Indeterminate Domain Proteins Regulate Rice Defense to Sheath Blight Disease

Qian Sun1†, Dan Dan Li1†, Jin Chu2†, De Peng Yuan1, Shuang Li3,4, Li Juan Zhong5, Xiao Han6* and Yuan Hu Xuan1*

Abstract

Background: Loose Plant Architecture 1 (LPA1), an indeterminate domain (IDD) protein, exhibits almost no expression in the leaves, but the overexpression of LPA1 significantly increases the resistance of rice to sheath blight disease (ShB) via the activation of PIN-FORMED 1a (PIN1a).

Results: In this study, we determined that Rhizoctonia solani infection significantly induced LPA1 expression in the leaves, and lpa1 was more susceptible to R. solani compared with the wild-type and revertant plants. In addition, infection with R. solani altered the expression of IDD3, IDD5, IDD10, and IDD13, and yeast two-hybrid, split-GFP, and coimmunoprecipitation assays showed that LPA1 interacts with IDD3 and IDD13. IDD13 RNAi plants were more susceptible, while IDD13 overexpressors were less susceptible to ShB compared with the wild-type. In parallel, idd3 exhibited no significant differences, while IDD3 overexpressors were more susceptible compared to the wild-type response to ShB. Additional chromatin-immunoprecipitation and electrophoretic mobility shift assay experiments indicated that IDD13 and IDD3 bound to the PIN1a promoter, and the transient assay indicated that IDD13 and IDD3 positively and negatively regulate PIN1a expression, respectively. Moreover, IDD13, IDD3, and LPA1 form a transcription factor complex that regulates PIN1a. A genetic study showed that the LPA1 repressor lines were similar to lpa1/IDD13 RNAi and were more susceptible than the lpa1 and IDD13 RNAi plants in response to ShB. The overexpression of IDD13 increased resistance to ShB in the idd1 background.

Conclusions: Taken together, our analyses established that IDD3, IDD13, and LPA1 form a transcription factor complex to regulate the defense of rice against ShB possibly via the regulation of PIN1a.

Keywords: Indeterminate domain protein, Sheath blight disease, Transcription activation, Defense, Rice

Background

Sheath blight disease (ShB) is one of the three major diseases that are caused by Rhizoctonia solani in rice (Oryza sativa) (Savary et al., 1995). The fungus damages rice during the whole period of the growth cycle and primarily infects the leaves, sheaths, and panicles. At the late stage of infection, the whole plant withers and lodges (Savary et al., 1995). ShB can reduce rice yield production up to 50% when the disease is severe (Savary et al. 2000). Since there is a lack of resistant cultivars against ShB, the application of fungicides is the current primary approach to control this disease (Savary et al. 2000). However, its use severely influences environmental conditions because of its effect on microbes in the environment, and the fungicides also increase the cost of cultivation. Thus, the isolation of resistant rice cultivars and the exploration of defense mechanisms against ShB have become an important issue. Previous studies have demonstrated that the overexpression of chitinase, β-1,3-glucanase, or polygalacturonase-inhibiting protein (OsPGIP1) enhances the resistance of rice to R. solani (Shah et al. 2009; Mao et al. 2014; Wang et al. 2015). The overexpression of an ethylene synthesis enzyme (OsACS2) promotes the resistance of rice to blast and sheath blight (Helliwell et al. 2013). The overexpression of BROAD-SPECTRUM RESISTANCE2 (BSR2) resulted in resistance to R. solani in Arabidopsis and rice (Maeda et al. 2019), and salicylic acid-dependent immunity contributes to resistance against R. solani in rice and Brachypodium.
distachyon (Kouzai et al. 2018). In addition, our recent studies identified that a mutation in Sugar Will be Eventually be Exported Transporter 11 (SWEET11) significantly promoted the defense of rice to ShB (Gao et al. 2018); related to ABI3/VP1-Like 1 (RAVL1) modulates rice defense against ShB via the activation of brassinosteroids and ethylene signaling genes (Yuan et al. 2018), and the overexpression of LPA1 (IDD14) promoted the defense of rice against ShB via the activation of PIN1a (Sun et al. 2019).

The indeterminate domain (IDD) consists of two C2H2 and two C3HC zinc finger motifs, and the IDD genes play diverse biological functions in plants. ID1 has been reported to control the flowering time in maize and rice (Colasanti et al. 1998; Park et al. 2008). Magpie (MAG)/AtIDD3 and jackdaw (JKD)/AtIDD10 regulate the fate of root cells (Welch et al. 2007). Enhydrous (ENY)/AtIDD1 regulates seed maturation (Feurtado et al. 2011). AtIDD8 modulates plant development (Seo et al. 2011). AtIDD14, AtIDD15, and AtIDD16 cooperatively regulate lateral organ morphogenesis and gravitropism by promoting auxin biosynthesis and transport in Arabidopsis (Cui et al. 2013). Loose plant architecture1 (LPA1)/IDD14 regulates shoot gravitropism and lamina joint angle (Wu et al. 2013; Liu et al. 2016). The regulator of CBF1 (ROCI)/IDD3 activates DREB1B/CBF1 to regulate chilling tolerance in rice (Dou et al. 2016). IDD2 regulates secondary cell wall formation in rice (Huang et al. 2018). In addition, the AtIDD4 repressor constitutively induces immunity in Arabidopsis (Volz et al. 2019). The binding motifs of the transcription factor IDD have been identified in maize (ID1, 5′-TTTGTCG/CTTTT-3′), Arabidopsis (AtIDD8, 5′-TTTTGTCC-3′), and rice (IDD10, 5′-TTTTGTCCG/3′) (Kozaki et al. 2004; Seo et al. 2011; Xuan et al. 2013). However, the function of IDD in plant defense, as well as the IDD target genes, remains largely unknown.

Auxin is one of the key phytohormones, and its polar transport is regulated by auxin influx AUX1/LAX and efflux protein PINs (Adamowski and Friml, 2015; Zazimalova et al. 2010). Auxin plays key roles in plant growth and development, as well as in controlling plant defense (Robert-Seilanianz et al. 2011; Naseem et al. 2012; Chen et al. 2007). More studies identified that auxin signaling regulates rice defense against the bacterial pathogen Xanthomonas oryzae (Fu et al. 2011) and the fungal pathogen Magnaporthe oryzae (Fu et al. 2011). Recently, we identified that exogenously treated auxin increases the resistance of rice to R. solani AG1-IA and revealed that LPA1 overexpression activates PIN1a to promote defense against R. solani in rice (Sun et al. 2019). However, whether other IDDs regulate the resistance of rice to ShB remains to be elucidated. In this study, we performed molecular, biochemical, and genetic studies to explore the function of IDD in rice defense. The results showed that IDD3 and IDD13 interact with LPA1 to regulate PIN1a expression and act to modulate the resistance of rice to ShB. Taken together, our analyses provide information on the role of the IDDs in the regulation of rice defense, as well as the regulatory mechanism for ShB in rice.

**Results**

**LPA1 Is Induced by Rhizoctonia solani, and lpa1 Is more Susceptible to Sheath Blight Disease**

Previously we demonstrated that the overexpression of LPA1 significantly promotes the resistance of rice to ShB via the activation of PIN1a (Sun et al. 2019). However, previous research indicated that LPA1 was expressed at very low levels in the leaves and sheath (Wu et al. 2013). Therefore, we analyzed the R. solani infection-dependent LPA1 expression in more detail. Interestingly, infection with R. solani significantly induced LPA1 expression after 72 h (Fig. 1a). Examination of the response of lpa1, LPA1 revertant (Rev.), and wild-type (WT) plants (Liu et al. 2016) showed that lpa1 was more susceptible to R. solani AG1-IA than the WT and revertant (Rev.) plants (Fig. 1b). The percentage of the leaf area covered with lesions was 41% in the WT, 56% in lpa1, and 39% in Rev. (Fig. 1c). Since LPA1 activates PIN1a transcription, the PIN1a expression level was examined in the wild-type, lpa1, and Rev. plants before and after inoculation with R. solani AG1-IA. The qRT-PCR results showed that PIN1a was induced by R. solani AG1-IA inoculation, and the level was lower in lpa1 than in the wild-type and Rev. plant leaves 72 h post-R. solani inoculation, but there were no significant differences without the inoculation (Fig. 1d).

**IDD13 and IDD3 Interact with LPA1**

Our transcriptome study discerned that several IDD genes, including IDD3, IDD5, IDD10, and IDD13, were differentially expressed upon R. solani infection (unpublished data). To verify the transcriptome data, qRT-PCR was performed to examine the expression of the IDD gene. The results showed that IDD5 was suppressed, while IDD3, IDD10, and IDD13 were induced by R. solani (Fig. 2a). To test whether IDD3, IDD10, or IDD13 interact with LPA1, yeast two-hybrid, split-GFP, and co-immunoprecipitation (co-IP) assays were performed. A yeast-two hybrid analysis indicated that LPA1 interacts with IDD3 and IDD13 but not with IDD10 (Fig. 2b). An additional split-GFP assay showed that LPA1 interacts with IDD3 or IDD13 at the nucleus in N. benthamiana leaves, but the negative control (LPA1-nYFP+cCFP) did not exhibit a visible signal (Fig. 2c). For the co-IP assay, LPA1-GFP was co-expressed with IDD3-Myc, IDD3-Myc or IDD10-Myc in N. benthamiana leaves, and the total proteins were immunoprecipitated using an anti-GFP antibody. The immunoprecipitated proteins were analyzed using an anti-Myc antibody. The results indicated that LPA1 interacts with IDD3 and IDD13 but not IDD10 in plants, and the interaction affinity in LPA1-IDD13 was higher than that in LPA1-IDD3 (Fig. 2d).
IDD3 Negatively and IDD13 Positively Regulated Resistance to Sheath Blight Disease

To analyze the function of IDD3 and IDD13 in response to ShB, idd3 mutants (idd3–1 and idd3–2), IDD3 over-expressors (OX), IDD13 RNAi, and IDD13 OX plants were tested. Before examining their response to ShB, the levels of expression of IDD3 and IDD13 were analyzed. The qRT-PCR results showed that the IDD3 transcript was not detected in two idd3 knock-out mutants (idd3–1 and idd3–2), while IDD3 was highly expressed in the IDD3 OX plants (#2, #3, #4, and #6) compared with the wild-type control (Fig. 3a). In addition, IDD13 was significantly suppressed in the IDD13 RNAi lines (#1, #2, #4, and #5), while it was obviously highly expressed in the IDD13 OX plants (#2, #3, #5, and #7) compared with the wild-type control (Fig. 3b). An additional R. solani infection test showed that the idd3 mutants were similar to the wild-type control and displayed a susceptible response to R. solani AG1-1A, but IDD3 OX exhibited more susceptible symptoms than those in the wild-type plants. The percentage of the leaf area covered with lesions was 41% in WT, 42% in idd3–1, 40.5% in idd3–2, 54.5% in IDD3 OX #2, and 56% in IDD3 OX #4 plants (Fig. 3c and d). In addition, the R. solani infection results indicated that the IDDI3 RNAi plants were more susceptible, while IDD13 OX plants were less susceptible to ShB than the wild-type control. The percentage of leaf area covered with lesions was 39% in the WT, 48% in IDDI3 RNAi #1, 49% in IDDI3 RNAi #4, 31% in IDDI3 OX #2, and 30% in IDDI3 OX #5 plants (Fig. 3e and f).

IDD3 and IDD13 Directly Regulate PIN1a Transcription

LPA1 promotes the resistance of rice to ShB via the activation of PIN1a (Sun et al., 2019), and IDD3 and IDD13 interact with LPA1 to regulate the resistance to ShB. Therefore, we tested the potential of IDD3 and IDD13 to bind to the PIN1a promoter in more detail using a chromatin immunoprecipitation (ChIP) assay. Before performing the ChIP assay, the IDD3-GFP and IDD13-GFP localization in the transgenic plants was evaluated. The GFP signal was detected in the nucleus of IDD3-GFP and IDD13-GFP transgenic lateral roots (Fig. 4a). In the PIN1a promoter region, a single IDD-binding motif was identified (Fig. 4b). To examine whether IDD3 and IDD13 bind to the IDD-binding motif, a ChIP assay was performed using 35S:IDD3:GFP or 35S:IDD13:GFP transgenic plant calli and an anti-GFP antibody. The samples without the application of the GFP antibody (−Ab) were used as the control for the GFP antibody (+Ab) to immunoprecipitate the DNA. The ChIP-PCR results showed that IDD3 and IDD13 bound to the P2 but not to the P1 (Fig. 4c). An electrophoretic mobility shift assay (EMSA) was performed to verify that IDD3 and IDD13 bound to the P2 fragment. The results indicated that IDD3 and IDD13 bound to P2, but the complex failed to bind to the mutated putative IDD-binding motif (TTTGTGG mutated to AAAAAAA) mP2 (Fig. 4d). To verify
the IDD3 and IDD13 activation of PIN1a via binding to the P2 region in the promoter, transient expression assays were conducted using the protoplast system. Protoplast cells were co-transformed with the 3SS:IDD3 or 3SS:IDD13 plasmid and the construct expressing the β-glucuronidase gene (GUS) under the control of pPIN1a or mpPIN1a. In the mutated promoter (mpPIN1a), the IDD-binding motif sequences TTTGTCG were replaced with AAAAAAAA. Protoplast cells
expressing IDD13 had approximately twice the levels of activated pPIN1a. However, IDD13 was unable to activate mpPIN1a. In parallel, IDD3 suppressed pPIN1a by approximately one third but did not affect mpPIN1a (Fig. 4e).

In addition, the PIN1a expression level was examined in the idd3 mutants and IDD3 OX, as well as in the IDD13 RNAi and IDD13 OX plants. The qRT-PCR results showed that the PIN1a level was obviously lower in IDD3 OX than in the wild-type and idd3 mutants, but there were no significant differences between the wild-type and idd3 mutants (Additional file 1: Figure S1a). Moreover, the PIN1a level was slightly lower in the IDD13 RNAi plants, while it was significantly higher in the IDD13 OX plants than in the wild-type control (Additional file 1: Figure S1b).

IDD3 Inhibits the LPA1-Mediated Activation of PIN1a Expression

IDD3 and IDD13 interact with LPA1, and IDD13 and LPA1 directly activate PIN1a transcription, while IDD3 suppresses it. Therefore, the effects of IDD3 on IDD13 and LPA1 regulation on PIN1a expression were examined. To verify the effect, 3SS:LPA1 was co-transformed with 3SS:IDD13 or 3SS:IDD3 and a vector expressing GLU1 under the control of pPIN1a. The results indicated that IDD13 and LPA1 activated pPIN1a, while IDD3 suppressed pPIN1a. In addition, co-expressing IDD13 and LPA1 increased the activation of pPIN1a compared to expressing the single IDD protein. However, the expression of IDD3 inhibited the LPA1 activation of pPIN1a (Fig. 5a). In addition, the possibility that IDD3, IDD13, and LPA1 form a transcriptional complex was tested. IDD3-HA and IDD13-Myc were expressed in N. benthamiana leaves and immunoprecipitated using an anti-HA antibody, but the western blot results indicated that IDD3 did not interact with IDD13 (Fig. 5b). Additional IDD3-HA, IDD13-Myc, and LPA1-GFP proteins were expressed in N. benthamiana leaves, and the total protein was immunoprecipitated with an anti-HA antibody. The Co-IP results showed that IDD3, IDD13, and LPA1 form a transcriptional complex in plants (Fig. 5b).

IDD13 Additively Functions with LPA1 in the Regulation of Resistance to Sheath Blight Disease

The IDD13 RNAi and lpa1 mutants were more susceptible to ShB, while the idd3 mutants exhibited no significant differences compared to the wild-type control, suggesting that IDD13 and LPA1 but not IDD3 might play a major role in the resistance of rice to ShB. To analyze whether IDD13 and LPA1 are functionally additive in the regulation of the resistance of rice to ShB, two genetic combinations were generated,
Fig. 4 IDD3 and IDD13 bind and activate the PIN1a promoter. a IDD3-GFP and IDD13-GFP were detected in the lateral roots. GFP signal and bright field are shown in the left and right, respectively. Bars = 20 μm. b Schematic diagram indicating the location of the putative IDD-binding motif (gray circle) within 1.5 kb of the PIN1a promoter and the probes (P) used for chromatin immunoprecipitation (ChIP) assays. c Relative ratios of immunoprecipitated DNA to input DNA were determined by qPCR. Input DNA was used to normalize the data. –Ab or + Ab: green fluorescent protein (GFP) antibody. Error bars represent the mean ± SE (n = 3). d An electrophoretic mobility-shift assay (EMSA) was conducted to evaluate GST-IDD3 and GST-IDD13 affinities to P2 and mutated probe mP2. e A transient expression assay was conducted by co-transfection with p35S:IDD3 or p35S:IDD13 and each of the vectors expressing GUS under the control of native (pPIN1a) and IDD-binding motif-mutated (mpPIN1a) PIN1a promoters in protoplast cells. The luciferase gene driven by the 35S promoter was used as an internal control to normalize GUS expression. Error bars represent the mean ± SE (n = 6). Different letters indicate significant differences at P < 0.05.

Fig. 5 IDD3, IDD13, and LPA1 form a complex to regulate PIN1a transcription. a A transient expression assay was conducted by co-transfection with p35S:IDD3, p35S:IDD13, p35S:LPA1, p35S:IDD3 + p35S:LPA1, p35S:IDD13 + p35S:LPA1 and the vector expressing the GUS under the control of native (pPIN1a) PIN1a promoters in protoplast cells. The luciferase gene driven by the 35S promoter was used as an internal control to normalize the GUS expression. Error bars represent the mean ± SE (n = 6). Different letters indicate significant differences at P < 0.05. b IDD3-HA + IDD13-Myc, IDD3-HA + IDD13-Myc + LPA1-GFP, or IDD3-HA + empty vector were transformed into tobacco leaves using Agrobacterium-mediated transformation. HA antibody-immunoprecipitated proteins were analyzed using western blot analysis with the Myc antibody. IDD3-HA, IDD13-Myc, and LPA1-GFP levels were analyzed by a western blot using HA, Myc, and GFP antibodies, respectively.
including IDD13 RNAi/lpa1 and lpa1/IDD13 OX. In addition, LPA1 repressor lines were examined (Wu et al. 2013, Liu et al. 2016). An R. solani infection test showed that IDD13 RNAi/lpa1 was more susceptible than the IDD13 RNAi, lpa1, and wild-type plants segregated from the same sibling, and IDD13 RNAi/lpa1 exhibited similar susceptible symptoms to the LPA1 repressor (Fig. 6a). The percentage of the leaf area covered with lesions was 41% in the WT, 51% in IDD13 RNAi, 54% in lpa1, 63% in IDD13 RNAi/lpa1, and 61.5% in the LPA1 repressor plants (Fig. 6b). In addition, R. solani infection results indicated that the lpa1/IDD13 OX plants were less susceptible to ShB than the lpa1 mutant and wild-type segregated from the same sibling, but they were more susceptible to ShB compared to the IDD13 OX plants segregated from the same sibling (Fig. 6c). The percentage of leaf area covered with lesions was 40.5% in WT, 53% in lpa1, 32% in IDD13 OX, and 37.5% in lpa1/IDD13 OX plants (Fig. 6d).

In addition, the level of expression of PIN1a was examined in the IDD13 RNAi/lpa1, lpa1/IDD13 OX, and LPA1 repressor lines. The qRT-PCR results showed that the PIN1a level was much lower in IDD13 RNAi/lpa1 than in the wild-type, IDD13 RNAi, and lpa1 and was similar between IDD13 RNAi/lpa1 and the LPA1 repressor after R. solani inoculation (Additional file 2: Figure S2a). In parallel, the PIN1a level was higher in lpa1/IDD13 OX than in lpa1 and higher than in the wild-type plants. The PIN1a level was noticeably higher in IDD13 OX than in the wild-type and lpa1/IDD13 OX after R. solani inoculation (Additional file 2: Figure S2b).

**IDD13 Overexpression Maintained Yield Production in Rice**

Since the IDD13 OX plants demonstrated increased resistance to ShB, yield factors were investigated further. The results demonstrated that IDD13 OX plants developed a similar tiller number, thousand-grain weight, and number of spikelets per panicle relative to the WT, but the overexpression of IDD13 slightly decreased the tiller angle compared with the wild-type (WT) plants (Fig. 7). LPA1 overexpression increased the content of 3-indole acetic acid (IAA), a natural form of auxin, and exogenous IAA treatment promoted the resistance of rice to ShB (Sun et al. 2019). Therefore, the endogenous IAA

**Fig. 6** IDD13 and LPA1 genetic combinations in response to sheath blight (Rhizoctonia solani). a Response of IDD13 RNAi, lpa1, IDD13 RNAi/lpa1, LPA1 repressor plants to R. solani AG1-IA compared with the wild-type (WT). b Percentage of the leaf area covered with lesions in the lines shown in (a). Data represent the means ± SE (n > 10). c Response of the lpa1, IDD13 OX, and lpa1/IDD13 OX plants to R. solani AG1-IA compared with the wild-type (WT). d Percentage of the leaf area covered with lesions in the lines shown in (c). Data represent the means ± SE (n > 10). Different letters indicate significant differences at P < 0.05.
levels in the WT, IDD13 OX2, and IDD13 OX5 plants were measured. The data demonstrated that IDD13 overexpressors contain higher levels of IAA than that of the WT plant leaves (Additional file 3: Figure S3).

Discussion
Sheath blight disease caused by *R. solani* is a major rice disease, which severely reduces grain yield. However, the host resistance mechanisms remain unknown. Previously, we identified that exogenous auxin treatment promoted resistance to ShB, and the overexpression of *LPA1/IDD14* promoted rice defense to ShB via the activation of the auxin poplar transporter *PIN1a* in rice (Sun et al. 2019). *PIN1a* RNAi and the *PIN1a* overexpressors were more and less susceptible to ShB, respectively (Sun et al. 2019), suggesting that LPA1 increases the local auxin content or the activation of auxin signaling by controlling *PIN1a* and enhancing the resistance of the rice to ShB. However, whether IDD proteins other than LPA1 regulate the resistance of rice remains unclear.

**IDDs Were Induced by *R. solani*, and LPA1 Interacts with IDD3 and IDD13**

The transcriptome analysis and additional qPCR verification showed that *IDD3, IDD10, IDD13*, and *LPA1* were up-regulated, while *IDD5* was down-regulated by *R. solani* in rice. In normal conditions, *LPA1* is barely expressed in the leaves and sheath of rice (Wu et al. 2013), but *R. solani* infection significantly induced the level of expression of *LPA1* in the leaves. Additional genetic study showed that the *lpa1* and *IDD13* RNAi mutant were more susceptible, but the *idd3* mutants exhibited a similar response to ShB compared with the wild-type. In addition, the overexpression of *IDD13* produced results similar to those of *LPA1*, whereas the overexpression of *IDD3* inhibited the resistance of rice to ShB, indicating that rice defense against ShB requires *LPA1* and *IDD13*, and *IDD3* negatively regulates the defense of rice to ShB. Since AtIDD15 functions in concert with AtIDD14 and AtIDD16 to directly activate auxin biosynthesis and transport-related genes in Arabidopsis (Cui et al. 2013), this suggests that IDD proteins are functionally additive in the regulation of auxin biosynthesis. Further biochemical and molecular assays identified that LPA1 interacts with IDD3 and IDD13, and the interaction affinity of LPA1 was higher with IDD13 than with IDD3. However, IDD3 did not directly interact with IDD13, while LPA1, IDD3, and IDD13 form a transcriptional complex, suggesting that these three IDDs may form a transcriptional complex to regulate the resistance of rice to ShB.

**IDD13 Positively and IDD3 Negatively Regulate *PIN1a***

*LPA1* and IDD10 were reported to localize to the nucleus and function as transcription factors (Wu et al. 2013; Xuan et al. 2013), and *IDD3-GFP* and *IDD13-GFP* were localized to the nucleus in the transgenic rice roots. Previously, we identified that *PIN1a* is a direct target of *LPA1*, which positively regulates the resistance of rice to ShB (Sun et al. 2019). Since LPA1 interacts with IDD3 and IDD13, the roles of IDD3 and IDD13 in the regulation of *PIN1a* transcription were analyzed. Additional ChIP and EMAS assays showed that IDD13 and IDD3 directly bound to the putative IDD-binding motif in the *PIN1a* promoter region. In addition, a transient assay
revealed that IDD13 acted similarly to LPA1 to directly activate PIN1a, but IDD3 directly bound to the PIN1a promoter and functioned as a transcriptional repressor. The transient assay results showed that the expression of IDD13 and LPA1 activated the level of transcription of PIN1a, while the expression of IDD3 suppressed the level of expression of PIN1a, suggesting that IDD13 and LPA1 function as transcriptional activators, while IDD3 functions as a transcriptional repressor to PIN1a.

The overexpression of LPA1 reduced the tiller angle and increased the contents of IAA in the leaves via the activation of PIN1a (Sun et al. 2019). IDD13 activates PIN1a, and the additional investigation of yield factors identified that the overexpression of IDD13 maintained yield production, while reducing the tiller angle compared with the wild-type plants. In addition, the IDD13 OX plants accumulated higher contents of IAA than were found in the wild-type plant leaves, suggesting that IDD13 OX acts similarly to the LPA1 OX plants to increase the resistance of rice to ShB by activating PIN1a without affecting rice production. However, the tiller angle of the LPA1 OX plants is smaller than that in the IDD13 OX plants, implying a dominant regulation of LPA1 compared with IDD13 in the transcriptional activation of PIN1a.

IDD13 and LPA1 Are Functionally Additive in the Regulation of the Resistance of Rice to Sheath Blight Disease

IDD3, IDD13, and LPA1 physically interact with and differentially regulate PIN1a. In addition, LPA1 positively regulates the resistance of rice to ShB. Next, a genetic study was performed to analyze the functions of IDD3 and IDD13 in the control of the resistance of rice to ShB. An R. solani infection assay indicated that the two idd3 mutants exhibited a similar susceptible response to ShB compared with the wild-type control. In addition, the level of expression of PIN1a was not changed in the idd3 mutants, which was similar to its expression in the wild-type plants. The overexpression of IDD3 significantly suppressed the PIN1a level compared with the wild-type plants, and IDD3 OX exhibited more susceptible symptoms to R. solani infection than the wild-type plants. The IDD13 RNAi plants were more susceptible to ShB, while the IDD13 OX plants were less susceptible compared with the wild-type control. Further genetic and pathology experiments indicated that an IDD13 RNAi/lpa1 double mutant was more susceptible to ShB compared with IDD13 RNAi and lpa1. In addition, the response of the LPA1 repressor plants to ShB was compared with that of IDD13 RNAi/lpa1. The results showed that expressing the LPA1 repressor to inhibit the transcription complex, including LPA1, produced a similar defect in response to ShB when compared with IDD13 RNAi/lpa1, and showed more susceptible symptom than in lpa1 and IDD13 RNAi, suggesting that IDD13 and LPA1 might be functionally additive. In parallel, the lpa1/IDD13 OX double mutants were more susceptible to ShB compared with the IDD13 OX plants, but they were less susceptible to ShB compared to lpa1 and the wild-type control, suggesting that IDD13 OX can partially rescue the defect from the LPA1 mutation in response to ShB. Additional expression level analyses indicated that the level of PIN1a was much lower in IDD13 RNAi/lpa1 or the LPA1 repressor than in IDD13 RNAi and lpa1, while it was higher in lpa1/IDD13 OX than in lpa1. These results suggest that IDD13 and LPA1 might be functionally additive in the regulation of the resistance of rice to ShB via the activation of PIN1a expression in rice.

Overall, this study identified a new IDD transcriptional complex and identified its function in the regulation of ShB via the regulation of PIN1a transcription. These results will broaden our understanding of the regulatory mechanism by which the IDDs regulate auxin transport and the resistance of rice to ShB.

Methods

Plant Growth and R. solani AG1-IA Inoculation

Wild-type (WT) control line (Oryza sativa Japonica, cultivar Dongjin), lpa1, LPA1 revertant (Rev.), IDD13 RNAi, IDD13-GFP overexpressor (IDD13 OX), idd3–1 (PFG_3A-09378), idd3–2 (PFG_3A-14,411), IDD3-GFP overexpressor (IDD3 OX), lpa1/IDD13 RNAi, lpa1/IDD13 OX, and LPA1 repressor plants were used. The plants were grown in a greenhouse at Shenyang Agricultural University, China, with a temperature of 23 °C–30 °C. One-month-old rice plants were inoculated with R. solani AG1-IA (Prasad and Eizenga, 2008). In brief, a 10-cm-long piece was cut from the second youngest leaf of the main tiller and placed on moistened filter paper in a Petri dish (diameter, 36 cm; height, 2.5 cm). Each replicate comprised six leaves, and four replicates per line were used in a completely randomized design. Colonized potato dextrose agar (PDA) blocks (diameter, 7 mm) were excised using a circular cutter and placed on the abaxial surface of each leaf piece. The leaves were incubated at 25 °C for 72 h in a chamber with continuous...
light. The filter paper was kept moist with sterile water. After 72 h, the length and width of the lesions within each leaf piece were measured using Image J Fiji software (NIH, Bethesda, MD, USA) and the approximate percentage of the leaf covered with lesions was calculated as previously described (Prasad and Eizenga, 2008; Eizenga et al. 2002). To analyze the *R. solani* AG1-IA infection-mediated expression of the *IDD* genes, one-month-old wild-type plants were inoculated with *R. solani* AG1-1A, and their leaves were sampled after 0, 24, 48, and 72 h of inoculation. The accession numbers in Genbank are as follows: IDD3 (EEC85036), IDD5 (XP_015647948), IDD10 (KAB8096499), IDD13 (XP_015610838), and LPA1 (IDD14) (XP_015629419).

**RNA Extraction and Quantitative Real-Time (qRT)-PCR Analysis**

Total RNA was isolated from the one-month-old rice leaves using the TRIzol reagent (Takara, Dalian, China), and the genomic DNA was removed by treatment with RQ-RNase free DNase (Promega, Madison, WI, USA). Complementary DNA was synthesized using the GoScript Reverse Transcription Kit (Promega) following the manufacturer’s instructions. A BIO-RAD CFX96 Real-time PCR system (Bio-Rad, Hercules, CA, USA) and SYBR-Green (Takara) were used for the qRT-PCR analyses. The gene expression levels were normalized to that of the level of *Ubiquitin*. The primers used for qRT-PCR are listed in Additional file 4: Table S1.

**Plasmid Construction**

To generate *IDD3-GFP* and *IDD13-GFP* overexpression transgenic plants, *IDD3* and *IDD13* ORF sequences were amplified and cloned into BglII and *Spe* I restriction enzyme sites of the pCAMBIA1302 binary vector, in which *IDD3* or *IDD13* coding sequences were N-terminally fused to the *GFP* coding sequences. To generate *IDD13 RNAi* plants, 300 bp of the *IDD13* coding region was cloned into *Swa* I and *Ascl* sites in the sense and *Xba* I and *BamH* I sites in the antisense orientation, respectively, in the pFGC5941 binary vector (ChromDB).

**Yeast Two-Hybrid Assay**

To test the interaction between *LPA1* and *IDD13*, *IDD3* or *IDD10* ORFs were cloned into the pGAD424 vector. The pair of *IDDs* was further transformed in the yeast strain P69-4A (Clontech, http://www.clontech.com/). Yeast cells carrying a pair of *IDDs* were grown on SD/Trp-/Leu- and SD/Trp-/Leu-His- plates. The sequences of the primers for cloning the *IDD13* ORF are listed in Additional file 3: Table S1.

**Split GFP Assay**

The N-proximal half of *YFP* (*nYFP*) and the C-proximal half of *CFP* (*cCFP*) sequences were fused to the C-terminal sequences of *LPA1* (IDD14) and C-terminal sequences of *IDD3* or *IDD13* in the pXNGW and pXCGW vectors, respectively. Agrobacterium cells (GV3101) harboring half of the *YFP* parts were mixed and then infiltrated into *Nicotiana benthamiana* leaves. Before observing the *YFP* signal using a confocal microscope (Olympus X1000, Japan), the tobacco plants were grown in a growth chamber for 36 to 48 h (Kim et al. 2009a).

**Co-Immunoprecipitation (co-IP) and Western Blot Analyses**

*IDD3-Myc + LPA1-GFP*, *IDD13-Myc + LPA1-GFP*, *IDD10-Myc + LPA1-GFP*, *IDD3-HA + IDD13-Myc*, or *IDD3-HA + IDD13-Myc + LPA1-GFP* were coexpressed in *N. benthamiana* leaves, respectively. After 36 h of expression, the protein was extracted, and Co-IP assays were performed as described previously (Kim et al. 2009b). Twenty micrograms of protein from each sample were separated on a 10% SDS-PAGE gel and electro-transferred onto Immobilon-P Transfer Membranes (MILLIPORE JAPAN, Tokyo, Japan). For the subsequent western blot analysis, the following primary antibodies were used: an anti-HA antibody (1:2000; Abcam, Cambridge, MA, USA), anti-GFP antibody (1:2000; Abcam), and anti-Myc antibody (1:2000; Abcam). The membranes were incubated for an additional hour with an anti-mouse or anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000; Cell Signaling Technology, Danvers, MA, USA) before the signal was detected using an ECL Western Blotting Detection System (GE Healthcare, Piscataway, NJ, USA).

**Chromatin-Immunoprecipitation (ChIP) Assay**

Eight grams of rice calli were collected from transgenic plants expressing 3SS:*IDD13-GFP* and 3SS:*IDD3-GFP* for the ChIP assay. The ChIP assay and subsequent ChIP-PCR assays were followed by a protocol described previously (Je et al. 2010). The primers used for the ChIP-PCR are listed in Additional file 3: Table S1.

**Electrophoretic Mobility Shift Assay (EMSA)**

To produce *IDD13* and *IDD3* recombinant proteins, the open reading frame sequences of *IDD13* and *IDD3* were sub-cloned into the pGEX *SX-1* expression vector, and the resulting pGEX *SX-1-IDD13* and pGEX *SX-1-IDD3* plasmids were used to transform *Escherichia coli* strain BL21 DE3. Recombinant proteins were harvested after a 4 h treatment with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 28 °C. The EMSA was performed as previously described (Je et al. 2010). The primers used to obtain the EMSA probes are listed in Additional file 3: Table S1.
**Transient Expression Assay**
For the transient assay, the effector plasmids (35S:LPA1, 35S:IDD13, and 35S:IDD3) and reporter (pPIN1a or mutated promoter, mppPIN1a), as well as an internal control plasmid (35S:LLUC), were co-transformed into protoplast cells (Yamaguchi et al. 2010). The GUS activity analyses were performed as previously described (Xuan et al. 2013). The luciferase assay was performed using a Luciferase Assay Kit (Promega), and PEG-mediated transformation and luciferase activity assays were performed as previously described (Yoo et al. 2007). The primers used for the transient assay are listed in Additional file 3: Table S1.

**IAA Measurement**
The leaves from 1-month-old IDD13 OX2, IDD13 OX5, and wild-type plants were used for IAA extraction. IAA extraction and calculation methods were followed as described by Pan et al. (2010). IAA-[α, α-D2] was used as an internal standard of IAA in the experiments.

**Statistical Analyses**
Statistical analyses were performed using Prism 5.0 (GraphPad, San Diego, CA, USA). For multiple lines comparison, a one-way analysis of variance (ANOVA) was performed, followed by Bonferroni’s multiple comparison tests. Differences among the samples were considered significant at $P < 0.05$.

**Supplementary information**
Supplementary information accompanies this paper at https://doi.org/10.1186/s12284-020-0371-1.

**Additional file 1: Figure S1.** PIN1α expression in IDD3 and IDD13 mutants and overexpressors. (A) Relative expression of PIN1α in wild-type (WT), idd3-1, idd3-2, IDD OX #2, and IDD OX #4 plant leaves. (B) Relative expression of PIN1α in wild-type (WT), IDD13 RNAi (#1 and #4), IDD OX #2, and IDD OX #5 plant leaves. The mRNA levels of the samples were normalized to that of Ubiquitin mRNA. Data represent the means ± standard error (n = 3). The expression of PIN1α in the WT was defined as “1.” Different letters indicate significant differences at P < 0.05.

**Additional file 2: Figure S2.** PIN1α expression in LPA1 and IDD13 genetic combinations. (A) Relative expression of PIN1α in the wild-type (WT), IDD13 RNAi, lpa1, IDD13 RNAi/lpa1 and LPA1 repressor plant leaves after 72 h of Rhizoctonia solani inoculation. (B) Relative expression of PIN1α in the wild-type (WT), lpa1, IDD3 OX, and lpa1/IDD3 OX plant leaves after 72 h of R. solanii inoculation. The mRNA levels of the samples were normalized to that of Ubiquitin mRNA. Data represent the means ± standard error (n = 3). The expression of PIN1α in WT was defined as “1.” Different letters indicate significant differences at P < 0.05.

**Additional file 3: Figure S3.** Measurement of the IAA content in WT and IDD13 overexpressors. The contents of IAA from the leaves of 1-month-old WT and IDD13 OX lines (OX2 and OX5) were measured. Vertical bars indicate average values ± SE (n = 3). Different letters indicate significant differences at $P < 0.05$.

**Additional file 4: Table S1.** Primer sequences

**Abbreviations**
ChiP: Chromatin-Immunoprecipitation; DIC: Differential Interference Contrast; EMISA: Electrophoretic Mobility Shift Assay; IDD: Indeterminate Domain; LPA1: Loose Plant Architecture 1; OX: Overexpressor; PIN1a: Pin-Formed 1a; ShB: Sheath Blight Disease; WT: Wild Type

**Acknowledgements**
The authors would thank Dr. Chang-deok Han at Gyeongsang National University, Korea for providing rice seeds.

**Authors’ Contributions**
Q Sun, and YH Xuan designed the experiment and wrote the manuscript; Q Sun, DD Li, J Chu, DP Yuan, and X Han conducted the experiments and performed data analysis. S Li and LJ Zhong participated in material development, sample preparation and data analysis. Q Sun, X Han, and YH Xuan corrected the manuscript. All authors read and approved the final manuscript.

**Funding**
This work was supported by an initiative grant (880416008) from Shenyang Agricultural University, the Support Plan for Innovative Talents in Colleges and Universities of Liaoning Province (LR2017037), and National Key R&D Program of China (2016YFD0101004).

**Availability of Data and Materials**
The datasets supporting the conclusions of this article are provided within the article and its additional files.

**Ethics Approval and Consent to Participate**
Not applicable.

**Consent for Publication**
Not applicable.

**Competing Interests**
The authors declare that they have no competing interests.

**Author details**
1College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, China. 2Institute of Plant Protection, Liaoning Academy of Agricultural Sciences, Shenyang 110161, China. 3Shanxi Key Laboratory of Chinese Jujube, Yan'an University, Yan'an 716000, Shaanxi, China. 4College of Life Science, Yan'an University, Yan'an 716000, Shaanxi, China. 5Microbial Research Institute, Liaoning Academy of Agricultural Sciences, Chaoyang 122000, China. 6College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China.

**Received:** 24 October 2019 **Accepted:** 28 January 2020

**Published online:** 06 March 2020

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