Altered Disease Development in the eui Mutants and Eui Overexpressors Indicates that Gibberellins Negatively Regulate Rice Basal Disease Resistance

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ABSTRACT  Gibberellins (GAs) form a group of important plant tetracyclic diterpenoid hormones that are involved in many aspects of plant growth and development. Emerging evidence implicates that GAs also play roles in stress responses. However, the role of GAs in biotic stress is largely unknown. Here, we report that knockout or overexpression of the \textit{Elongated uppermost internode} (Eui) gene encoding a GA deactivating enzyme compromises or increases, respectively, disease resistance to bacterial blight (\textit{Xanthomonas oryzae pv. oryzae}) and rice blast (\textit{Magnaporthe oryzae}). Exogenous application of GA\textsubscript{3} and the inhibitor of GA synthesis (uniconazol) could increase disease susceptibility and resistance, respectively, to bacterial blight. Similarly, uniconazol restored disease resistance of the eui mutant and GA\textsubscript{3} decreased disease resistance of the Eui overexpressors to bacterial blight. Therefore, the change of resistance attributes to GA levels.

In consistency with this, the GA metabolism genes \textit{OsGA20ox2} and \textit{OsGA2ox1} were down-regulated during pathogen challenge. We also found that \textit{PR1a} induction was enhanced but the SA level was decreased in the \textit{Eui} overexpressor, while the JA level was reduced in the \textit{eui} mutant. Together, our current study indicates that GAs play a negative role in rice basal disease resistance, with EUI as a positive modulator through regulating the level of bioactive GAs.

INTRODUCTION

Plants encounter various abiotic and biotic environmental stresses during their lifetimes. They have to modulate growth and development to adapt to different stress conditions. Many constitutive defense mutants, such as \textit{cpr1}, \textit{mpk4}, \textit{bon1}, and \textit{mekk1}, reduce growth (Bowling et al., 1994; Petersen et al., 2000; Yang and Hua, 2004; Ichimura et al., 2006), indicating that defense response may down-regulate growth and development. This hypothesis is further supported by the \textit{snc1 yucca} and \textit{cpr6 yucca} double mutants in which the phenotypes of \textit{yucca} are mostly suppressed by \textit{snc1} or \textit{cpr6}, which are involved in disease resistance (Wang et al., 2007a). On the other hand, mutation of genes that are involved in growth and development, like \textit{axr2} (Wang et al., 2007a) and \textit{as1} (Nurmberg et al., 2007), enhance disease resistance; thus, defense response antagonizes development and growth in many cases.

It is well known that salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the phytohormones involved in disease resistance. Recently, the development-regulating hormones abscisic acid (ABA) (de Torres-Zabala et al., 2007; Adie et al., 2007), cytokinin (Siemens et al., 2006), auxin (Navarro et al., 2006; Wang et al., 2007a; Zhang et al., 2007), and brassinosteroid (Nakashita et al., 2003) were also reported to be involved in disease resistance. ABA is an important hormone that is involved in many aspects of plant development and responses to abiotic stress, such as drought, low temperature, and salinity, and also acts as a negative regulator in many defense responses (Mauch-Mani and Mauch, 2005; de Torres-Zabala et al., 2007).

Auxin regulates almost all growth and development processes in plants. It has been shown that \textit{Agrobacterium tumefaciens}, \textit{Agrobacterium rhizogenes}, and other gall-forming bacteria can produce the hormone for their fitness in the host (Robert-Seilaniantz et al., 2007). \textit{Pseudomonas syringae} (\textit{P. syringae}) secretes the type III effector AvrRpt2 to modulate the host auxin physiology to promote disease (Chen et al., 2007). Interestingly, a member of the GH3 family of early auxin-responsive genes, \textit{GH3.5}, acts as a bifunctional modulator in
both SA and auxin signaling during pathogen infection in *Arabidopsis* (Zhang et al., 2007). Overexpression of the GH3.5 gene led to elevated accumulation of both SA and IAA, and increased susceptibility to avirulent *P. syringae* pathogens. The repression of auxin signaling, through down-regulating the auxin receptor genes by the microRNA mir393a that is induced by the pathogen flagellin-derived peptide flg22, resulted in increased resistance (Navarro et al., 2006). These studies indicate that auxin plays an important role in disease susceptibility pathways. Consistent with these observations, recently, it has been shown that SA enhances disease resistance partially through repression of auxin signaling (Wang et al., 2007a).

Cytokinin homeostasis genes were down-regulated during the *Arabidopsis–Plasmodiophora brassicae* interaction; overexpression of the cytokinin degradation genes could enhance disease resistance to the pathogen (Siemens et al., 2006). By contrast, another growth-regulating hormone, brassinosteroid, could increase disease resistance in rice and tobacco (Nakashita et al., 2003). These data suggest that plants might employ different hormone signaling pathways to manipulate growth, development, and defense responses during pathogen attack.

Gibberellins (GAs) are a group of isoprenoid plant hormones that regulate many aspects of growth and development in plants, including seed germination, stem elongation, and flowering. GAs were originally identified from the fungal pathogen *Gibberella fujikuroi* (Yabuta and Sumiki, 1938), which causes super-elongated rice, named bakanae rice. GA-deficient fungal mutants do not affect fungal development, suggesting that GAs produced by fungal pathogens may have a pathogenicity role in plants (reviewed by Robert-Seilaniantz et al., 2007). A direct link of GA biosynthesis with plant disease symptoms came from the rice dwarf virus (RDV) study showing that the outer capsid protein P2 of RDV interacts with the host ent-kaurene oxidases (KAO), which play a key role in the biosynthesis of GAs (Zhu et al., 2005). They also found that KAO expression was reduced in the infected plants, leading to decreased levels of GA1 in the infected plants. Moreover, the interaction between P2 and rice ent-kaurene oxidase-like proteins may decrease phytoalexin biosynthesis and make plants more competent for virus replication. ELONGATED UPPERMOST INTERNODE (EUI) is a P450–kaurene oxidase-like proteins may decrease phytoalexin biosynthesis and make plants more competent for virus replication. ELONGATED UPPERMOST INTERNODE (EUI) is a P450 biocatalyst that deactivates biologically active GAs through a novel reaction (Zhu et al., 2006). The loss-of-function mutants of EUI accumulate large amounts of bioactive GAs and extremely elongate the uppermost internode, whereas overexpression of EUI resulted in the GA-deficient phenotype with severe dwarfing, dark-green leaves, and male sterility (Xu et al., 2004; Zhu et al., 2006). Interestingly, the GA-overproducing *eui* rice seems more susceptible to bacterial and fungal pathogens in the field (Yang and He, unpublished data), implying that GA homeostasis may cross-talk with disease resistance. However, the underlying mechanism of this GA-related susceptibility is elusive.

In this work, we compared disease resistance in the *eui* mutants and the *Eui* overexpressors (*Eui–OX*) with the corresponding wild types, and the effects of exogenous application of GA and the GA synthesis inhibitor on rice basal disease resistance to bacterial blight (*Xanthomonas oryzae pv. oryzae*, Xoo) and fungal blast (*Magnaporthe oryzae*, *M. oryzae*)—two of the most destructive diseases in rice. We demonstrate that the GA-mediated development actively impacts in disease resistance in rice, revealing another dimension of the complex cross-talk between development and defense.

**RESULTS**

*eui* Mutants Are More Susceptible to Bacterial Blight

In order to examine the role of *Eui*-mediated GA homeostasis in disease resistance, three alleles of the *eui* mutation and their corresponding wild-type plants (Zhu et al., 2006) were inoculated with *Xoo* Philippine race 6 (strain PXO99A) and *Korean race 1* (strain DY89031) using the leaf-clipping method. Lesion lengths of more than 50 leaves for each line were recorded at 14 d post inoculation (dpi). As shown in Figure 1, the mutants displayed significantly longer lesion to PXO99A than their controls. Similar results were obtained with infection by DY89031 (data not shown). Similarly, the representative transgenic lines S73 and S74, in which *Eui* expression was knocked down with RNAi (Zhang et al., 2008), phenocopied the susceptibility of the *eui* mutants to both the strains (Figure 2A and 2B and Supplemental Figure 1A). We also observed slightly enhanced resistance to *Xoo* in semi-dwarf rice containing the mutant ‘Green Revolution’

![Figure 1. Enhanced Susceptibility to Xoo in the eui Mutants.](image)

Eight-week-old plants were inoculated with *Xoo* strain PXO99A using the leaf-clipping method in the *eui* mutants and the corresponding wild types. The lesion length of more than 50 leaves was recorded after 2 weeks. Bars indicate standard error. ** indicates that lesions of the *eui* mutants were significantly longer than their wild types (student’s t-test: *P* = 0.004 with *eui-1* and ZS97, *P* = 4.1E–12 with *eui-3* and ZH11, *P* = 1E–8 with *eui-4* and 02428). These experiments were repeated three times, with similar results.
gene \textit{sd1}, in comparison with the near-isogenic tall rice carrying the wild-type \textit{Sd1} gene that encodes a GA20 oxidase (data not shown; Sasaki et al., 2002). Therefore, the loss of function of \textit{Eui} decreased disease resistance, suggesting that \textit{EUI} might be a positive modulator of basal disease resistance in rice.

\textbf{Overexpression of \textit{Eui} Increases Disease Resistance to Bacterial Blight}

We further analyzed disease resistance of the \textit{Eui} overexpressors, \textit{Eui–OX} (Zhu et al., 2006). These dwarf \textit{Eui–OX} lines exhibited significantly increased disease resistance as compared with the wild-type and the separated negative transgenic plants (Figure 2 and Supplemental Figure 1B). We also observed that dwarf severity was somehow correlated with resistance degree, since lines OX-21, OX-22, OX-39, and OX-47 were more dwarf and appeared more resistant than OX-7, OX-11, and OX-15 (Figure 2B and data not shown). In consistency with disease symptom, a three- to five-fold reduction in bacterial growth was measured in OX-39 compared with the wild-type at 8–14 dpi. These results further support the notion that \textit{EUI} positively regulates disease resistance against bacterial blight.

\textbf{Silence of \textit{Eui} Leads to Reduction of Resistance and Overexpression of \textit{Eui} Enhances Resistance to Rice Blast Fungus}

We have shown that the \textit{eui} mutants and \textit{Eui} overexpressors exhibited opposite resistance phenotypes to bacterial blight. We next determined whether the \textit{EUI}-mediated development process also modulates disease resistance to rice blast. The RNAi lines S73 and S74 exhibited slightly severer symptoms than the wild-type (Figure 3A and 3B). For grade 5 disease index (the most susceptible symptom), S73 and S74 displayed 20 and 19%, respectively, in comparison with 9% in the wild-type (Figure 3B). Similar reduction of blast resistance was also observed in the \textit{eui-1} mutant (data not shown). In contrast, the overexpression lines OX-7, OX-11, OX-21, and OX-39 exhibited...
significantly enhanced resistance to the fungus, with 70–83% of grade 0 and 1, in comparison with 18% in the wild-type, while the percentages for grades 4 and 5 were greatly decreased in these lines (Figure 3B). The further determination of fungal growth in the host using Southern hybridization with the fungal 28S rDNA probe (Qi and Yang, 2002) confirmed that fungal growth was greatly limited in the Eui overexpressors, and slightly increased in the RNAi lines compared with the wild-type (Figure 3C). These data demonstrated that Eui also positively regulates resistance to rice blast.

**GAs Negatively Regulate Disease Resistance**

EUI is an enzyme that deactivates bioactive GAs, the eui mutants and the overexpression transgenic plants accumulate high or low levels of bioactive GAs, respectively (Zhu et al., 2006). Therefore, we logically speculate that GA accumulation negatively regulates basal defense. To further examine this hypothesis, we treated rice with either GA$_3$ (10 μM) or the GA biosynthesis inhibitor uniconazol (10 μM) before inoculation with Xoo. As expected, the plants treated with GA$_3$ displayed longer lesions, and those treated with uniconazol exhibited shorter lesions in comparison with the non-treated control (Figure 4A). To exclude the possibility that the EUI protein itself but not its GA-deactivating activity plays a direct role in disease resistance, we applied GA$_3$ or uniconazol on the Eui overexpressors and the RNAi lines before inoculation with Xoo. GA$_3$ suppressed disease resistance of the Eui overexpressors, while uniconazol restored basal resistance in the RNAi lines (Figure 4). Taken together, GAs negatively regulate rice disease resistance, and the changes of resistance in the eui mutants and Eui overexpressors most likely attributed to the endogenous GA-level alteration.

**SA and JA Levels Decrease Respectively in the Eui Overexpressors and eui Mutant**

The antagonistic interaction between the SA-dependent and JA/ET-dependent defense pathways in Arabidopsis is well known (Thomma et al., 1998; Petersen et al., 2000; Spoel et al., 2003; Li et al., 2004). Interestingly, the loss-of-function mutants in DELLAs, the suppressors of the GA signaling
pathway, up-regulate the SA-mediated defense and down-regulate the JA/ET-mediated defense in Arabidopsis (Robert-Seilaniantz et al., 2007). In rice, endogenous SA protects rice from oxidative stress and the SA-deficient transgenic NahG plants are more susceptible to M. oryzae (Yang et al., 2004), while enhancing JA generation could increase PR expression and blast resistance (Mei et al., 2006). This led us to analyze SA and JA levels in the eui mutant and Eui overexpressors. The SA level did not change in the eui mutant but significantly decreased in the overexpressors (Figure 5A), similarly to the previous result that the transgenic poplar plants overproducing mutant DELLA protein gai1 or rgl1 accumulated less SA (Busov et al., 2006). However, the decreased SA level seemed not to compromise disease resistance in the Eui overexpressors, in contrast to the NahG plants that mostly deprive SA (Yang et al., 2004). The JA level was reduced in the eui mutant but maintained the wild-type level in the overexpressors (Figure 5B). This result suggests that the enhanced disease susceptibility of the eui mutant might partially be attributed to the reduced JA level.

Expression Patterns of Pathogenesis- and GA-Related Genes in the Eui Overexpression Plants

In order to investigate the potential molecular mechanisms of the GA-mediated disease susceptibility, we first analyzed the induction of pathogenesis-related (PR) genes in wild-type and Eui overexpressor and RNAi plants during infection by M. oryzae. As shown in Figure 6A, PR1a induction was enhanced at 12 h but greatly decreased to the control level at 96 h post inoculation (hpi) in the overexpressor compared with its pattern in the wild-type. Its induction appeared delayed in the RNAi S73 compared with the wild-type. We did not observe differential expression of PR1b and PR10 in these plants (Figure 6A). These results implied that GA homeostasis might modulate the induction dynamics of specific PR genes in rice.

We also analyzed the GA biosynthesis gene GA20ox2 (Sd1) and the GA catabolism gene GA2ox1 during blast infection. Interestingly, both GA20ox2 and GA2ox1 were down-regulated with similar expression patterns in the wild-type and the Eui overexpressor (Figure 6B). GA2ox1 is also down-regulated, while GA20ox2 seems less affected in S73 with unknown mechanism. This result was reminiscent of cytokinin synthases and oxidases, which were both down-regulated during the Arabidopsis–Plasmopara brassicae interaction, while overexpression of cytokinin oxidases enhanced resistance (Siemens et al., 2006). Consistently with their pathogen-mediated expression dynamics, cis-element analysis revealed several pathogen-responsive W-boxes within the 1-kb promoter regions of GA20ox2 and GA2ox1 (Figure 6C). These results suggest that GA homeostasis could be regulated during the rice–M. oryzae interaction. In addition, we did not observe expression change of SLR1 during blast infection, which encodes a DELLA protein in rice GA signaling (Ikeda et al., 2001). Whether GA signaling changes during this pathogenesis process requires further investigation.

DISCUSSION

GA-Mediated Development Cross-Talks with Defense Response

Gibberellins are widely recognized as phytohormones that regulate plant growth and development. Although the GA level is observed as reduced during virus infection in rice (Zhu et al., 2005), there is a lack of fundamental information on GA effects on disease resistance. Here, we have demonstrated that GAs exhibit a negative effect on basal disease resistance through genetic and physiological analysis. Our current study would shed light on obscure roles of the diterpenoid hormone on biotic stress.

The mutations of the DELLA proteins cause Arabidopsis to be more resistant to the biotrophic pathogen Pst DC3000 and more susceptible to the necrotrophic pathogen A. brassicicola (Robert-Seilaniantz et al., 2007). These studies implicate that GAs may have either a positive or a negative role in resistance to different pathogens in Arabidopsis. Our results indicate that GAs display negative roles in resistance to both biotrophic (Xoo) and hemibiotrophic (M. oryzae) pathogens in rice. These observations suggest that GAs, in addition to auxin...
Zheng et al. (2007), may be another virulent factor in disease susceptibility. It would be interesting to further examine resistance phenotypes of the GA signaling mutants such as slr1, gid1, and gid2 mutants in rice. Many pathogens can produce phytohormones. Gall-forming bacteria produce auxin and cytokinin that are required for disease development (Robert-Seilaniantz et al., 2007). ABA is also produced by saprophytic and parasitic fungi (Mauch-Mani and Mauch, 2005). The functional analog of JA, coronatine, is secreted by P. syringae to suppress the SA-mediated defense (Kloek et al., 2001) and ABA-mediated stomatal closure (Melotto et al., 2006). GAs were first identified from the fungus G. fujikuroi, causing bakanae disease in rice. Another phytopathogenic fungus, Sphaceloma sp., can also produce GAs and causes super-elongation disease (Hiroshi, 2006). However, there is no information on pathogenicity of those fungal mutants defective in GA biosynthesis. In our study, both endogenous and exogenous GAs have negative roles in disease resistance to bacterial and fungal pathogens. We propose that GAs produced by the pathogenic fungi may also have a virulent role. Moreover, some host GA synthesis and signaling components may be potential targets of pathogen virulence effectors, as discovered with the virus P2 protein (Zhu et al., 2005).

### Antagonism between Disease Resistance and Development

Growth versus retardation of plants is fine-tuned under different conditions. Plants relocate the resource and produce defensive components upon pathogen attack. Therefore, growth could be limited in defensive situations (Purrington, 2000). Many Arabidopsis mutants constitutively expressing defense-related genes, such as mpk4, cpr1, scn1, bon1, and mekk1, exhibit growth defects and dwarfism. Interestingly, other mutants, such as pad4, sid2, and rar1, that compromise disease resistance can rescue the growth retardation of these growth-defective mutants, while reducing the resistance ability (Petersen et al., 2000; Jirage et al; 2001; Zhang et al., 2003; Yang and Hua, 2004; Ichimura et al., 2006). On the other hand, some mutants defective in growth can enhance disease resistance. For example, the gain-of-function mutation of AXR2/IAA7 results in growth and development defects (Nagpal et al., 2000), but enhances disease resistance (Wang et al., 2007a). ASYMMETRIC LEAVES 1 (AS1) is a key regulator of leaf development and stem cell function (Byrne et al., 2000); its loss-of-function mutant enhances resistance against necrotophic fungi (Nurmberg et al., 2007). These data indicate that disease resistance can antagonize plant growth, as proposed previously (Purrington, 2000).

In contrast, some components are required for both defense and normal development. BAK1 (BRI1-associated kinase 1) regulates both brassinosteroid-dependent development and brassinosteroid-independent defense response (Chinchilla et al., 2007; He et al., 2007; Heese et al., 2007; Kemmerling et al., 2007). The MAPK signaling pathway regulates innate immunity in plants; the components MKK4/MKKS-MPK3/MPK6 were found to be involved in stomatal differentiation (Wang et al., 2007b). Because diverse signaling pathways orchestrate the development in plants, it is reasonable to speculate that cross-talks widely exists between defense responses and development.
Crop ‘Green Revolution’, which largely increases grain production, was achieved by the application of the GA malfunction mutant sd1 in rice and rht in wheat. sd1 encodes GA20ox2 that synthesizes bioactive GAs and its loss-of-function mutants result in high-yield semi-dwarf phenotypes (Sasaki et al., 2002). RHT is a DELLA protein that represses the GA signaling pathway; its gain-of-function is also desirable semi-dwarf (Peng et al., 1999). We observed that tall eui rice plants were more susceptible and dwarf plants exhibited enhanced disease resistance (Figures 1 and 2 and data not shown). Thus, the increase in crop production in ‘Green Revolution’ is probably attributed partially to increased disease resistance in rice and wheat. This might further provide a practical approach to crop design breeding for a better yield potential and disease resistance.

Possible Mechanisms of GA-Modulated Disease Resistance

Although we detected the down-regulation of the GA biosynthesis and metabolism genes, changes of SA or JA levels, and different expression of PR genes, including PR1a in the eui mutant and Eui overexpressors, the mechanism of the GA involvement in disease resistance remains largely unknown currently. One possibility is that GA has negative regulatory roles on expression of a specific set of PR genes, including PR1a, to support pathogen growth. Another possibility is that GA may modify plant metabolism profiles, resulting in decreased antimicrobial substances or increased nutrient efflux favoring microbes, as observed in the auxin-mediated susceptibility (Zhang et al., 2007). In supporting the latter possibility, the evidence showed that the transgenic poplar overexpressing the mutant DELLA proteins ga1 and rgl1, which constitutively repress GA signaling and are GA-insensitive, accumulated less glucose, fructose, and galactose (Busov et al., 2006). Metabolism changes may also result in cell wall modification, known to be a critical aspect of the plant basal defense. Many mutants defective in cell wall formation, such as the callose synthesis gene powdery mildew resistant 4 (Nishimura et al., 2003), powdery mildew resistant 5 affecting pectin composition (Vogel et al., 2002), botrytis-resistant 1 encoding long-chain acyl–CoA synthetase 2 (LAC52) (Bessire et al., 2007), three cellulose synthetase genes (CESA4/IRREGULAR XYLEMS, CES2/IRX3, and CES2/IRX1) for secondary cell wall formation (Hernández-Blanco et al., 2007), are more resistant to pathogens. Similarly to overexpression of AtGA2ox in tobacco (Biemelt et al., 2004), we observed that Eui overexpression also decreased lignin content (data not shown). The variation of cell wall composition may modulate disease resistance in Eui–OX.

There is also the third possibility that GA may directly modulate SA and/or JA homeostasis. However, rice plants accumulate high levels of SA (Silverman et al., 1995; Chen et al., 1997; Yuan et al., 2007); a slight change in the SA level unlikely results in the altered disease resistance observed in this study. It will be informative to conduct detailed microarray analysis of GA-, SA-, JA-, and pathogen-induced global expression profiles of rice genes in which defense signaling keeps being established, which might provide molecular clues of how GA regulates defense responses.

METHODS

Rice Materials

Three alleles of eui mutations (eui-1 and the wild-type ZS97, eui-3 and the wild-type ZH11, eui-4 and the wild-type 02428), the RNAi lines S73 and S74 (Zhang et al, 2008) and overexpression lines OX-7, OX-11, OX-15, OX-21, OX-24, OX-39, and OX-47 (Zhu et al., 2006) and the wild-type TP309 were used in the study.

Pathogen Inoculation and Disease Index

For Xoo resistance assays, rice plants were planted in an isolated paddy field. Three independent inoculation experiments were conducted with 8-week-old adult plants. Xoo strains Philippine race 6 (PXO99A) and Korea race 1 (DY89031) were used for inoculation. Bacteria were incubated on a potato-agar medium at 28°C for 3 d. Inoculum was prepared by suspending the bacterial mass in sterilized water at a concentration of OD600 = 1.0. Lesion length was recorded at 14 dpi. Bacterial growth was record for PXO99A.

For M. oryzae resistance, rice seedlings were planted in greenhouses at a temperature of 28°C/24°C (day/night). The strain of blast CH14 (ZB1) was used in the experiments. Two-week-old seedlings were spray-inoculated with spore suspensions (1 × 10^5 spores ml⁻¹) in a dew growth chamber for 24 h in darkness at 26°C, and were subsequently kept at 12 h/12 h (day/night), 26°C and 90% relative humidity for 5 d. Then, lesion types on leaves were scored from 0 (resistant) to 5 (susceptible) according to the standard scale (Bonman et al., 1986), with the criterion of disease severity: grade 0, no visible lesion; grade 1, diseased lesion diameter smaller than 0.5 mm, yellow brink; grade 2, diseased lesion 0.5–1.0 mm in diameter, black-brown brink; grade 3, diseased lesion 1.0–1.5 mm in diameter, yellow-brown brink; grade 4, diseased lesion 1.5–2.0 mm in diameter, water soaking, gray or brown brink; grade 5, diseased lesion much more than 2.0 mm in diameter or cover across two small leaf veins.

Statistical analysis was performed for Xoo and M. oryzae disease index with large sample size (>50 leaves for each inoculation).

DNA Extraction and Southern Hybridization

Total DNA of rice with fungal mycelia was extracted according to the method described previously (Qi and Yang, 2002). Briefly, infected rice leaves were grounded with liquid nitrogen and transferred into extraction buffer (0.3 M NaCl, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, 2% sarkosyl, 0.5% sodium dodecylsulfate, 5 M urea, and 5% Phenol), followed by the extraction by phenol/chloroform solution (pH 8.0). After centrifugation, the supernatants were precipitated with 0.7 volume of isopropanol by centrifugation for 5 min
at 12 000 rpm. After being washed with 70% ethanol, the resultant DNA samples were dissolved in Tris-EDTA (TE) buffer containing RNase. DNA (20 μg) was transferred to Hybond-N* membranes (Amersham) for Southern blot. A 330-bp fragment of *M. oryzae* 28S rDNA was amplified from fungal genomic DNA using the primers 5’–TACGAGGAAACCCTCATTAGATAATAATTA–3’ and 5’–TCAGCAGATCGATAACGATGAAAGCTGC–3’, as described previously (Qi and Yang, 2002), and labelled with [α-32P] dCTP using a random primer labelling kit (TaKaRa) for hybridization and autoradiography.

RNA Extraction and Reverse PCR

Total RNA was extracted using TRIzol reagent from leaves of rice at different inoculation times. Reverse transcription–PCR (RT–PCR) was conducted using SuperScript III First-Strand Synthesis System (Invitrogen). Primers used in semi-quantitative PCR are GA20ox2F: 5’–CGCACGTTCTTCCAGGTGC–3’; GA20ox2R: 5’–TTTTCTCCAGGAGTTCCATGATCGTCAGC–3’; SLR1F: 5’–GGTCCGGCCCAAGGATGCATCA–3’; SLR1R: 5’–AGGAGCGTGCTCGCCTGTTT–3’; PR1aR: 5’–CGCACGGGTTCTTCCAGGTGTC–3’; GA2ox1F: 5’–CGAGCAGGATGGAAGGCTACAGG–3’; PR10F: 5’–TAACCAAGCTGGCCATTG–3’; PR10R: 5’–TGCCGCTCATCTTCAGATAATTA–3’ and 5’–TCAGCAGATCGATAACGATGAAAGCTGC–3’, as described previously (Qi and Yang, 2002), and labelled with [α-32P] dCTP using a random primer labelling kit (TaKaRa) for hybridization and autoradiography.

SA and JA Assay

Free and total SA were extracted from the leaves of 2-week-old plants grown in a growth chamber at 28°C/22°C and 16 h/8 h (day/night), and measured as described previously (Zhang et al., 2007). For JA assay, 2-week-old rice leaves were harvested. JA was analyzed by gas chromatography–mass spectrometry (GC–MS) with the labeled internal standard D3-JA, as described previously (Lou and Baldwin, 2003), and JA levels were determined with three biological repeats.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant* Online.

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