH 5.5. Whole roots were harvested at 0, 1, 3, and 6 h following the provision of $(NH_4)_2SO_4$. To examine the effects of auxin and an auxin inhibitor on *IDD10* expression, plants were grown in distilled water (dH_2O) for 7 days and transferred to dH_2O containing 1 μM naphthalene-1-acetic acid (NAA) or the auxin inhibitor 1-N-naphthylphthalamic acid (NPA). Whole roots were sampled 0, 3, 6, and 9 h after NAA or NPA application.

To analyze the gravity response in root tips, 3-day-old wild-type seedlings grown in water were transferred to water or 0.5 mM $(NH_4)_2SO_4$ solution and reoriented so that the root tips were set at an angle 90° away from the direction of gravity. The angle between a horizontal line and the direction of root tip growth was measured every 30 min. To analyze the effect of the auxin efflux inhibitor on root growth in *idd10*, wild-type and *idd10* plants were grown in 0.5x MS medium containing 50 nM NPA for 3 days.

RNA Extraction and Quantitative RT-PCR Analysis

Total cellular RNA was isolated with an RNeasy Plant Mini Kit (QIAGEN, GmbH, Hilden, Germany) and subsequently treated with RQ-RNase free DNase (Promega, Madison, WI, USA) to eliminate genomic DNA contamination. For cDNA synthesis, a reverse transcriptase RNaseH (Toyobo, http://www.toyobo-global.com/) transcription kit was used following the manufacturer's instructions (Promega, Madison, WI, USA). The RT-PCR products were quantified using Eco 3.0 software (Illumina, San Diego, California, USA), and values were normalized against *UBIQUITIN* levels from the same samples. The primers used for qRT-PCR are listed in Table S1.

Statistical Analysis

Statistical calculations were performed using prism 5 (GraphPad, San Diego, CA). All data are expressed as mean ± SE. Comparisons between two groups were performed by *t* test ($*P<0.05$).

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Author's Contributions

YHX and CDH designed experiments for screen and analysis of materials; YHX and VK performed qPCR and physiology experiments; XFZ, BIJ, CMK, JH, JHC, and GHY propagated and maintained plants in the field, YHX and CDH analyzed the data and wrote the manuscript. All the authors agreed on the contents of the paper and have no conflicting interests to declare.

Supporting Information

Table S1. Primer sequences
Fig. S1. The effects of NO_3^- on the gravity response in roots.
Fig. S2. Gravity responses in the coleoptiles and shoots of wild-type, *idd10*, and *IDD10 overexpression* (*OX4*) seedlings.

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