The Systemic Acquired Resistance Regulator OsNPR1 Attenuates Growth by Repressing Auxin Signaling through Promoting IAA-Amido Synthase Expression[1[OPEN]]

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Systemic acquired resistance is a long-lasting and broad-spectrum disease resistance to pathogens. Our previous study demonstrated that overexpression of *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1* (*OsNPR1*), a master gene for systemic acquired resistance in rice (*Oryza sativa*), greatly enhanced resistance to bacterial blight caused by *Xanthomonas oryzae* pv *oryzae*. However, the growth and development of the *OsNPR1* overexpression (*OsNPR1*-OX) plants were restrained, and the mechanism remained elusive. In this study, we dissected the *OsNPR1*-induced growth inhibition. We found that the *OsNPR1*-OX lines displayed phenotypes mimicking auxin-defective mutants, with decreases in root system, seed number and weight, internode elongation, and tiller number. Whole-genome expression analysis revealed that genes related to the auxin metabolism and signaling pathway were differentially expressed between the *OsNPR1*-OX and wild-type plants. Consistently, the indole-3-acetic acid (IAA) content was decreased and the auxin distribution pattern was altered in *OsNPR1*-OX plants. Importantly, we found that some *GH3* family members, in particular *OsGH3.8* coding IAA-amido synthetase, were constitutively up-regulated in *OsNPR1*-OX plants. Decreased *OsGH3.8* expression by RNA interference could partially restore IAA level and largely rescue the restrained growth and development phenotypes but did not affect the disease resistance of *OsNPR1*-OX plants. Taken together, we revealed that *OsNPR1* affects rice growth and development by disrupting the auxin pathway at least partially through indirectly up-regulating *OsGH3.8* expression.

Rice (*Oryza sativa*) is a major staple food crop for more than half of the world's population. However, diseases are a major challenge to rice production. Thus, breeding for disease resistance has been a long-lasting goal of rice breeding programs. Application of major disease resistance (R) genes and quantitative trait loci has contributed greatly to increasing rice resistance against diverse pathogens (McDonald and Linde, 2002; Kou and Wang, 2010). However, due to the arms race between pathogens and plants, plants frequently lose their resistance against specific pathogen strains (Jones and Dangl, 2006; Dangl et al., 2013). Therefore, breeding for varieties with durable and broad-spectrum disease resistance is critical to sustainable agricultural development (Zhang, 2007).

Recent progress in plant defense signaling and rice functional genomics provides new approaches for developing rice varieties with broad-spectrum resistance. It is well recognized that during long-term coevolution between plant hosts and pathogens, plants have developed a complicated immune system composed of both preformed and inducible defense mechanisms (Jones and Dangl, 2006; Spoel and Dong, 2012). The preformed defense system includes physical and chemical barriers consisting of antimicrobial molecules, such as saponins and quinines (Bednarek and Osbourn, 2009). The inducible defense mechanisms mainly include

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X.L., D.-L.Y., and Z.H. conceived and designed the experiments; X.L., D.-L.Y., L.S., and B.M. performed the experiments; Q.L. conducted the microarray analysis; X.L., D.-L.Y., and Z.H. wrote the article.

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pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity. Pathogen-associated molecular pattern-triggered immunity offers nonspecific basal defense and controls the extent of pathogen proliferation, while effector-triggered immunity inhibits pathogen infection and growth in a more pronounced manner (Jones and Dangl, 2006). In addition to restricting pathogens locally, effector-triggered immunity also can initiate systemic acquired resistance (SAR) via the production of signaling molecules, such as salicylic acid (SA) and methyl salicylic acid (Fu and Dong, 2013; Shah and Zeier, 2013). The initiation of SAR leads to systemic expression of pathogenesis-related (PR) genes in the uninoculated distal tissues to protect the rest of the plant against future pathogen attacks. SAR not only provides broad-spectrum resistance against various pathogens but also lasts for days to weeks/months, possibly extending through the entire growing season (Fu and Dong, 2013). Because of the nature of its long-lasting and broad-spectrum resistance, SAR is promising for breeding disease-resistant crops through genetic modifications of the signaling pathway (Wally and Punja, 2010).

Over the past two decades, significant progress has been made in understanding the SAR signaling pathway. A milestone is the cloning of Arabidopsis (*Arabidopsis thaliana*) *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1* (*NPR1*; herein referred to as *AtNPR1*), also known as *NONIMMUNITY1*, a key regulator of SAR (Cao et al., 1994; Ryals et al., 1997). To date, *NPR1* orthologs have been characterized from many plant species, and overexpression of *AtNPR1* or other orthologs has resulted in increased broad-spectrum resistance to pathogens in a variety of plant species, which functions as a transcriptional coactivator to activate defense gene expression (Cao et al., 1998; Friedrich et al., 2001; Lin et al., 2004; Malnoy et al., 2007; Wally et al., 2009; Parkhi et al., 2010; Zhang et al., 2012). Similarly, the rice ortholog of *AtNPR1* (*OsNPR1*; also known as *NH1*) also has been cloned and functionally identified (Chern et al., 2005; Yuan et al., 2007). Therefore, *NPR1* provides a promising candidate for genetic engineering of broad-spectrum disease resistance in crops.

It is well documented that cross talk between defense and development fine-tunes disease resistance and growth, indicating the fitness cost of disease resistance (López et al., 2008; Spoel and Dong, 2008; Robert-Seilaniantz et al., 2011; Yang et al., 2013). In our previous work, we found that transgenic rice constitutively expressing *OsNPR1* greatly increased disease resistance to *Xanthomonas oryzae* pv *oryzae* (Xoo; Yuan et al., 2007). However, these *OsNPR1* overexpression lines (*OsNPR1*-OX) displayed a dwarf phenotype in the paddy field. Another group observed a similar dwarf phenotype of *OsNPR1/NH1* overexpression plants in a growth chamber (Chern et al., 2005). Apparently, this defect limits the application of *OsNPR1* in improving elite rice varieties for enhancing disease resistance. Therefore, it is necessary to dissect the underlying mechanism of *OsNPR1*-mediated growth retardation. In this study, we investigated the *OsNPR1*-induced dwarf phenotype and documented that *OsNPR1* regulates rice growth and development through repressing auxin signaling, including *OsGH3.8* activation, shedding light on the cross talk between the SA-mediated defense pathway and growth.

RESULTS

OsNPR1 Overexpression Inhibits Rice Growth and Development

We functionally characterized the rice ortholog *OsNPR1* and showed that *OsNPR1* overexpression increased disease resistance to *Xoo* in our previous study (Yuan et al., 2007). However, we observed that growth and development were strongly inhibited in the *OsNPR1*-OX transgenic plants. Compared with the wild-type Taipei 309 (TP309), the two representative *OsNPR1*-OX lines G312 and G316 were smaller in both aboveground and belowground morphology through their entire lifetimes, with decreases in plant height, tiller number (Fig. 1, A–C), root system (Fig. 1, D–F), and grain number and seed setting (Fig. 1, G–I). To determine the *OsNPR1* overexpression-mediated phenotypes, we screened for spontaneous transgene-silenced plants in a progeny population and found that transgene silencing occurred in some individuals in the progeny of the *OsNPR1*-OX line (Fig. 2A; Supplemental Fig. S1). Consequently, those transgene-silenced plants lost disease resistance against *Xoo* (Fig. 2, B and C). Simultaneously, the transgene-silenced plants also restored growth phenotypes (Fig. 2, D–F). The results further supported the hypothesis that *OsNPR1* overexpression was responsible for growth inhibition.

To further shape the effect of *OsNPR1* overexpression on morphological changes, we performed cellular observations with sections of the uppermost internodes and observed that cells were smaller and abnormally shaped in the *OsNPR1*-OX plants in comparison with the wild-type control (Fig. 3). Therefore, *OsNPR1* likely inhibited cell expansion when overexpressed.

OsNPR1 Overexpression Alters the Expression of Hormone-Related Genes

To investigate the mechanism of *OsNPR1*-mediated growth retardation, we first compared the global gene expression of *OsNPR1*-OX and wild-type plants at heading stage by microarray analysis using the Affymetrix oligonucleotide chip GeneChip Rice Genome Array with three biological replicates. Reproducible differentially expressed probe sets were selected (fold change ≥ 2 and $P < 0.05$) from total normalized data using Data Mining Tool version 3.0. A total of 895 genes showed differential expression between the two plant groups, of which 729 and 166 were up- and down-regulated, respectively (Supplemental Table S1). Functional classification of the genes was performed according to the Rice Genome Annotation Project Release 7.0 (Kawahara et al., 2013; http://rice.plantbiology.msu.edu). Microarray data

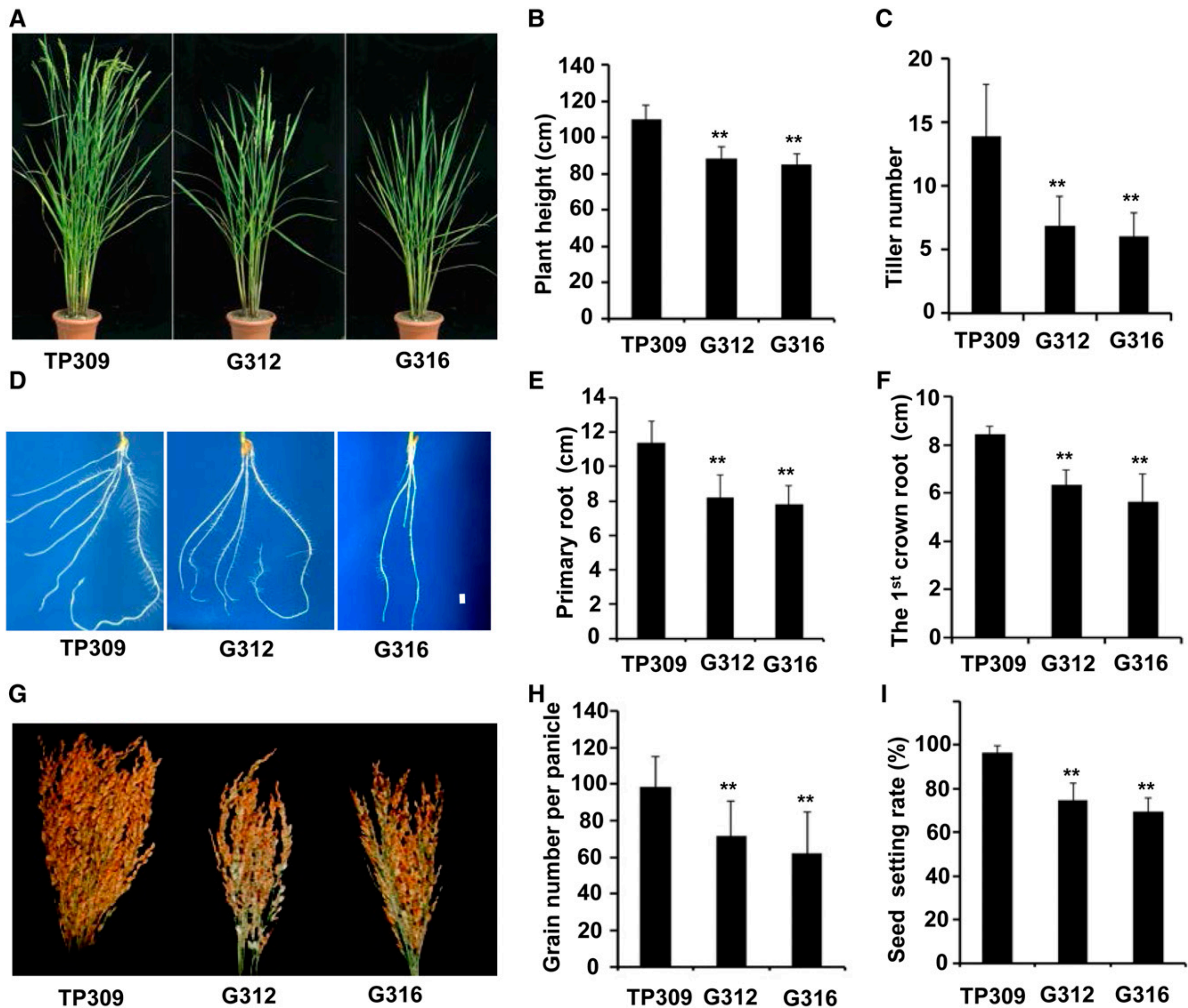

Figure 1. *OsNPR1* overexpression plants exhibited growth and developmental defects. A, Morphological phenotypes of adult *OsNPR1*-OX (G312 and G316) and wild-type (TP309) plants during the heading stage, grown at the Hainan station. Note that G312 and G316 were dwarf and delayed in flowering. B and C, Plant heights and tiller numbers of *OsNPR1*-OX and wild-type plants. Data shown are means ± SD ($n = 20$). D, Root architecture of *OsNPR1*-OX and wild-type plants. E and F, Lengths of primary (E) and lateral (F) roots. Root lengths were measured at 21 d after seed germination. Data shown are means ± SD ($n \geq 24$). G, Mature panicles of *OsNPR1*-OX and wild-type plants as the harvest of each plant. H and I, Grain number per panicle (H) and seed-setting rate (I) were decreased significantly in *OsNPR1*-OX plants compared with wild-type plants. Data were collected from all panicles of whole plants ($n \geq 5$). Asterisks indicate significant differences in comparison with the wild-type TP309 (**, $P < 0.01$, Student's *t* test).

were validated for some genes using qRT-PCR analysis (Supplemental Fig. S2).

A set of metabolism, transcriptional regulation, kinase, calcium signaling, and hormone genes are differentially expressed (Supplemental Table S1). Because plant hormones play vital roles in cross talk between defense and growth in rice (Yang et al., 2013) and those genes are likely related to diverse hormone pathways, we focused on the genes involved in hormone signaling pathways. We found that 20 genes involved in hormone signaling pathways that regulate development and growth, including auxin, cytokinin, brassinolides (BRs), and gibberellic acids (GAs), were differentially expressed in *OsNPR1*-OX plants compared with wild-type plants (Table I). Five differentially expressed genes related to cytokinin, including two putative cytokinin-*O*-glucosyl transferase genes (Os02g51930 and Os06g11720; Kawahara et al., 2013), were up-regulated. These genes encode enzymes that can convert active cytokinin to its *O*-glucoside (Pineda Rodo et al., 2008; Kudo et al., 2012). The other three cytokinin response genes (Os02g35180, Os04g36070, and Os11g04720; Jain

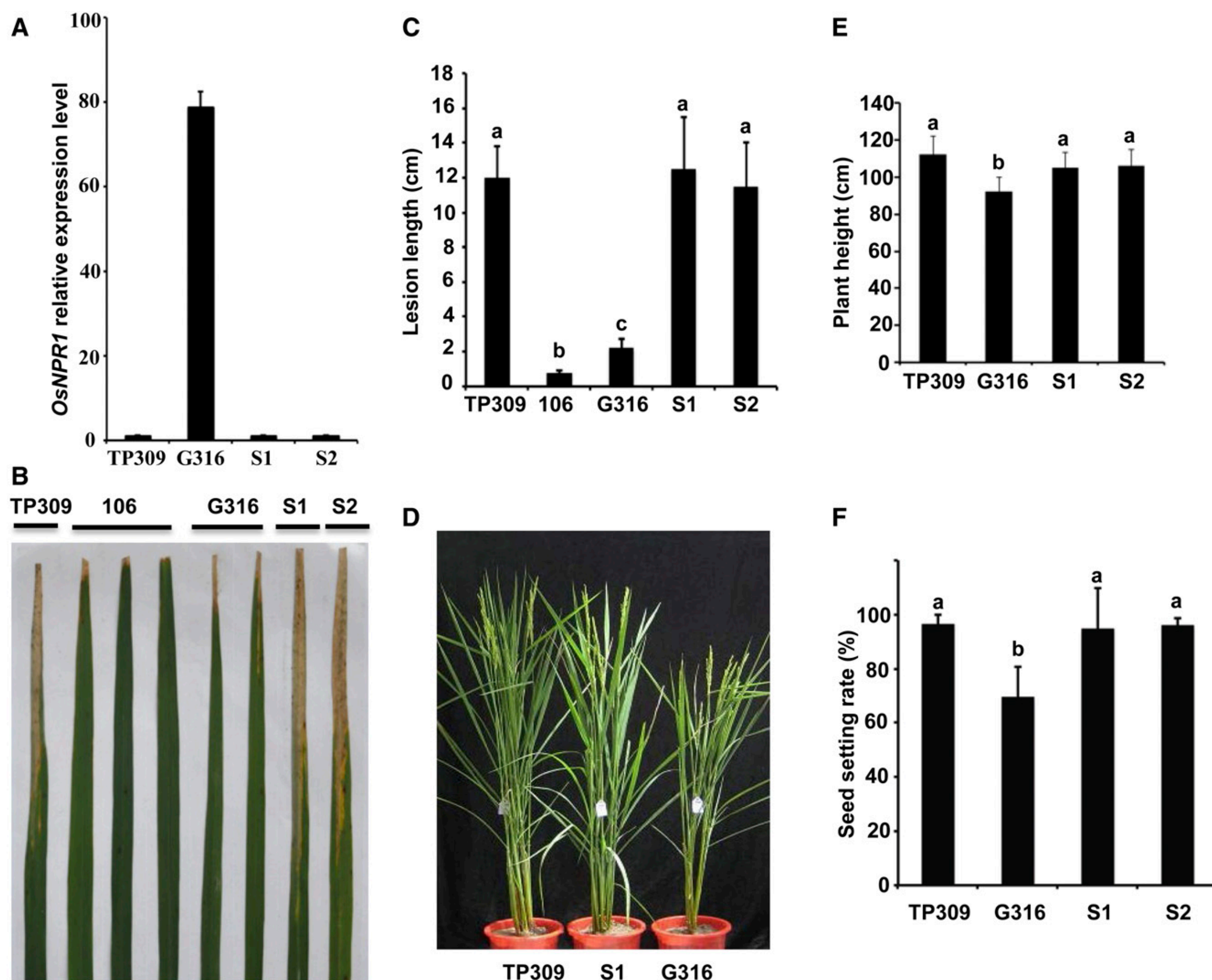

Figure 2. Transgene silence of *OsNPR1*-OX restored growth and development. A, Expression levels of *OsNPR1* measured by qRT-PCR in wild-type TP309, G316, and two transgene-silenced G316 plants (S1 and S2) with the rice *UBIQUITIN* gene (Os03g13170) as an internal control, using primers listed in Supplemental Table S2. B and C, Decreased disease resistance of the transgene-silenced plants to *Xoo* (strain PXO99A) in comparison with TP309, G316, and the fully resistant *Xa21* line 106 control (Song et al., 1995). D, Normal morphological phenotype of the transgene-silenced line (S1) compared with the wild type and G316 during the heading stage. E and F, Statistical analysis of plant heights (E) and seed-setting rates (F) of TP309, G316, S1, and S2 plants grown at the Shanghai station. Data were collected from adult plants grown in the field. Different letters indicate significant differences at $P < 0.05$ ($n = 20$; ANOVA followed by Duncan's multiple range test).

et al., 2006b; Kawahara et al., 2013) were down-regulated. This finding suggested that cytokinin levels or signaling might decrease in *OsNPR1*-OX plants compared with wild-type plants. In fact, cytokinin-deficient mutants displayed increased root mass and branching but also shorter stature, thinner stems, narrower leaves, and smaller meristems compared with wild-type plants (Werner et al., 2003; Pineda Rodo et al., 2008; Kudo et al., 2012). Based on the *OsNPR1*-OX phenotypes of decreased root system and branching (Fig. 1D), the possible disruption of cytokinin signaling might not be responsible for the *OsNPR1*-inhibited development and growth. Three genes possibly involved in BR signaling were differentially expressed in *OsNPR1*-OX plants, including Os06g16300 (annotated to encode putative BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1 PRECURSOR; Kawahara et al., 2013), Os06g12120 (*OsSERL6*/*OsBAK1-3*; Singla et al., 2009; Khew et al., 2015), and Os07g40630 (annotated to encode putative BRASSINOSTEROID INSENSITIVE1 precursor). The three genes were up-regulated in *OsNPR1*-OX plants, likely indicating enhanced BR signaling. However, we did not observe enhanced BR phenotypes that should display increases in leaf length and angle (Tong et al., 2012) and twisted leaves (Khew et al., 2015).

At first glance, the dwarf phenotype of *OsNPR1*-OX plants might imply that *OsNPR1* overexpression could

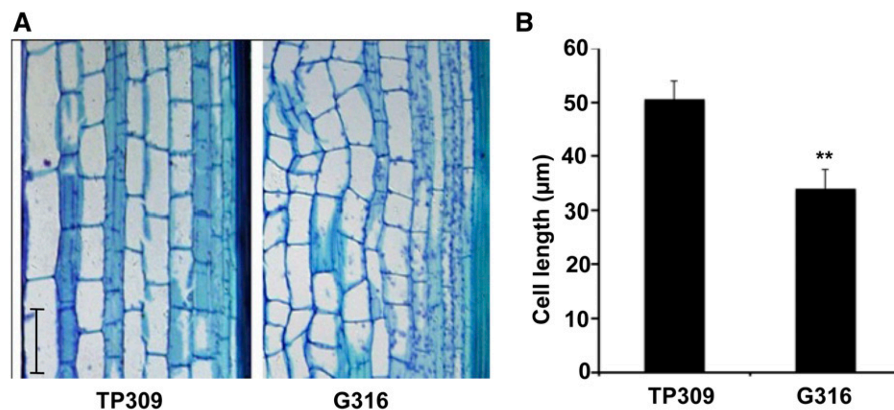

Figure 3. Cell development was repressed in *OsNPR1*-OX plants. A, Cell morphology in the longitudinal section of panicle necks of TP309 and G316 at the heading stage. Sections were stained with Toluidine Blue and observed with a light microscope (Leica). Bar = 50 μm. B, Statistical analysis of cell lengths in the parenchyma near the sclerenchyma. More than 100 cells from five individual stem segments were measured for both TP309 and G316. Asterisks indicate significant differences (**, $P < 0.01$, Student's *t* test).

suppress the GA signaling pathway. Intriguingly, five GA signaling genes were up-regulated in *OsNPR1*-OX plants, of which Os03g57640 and Os07g06830 were predicted to belong to the GA receptor *GID1 L2* family (Kawahara et al., 2013; Jiang et al., 2014); Os06g15620 was annotated to encode a GA-regulated GASA/GAST/Snakin family protein, Os07g36170 was annotated to encode a chitin-inducible GA-responsive protein, and Os07g39470 was annotated to encode a GA response modulator protein (Kawahara et al., 2013). However, *OsNPR1*-OX plants showed the same sensitivity to GA as wild-type plants (Supplemental Fig. S3A). Furthermore, not much similarity was found between the *OsNPR1*-OX and the GA-deficient *EUI*-OX plants that were dwarf with more tillers compared with wild-type plants (Zhu et al., 2006; Zhang et al., 2008a, 2011; Supplemental Fig. S3, B–D). Therefore, we concluded that the stunted phenotypes caused by *OsNPR1* overexpression were unlikely to be caused by disruption in cytokinin, BR, or GA signaling and that these signaling pathways might be somewhat affected in *OsNPR1*-OX plants.

Interestingly, among the 20 differentially expressed hormone-related genes, seven genes were predicted to involve the auxin pathway (Table I); among these candidates, a putative auxin synthesis gene, *OsYUCCA7* (Os04g03980; Yamamoto et al., 2007), was down-regulated (Fig. 4A), and an auxin catabolism gene, *OsGH3.8* (Os07g40290), whose function in plant defense and growth and development had been identified by Ding et al. (2008), was up-regulated (Fig. 4B). The changes in the two genes might result in decreased free auxin levels. Consequently, it was indeed found that the auxin response genes *OsIAA14* and *OsIAA24* were down-regulated (Fig. 4, C and D). Based on the auxin-related phenotypes of *OsNPR1*-OX plants (Fig. 1), the

Table I. *Differentially expressed genes related to hormone signaling in OsNPR1-OX compared with the wild type*
R1, R2, and R3 indicate three replicates.

Hormone	Locus Identifier	Gene Product Name	Signal Log2 Ratio		
			R1	R2	R3
Auxin	LOC_Os01g56240	OsSAUR2, auxin-responsive SAUR gene family member, expressed	5.5	4.0	2.9
	LOC_Os03g58350	OsIAA14, auxin-responsive Aux/IAA gene family member, expressed	−2.1	−4.7	−3.4
	LOC_Os04g03980	OsYUCCA7, flavin monooxygenase, putative, expressed	−2.7	−5.9	−1.8
	LOC_Os07g08460	OsIAA24, auxin-responsive Aux/IAA gene family member, expressed	−3.1	−3.8	−1.8
	LOC_Os07g40290	OsGH3.8, probable IAA-amido synthetase, expressed	3.8	2.4	2.0
	LOC_Os09g37330	OsSAUR39, auxin-responsive SAUR gene family member, expressed	−3.4	−1.5	−2.3
	LOC_Os11g14190	ARGOS, putative, expressed	−1.5	−1.1	−2.2
Cytokinin	LOC_Os02g35180	OsRR2 type A response regulator, expressed	−1.1	−1.2	−1.9
	LOC_Os02g51930	Cytokinin-*O*-glucosyltransferase2, putative, expressed	1.2	2.7	1.0
	LOC_Os04g36070	OsRR1 type A response regulator, expressed	−1.7	−1.1	−2.4
	LOC_Os06g11720	Cytokinin-*O*-glucosyltransferase2, putative, expressed	5.0	2.3	3.2
	LOC_Os11g04720	OsRR10 type A response regulator, expressed	−1.1	−1.0	−1.4
GA	LOC_Os03g57640	GA receptor GID1L2, putative, expressed	1.6	1.9	2.8
	LOC_Os06g15620	GASR7, GA-regulated GASA/GAST/Snakin family protein precursor, expressed	1.4	2.3	4.3
	LOC_Os07g06830	GA receptor GID1L2, putative, expressed	3.3	1.3	1.9
	LOC_Os07g36170	Chitin-inducible GA-responsive protein, putative, expressed	1.9	1.5	1.9
	LOC_Os07g39470	GA response modulator protein, putative, expressed	1.9	1.3	1.7
Brassinosteroid	LOC_Os06g12120	BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1	2.7	1.2	1.1
	LOC_Os06g16300	BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1	1.3	1.4	1.0
	LOC_Os07g40630	BRASSINOSTEROID INSENSITIVE1 precursor	3.1	2.1	4.9

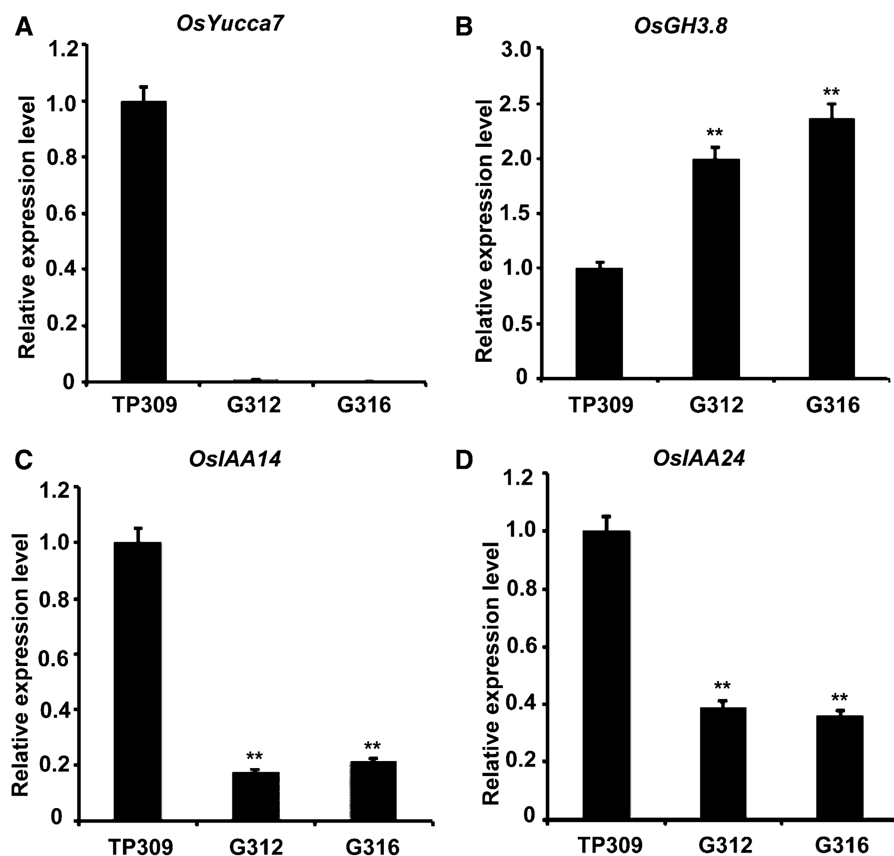

Figure 4. Expression of differentially expressed genes related to the auxin pathway. RNA was extracted from young uppermost internodes at the heading stage. The expression levels of *OsYUCCA7* (A), *OsGH3.8* (B), *OsIAA14* (C), and *OsIAA24* (D) were analyzed by qRT-PCR. Note that *OsGH3.8* was activated in G312 and G316. The oligonucleotide sequences used for qRT-PCR are listed in Supplemental Table S2. The rice *OsACTIN1* gene (Os03g50885) was used as an internal control. Asterisks indicate significant differences in comparison with TP309 (**, $P < 0.01$, Student's *t* test).

results implied that *OsNPR1* overexpression likely suppressed auxin signaling by disturbing auxin metabolism.

OsNPR1 Overexpression Changes Auxin Homeostasis

The final output of auxin signaling is determined by its levels, distribution, and perception system. To determine whether the auxin pathway indeed contributes to the *OsNPR1*-OX phenotypes, the free indole-3-acetic acid (IAA) levels were measured using an Accurate-Mass Q-TOF LC/MS device (Agilent 6520) with IAA-[α,α-D2] as an internal standard. The free IAA levels were significantly lower in *OsNPR1*-OX G312 and G316 plants than in TP309 plants (Fig. 5A), consistent with changes in the expression levels of the two auxin genes, *OsYUCCA7* and *OsGH3.8* (Fig. 4, A and B). Therefore, we concluded that *OsNPR1* overexpression indeed decreased auxin levels through either down- or up-regulating the genes responsible for auxin biosynthesis and metabolism, respectively. To determine whether auxin perception, signaling, and transport systems also were affected by *OsNPR1* overexpression, a growth inhibition assay with exogenous auxin application was conducted using root length as a parameter. The *OsNPR1*-OX overexpression lines exhibited lower sensitivity to naphthaleneacetic acid (NAA) than the wild-type plants (Fig. 5B). Because NAA is a substrate of *GH3*-encoded IAA-amido synthases, *OsGH3.8* up-regulation might partially contribute to the reduced sensitivity to NAA in the *OsNPR1*-OX lines (Staswick et al., 2005).

To further examine auxin homeostasis in *OsNPR1* overexpression plants, we took advantage of the DR5-GUS reporter, which is often used to detect active auxin levels and distribution in Arabidopsis (Nakamura et al., 2003) and also works well in rice (Zhao et al., 2009). We introduced the *OsNPR1*-OX transgene into the DR5-GUS background (variety Zhonghua 11 [ZH11]; Supplemental Fig. S4A). The ZH11 *OsNPR1*-OX plants also exhibited developmental defects and dramatically enhanced disease resistance (Supplemental Fig. S4, A and B). Histochemical staining of GUS activity revealed that both shoots and roots showed dramatically weaker DR5-GUS activity in *OsNPR1*-OX plants compared with the DR5-GUS control plants at the seedling stage (Fig. 5C). The weaker GUS activity caused by *OsNPR1* overexpression was attributed to the lower *GUS* transcript level (Fig. 5D), indicating auxin deficiency in *OsNPR1*-OX plants. Consistent with the smaller adult plant height (Fig. 1, A and B), the uppermost node and the two internodes were more weakly stained in *OsNPR1*-OX plants compared with DR5-GUS control plants (Fig. 5E). The evidence of the exogenous auxin response and DR5-GUS reporter activity allowed us to conclude that *OsNPR1* overexpression changed auxin homeostasis most likely through auxin metabolism, which resulted in decreased auxin levels in *OsNPR1*-OX plants. Consistent with our hypothesis, exogenous

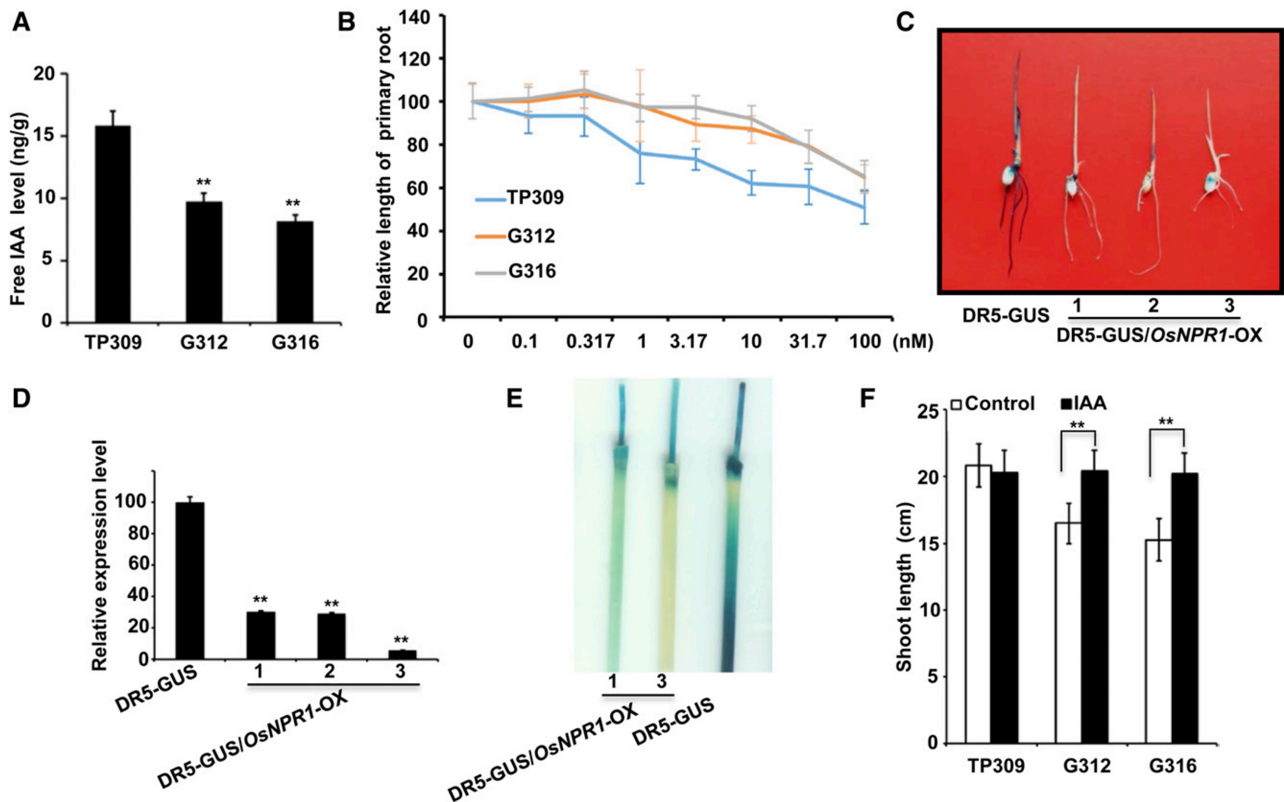

Figure 5. *OsNPR1*-OX changed auxin biosynthesis and response. A, *OsNPR1* overexpression decreased free IAA levels in G312 and G316. Young uppermost internodes at the heading stage were collected for IAA extraction. B, Root growth inhibition by NAA in *OsNPR1*-OX and wild-type plants recorded and analyzed (n = 24) at day 7 after treatment. C, GUS staining and qRT-PCR analysis of GUS expression levels in the wild type (ZH11) and three DR5-GUS/*OsNPR1*-OX lines. Seedlings at 5 d after germination were incubated in GUS staining buffer for 4 h, indicating the decrease in GUS staining in DR5-GUS/*OsNPR1*-OX plants. D, Expression levels of *GUS* detected by qRT-PCR in RNA extracted from 3-week-old seedlings. The rice *OsACTIN1* gene (Os03g50885) was used as an internal control. E, GUS staining of young uppermost nodes at the heading stage. Tissues were incubated in GUS staining buffer for 12 h. Note that GUS staining was decreased in the internodes and nodes of DR5-GUS/*OsNPR1*-OX. F, Exogenous application of IAA (1 nM) could partially restore the growth of *OsNPR1*-OX plants. Asterisks indicate significant differences in comparison with TP309 (A and F) or DR5-GUS (D; **, $P < 0.01$, Student's t test).

application of IAA could largely restore the growth of G316 plants (Fig. 5F).

OsNPR1 Overexpression Also Enhances *OsGH3.8* Expression at the Seedling Stage

We have shown that *OsYUCCA7* and *OsGH3.8*, genes involved in auxin biosynthesis and metabolism, respectively, were differentially regulated in *OsNPR1*-OX at the heading stage, suggesting that *OsNPR1* might regulate the expression of the two auxin genes. Because *OsNPR1* overexpression repressed rice growth and development over the plant's entire lifetime, we then compared the expression levels of the two genes between *OsNPR1*-OX and wild-type plants at the seedling stage. We found that *OsGH3.8* also was significantly up-regulated at the seedling stage (Fig. 6A); however, *OsYUCCA7* expression was not changed at this stage (Supplemental Fig. S5). We also analyzed other members of the rice *GH3* family (Jain et al., 2006a) at the seedling stage and found that two other *GH3* members, *OsGH3.1* and *OsGH3.11*, also were significantly but not dramatically up-regulated in the *OsNPR1*-OX lines compared with the wild type (Fig. 6A). Moreover, we found that the expression level of *OsGH3.8* was correlated with that of *OsNPR1*, since the transgene-silenced plants in G316 reduced the expression of *OsGH3.8* (Fig. 6B). Therefore, we propose that *OsNPR1* overexpression indirectly up-regulates some *GH3* genes, in particular *OsGH3.8*, triggering a defense cost process that links with the auxin signaling pathway. Consistent with this hypothesis, *OsGH3.8* overexpression also caused abnormal plant morphology and retarded growth and development (Ding et al., 2008).

Decrease of *OsGH3.8* Expression Partially Restores *OsNPR1*-OX Growth But Not Disease Resistance

To further confirm whether the activation of *OsGH3.8* was responsible for the stunted phenotypes

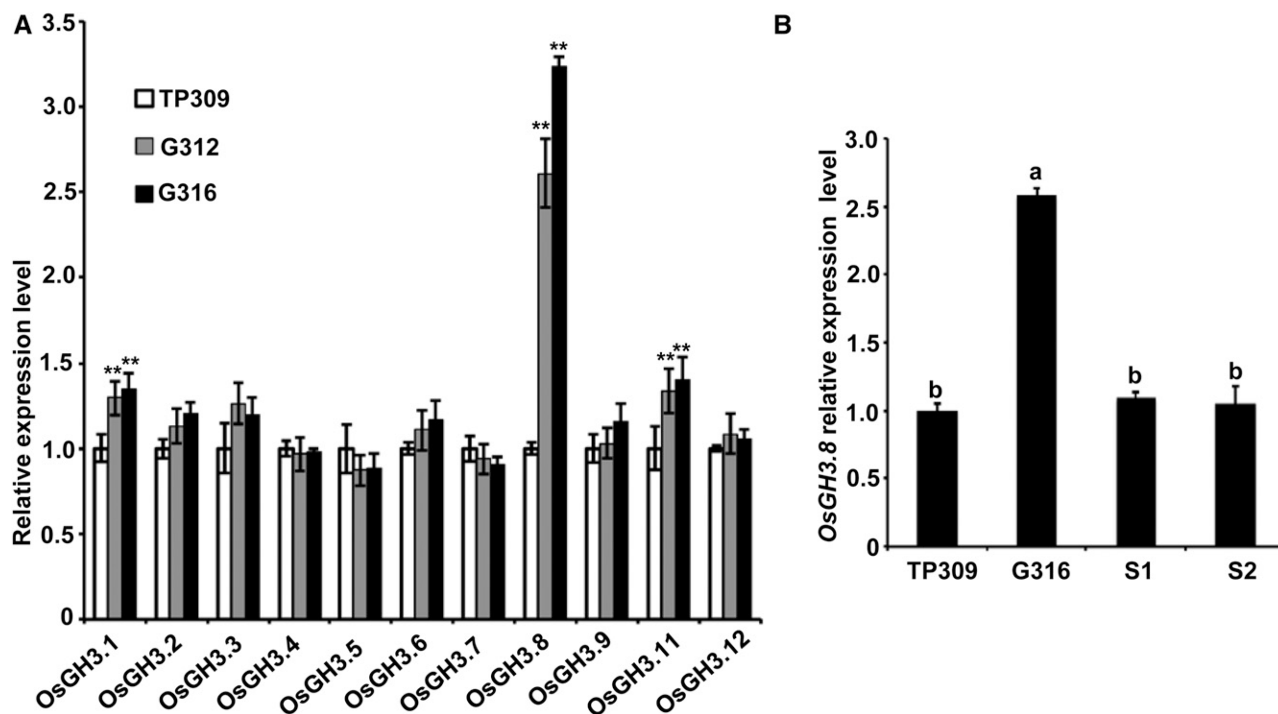

Figure 6. *OsNPR1* up-regulated *OsGH3.8*. A, Expression levels of *GH3* family genes in the wild type and *OsNPR1* overexpression lines G312 and G316. RNA was extracted from 3-week-old seedlings. qRT-PCR was performed with the *OsACTIN1* gene (Os03g50885) as an internal control, and expression levels of the genes in wild-type TP309 were set as 1. Asterisks indicate significant differences in comparison with wild-type TP309 (**, $P < 0.01$, Student's *t* test). B, Expression levels of *OsGH3.8* in *OsNPR1* overexpression line G316 and two transgene-silenced G316 plants (S1 and S2). Different letters indicate significant differences ($P < 0.05$, ANOVA followed by Duncan's multiple range test).

of *OsNPR1*-OX plants, we first developed *OsGH3.8* RNA interference lines (*OsGH3.8*-RNAi) in the TP309 background (Supplemental Fig. S6A), which exhibited a significantly lower seed-setting rate compared with wild-type plants, as reported (Supplemental Fig. S6B; Yadav et al., 2011). The stable *OsGH3.8*-RNAi line was crossed with *OsNPR1*-OX plants to generate *OsNPR1*-OX/*OsGH3.8*-RNAi plants. However, we could not obtain lines with strong RNA interference; rather, plants exhibited only partially reduced *OsGH3.8* expression in the *OsNPR1*-OX background, using an unknown mechanism (Supplemental Fig. S6, C and D). We found that the decrease in *OsGH3.8* expression partially restored IAA contents in the *OsNPR1*-OX (G316)/*OsGH3.8*-RNAi plants (Fig. 7A). As expected, knockdown of *OsGH3.8* also rescued the *OsNPR1*-OX growth phenotypes, including plant height (Fig. 7, B and C) and grain productivity (Fig. 7, D–G). Importantly, the *OsNPR1*-mediated disease resistance was not attenuated by the *OsGH3.8* knockdown (Fig. 7H). Therefore, we genetically confirmed that *OsGH3.8* is an important downstream target of the *OsNPR1*-mediated defense signaling pathway, which regulates auxin signaling to shape rice growth and development, providing a link between defense and fitness cost. However, the mechanism by which *OsNPR1* activates *OsGH3.8* remains elusive, and we could not provide direct molecular evidence for a causal link between NPR1 protein activity and *OsGH3.8* gene expression.

DISCUSSION

OsNPR1 Overexpression Represses Rice Growth and Development by Disturbing Auxin Homeostasis

Plant defense and growth are largely governed by diverse phytohormones. Auxins, with IAA as a main active form in higher plants, can regulate many aspects of plant development, such as gravitropism, root, leaf, and flower formation, plant vasculature development, and seed germination (Kieffer et al., 2010; Zhao, 2010; Liu et al., 2013). This study reveals that the auxin signaling pathway impacts the *OsNPR1*-mediated disease resistance in rice partly through the IAA-amido synthetase gene *OsGH3.8*, which has been reported to play important roles in rice growth and basal defense through modifying cell wall structure (Ding et al., 2008). Our study provides another genetic model indicating that the plant defense machinery interacts with growth and development and further supports our previous claim that rice defense is usually prioritized over growth when defense hormones encounter growth hormones in the cereal model crop (Yang et al., 2008, 2012, 2013).

Auxin, as a negative regulator of plant immunity, has long been known to actively modify plant defense

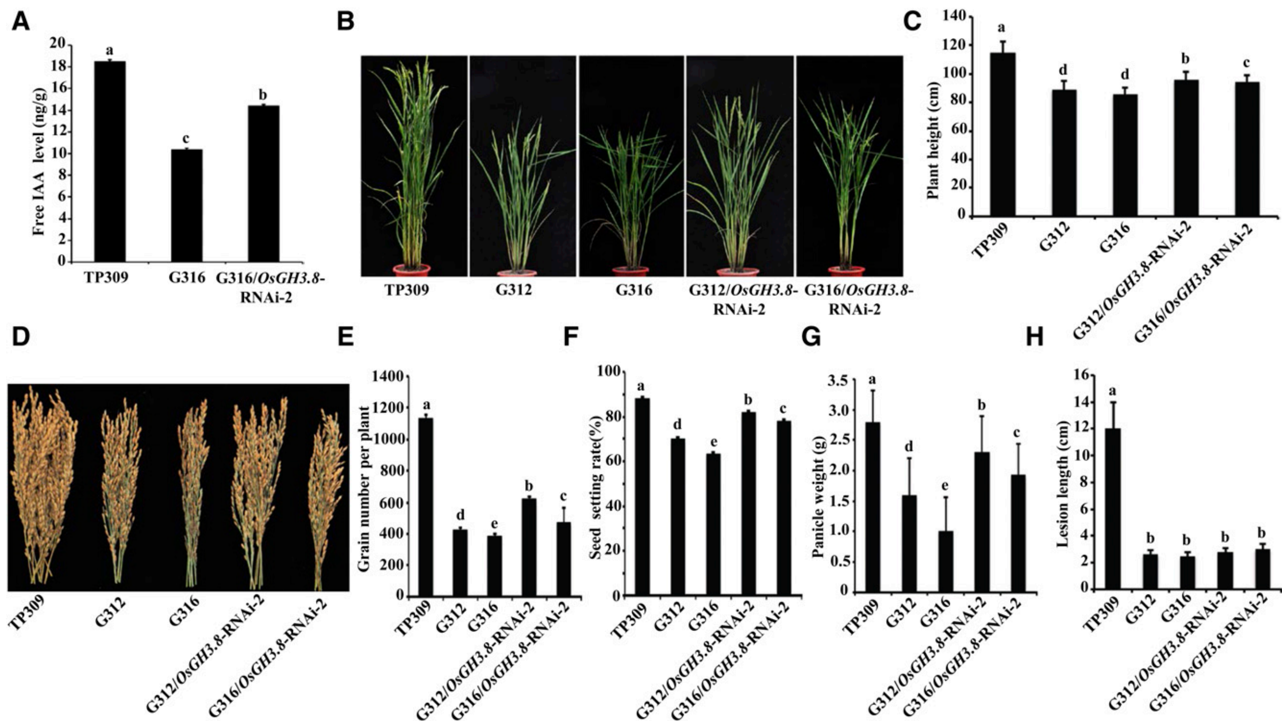

Figure 7. Decreased *OsGH3.8* expression partially rescued *OsNPR1*-OX developmental defects. A, Free IAA levels measured in young uppermost internodes at the heading stage. B, Morphological phenotypes of adult plants at the heading stage, grown at the Hainan station. C, Statistical analysis of plant heights. Note that *OsNPR1*-OX/*OsGH3.8*-RNAi-2 partially restored the phenotype of *OsNPR1*-OX in comparison with wild-type TP309. D, Mature panicles of the indicated genotypes. All panicles were harvested for individual plants. E to G, Statistical analysis of grain number per plant (E), seed-setting rate (F), and panicle weight (G). Data were collected from more than five adult plants of each genotype grown in the field. H, Statistical analysis of lesion lengths after inoculation with *Xoo* PXO99A. Different letters (A, C, and E–H) indicate significant differences at $P < 0.05$ (ANOVA followed by Duncan's multiple range test).

(Navarro et al., 2006; Chen et al., 2007). A set of evidence has demonstrated that auxin plays roles in balancing plant defense responses and growth in both monocotyledonous and dicotyledonous plants. For example, auxin signaling repression through negatively regulated mRNAs for the F-box receptor was reported to contribute to antibacterial resistance in Arabidopsis (Navarro et al., 2006). Interestingly, increased auxin levels even could counteract the effect of activated SA signaling on disease resistance (Zhang et al., 2007). Wang et al. (2007) demonstrated that auxin might down-regulate the defense response by inhibiting the full induction of SA-mediated signaling and that inhibition of auxin signaling is part of the SA-mediated disease resistance in Arabidopsis (Wang et al., 2007). In rice, reducing auxin content by constitutively expressing *OsGH3.8* and *OsGH3.1* enhanced disease resistance to both fungal and bacterial pathogens (Ding et al., 2008; Domingo et al., 2009). Therefore, auxin signaling is likely subjected to strict monitoring and modification during plant-pathogen interactions.

OsGH3.8 Is a Downstream Target of *OsNPR1*

The intertwining relationships between the SA and auxin pathways have been widely reported (Navarro et al., 2006; Wang et al., 2007; Zhang et al., 2007, 2008b). Some members of the *GH3* gene family are actively involved in fine-tuning plant defense and growth. Our previous study with the *GH3.5* activation-tagged mutant *gh3.5-1D* documented that GH3.5 acts as a bifunctional modulator in both SA and auxin signaling pathways during pathogen infection in Arabidopsis. *GH3.5* overexpression in *gh3.5-1D* plants resulted in elevated SA accumulation and high levels of free IAA after pathogen infection, simultaneously augmenting the SA and auxin pathways and impairing different R gene-mediated resistance (Zhang et al., 2007). Interestingly, *GH3.5* also modulated abiotic stress adaptation by changing auxin homeostasis (Park et al., 2007). Another Arabidopsis *GH3* gene, *GH3.3*, was up-regulated using benzothiadiazole, an SA analog (Wang et al., 2007). In rice, reducing auxin levels by constitutively expressing *OsGH3.8* (Ding et al., 2008) and *OsGH3.1* (Domingo et al., 2009) also enhanced disease resistance to fungal and bacterial pathogens. Intriguingly, however, the enhanced disease resistance conferred by *OsGH3.8* up-regulation is suggested to be independent of the SA pathway, given that *PR1* gene induction and SA accumulation were slightly suppressed in *OsGH3.8*-overexpressing plants (Ding et al., 2008).

NPR1 is a key regulator of the SA-mediated pathway and acts as a transcriptional coactivator in plant innate immunity (Cao et al., 1998; Friedrich et al., 2001; Malnoy et al., 2007; Wally et al., 2009; Parkhi et al., 2010; Zhang et al., 2012). Our results here indicate that *OsGH3.8* was up-regulated in *OsNPR1*-OX plants and consequently decreased IAA levels (Fig. 5). We propose that *OsGH3.8* is a downstream target of *OsNPR1* for modifying plant growth and development based on the following lines of evidence. First, *OsGH3.8* is up-regulated in *OsNPR1*-OX. Second, *OsGH3.8* is greatly inducible by SA (Ding et al., 2008). Third, the decrease in *OsGH3.8* expression partially restored the growth and developmental defects of *OsNPR1*-OX plants. Because the *OsGH3.8* expression level in the *OsNPR1*-OX/*OsGH3.8*-RNAi line could not be decreased to the level in the original *OsGH3.8*-RNAi lines (Supplemental Fig. S6, A and D), we proposed that the *OsNPR1*-OX stunted phenotype would be more profoundly rescued if *OsGH3.8* expression could be further knocked down. Nevertheless, it is interesting that *OsGH3.8* knockdown did not affect *OsNPR1*-enhanced disease resistance, regardless of the role that *OsGH3.8* plays in basal defense in rice. These observations suggest that the strong *OsNPR1*-dependent enhancement of the SA defense pathway could not be compromised by the potential loss of *OsGH3.8*-mediated basal defense. Alternatively, as suggested previously, the functional redundancy of the *GH3* family led to a nondecrease of disease resistance in *GH3.8* suppression or knockout plants (Ding et al., 2008). NPR1 is a master transcription regulator that interacts with transcription factors to activate target genes. Chern et al. (2014) showed that OsNPR1 (NH1) is capable of interacting strongly with rTGA2.1, rTGA2.2, rTGA2.3, rLG2, TGAL2, and TGAL4, which belong to the TGA family of basic-region Leu zipper transcription factors involved in defense responses. Therefore, OsNPR1 likely indirectly regulates *OsGH3.8* through an unrecognized transcriptional mechanism. We propose that other unknown transcription factor(s) may be regulated directly by *OsNPR1* and may play an important role in *OsGH3.8* activation. The transcription factor(s) could be identified through extensive screenings of OsNPR1-interacting proteins or genetic suppressors of *OsNPR1*-OX disease resistance or developmental phenotypes.

Potential Strategy for *OsNPR1* Application in Rice Breeding for Broad-Spectrum Disease Resistance

OsNPR1 is a key regulator of innate immunity in rice and has been regarded as an ideal candidate for durable and broad-spectrum disease resistance breeding by transgenic ectopic expression (Chern et al., 2005; Yuan et al., 2007). The fact that *OsNPR1* overexpression inhibits rice growth and development by repressing auxin signaling partially by activating *OsGH3.8* makes it difficult to utilize this gene directly in improving disease resistance in elite rice varieties. Our results have shown that the auxin pathway is the target of OsNPR1-dependent growth and development, which likely does not affect OsNPR1-mediated disease resistance. Therefore, one strategy of *OsNPR1* application in rice molecular breeding is to block the downstream auxin signal in growth and development by mutating the *OsNPR1*-interacting gene(s) or its promoter, such as *GH3.8*, using CRISPR/Cas9 technology, provided that the precise mechanism or transcription factor(s) has been recognized. Another strategy is to promote growth and development and thereby increase yield potential in *OsNPR1*-OX plants by introducing some auxin-related yield regulators, such as the *OsSPL14* elite alleles, *IPA1* and *WFP* (Jiao et al., 2010; Miura et al., 2010), into *OsNPR1*-OX plants, which would promote plant growth and development with stronger and elongated culms and higher grain productivity and likely would overcome the negative effects of *OsNPR1* on yield traits.

CONCLUSION

In this study, the SAR master gene *OsNPR1* was recognized to inhibit plant growth and development through repressing the auxin signaling pathway in overexpression transgenic rice. This finding provides further insight into the fine-tuned hormone network of defense and growth interactions.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The rice (*Oryza sativa*) varieties TP309 and ZH11 were used in rice transformation. The *OsNPR1* overexpression lines (G312 and G316) and the *EUI1* overexpression line all were developed in our laboratory (Zhu et al., 2006; Yuan et al., 2007). The transgene-silenced plants of *OsNPR1*-OX, S1 and S2, were isolated from the progeny of G316. The presence of transgene in S1 and S2 plants was confirmed by PCR using forward primer that located in LacZ on the plasmid of 1301-35SN (Yuan et al., 2007) and reverse primer that located in *OsNPR1* (Supplemental Table S2). The *Xa21* transgenic line 106 was provided by Dr. Pamela Ronald (University of California, Davis; Song et al., 1995). The DR5-GUS transgenic rice line was provided by Dr. Yu Zhao (Huazhong Agricultural University). The *OsNPR1* overexpression line in ZH11 was developed as described below and then was crossed with DR5-GUS to obtain the DR5-GUS/*OsNPR1*-OX line. The *OsGH3.8* RNA interference lines (*OsGH3.8*-RNAi) were developed as described below. The *OsNPR1*-OX/*OsGH3.8*-RNAi double transformants were obtained by crossing G312, G316, and *OsGH3.8*-RNAi plants. Rice plants were grown in experimental fields (the Shanghai station and/or the Hainan station) or in growth chambers under growth conditions with 12-h day, 28°C, and 80% relative humidity followed by 12-h night, 26°C, and 60% relative humidity.

Plasmid Construction and Rice Transformation

To develop *OsNPR1*-OX in the ZH11 background, the *OsNPR1* transgene (Yuan et al., 2007) was transformed into ZH11 calli. To generate *OsGH3.8*-RNAi plants, a hairpin structure was constructed with a specific fragment of this gene coding region (nucleotides 69–700) and its partial antisense fragment (nucleotides 75–508). The sense fragment was amplified using the primers 5′-TGGATCCCGACGCCTCTTCCTCT-3′ and 5′-AGGGCCCAATGATTCATGGCCAT-3′ (*Bam*HI and *Apa*I sites), and the antisense fragment was amplified using the primers 5′-AGGGCCCGGTGAGGAACTCGGAG-3′ and 5′-AGGTACCTTCCTCTCGCTACTAC-3′ (*Apa*I and *Kpn*I sites). Both fragments were fused using *Apa*I digestion and ligation, and the resultant fragment was inserted into the expression vector pUN1301 (*Bam*HI and *Kpn*I sites) under the control of the maize (*Zea mays*) ubiquitin promoter. Rice transformation was

performed using the *Agrobacterium tumefaciens*-mediated method to generate more than 15 independent transgenic plants.

Microarray Data Analysis

The young uppermost internodes of *OsNPR1*-OX G316 and wild-type TP309 plants at the heading stage were harvested and stored in liquid nitrogen for RNA isolation. Microarray analysis was performed using the oligonucleotide chip GeneChip Rice Genome Array developed by Affymetrix. Microarray experiments were performed at an Affymetrix-authorized center (GeneTech Biotechnology) following the protocol of the Affymetrix GeneChip Rice Genome Array with three biological replicates. Raw data analysis was conducted as described previously (Wang et al., 2008). Reproducibly differentially expressed probe sets were selected (fold change ≥ 2 and $P < 0.05$) from total normalized data using Data Mining Tool version 3.0. Functional classification of the genes was performed according to the Rice Genome Annotation Project Release 7.0 (Kawahara et al., 2013; http://rice.plantbiology.msu.edu) and some publications involved in gene function analysis, such as Jain et al. (2006a). Microarray data have been deposited in the NCBI Gene Expression Omnibus and are accessible through GEO series accession number GSE76178 (the gene expression data of OsNPR1 overexpression and its wild-type TP309 plants).

Auxin and GA Response Experiments

Sterile TP309, G312, and G316 seeds were germinated in the dark for 2 d at 28°C and then were incubated in 0.5× MS liquid medium supplied with different concentrations of NAA. After 7 d, the lengths of the primary roots of seedlings were measured. Thirty seedlings of each line were analyzed with three biological repeats. For GA treatment, seeds after germination were grown in one-half-strength MS medium with different concentrations of GA_3. The shoot lengths were measured at day 17 after treatment ($n \geq 24$). For exogenous application of IAA, 6-d-old seedlings grown in one-half-strength MS liquid medium were incubated with 1 nM IAA for another 10 d. Shoot length was measured ($n = 20$).

Auxin Extraction and Quantification

The young uppermost internodes of *OsNPR1*-OX G316, G316/*OsGH3.8*-RNAi, and wild-type (TP309) plants at heading stage were harvested for IAA extraction. IAA extraction and quantification were performed according to Pan et al. (2010) using an Agilent 6520 Accurate-Mass Q-TOF LC/MS device. IAA-[α,α-D2] (International Laboratory) was used as an internal standard of IAA.

Gene Expression Analysis

Total RNA was extracted using TRIzol reagent and treated with DNase I using a DNA-free kit (Invitrogen; http://www.invitrogen.com/). cDNA was synthesized from 3 μg of total RNA using an oligo(dT) primer and SuperScript III reverse transcriptase according to the manufacturer's instructions (Invitrogen). Real-time reverse transcription-PCR analysis was performed using SYBR Premix Ex Taq (TaKaRa; http://www.takara.com.cn/) and gene-specific primers with *OsACTIN1* as the internal control. Semiquantitative reverse transcription-PCR was conducted with a rice ubiquitin gene (*Os03g13170*) as the internal control. All of the primer sequences are listed in Supplemental Table S2.

Histological Analysis

The elongating zones of the uppermost internodes were cut into 0.2-cm sections and then immersed in hydrofluoric acid, followed by paraffin section preparation using a previously described protocol (Yang et al., 2011). Sections were stained with 0.05% Toluidine Blue O (Chroma Gesellshaft Shaud) and observed with a light microscope (Leica).

DR5-GUS Staining

For GUS staining, the materials were placed in staining solution (50 mM $NaPO_4$, pH 7.2, 2 mM X-gluc, 0.5 mM K3Fe[CN]6, and 0.5 mM K4Fe[CN]6), vacuum infiltrated for 5 min, and incubated at 37°C. Five-day-old whole seedlings of DR5-GUS and DR5-GUS/*OsNPR1*-OX were incubated in GUS staining buffer for 4 h. Young uppermost internodes at the heading stage were incubated in GUS staining buffer for 12 h.

Pathogen Inoculation

Rice plants were inoculated with *Xanthomonas oryzae* pv *oryzae* strain PXO99A using the leaf clip method (Song et al., 1995). After 14 d, the lesion lengths were recorded. More than five individual plants, with five to six leaves of each plant, were inoculated for each genotype.

Statistical Analysis

For gene expression levels detected by qRT-PCR with three replicates, and plant growth and yield components measured with 20 or 24 plants, Student's *t* test or ANOVA followed by Duncan's multiple range test was performed.

The complete set of microarray data has been deposited in a MIAME-compliant format under accession number GSE76178 (http://www.ncbi.nlm.nih.gov/geo).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Genotyping of two transgene-silenced G316 plants, S1 and S2.

Supplemental Figure S2. qRT-PCR validation of differentially expressed genes revealed by microarray assay.

Supplemental Figure S3. *OsNPR1* overexpression has no effect on the GA response.

Supplemental Figure S4. Generation of *OsNPR1* overexpression lines in ZH11.

Supplemental Figure S5. *OsYUCCA7* expression levels at the seedling stage.

Supplemental Figure S6. Generation of *OsNPR1*-OX/*OsGH3.8*-RNAi.

Supplemental Table S1. Differentially expressed genes revealed by microarray between TP309 and G316.

Supplemental Table S2. Primers used for PCR analysis.

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LITERATURE CITED

Bednarek P, Osbourn A (2009) Plant-microbe interactions: chemical diversity in plant defense. Science **324:** 746–748

Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell **6:** 1583–1592

Cao H, Li X, Dong X (1998) Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proc Natl Acad Sci USA **95:** 6531–6536

Chen Z, Agnew JL, Cohen JD, He P, Shan L, Sheen J, Kunkel BN (2007) *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. Proc Natl Acad Sci USA **104:** 20131–20136

Chern M, Bai W, Ruan D, Oh T, Chen X, Ronald PC (2014) Interaction specificity and coexpression of rice *NPR1* homologs 1 and 3 (NH1 and NH3), TGA transcription factors and Negative Regulator of Resistance (NRR) proteins. BMC Genomics **15:** 461

Chern M, Fitzgerald HA, Canlas PE, Navarre DA, Ronald PC (2005) Overexpression of a rice *NPR1* homolog leads to constitutive activation of defense response and hypersensitivity to light. Mol Plant Microbe Interact **18:** 511–520

Dangl JL, Horvath DM, Staskawicz BJ (2013) Pivoting the plant immune system from dissection to deployment. Science **341:** 746–751

Ding X, Cao Y, Huang L, Zhao J, Xu C, Li X, Wang S (2008) Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin

expression and promotes salicylate- and jasmonate-independent basal immunity in rice. Plant Cell **20:** 228–240

Domingo C, Andrés F, Tharreau D, Iglesias DJ, Talón M (2009) Constitutive expression of *OsGH3.1* reduces auxin content and enhances defense response and resistance to a fungal pathogen in rice. Mol Plant Microbe Interact **22:** 201–210

Friedrich L, Lawton K, Dietrich R, Willits M, Cade R, Ryals J (2001) *NIM1* overexpression in Arabidopsis potentiates plant disease resistance and results in enhanced effectiveness of fungicides. Mol Plant Microbe Interact **14:** 1114–1124

Fu ZQ, Dong X (2013) Systemic acquired resistance: turning local infection into global defense. Annu Rev Plant Biol **64:** 839–863

Jain M, Kaur N, Tyagi AK, Khurana JP (2006a) The auxin-responsive *GH3* gene family in rice (*Oryza sativa*). Funct Integr Genomics **6:** 36–46

Jain M, Tyagi AK, Khurana JP (2006b) Molecular characterization and differential expression of cytokinin-responsive type-A response regulators in rice (*Oryza sativa*). BMC Plant Biol **6:** 1

Jiang G, Xiang Y, Zhao J, Yin D, Zhao X, Zhu L, Zhai W (2014) Regulation of inflorescence branch development in rice through a novel pathway involving the pentatricopeptide repeat protein sped1-D. Genetics **197:** 1395–1407

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, et al (2010) Regulation of *OsSPL14* by *OsmiR156* defines ideal plant architecture in rice. Nat Genet **42:** 541–544

Jones JD, Dangl JL (2006) The plant immune system. Nature **444:** 323–329

Kawahara Y, de la Bastide M, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Schwartz DC, Tanaka T, Wu J, Zhou S, et al (2013) Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice (N Y) **6:** 4

Khew CY, Teo CJ, Chan WS, Wong HL, Namasivayam P, Ho CL (2015) Brassinosteroid insensitive 1-associated kinase 1 (OsI-BAK1) is associated with grain filling and leaf development in rice. J Plant Physiol **182:** 23–32

Kieffer M, Neve J, Kepinski S (2010) Defining auxin response contexts in plant development. Curr Opin Plant Biol **13:** 12–20

Kou Y, Wang S (2010) Broad-spectrum and durability: understanding of quantitative disease resistance. Curr Opin Plant Biol **13:** 181–185

Kudo T, Makita N, Kojima M, Tokunaga H, Sakakibara H (2012) Cytokinin activity of cis-zeatin and phenotypic alterations induced by overexpression of putative cis-zeatin-*O*-glucosyltransferase in rice. Plant Physiol **160:** 319–331

Lin WC, Lu CF, Wu JW, Cheng ML, Lin YM, Yang NS, Black L, Green SK, Wang JF, Cheng CP (2004) Transgenic tomato plants expressing the Arabidopsis *NPR1* gene display enhanced resistance to a spectrum of fungal and bacterial diseases. Transgenic Res **13:** 567–581

Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li J, He ZH (2013) Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated *ABI3* activation in Arabidopsis. Proc Natl Acad Sci USA **110:** 15485–15490

López MA, Bannenberg G, Castresana C (2008) Controlling hormone signaling is a plant and pathogen challenge for growth and survival. Curr Opin Plant Biol **11:** 420–427

Malnoy M, Jin Q, Borejsza-Wysocka EE, He SY, Aldwinckle HS (2007) Overexpression of the apple *MpNPR1* gene confers increased disease resistance in Malus × domestica. Mol Plant Microbe Interact **20:** 1568–1580

McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol **40:** 349–379

Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M (2010) *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nat Genet **42:** 545–549

Nakamura A, Higuchi K, Goda H, Fujiwara MT, Sawa S, Koshiba T, Shimada Y, Yoshida S (2003) Brassinolide induces *IAA5*, *IAA19*, and DR5, a synthetic auxin response element in Arabidopsis, implying a cross talk point of brassinosteroid and auxin signaling. Plant Physiol **133:** 1843–1853

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science **312:** 436–439

Pan X, Welti R, Wang X (2010) Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. Nat Protoc **5:** 986–992

Park JE, Park JY, Kim YS, Staswick PE, Jeon J, Yun J, Kim SY, Kim J, Lee YH, Park CM (2007) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. J Biol Chem **282:** 10036–10046

Parkhi V, Kumar V, Campbell LM, Bell AA, Shah J, Rathore KS (2010) Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis *NPR1*. Transgenic Res **19:** 959–975

Pineda Rodo A, Brugière N, Vankova R, Malbeck J, Olson JM, Haines SC, Martin RC, Habben JE, Mok DW, Mok MC (2008) Over-expression of a zeatin O-glucosylation gene in maize leads to growth retardation and tasselseed formation. J Exp Bot **59:** 2673–2686

Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol **49:** 317–343

Ryals J, Weymann K, Lawton K, Friedrich L, Ellis D, Steiner HY, Johnson J, Delaney TP, Jesse T, Vos P, et al (1997) The *Arabidopsis* NIM1 protein shows homology to the mammalian transcription factor inhibitor I kappa B. Plant Cell **9:** 425–439

Shah J, Zeier J (2013) Long-distance communication and signal amplification in systemic acquired resistance. Front Plant Sci **4:** 30

Singla B, Khurana JP, Khurana P (2009) Structural characterization and expression analysis of the SERK/SERL gene family in rice (*Oryza sativa*). Int J Plant Genomics **2009:** 539402

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, et al (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science **270:** 1804–1806

Spoel SH, Dong X (2008) Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe **3:** 348–351

Spoel SH, Dong X (2012) How do plants achieve immunity? Defence without specialized immune cells. Nat Rev Immunol **12:** 89–100

Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W (2005) Characterization of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. Plant Cell **17:** 616–627

Tong H, Liu L, Jin Y, Du L, Yin Y, Qian Q, Zhu L, Chu C (2012) DWARF AND LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-like kinase to mediate brassinosteroid responses in rice. Plant Cell **24:** 2562–2577

Wally O, Jayaraj J, Punja ZK (2009) Broad-spectrum disease resistance to necrotrophic and biotrophic pathogens in transgenic carrots (*Daucus carota* L.) expressing an Arabidopsis *NPR1* gene. Planta **231:** 131–141

Wally O, Punja ZK (2010) Genetic engineering for increasing fungal and bacterial disease resistance in crop plants. GM Crops **1:** 199–206

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol **17:** 1784–1790

Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, He W, Lu B, Lin H, et al (2008) Control of rice grain-filling and yield by a gene with a potential signature of domestication. Nat Genet **40:** 1370–1374

Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H, Schmülling T (2003) Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell **15:** 2532–2550

Yadav SR, Khanday I, Majhi BB, Veluthambi K, Vijayraghavan U (2011) Auxin-responsive *OsMGH3*, a common downstream target of *OsMADS1* and *OsMADS6*, controls rice floret fertility. Plant Cell Physiol **52:** 2123–2135

Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M, Sazuka T (2007) Auxin biosynthesis by the *YUCCA* genes in rice. Plant Physiol **143:** 1362–1371

Yang DL, Li Q, Deng YW, Lou YG, Wang MY, Zhou GX, Zhang YY, He ZH (2008) Altered disease development in the *eui* mutants and Eui overexpressors indicates that gibberellins negatively regulate rice basal disease resistance. Mol Plant **1:** 528–537

Yang DL, Yang Y, He Z (2013) Roles of plant hormones and their interplay in rice immunity. Mol Plant **6:** 675–685

Yang DL, Yao J, Mei CS, Tong XH, Zeng LJ, Li Q, Xiao LT, Sun TP, Li J, Deng XW, et al (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci USA **109:** E1192–E1200

Yang W, Ren S, Zhang X, Gao M, Ye S, Qi Y, Zheng Y, Wang J, Zeng L, Li Q, et al (2011) *BENT UPPERMOST INTERNODE1* encodes the class II formin FH5 crucial for actin organization and rice development. Plant Cell **23:** 661–680

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, et al (2007) Functional analysis of rice *NPR1*-like genes reveals that *OsNPR1/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J **5:** 313–324

Zhang JY, Qiao YS, Lv D, Gao ZH, Qu SC, Zhang Z (2012) *Malus hupehensis NPR1* induces pathogenesis-related protein gene expression in transgenic tobacco. Plant Biol (Stuttg) (Suppl 1) **14:** 46–56

Zhang Q (2007) Strategies for developing green super rice. Proc Natl Acad Sci USA **104:** 16402–16409

Zhang Y, Zhang B, Yan D, Dong W, Yang W, Li Q, Zeng L, Wang J, Wang L, Hicks LM, et al (2011) Two Arabidopsis cytochrome P450 monooxygenases, CYP714A1 and CYP714A2, function redundantly in plant development through gibberellin deactivation. Plant J **67:** 342–353

Zhang Y, Zhu Y, Peng Y, Yan D, Li Q, Wang J, Wang L, He Z (2008a) Gibberellin homeostasis and plant height control by *EUI* and a role for gibberellin in root gravity responses in rice. Cell Res **18:** 412–421

Zhang Z, Li Q, Li Z, Staswick PE, Wang M, Zhu Y, He Z (2007) Dual regulation role of GH3.5 in salicylic acid and auxin signaling during Arabidopsis-*Pseudomonas syringae* interaction. Plant Physiol **145:** 450–464

Zhang Z, Wang M, Li Z, Li Q, He Z (2008b) Arabidopsis GH3.5 regulates salicylic acid-dependent and both *NPR1*-dependent and independent defense responses. Plant Signal Behav **3:** 537–542

Zhao Y (2010) Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol **61:** 49–64

Zhao Y, Hu Y, Dai M, Huang L, Zhou DX (2009) The WUSCHEL-related homeobox gene *WOX11* is required to activate shoot-borne crown root development in rice. Plant Cell **21:** 736–748

Zhu Y, Nomura T, Xu Y, Zhang Y, Peng Y, Mao B, Hanada A, Zhou H, Wang R, Li P, et al (2006) *ELONGATED UPPERMOST INTERNODE* encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. Plant Cell **18:** 442–456