OsRAR1 and OsSGT1 Physically Interact and Function in Rice Basal Disease Resistance

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The RAR1 and SGT1 proteins function synergistically or antagonistically in plant innate immune responses. Here, we show that the rice orthologs OsRAR1 and OsSGT1 physically interact in vivo and in yeast. They displayed conserved roles in Arabidopsis disease resistance through ectopic expression in the Arabidopsis rar1 and sgt1 mutants. Overexpression of OsRAR1 and OsSGT1 in rice significantly increased basal resistance to a virulent bacterial blight Xanthomonas oryzae pv. oryzae PXO99 but not to another virulent strain DY89031, suggesting race-specific-like basal resistance conferred by OsRAR1 and OsSGT1. OsRar1-OE and OsSGT1-OE plants also enhanced resistance to all four virulent blast fungal Magnaporthe oryzae races. Overexpression of the OsSGT1-green fluorescent protein (GFP) fusion most likely caused a dominant negative phenotype which led to race-specific-like basal resistance. Transgenic plants overexpressing OsSGT1-GFP show enhanced resistance to DY89031 but decreased resistance to PXO99, implying that OsSGT1 might be the target of a component required for DY89031 virulence or OsSGT1-GFP might stabilize weak resistance proteins against DY89031. Consistent with the hypothesis of the dominant negative regulation, we observed the reduced sensitivity to auxin of OsSGT1-GFP plants compared with the wild-type ones, and the curling-root phenotype in OsSGT1-OE plants. These results collectively suggest that OsRAR1 and OsSGT1 might be differentially required for rice basal disease resistance. Our current study also provides new insight into the roles of OsSGT1 in basal disease resistance.

Additional keywords: development, R-mediated resistance.

Although it does not lead to strong disease resistance compared with R-mediated specific resistance, basal defense provides the first line of defense against pathogen attack. One such PRR is the well-characterized flagellin receptor kinase FLS2 that recognizes the highly conserved flg22 peptide, leading to immediate defense responses (Asai et al. 2002; Gomez-Gomez et al. 2001; Zipfel et al. 2004). Moreover, the recognition of flg22 induces FLS2 internalization associated with defense responses (Robatzek et al. 2006). In addition, some other important components involved in basal defense have been identified, of which EDS1 and its interacting protein PAD4 are required by both R resistance and basal defense (Falk et al. 1999; Feys et al. 2001; Jirage et al. 1999; Parker et al. 1996). Recently, a key regulator of R resistance, RIN4, also has been shown to modulate flg22 signaling (Kim et al. 2005). Collectively, both basal and specific immune signaling pathways appear to be continuum-linked.

As a key component involved in diverse R resistance, RAR1 is a novel eukaryotic zinc-binding protein. It is required for race-specific resistance to powdery mildew in barley (Haltermann et al. 2001; Haltermann and Wise 2004; Lahaye et al. 1998; Shen et al. 2003; Shirasu et al. 1999). Genetic screening also discovered that RAR1 is a key component for diverse R-mediated resistance in Arabidopsis (Holt et al. 2005; Muskett et al. 2002; Tornero et al. 2002). The RAR1 gene also plays an important role in plant basal defense: mutations in RAR1 enhance susceptibility to virulent pathogens in Arabidopsis and barley (Holt et al. 2005; Jarosch et al. 2005). Furthermore, AIRAR1 is a target of the Pseudomonas syringae effector AvrB that suppresses PAMP-triggered immunity (Shang et al. 2006). RAR1 contains two conserved CHORD domains required for physical interaction with the partner protein SGT1, which also plays an essential role in Mla12-mediated specific powdery mildew resistance in barley (Azevedo et al. 2002). Similarly, SGT1 protein is required by multiple R-mediated resistance and nonhost resistance in many species, including Arabidopsis, tobacco, wheat, pepper, and tomato (Austin et al. 2002; Leister et al. 2005; Liu et al. 2002; Peart et al. 2002; Schornack et al. 2004; Scofield et al. 2005; Tor et al. 2002; Zhang et al. 2004). It has been demonstrated that RAR1 and SGT1 directly interact with each other in planta, and the interaction of RAR1 and SGT1 is suggested to be essential for their functions in plant defense (Austin et al. 2002; Liu et al. 2002; Tor et al. 2002). RAR1 and SGT1 function synergistically in most R resistance. However, the Arabidopsis AtRAR1 and AtSGT1b play antagonistic roles in accumulation of some R proteins and in basal defense (Holt et al. 2005). A recent report further suggested that the antagonis-
tic roles of AtRAR1 and AtSGT1b might result from the presence of two copies of SGT1, AtSGT1a and AtSGT1b, in Arabidopsis. AtSGT1a and AtSGT1b accumulate at different steady-state levels and diverse R proteins require different threshold of SGT1 to trigger effective resistance (Azevedo et al. 2006). Therefore, arguments arise on the precise mechanisms of RAR1 and SGT1 roles in plant disease resistance.

Silencing of the Rar1 homolog chp in Caenorhabditis elegans by RNA interference (RNAi) resulted in semisterility and embryo lethality, indicating a role of RAR1 in development (Shirasu et al. 1999). In tobacco, both NbRAR1 and NbSGT1 are associated with the COP9 signalosome and the components of the Skp1/Cullin/F-box protein (SCF)-type E3 ubiquitin ligase complex, which is involved in protein degradation mediated by the COP9 signalosome (Liu et al. 2006). Furthermore, AtSGT1 is required for SCFTRI-mediated degradation of Aux/indole-3-acetic acid (IAA) proteins and is involved in the auxin-related processes, including auxin inhibition of root growth, lateral root development, hypocotyl elongation at high temperature, and apical dominance (Gray et al. 2003). Therefore, RAR1 and SGT1 link disease resistance to developmental processes. However, questions still remain as to the regulation of RAR1 and SGT1 in the cross-talk of plant defense and development.

Rice, as a monocot plant with known genome sequence, has been a model for studying the molecular mechanism of defense responses in cereal crops. The rice genome contains single-copy Rar1 and one SGT1 homologous genes, OsRar1 and OsSGT1. Whether the two genes are involved in disease resistance remains unknown. Here, we report that the rice orthologs OsRAR1 and OsSGT1 physically interact with each other and function in basal disease resistance to rice bacterial leaf blight caused by Xanthomonas oryzae pv. oryzae and fungal blast caused by Magnaporthe oryzae in a race-specific-like basal resistance manner. We also present evidence that OsSGT1 functions in the cross-talk between defense and development, and that expression of the OsSGT1-green fluorescent protein (GFP) fusion might dominate or negatively regulate defense and root growth.

RESULTS
OsRAR1 and OsSGT1 interact with each other in vivo and in yeast.

It was reported that the barely and Arabidopsis RAR1 and SGT1 proteins interact with each other via their CHORDII and CS domains (Azevedo et al. 2002). We first determined subcellular localization of the OsRAR1 and OsSGT1 proteins. Both DsRED-OsRAR1 and OsSGT1-GFP fusion proteins localize ubiquitously in the cytoplasm and nucleus (Fig. 1A and B). Furthermore, co-expression of OsSGT1-GFP and DsRED-OsRAR1 showed that they were co-localized (Fig. 1C), suggesting that they might physically interact with each other in vivo. We further performed a bimolecular fluorescence complementation (BiFC) assay in which the yellow fluorescent protein (YFP)
was split into two polypeptides (Fig. 1D), with the N terminus fused with OsSGT1 (YN-OsSGT1) and the C terminus with OsRAR1 (YC-OsRAR1). Co-expression of YN-OsSGT1 and YC-OsRAR1 reconstituted YFP fluorescence (Fig. 1E), whereas co-expression of YN-OsSGT1 and YC or YC-OsRAR1 and YN did not produce any fluorescence (data not shown). Collectively, we demonstrated that OsRAR1 and OsSGT1 physically interact with each other in vivo. We further examined the interacting domains of OsSGT1 and OsRAR1 using the yeast two-hybrid system. The result showed that OsRAR1 and OsSGT1 interact

![Fig. 2. Determination of interaction domains of OsRAR1 and OsSGT1 in yeast two-hybrid experiments. The full-length and partial coding sequences of OsSGT1 and OsRAR1 were inserted into the vectors pGAD-T7 (activator domain fusion, AD), pGBK-T7 (binding domain fusion, BD), or both, as indicated. These constructs were introduced into yeast AH109 cells with empty vectors as controls, and interactions were detected in high stringent screen medium with X-α-gal according to the protocol for the GAL4 system.](image)

![Fig. 3. Conserved function of OsRAR1 and OsSGT1 in Arabidopsis. A, Levels of OsRAR1 and OsSGT1 were detected by Western blot in the transformed Arabidopsis rar1 and sgt1 mutants. Note that OsRAR1 accumulated to only a trace amount. B, Resistance gene–mediated resistance to DC3000 (AvrRpt2) was restored in the rar1 mutant expressing the AtRar1-OsRar1 chimera. C, Basal resistance to DC3000 was restored in the rar1 mutant expressing the AtRAR1-OsRAR1 chimera. D, Significantly (P < 0.05) enhanced basal resistance to DC3000 in the sgt1b mutant overexpressing OsSGT1. Each two representative lines of OsRar1 and OsSGT1 transformants were shown. All experiments were repeated at least once with similar results.](image)
with each other via the CHORDII domain of OsRAR1 and the CS domain of OsSGT1 (Fig. 2), consistent with the previous observation (Azevedo et al. 2002).

**OsRar1 and OsSGT1 exhibit conserved functions in Arabidopsis disease resistance.**

Mutations in AtRAR1 lead to impairment of multiple R-mediated resistance and basal defense (Holt et al. 2005; Muskett et al. 2002; Tornero et al. 2002). High identity in sequences predicts that OsRar1 and AtRAR1 could be functionally conserved. To test this hypothesis, we expressed OsRar1 driven by the AtRAR1 promoter in the Atrar1-11 mutant, as indicated by protein levels (Fig. 3A). The introduction of OsRar1 restored the RPS2-mediated resistance of Atrar1 to avirulent *P. syringae pv. tomato* DC3000 (AvrRpt2) to the same level of the wild-type Ler (Fig. 3B). In addition, OsRar1 rescued basal disease resistance of Atrar1 to virulent *P. syringae pv. tomato* DC3000 (Fig. 3C), indicating that OsRar1 functions in basal defense as well.

Single mutation of neither AtSGT1a nor AtSGT1b affects the basal defense of Arabidopsis. We constitutively expressed OsSGT1 (OsSGT1-OE) in the Atsgt1b-1 mutant (Fig. 3A). Inoculation with *P. syringae pv. tomato* DC3000 showed that the OsSGT1-OE lines statistically significantly decreased the growth of bacteria compared with the wild-type and Atsgt1b mutant (Fig. 3D). We also observed that the OsSGT1-OE lines restored the RPP5-mediated resistance to *Peronospora parasitica* race Noco2 (Table 1). Therefore, OsSGT1, like OsRar1, is also the rice ortholog and functions in both R and basal disease resistance in transgenic Arabidopsis. These results demonstrate that the rice ortholog OsRar1 and OsSGT1 have conserved functions in Arabidopsis defense.

Fig. 4. Enhanced basal disease resistance to bacterial blight in OsRar1-OE and OsSGT1-OE plants. A, Northern blot detection of the OsRar1 and OsSGT1 transcripts in transgenic plants. B, Levels of the OsRAR1 and OsSGT1 proteins detected by Western blot in each two representative transgenic lines, with Rubisco staining as a control. Note that both proteins were hardly detectable in the wild-type TP309. Disease resistance to virulent PXO99 was significantly enhanced (*P < 0.05*) in C, OsRar1-OE and D, OsSGT1-OE plants compared with the wild-type and vector control. Basal disease resistance in E, OsRar1-OE and F, OsSGT1-OE plants to virulent *Xanthomonas oryzae pv. oryzae* DY89031. No reliable difference was observed in disease resistance in these plants compared with the wild-type and vector control.

**OsRar1 and OsSGT1 function in rice basal disease resistance to bacterial blight.**

To determine roles of OsRar1 and OsSGT1 in rice disease resistance, we generated transgenic rice constitutively expressing...
OsRar1 and OsSGT1 (OsRar1-OE and OsSGT1-OE). RNA and Western blotting revealed that both OsRar1 and OsSGT1 were overexpressed in the homozygous progeny plants at the transcript and protein levels (Fig. 4A and B). We inoculated transgenic plants with virulent X. oryzae pv. oryzae PXO99 and DY89031, in comparison with the wild-type TP309, vector control, and the transgenic TP309 line 106 (Xa21-106) harboring the resistance gene Xa21 to PXO99 but not DY89031 (Song et al. 1995; Yuan et al. 2007). All OsRar1-OE lines exhibited a statistically significant (P < 0.05) alleviation of disease symptom to PXO99 in comparison with the controls (Fig. 4C). Enhanced resistance to PXO99 was also observed for the OsSGT1 overexpressing lines (Fig. 4D). Intriguingly, none of the OsRar1-OE and OsSGT1-OE lines showed significant change in resistance to another virulent X. oryzae pv. oryzae strain, DY89031, with repeated experiments of two generations (Fig. 4E and F). These results suggest that OsRar1 and OsSGT1 function in rice basal defense to bacterial blight in a race-specific-like manner (discussed below). Together with the data from transgenic Arabidopsis, OsRar1 and OsSGT1 function in rice basal disease resistance to blast fungus.

We further tested basal disease resistance of OsRar1-OE and OsSGT1-OE plants to blast fungus (M. oryzae). Unlike to X. oryzae pv. oryzae, both types of transgenic plants exhibited enhanced resistance to all the four tested virulent fungal races compared with the wild-type and vector transgenic controls (Fig. 5A and B), suggesting different requirements for OsRar1 and OsSGT1 in basal resistance to X. oryzae pv. oryzae and blast. Because there is no available Osrar1 and Ossgt1 knockout mutants, we also extensively conducted RNA interference with both double-stranded RNA technology that has been successfully used to knockdown the OsNPR1 gene in our previous study (Yuan et al. 2007) and antisense expression, but we did not manage to generate OsRar1 and OsSGT1 knockout plants (Supplemental Figure 1). It is possible that the regions of OsRAR1 and OsSGT1 chosen do not work well with the interference approach. It remains unknown what phenotypes, if any, are associated with knockout mutants of OsRar1 and OsSGT1.

OsSGT1-GFP fusion probably acts as a dominant negative form and confers a race-specific-like basal resistance.

We generated rice plants (OsSGT1-GFP) constitutively expressing the OsSGT1-GFP fusion, in which both the OsSGT1-GFP fusion protein and the endogenous OsSGT1 protein were

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**Table 1. OsSGT1 restores RPP5-mediated resistance to Peronospora parasitica Noco2 in the Atsgt1b mutant**

<table>
<thead>
<tr>
<th>Line</th>
<th>Diseased plants/total plants</th>
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<tr>
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<td>0/24</td>
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<tr>
<td>Atsgt1b-1</td>
<td>24/24</td>
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<tr>
<td>Atsgt1b/OsSGT1-OE 2</td>
<td>0/13</td>
</tr>
<tr>
<td>Atsgt1b/OsSGT1-OE 50</td>
<td>0/24</td>
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<tr>
<td>Atsgt1b/OsSGT1-OE 106</td>
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<tr>
<td>Atsgt1b/OsSGT1-OE 170</td>
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**Fig. 5.** Enhanced basal disease resistance to *Magnaporthe oryzae* in OsRar1-OE and OsSGT1-OE plants. Leaves were spot inoculated with spore suspensions of four blast races (CH131, OS9649, CH155, and SY22) and disease symptoms were recorded at 5 days postinoculation. Each two representative lines of A, OsRar1-OE and B, OsSGT1-OE plants were shown, together with the controls. Note that lesion development was inhibited in the transgenic plants compared with the wild-type and vector controls in independent inoculation experiments.
produced (Fig. 6A, upper panel). OsSGT1-GFP accumulation was further confirmed by Western blotting with a commercial anti-GFP antibody (Fig. 6A, lower panel). The OsSGT1-GFP fusion also ubiquitously localized in the cytoplasm and nucleus of transgenic rice root cells (Fig. 1F). We observed that the basal disease resistance to PXO99 was significantly compromised in OsSGT1-GFP plants (Fig. 6B). Surprisingly, resistance to DY89031 was greatly increased in all lines to a level similar to Xa21-triggered resistance to PXO99 with repeated experiments of two generations (Fig. 6C and D). Because TP309 does not contain known R genes to either PXO99 or DY89031, we propose that OsSGT1-GFP most likely acts as a dominant negative form that inhibits the endogenous OsSGT1-mediated resistance to PXO99 but positively regulates resistance to DY89031. We also generated transgenic rice lines (DsRED-OsRar1) constitutively expressing the DsRED-OsRAR1 fusion that express both the DsRED-OsRAR1 and endogenous OsRAR1 proteins (Fig. 7A). The DsRED-OsRAR1 fusion protein also was co-localized with OsSGT1-GFP fusion in the root cells of the transgenic hybrid plant (Fig. 1F), suggesting that the fusions do not change their physical interaction in the rice cell as in the onion cell. However, no changes were observed in resistance to both X. oryzae pv. oryzae strains in DsRED-OsRar1 plants (Fig. 7B and C). Whether the DsRED-OsRAR1 fusion has lost its function remains to be investigated.

**DISCUSSION**

Our study has demonstrated the conserved functions of OsRAR1 and OsSGT1 in rice immunity. The functions of RAR1 and SGT1 in defense responses likely are fulfilled through their physical interaction in a complex (Azevedo et al. 2002; Dodds and Schwechheimer 2002; Muskett and Parker 2003). We performed detailed experiments to visualize the molecular interaction between OsRar1 and OsSGT1 (Fig. 1). We also showed that this interaction is via the CHORD II and CS do-

**Fig. 6.** Race-specific-like basal disease resistance in OsSGT1-green fluorescent protein (GFP) plants. A, Both the endogenous OsSGT1 protein and the OsSGT1-GFP fusion were expressed in two representative OsSGT1-GFP lines. B, Significant decreased (P < 0.05) resistance to virulent *Xanthomonas oryzae* pv. oryzae PXO99 in the OsSGT1-GFP lines compared with TP309, with Xa21-106 as the resistant control. C, High resistance to virulent *X. oryzae* pv. oryzae DY89031 in the OsSGT1-GFP lines, in comparison with TP309, Xa21-106, and OsSGT1-OE plants. D, Lesion lengths of OsSGT1-GFP, TP309, and Xa21-106 inoculated with *X. oryzae* pv. oryzae DY89031.
mains of OsRAR1 and OsSGT1 (Fig. 2). Therefore, our study supports that the physical interaction is a common feature of RAR1 and SGT1 in diverse plant species.

RAR1 is required for basal defense in Arabidopsis and barley, probably through its contribution to R protein accumulation (Holt et al. 2005; Jarosch et al. 2005). We showed that overexpression of OsRar1 and OsSGT1 restricted growth of one of the two virulent bacterial strains and all fungal races tested (Figs. 4 and 5). The difference in basal resistance of OsRar1-OE and OsSGT1-OE plants to X. oryzae pv. oryzae and M. oryzae probably resulted from distinct pathways by which rice responds to X. oryzae pv. oryzae and M. oryzae, as suggested by our microarray assay (Li et al. 2006). Furthermore, we found that constitutively expressing OsSGT1 in an Arabidopsis sgt1b mutant also increased resistance to its virulent pathogen (Fig. 3D), indicating that SGT1, like Rar1, also plays a positive role in plant basal defenses in addition to its involvement in R and nonhost disease resistance (Peart et al. 2002).

It is surprising that OsSGT1-OE and OsRar1-OE plants exhibited race-specific-like basal resistance to PXO99 and DY89031, suggesting that the two strains acquire divergent virulent components. Furthermore, when overexpressed as a GFP fusion, OsSGT1 appeared to cause a dominant negative phenotype that impaired basal disease resistance to PXO99 but induced a race-specific-like resistance to DY89031 (Fig. 6).

![Anti-OsRAR1](image)

A

![Lesion length (cm)](image)

B

![Lesion length (cm)](image)

C

**Fig. 7.** Basal disease resistance in DsRED-OsRar1 plants. **A,** Both endogenous OsRAR1 and DsRED-OsRAR1 lines. Asterisks indicate nonspecific proteins detected by the OsRAR1 antibodies. **B,** Disease resistance to PXO99 in DsRED-OsRar1, TP309, and Xa21-106 plants. **C,** Disease resistance to DY89031 in DsRED-OsRar1, TP309, and vector control plants. No reliable difference was observed in disease resistance in these plants compared with the wild-type and vector control.

Similar dominant negative effects of GFP fusion proteins were observed in the GFP-phragmoplastin fusion protein on cell plate formation of tobacco BY-2 cells (Gu and Verma 1997) and the ASK1-GFP on Arabidopsis growth and fertility (Wang and Yang 2006).

These observations have not been reported in plants. The complicated phenotype may indicate different requirements for the SGT1 protein or its complex in basal defense to diverse bacterial strains, because SGT1 is required differently for diverse R genes and nonhost resistance (Austin et al. 2002; Leister et al. 2005; Schornack et al. 2004; Scofield et al. 2005; Tor et al. 2002). Most likely, the OsSGT1-GFP fusion titrates out and the wild-type OsSGT1 (as well as OsRAR1) stabilizes weak R proteins that recognize uncharacterized effectors or pathogen determinants of PXO99. In this scenario, weak R proteins recognizing effectors of DY89031 may be different from those of PXO99 and the OsSGT1-GFP fusion might stabilize weak R proteins against DY89031. Alternatively, OsSGT1 may be the target of a component required for DY89031 virulence, and the OsSGT1-GFP fusion could have titrated out the component, similar to ArRAR1 as the target of the P. syringae AvrB (Shang et al. 2006). Consistent with our hypothesis, DY89031 contains mutations in the genes required for AvrXa21 activity to XA21 (Goes da Silva et al. 2004), which is proposed to be a secreted peptide acting as a quorum-sensing molecule with features of PAMP and Avr elicitors (Burdman et al. 2004; Lee et al. 2006). Further evidence that PAMPs derived from different bacterial strains induce strain-specific defense responses comes from the study that flagellin of P. avenae functions as a specific elicitor in rice (Che et al. 2000). A recent report also showed that sequence variation of flagellin among X. campestris pv. campestris strains affects FLS2-dependent pathogen recognition by Arabidopsis (Sun et al. 2006).

Our current study indicates that, although OsRAR1 and OsSGT1 physically interact with each other in planta and both function in basal disease resistance, mechanisms by which OsRAR1 and OsSGT1 regulate basal defense might be different, because the DsRED-OsRAR1 fusion protein appeared to not alter resistance to X. oryzae pv. oryzae (Fig. 7). Consistent with this idea, RAR1 and SGT1 in other plant species also function differently or even antagonistically in some R-gene-mediated resistance (Holt et al. 2005; Leister et al. 2005). It will be interesting to further investigate the mechanism of fine-tuning the RAR1–SGT1 interaction and additional components in the RAR1–SGT1 complex as previously proposed (Dodds and Schwechheimer 2002; Musckett and Parker 2003). OsSGT1 loss-of-function data is not available currently; therefore, the dominant negative-like phenotype of OsSGT1-GFP needs to be further characterized genetically.

The OsSGT1–GFP fusion function is supported by the developmental phenotype. Because SGT1 has been shown to act cooperatively with the SCFTIR1-mediated auxin pathway and the COP9 signalosome (Gray et al. 2003; Liu et al. 2006), our data point strongly to the possibility that OsSGT1 and OsSGT1-GFP differently regulate the rice COP9 signalosome and SCFTIR1 complex that interactively play key roles in plant development through ubiquitin-proteasome-mediated protein degradation (Wei and Deng 2003). Due to lacking functional information of the rice COP9 and SCFTIR1 complexes (Yamamoto et al. 2003), we currently do not know how the OsSGT1-GFP-mediated dominant negative regulation occurs. Our preliminary data showed that OsSGT1-GFP roots were less sensitive to 2,4-D but not to IAA in comparison with wild-type roots. It is possible that the OsSGT1-GFP fusion might affect a particular auxin transport system differentially required for 2,4-D and IAA responses, as previously reported (Zhuang et al. 2006). Interestingly, the
curling root phenotype also is associated with the Atmlo mutants (Sandra et al. 2007). Overexpression of OsSGT1 leads to this phenotype in rice, probably through regulating the interaction between SGT1 and RAR1, because the latter has been shown to be associated with the MLO function and rice contains a functional MLO ortholog (Elliott et al. 2002). The detailed proteomic and genetic analysis, and the identification of the SGT1-interacting components that is required for DY89031 virulence and probably for COP9/SCE2TR1-mediated development as well, would provide a clue for the OsSGT1-mediated phenotypes.

**MATERIALS AND METHODS**

**Gene cloning and plasmid construction.**

Using publicized genome sequences of rice, we isolated the 1.2-kb full-length OsSGT1 cDNA (GenBank accession number AF192467) from a rice cDNA library, which was inserted into Kan1 and BamHI sites of the binary vector 35S-C1301 (provided by P. Ronald) to form the OsSGT1 overexpression construct OsSGT1-OE. The full-length cDNA of OsRar1 (GenBank accession number C28356) was obtained from the National Institute of Agrobiological Sciences (Tsukuba, Japan) and was inserted into ApaI and SacI sites of 35S-C1301 to form the overexpression construct OsRar1-OE. For complementation of the Arabidopsis rar1 mutant, the OsRar1 cDNA was placed under the promoter of AtRar1 to generate the AtRar1-OsRar1 chimera that was inserted into pCAMBIA1301 for Arabidopsis transformation. The construct OsSGT1-OE also was transformed into the Atsgt1b mutant. For RNAi, a 600-bp OsRar1 or OsSGT111 cDNA fragment was reversely inserted into 35S-C1301 to generate antisense expression plasmid, or was inserted into the double-stranded RNA interference vector 1300S to form RNAi plasmid (Yuan et al. 2007).

**Rice and Arabidopsis transformation.**

Rice and Arabidopsis transformation was performed with the Agrobacterium-mediated method. The rice cv. Taipei 309 (TP309) was used as recipient to produce more than 20 independent transgenic lines for each construct. All transformants (TP309) was used as recipient to produce more than 20 independent transgenic lines for each construct. The bacterial pathogen growth curve was measured on record at 5 and 7 days postinoculation. Inoculated leaves were kept in a growth chamber with light intensity of 240 μmol m–2 s–1 at 26°C, and disease symptoms were recorded at 5 and 7 days after inoculation. Root length was measured at day 7 after inoculation.

**Pathogen inoculation.**

Seven-week-old plants of independent transgenic lines and controls were inoculated with X. oryzae pv. oryzae races P6 (strain PX099) and K1 (strain DY89031) using the leaf clip method (Song et al. 1995). After 2 weeks, lesion lengths were recorded. For blast inoculation, four virulent races (OS9649, Sy22, CH155, and CH131) and a spot-inoculation method were applied (Xiong and Yang 2003). Leaves were pierced with a needle, and a droplet of spore suspension (10 μl containing approximately 50 spores) was inoculated on the punctured sites. Inoculated leaves were kept in a growth chamber with light intensity of 240 μmol m–2 s–1 at 26°C, and disease symptoms were recorded at 5 and 7 days postinoculation. P. syringae inoculation on Arabidopsis was performed with the syringe injection method. The bacterial pathogen growth curve was measured with independent assays. Arabidopsis inoculation with Peronospora parasitica race Noco2 was performed as previously described (Bowling et al. 1997).

**Subcellular localization and BiFC analysis.**

The full-length coding regions of OsSGT1 and OsRar1 were in-frame fused with mGFP (GenBank accession number U87973) and DsRED (GenBank accession number AF168419) in 35S-C1301 with appropriate sites (Supplemental Table 1). The OsSGT1-GFP and DsRED-OsRar1 fusions were expressed transiently in onion epidermal cells by particle bombardment. Transgenic rice also was generated with the fusion constructs. For analysis of BiFC of OsSGT1 and OsRar1 interaction, the fragments corresponding to the N (YNI-155) and C (YCI56-239) termini of YFP (GenBank accession number AF292557) were fused in-frame with OsSGT1 and OsRar1, respectively, to generate BiFC expression constructs YN-OsSGT1 and YC-OsRar1 in 35S-C1301 with appropriated sites, which were co-introduced into onion epidermal cells by particle bombardment. Fluorescent images were observed with a confocal laser microscopy (Zeiss LSM510).

**RNA preparation and Northern blotting.**

Total RNA was prepared from leaf tissues using TRIzol reagent according to the manufacturer’s instructions (Gibco BRL, Gaithersburg, MD, U.S.A.). RNA samples (20 μg) were separated on a 1% formaldehyde-agarose gel, and then blotted onto Hybond-N membranes (Amersham, Tokyo). The 1.1-kb OsRar1 and a 1.2-kb OsSGT1 fragments were labeled with [α-32P]dCTP using a random primer labeling kit (Takara) for hybridization and autoradiography.

**Protein analysis.**

The full-length coding regions of OsRar1 and OsSGT1 were ligated into pET-32a to produce the fusion proteins in the Escherichia coli BL21(DE3). Antibodies (antisum) against the OsRAR1 and OsSGT1 fusion proteins were raised with standard procedure. Western blotting was performed using the SuperSignal West chemiluminescence kit according to the manufacturer’s protocol (Pierce, Rockford, IL, U.S.A.).

**Auxin responses.**

For analysis of auxin responses, 2,4-D and IAA of different concentrations were added to 1/2 Murashige Skoog medium. Sterilized rice seed were germinated on the media at 60 μm2 s–1 light intensity, 26°C, and day and night length of 14 and 10 h, respectively. Root length was measured at day 7 after germination.

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**LITERATURE CITED**


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