Abscisic Acid Interacts Antagonistically with Salicylic Acid Signaling Pathway in Rice–*Magnaporthe grisea* Interaction

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Plant hormones play pivotal signaling roles in plant–pathogen interactions. Here, we report characterization of an antagonistic interaction of abscisic acid (ABA) with salicylic acid (SA) signaling pathways in the rice–*Magnaporthe grisea* interaction. Exogenous application of ABA drastically compromised the rice resistance to both compatible and incompatible *M. grisea* strains, indicating that ABA negatively regulates both basal and resistance gene-mediated blast resistance. ABA markedly suppressed the transcriptional upregulation of *WRKY45* and *OsNPR1*, the two key components of the SA signaling pathway in rice, induced by SA or benzothiadiazole or by blast infection. Overexpression of *OsNPR1* or *WRKY45* largely negated the enhancement of blast susceptibility by ABA, suggesting that ABA acts upstream of *WRKY45* and *OsNPR1* in the rice SA pathway. ABA-responsive genes were induced during blast infection in a pattern reciprocal to those of *WRKY45* and *OsNPR1* in the compatible rice–blast interaction but only marginally in the incompatible one. These results suggest that the balance of SA and ABA signaling is an important determinant for the outcome of the rice–*M. grisea* interaction. ABA was detected in hyphae and conidia of *M. grisea* as well as in culture media, implying that blast-fungus-derived ABA could play a role in triggering ABA signaling at host infection sites.

Plants have evolved a battery of defense mechanisms to protect themselves from microbial pathogen attacks, in which plant hormones salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) play central signaling roles (Grant and Jones 2009; Lopez et al. 2008; Robert-Seilanianz et al. 2007). Each of these hormones generates and transmits a distinct defense signal, while cooperative or antagonistic crosstalks between them are emerging as pivotal for outcomes of disease resistance. Microbial pathogens, on the other hand, have also evolved virulence mechanisms to avoid plant defenses by manipulating hormone balances (Asselbergh et al. 2008 b; Lopez et al. 2008). The most well-known example of such interaction is the perturbation of the SA/JA balance in

Arabidopsis by a biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (DC3000) (Brooks et al. 2005; Laurie-Berry et al. 2006). SA and JA mediate defense signaling against biotrophic and necrotrophic pathogens, respectively (Loake and Grant 2007). In most cases, these two signaling pathways mutually oppose each other (Loake and Grant 2007). This antagonism between SA and JA is targeted by some *P. syringae* strains that produce the phytotoxin coronatine, a JA-isoleucine analog that functionally mimics JA and suppresses SA signaling, thereby compromising host resistance to the biotrophic pathogen (Brooks et al. 2005; Laurie-Berry et al. 2006; Lopez et al. 2008). Many other fungal and bacterial pathogens have also been found to be capable of manipulating host hormone signaling pathways to their own advantage by either producing plant hormones or plant hormone-like substances by themselves or altering the hormone synthesis pathways of host plants (de Torres-Zabala et al. 2007; Robert-Seilanianz et al. 2007). Therefore, it is proposed that “successful defense likely requires that the plant attenuate any microbe-induced hormonal perturbation” (Grant and Jones 2009).

SA is involved in the activation of systemic acquired resistance (SAR) in many dicots such as *Arabidopsis* and tobacco (Durrant and Dong 2004; Vallad and Goodman 2004). A transcriptional cofactor, NPR1, plays a key role in mediating the SA signaling pathway in *Arabidopsis* (Dong 2004). Rice also has an SA signaling pathway that shows some similarities to that in *Arabidopsis* and the rice ortholog of NPR1, *OsNPR1/NH1*, plays a role in rice resistance to the leaf blight bacteria *Xanthomonas oryzae* pv. *oryzae* (Chern et al. 2001, 2005; Fitzgerald et al. 2004; Yuan et al. 2007). We previously identified a rice transcription factor, WRKY45, whose gene is highly, specifically induced by SA and a chemical defense inducer, benzothiadiazole (BTH). WRKY45 was also found to be a key regulator of the SA signaling pathway and involved in BTH-induced resistance to *Magnaporthe grisea* (Shimono et al. 2007) and *X. oryzae* pv. *oryzae* (Shimono, unpublished). Notably, the SA signaling pathway in rice was found to branch into the *OsNPR1*-dependent and the WRKY45-dependent pathways downstream of SA (Shimono et al. 2007), unlike the SA signaling pathway in *Arabidopsis*, which is primarily mediated by NPR1 (Wang et al. 2006).

ABA is an important plant hormone that plays signaling roles in diverse processes of plant growth and development as well as in plant responses to various abiotic stresses (Yamaguchi-Shinozaki and Shinozaki 2006). Recently, ABA has also emerged as a key signaling molecule in plant–pathogen inter-

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actions (Asselbergh et al. 2008b; Mauch-Mani and Mauch 2005). Exogenous application of ABA or ABA accumulation due to genetic defects has been shown to enhance the susceptibility of various plant species to bacterial and fungal pathogens (Achuo et al. 2006; Fan et al. 2009; Henfling et al. 1980; Koga et al. 2004; Matsumoto et al. 1980; Mohr and Cahill 2003; Ward et al. 1989). Conversely, ABA-deficient mutation results in an enhanced resistance to various pathogens in tomato (Achuo et al. 2006; Asselbergh et al. 2007, 2008a; Audenaert et al. 2002) and *Arabidopsis* (de Torres-Zabala et al. 2009; Mohr and Cahill 2003). These mutant plants display stronger SA-dependent defense responses than do wild-type plants, suggesting that the antagonistic interaction of ABA on the SA signaling pathway underlies the negative impact of ABA on plant defense responses. This notion is also supported by a recent study that has shown antagonistic crosstalk between SA-mediated activation of SAR and ABA-mediated abiotic stress responses (Yasuda et al. 2008). Interestingly, de Torres-Zabala and associates (2007) have shown that *P. syringae* type III effectors (T3Es) hijack the *Arabidopsis* ABA biosynthetic pathway to antagonize SA levels and suppress plant basal defenses, suggesting an important role of ABA in the patho-

genicity. On the other hand, some studies have shown positive roles of ABA in plant defense (e.g., via regulating stomatal closure [Melotto et al. 2006] and priming callose deposition [Flors et al. 2008; Ton and Mauch-Mani 2004]). Thus, ABA appears to play different roles in defense, depending upon pathosystems and infection stages. (Asselbergh et al. 2008b; Mauch-Mani and Mauch 2005).

The negative impact of ABA has also been reported in rice defenses to *M. grisea* (Koga et al. 2004; Matsumoto et al. 1980). Rice genes responsive to ABA and dehydration stresses were noted to be induced during infection by a compatible race of *M. grisea* (Ribot et al. 2008). Bailey and associates (2009) reported that ABA antagonistically interacts with the ET signaling pathway and, consequently, increases blast susceptibility in a compatible rice-*M. grisea* interaction. These reports support the involvement of ABA in rice-*M. grisea* interaction; however, the manner in which ABA signaling is generated and integrated into the rice defense pathway during rice-pathogen interactions remains largely unknown.

In the present study, we investigated the molecular mechanisms underlying the effects of ABA in enhancing the susceptibility of rice to *M. grisea*, focusing on the antagonistic interaction of ABA with the SA signaling pathway.

RESULTS

Exogenous ABA compromises rice defense to *M. grisea*.

To confirm the effect of ABA on rice blast resistance and expand the scope of the investigation, we tested the effects of ABA application on both compatible (Fig. 1A and B) and incompatible (Fig. 1C and D) rice-*M. grisea* interactions. Foliar spray treatment of Nipponbare rice seedlings with ABA rendered the seedlings more susceptible to a compatible *M. grisea* race (race 007.0) (Fig. 1A and B). The rice cv. Nipponbare has resistance genes *Pish* (Imbe and Matsumoto 1985) and *Pi19* (Hayashi et al. 1998) that confer race-specific resistance to avirulent races of blast fungus 102.0 and 002.0, respectively. Inoculation with race 102.0 caused numerous small necrotic lesions in the leaves of control plants, probably due to hypersensitive response (HR) cell death (Fig. 1C), whereas race 002.0 caused fewer visible necrotic lesions (Supplementary Fig. S1A). Pretreatment by ABA caused a compromise in the resistance of Nipponbare rice to both the avirulent blast fungus races, 102.0 (Fig. 1C and D) and 002.0, giving rise to severe disease symptoms. These data indicate that ABA has a negative impact on both basal and resistance (*R*) gene-mediated rice defense to *M. grisea*. Soil drenching with ABA resulted in similar enhancement in disease severity in a dose-dependent manner (Supplementary Fig. S2).

ABA suppresses SA-induced transcriptional upregulation of *WRKY45* and *OsNPR1*.

In *Arabidopsis*, ABA has been shown to antagonize the SA signaling pathway, and this antagonism is considered responsible for the enhancement of disease susceptibility by ABA (de Torres-Zabala et al. 2007; Yasuda et al. 2008). These studies prompted us to test the effects of ABA, as well as of other hormones, on the expression of *WRKY45* and *OsNPR1*, the two key regulators in the rice SA pathway, with each regulating one of two pathways that branch downstream of SA. *WRKY45* upregulates highly specifically in response to SA and BTH; moreover, *OsNPR1* is also responsive to BTH (Shimono et al. 2007). Leaf blade segments were treated with SA alone or SA plus various plant hormones, and examined for the expression of *WRKY45* and *OsNPR1*. *WRKY45* expression was upregulated by SA and this upregulation was specifically and strongly suppressed by ABA (Fig. 2A). On the other hand, *OsNPR1* ex-

Fig. 1. Effects of exogenous abscisic acid (ABA) on the susceptibility of rice plants to compatible and incompatible blast fungus. **A** and **B**, Compatible race 007.0 and **C** and **D**, incompatible race 102.0 of *Magnaporthe grisea* were spray inoculated onto Nipponbare rice. **A** and **C**, Disease symptoms at 6 days postinoculation (dpi) in fungus-inoculated rice plants after pretreatment by spraying with mock (–ABA) or 0.1 mM ABA (+ABA) solutions. **B**, Average lesion numbers per 10-cm middle regions of leaf blades (6 dpi) are shown with standard deviations (s.d.). **D**, Severity of blast disease was evaluated by the amounts of *M. grisea* genomic 28S rDNA relative to rice genomic *Rubq1* DNA (6 dpi). Mean values of three determinations with s.d. are shown. CON, leaf blades of noninoculated rice seedlings. Asterisks indicate a significant difference from the mock control (*t* test, $P < 0.01$).

pression was upregulated in the samples submerged in aqueous solvent (mock), indicating that mere submergence can induce *OsNPR1* expression in leaf blade segments (Fig. 2B). Thus, the effect of submergence obscured the effects of SA on *OsNPR1* expression. However, ABA was found to strongly suppress *OsNPR1* expression in submerged leaf blade segments to a level below that in the control sample without submergence (CON) (Fig. 2B). The induction of *OsNPR1* expression in aqueous solutions may be attributed to decreased ABA levels due to submergence, because ABA becomes rapidly oxidized to phaseic acid by ABA 8'-hydroxylase in submerged rice plants (Kende et al. 1998; Saika et al. 2007). This result also implies that the basal expression of *OsNPR1* is also negatively regulated by ABA. The effectiveness of ABA treatment was validated by examining the induction of an ABA-responsive gene, *SalT* (Rabbani et al. 2003) (Figs. 2C and 3C).

To assess the relevance of the expression levels of *WRKY45* and *OsNPR1*—which were modified by BTH or ABA treatments—with the development of blast disease, we pretreated rice seedlings with BTH, ABA, or BTH plus ABA for 24 h and then inoculated with compatible blast fungus (race 007.0). Both *WRKY45* and *OsNPR1* were induced by BTH after 24 h of the treatment, and the effect persisted until day 5 after blast inoculation (Fig. 3A and B). On the other hand, ABA largely offset the effects of BTH on the upregulation of *WRKY45* and *OsNPR1*, and also decreased the basal level of *OsNPR1* transcripts (Fig. 3A and B). Moreover, ABA also strongly suppressed the expression of *WRKY45* and *OsNPR1* induced by blast inoculation, which was partially alleviated by cotreatment with BTH (Fig. 3A and B). Interestingly, the ABA-responsive gene *SalT* was induced by blast inoculation at later time points, and this induction was enhanced by pretreatment with ABA (Fig. 3C). Meanwhile, BTH moderately suppressed the upregulation of *SalT* induced by blast inoculation. These results indicate that SA and ABA mutually antagonize each other's signaling pathways in rice. We also observed the antagonism between SA and ABA in the expression of rice ABA-responsive genes, *RAB16b* (Yamaguchi-Shinozaki et al. 1989), *OsWsi18* (Joshee et al. 1998), and *SalT*, after treatment of rice leaf segments with ABA or ABA plus SA (Supplementary Fig. S3). Determination of blast fungal DNA revealed that the growth of blast fungus was inversely correlated with the expression levels of *WRKY45* and *OsNPR1* in rice plants (Fig. 3D). Thus, BTH upregulated both *WRKY45* and *OsNPR1* and reduced fungal growth; meanwhile, ABA antagonized the effects of BTH on both gene expression and fungal growth. These data strongly suggest that the suppression of SA-responsive upregulation of—as well as the basal—expression of *WRKY45* and *OsNPR1* underlies the reduction in the BTH-induced and basal resistance of rice to *M. grisea* by ABA application. It should be noted that ABA had no direct effect on the growth and morphology of *M. grisea* (Koga et al. 2004).

Recently, Qiu and Yu (2009) have reported that *WRKY45* is constitutively expressed in various rice tissues and that ABA treatment induced *WRKY45* expression. In this report, submerging whole seedlings in 100 μ M ABA solution induced a transient expression of *WRKY45* at 1 h. In our time-course experiment using leaf segments, however, we did not observe ABA-responsive changes in *WRKY45* expression except for a small, nonspecific increase at 0.5 h (Supplementary Fig. S4).

SA and ABA signaling are inversely activated during blast infection.

To further investigate the interaction between SA and ABA signaling pathways during blast infection, we examined the temporal expression patterns of SA-responsive *WRKY45* and ABA-responsive gene *SalT*, following inoculation with com-

patible (007.0) or incompatible (102.0) blast fungus races (Fig. 4). In the compatible interaction, *WRKY45* began to be upregulated at 1.5 days postinoculation (dpi) and reached a peak of expression at 3 dpi, then declined to near the basal level by 5 dpi. In contrast, *SalT* expression began to increase at 3 dpi, and this upregulation continued until the end of the time course. On the other hand, the incompatible interaction caused only a minor induction of *SalT* expression, whereas it induced a much stronger and persistent expression of *WRKY45* compared with that in the compatible interaction. Other ABA-responsive genes, *OsWsi18* and *RAB16b*, showed expression patterns

Fig. 2. Abscissic acid (ABA) suppresses salicylic acid (SA)-induced upregulation of *WRKY45* and *OsNPR1*. Leaf segments from rice seedlings were treated in solutions containing various plant hormones under light for 8 h at 30°C. Transcript levels of **A**, *WRKY45*; **B**, *OsNPR1*; and **C**, *SalT* were determined by quantitative reverse-transcription polymerase chain reaction analyses using *Rubq1* as an internal control. Mean values from three determinations with standard deviations are shown. SA (1 mM); IAA, indole-3-acetic acid (50 μ M); GA, gibberellin (50 μ M); CK, kinetin (50 μ M); ACC, 1-aminocyclopropane-1-carboxylic acid (50 μ M); ABA (50 μ M); BR, brassinolide (2 μ M); JA, jasmonic acid (50 μ M); CON, leaf blades sampled directly from untreated rice seedlings.

Fig. 3. Suppression of benzothiadiazole (BTH)-induced *WRKY45*/*OsNPR1* expression by abscisic acid (ABA) and its effects on blast susceptibility. Rice seedlings at the four-leaf stage were pretreated for 24 h by spraying mock, BTH (0.5 mM), ABA (0.1 mM), or BTH + ABA solutions, and then inoculated with *Magnaporthe grisea* (race 007.0). Transcript levels of **A**, *WRKY45*; **B**, *OsNPR1*; and **C**, *SalT* in pretreated plants (0 h postinoculation [hpi]) and blast-inoculated plants at indicated time points (hpi) were determined by quantitative reverse-transcription polymerase chain reaction analyses using *Rubq1* as an internal control. **D**, Severity of blast disease was evaluated by quantifying *M. grisea* genomic 28S rDNA relative to rice genomic *Rubq1* DNA (6 days postinoculation). Mean values of three determinations with standard deviations are shown. The asterisks indicate a significant difference from the mock control (*t* test, $P < 0.01$).

similar to that of *SalT* (Supplementary Fig. S5). *OsPR1b* showed upregulation concomitant with *WRKY45*, with higher expression levels observed during the incompatible interaction than the compatible one. These results strongly suggest the occurrence of a mutually antagonistic interaction between SA and ABA signaling pathways during blast infection, which is potentially important to the rice-*M. grisea* interaction.

Overexpression of *WRKY45* or *OsNPR1* negates the effects of ABA.

Overexpression of *WRKY45* in rice enhances rice resistance to *M. grisea* by strengthening the SA pathway (Shimono et al. 2007). To further validate the antagonistic interaction of ABA with the rice SA pathway and to determine the point at which ABA acted in the SA signaling pathway, we tested the effects of ABA application on the blast resistance of the transgenic lines overexpressing *WRKY45*. The plant seedlings were pretreated with ABA for 24 h by foliar spraying and then inoculated with compatible blast fungus (race 007.0). ABA application resulted in only a minor reduction in the blast resistance in the two transgenic rice lines overexpressing *WRKY45* but did not restore blast susceptibility comparable with that of control wild-type plants (Fig. 5A). These results indicate that the negative impact of ABA on the SA-mediated defense signaling leading to the downregulation of *WRKY45* was partially suppressed by *WRKY45* overexpression. *OsNPR1* also plays a key role in the rice SA pathway by regulating one of the branched pathways and its overexpression enhances rice resistance to *M. grisea* and *X. oryzae* (S. Sugano, unpublished data). Similar experiments using *OsNPR1*-overexpressing rice plants demonstrated that the negative impact of ABA on the *OsNPR1* sub-pathway was also suppressed by *OsNPR1* overexpression. Collectively, these results further support the negative interaction of ABA on the rice SA pathway and that the action sites of ABA lie upstream of *WRKY45* and *OsNPR1*.

M. grisea produces and secretes ABA.

The strong negative impact of ABA on the SA signaling pathway and its subsequent effects on blast resistance led us to

Fig. 4. Expression of marker genes for salicylic acid (SA) and abscisic acid (ABA) signaling pathways is inversely correlated during blast infection. Rice seedlings at the four-leaf stage were inoculated with compatible (race 007.0) or incompatible (race 102.0) blast fungus *Magnaporthe grisea*. Transcript levels of SA-responsive *WRKY45* (solid lines) and ABA-responsive *SalT* (gray lines) genes were determined at the indicated time points (days postinoculation [dpi]) by quantitative reverse-transcription polymerase chain reaction analyses using *Rubq1* as an internal control. Mean values of three determinations with standard deviations are shown.

examine the possibility that ABA is derived from the fungus. Hormone analysis revealed that substantial amounts of ABA were accumulated in both hyphae (mycelium) and conidia, with higher levels detected in conidia compared with hyphae (Table 1). ABA was also detected in culture filtrates, indicating that the ABA produced in blast fungus was secreted into the culture medium. Whether fungus-derived ABA is responsible for the suppression of the rice SA pathway should be addressed in the future.

DISCUSSION

Rice blast disease, caused by the fungal pathogen *M. grisea*, is one of the most devastating diseases affecting cultivated rice that causes chronic yield losses and destabilizes annual production. It has often been observed that the incidence and severity of rice blast disease is influenced by environmental conditions. Generally, abiotic stresses, such as low temperature and drought, render rice plants more susceptible to blast disease (Bonman et al. 1988; Gill and Bonman 1988; Lai et al. 1999; Koga et al. 2004). Previous studies have suggested the possible linkage of ABA to this stress-associated rice susceptibility to blast disease (Kahn and Libby 1958; Kende et al. 1998; Lai et al. 1999; Saika et al. 2007; Bailey et al. 2009). In addition, exogenous application of ABA compromised rice resistance to *M. grisea* (Fig. 1) (Matsumoto et al. 1980; Koga et al. 2004; Bailey et al. 2009). On the other hand, ABA is also known to play a key role in plant responses to various abiotic stresses (Lee et al. 1995; Yamaguchi-Shinozaki and Shinozaki 2006). Hence, it appears that ABA plays an important regulatory role in the crosstalk between abiotic and biotic responses in rice to shift defense resources to the most life-threatening stress (Fujita et al. 2006; Asselbergh et al. 2008b).

The effects of ABA on plant defense pathways have been shown to be profound and drastic (Asselbergh et al. 2008b). In potato, for example, ABA pretreatment rendered potato slices vulnerable to infection by an incompatible isolate of *Phytophthora infestans*, and also even by the fungus *Cladosporium cucumerinum* that is normally nonpathogenic to potato (Henfling et al. 1980). It was also shown in soybean that ABA treatment compromised resistance to the incompatible isolates of *P. sojae* (McDonald and Cahill 1999). We showed that ABA treatment drastically enhanced disease susceptibility not only to compatible but also to incompatible blast fungus strains (Fig. 1). These results suggest that ABA negatively regulates both basal and *R*-gene-mediated defense pathways of rice plants to *M. grisea*. SA-mediated defense responses are an important part of both basal and *R*-gene-mediated resistance in *Arabidopsis* (Glazebrook 2001; Xiao et al. 2005; Venugopal et al. 2009). Therefore, it appears reasonable that ABA suppresses both types of resistance by suppressing the SA signaling pathway. The degrees of the effect of ABA on different incompatible rice–blast fungus combinations were considerably different (Fig. 1): ABA treatment resulted in severe blast disease symptoms in rice seedlings infected with blast fungus race 102.0 (Fig. 1C and D) but much lesser symptoms in those infected

with race 002.0. Moreover, Koga and associates (2004) previously reported that, in an experiment using leaf sheaths, ABA treatment markedly suppressed the basal resistance of rice—referred to as whole-plant-specific resistance—in a compatible interaction, whereas no significant effect of ABA was observed in the incompatible rice (ZTR, *Pi-z*)–blast fungus (isolate Kita 1) interaction. In the light of our present study showing that ABA antagonizes the SA pathway, these data imply that different *R*-gene-mediated defenses depend upon the SA pathway to different extents. Alternatively, differences in the genetic background of fungal races used may be responsible for the observed difference.

In this study, we have shown that SA and ABA mutually antagonize each other's signaling pathways in rice. ABA suppressed SA (BTH)-induced expression of *WRKY45* and

Fig. 5. Overexpression of *WRKY45* or *OsNPR1* largely negates the abscisic acid (ABA)-enhanced susceptibility to *Magnaporthe grisea*. Control wild type (WT) and two lines each of **A**, *WRKY45-ox* and **B**, *OsNPR1-ox* transgenic rice plants at the four-leaf stage were sprayed with mock or ABA (0.1 mM) solution and then inoculated with *M. grisea* (race 007.0) after 2 days. Mean values for the lesion number per 10-cm middle region of leaf blades with standard deviations are shown. Asterisks indicate significant difference from the mock-treated WT plants (*t* test, $P < 0.01$).

Table 1. Abscisic acid (ABA) contents in hyphae and conidia of *Magnaporthe grisea* and culture medium

Blast race	ABA (pmol per gram of fresh weight) ^a		
	Hyphae	Filtrate	Conidia
007.0	26.80 ± 5.21	2.76 ± 0.69	120.19 ± 26.48
102.0	16.26 ± 1.55	1.61 ± 0.37	195.93 ± nc

^a Values are represented as mean ± standard deviation ($n = 4$); nc = standard deviation was not calculated because there were only two samples.

OsNPR1, the two key components of the SA signaling pathway in rice, accompanied by an enhanced susceptibility to *M. grisea* infection (Figs. 2 and 3). Conversely, SA (BTH) suppressed ABA-responsive gene expression (Fig. 3). Overexpression of *WRKY45* or *OsNPR1* largely negated the negative impact of ABA on blast resistance (Fig. 5). We have also shown that the expression of marker genes for SA and ABA signaling pathways are inversely correlated during blast infection (Fig. 4). These data, taken together, strongly suggest that the balance of interaction between SA and ABA signaling is an important determinant for the outcome of rice–*M. grisea* interaction. The incompatible rice–*M. grisea* interaction induced only a minor upregulation of ABA-responsive genes, whereas it induced a much stronger expression of *WRKY45* and *OsPR1b* compared with the compatible interaction (Fig. 4). This suggests that the incompatible interaction strongly activates the SA pathway and causes the concomitant downregulation of the ABA pathway, resulting in strong blast resistance. The induction of ABA-responsive genes appeared to be preceded by *WRKY45* expression during blast infection (Fig. 4). This may suggest that the antagonistic effects of ABA on the SA pathway act relatively later during blast infection, after transient activation of the SA pathway. However, we cannot rule out the possibility that early local activation of ABA signaling in host cells at the infection sites was missed because such local activation would be difficult to detect. Indeed, we found that ABA application later than 24 h after inoculation caused only a small increase in blast susceptibility (data not shown), implying that ABA action at earlier stages could be important. A transgenic rice line containing a reporter gene (e.g., β -glucuronidase) driven by an ABA-responsive promoter would aid in the detailed investigation of ABA signaling dynamics in the early stages of blast infection.

Recently, Bailey and associates (2009) have reported that the exogenous application of ABA drastically reduces ET levels in rice and enhances rice blast susceptibility. In addition, RNAi-

mediated suppression of *OsEIN2b*—a central component of ET signal transduction—resulted in ABA-hypersensitivity, reduced defense gene expression, and enhanced blast susceptibility. These data suggest that ABA antagonistically interacts with the ET signaling pathway and, consequently, enhances susceptibility of rice to *M. grisea*. Taken together, it appears that ABA opposes both SA and ET signaling pathways to suppress the defense response against *M. grisea* in rice (Fig. 6).

Many fungal and bacterial phytopathogens have developed various mechanisms to interfere with hormonal balances in host plants for efficient infection or growth, either by producing hormones by themselves or by modulating hormone synthesis pathways in host plants (Robert-Seilanianantz et al. 2007; Grant and Jones 2009). For example, *Pseudomonas syringae* secretes T3Es to induce ABA synthesis in planta during the interaction with *Arabidopsis*, thereby suppressing SA production and the basal defense of the host plants (de Torres-Zabala et al. 2007). Several fungal pathogens, such as *Botrytis cinerea*, *Ceratocystis coerulescens*, *Fusarium oxysporum*, and *Rhizoctonia solani*, can themselves produce ABA (Dörffling et al. 1984; Kettner and Dörffling 1987; Oritani and Kiyota 2003), although the relevance of the ABA production of these pathogens to their pathogenicity remains unknown. In this study, the blast fungus *M. grisea* and its culture media were found to contain ABA (Table 1), indicating that the fungus produces and secretes ABA. These results imply that the fungus may use its own ABA to trigger ABA signaling at host infection sites such that it suppresses SA and ET signaling pathways to alleviate rice defense responses.

MATERIALS AND METHODS

Plant materials and growth conditions.

The Japonica rice *Oryza sativa* subsp. *japonica* cv. Nipponbare was grown in a greenhouse on soil (Bonsol No. 2; Sumitomo Chemical Corp., Tokyo) at 28°C in the day and 23°C at night. The relative humidity in the greenhouse was approximately 70%.

Chemical treatments.

All stock solutions, except brassinolide (BR), were prepared at a concentration of 100 mM as described previously (Jiang et al. 2008). BR was prepared at 20 mM concentration. Indole-3-acetic acid (IAA) (Sigma-Aldrich, St. Louis), gibberellin A3 (GA₃) (Wako, Osaka, Japan), 1-aminocyclopropane-1-carboxylic acid (ACC) (Wako), ABA ([$\pm$]-*cis-trans*) (Sigma-Aldrich), methyl jasmonate (ME-JA) (Wako), and (BR) (Wako) were dissolved in ethanol. Kinetin (CK) (Sigma-Aldrich) and BTH *S*-methyl ester (Wako) were dissolved in dimethyl sulfoxide (DMSO); and ethephon (ET) (Sigma-Aldrich) and SA (Nacalai Tesque, Tokyo) in H₂O. The solvents did not exceed a final concentration of 0.1% in the solutions used for plant treatments and had no effect on the rice gene expressions examined in this work.

Leaf blades from rice plants at the four-leaf stage were cut into segments of approximately 0.5 cm in length and submerged in a solution containing the chemicals prepared in 0.01% Silwet L-77. The leaf segments were then incubated under light for 8 h at 30°C. In some experiments, rice plants at the four-leaf-stage were foliar sprayed with a chemical solution prepared in 0.02% Silwet L-77.

RNA analyses.

Total RNA was isolated from leaf blades of the fourth leaf of rice seedlings using the Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was run on a Thermal Cycler Dice TP800 system (Takara Bio, Tokyo) using SYBR premix Ex

Fig. 6. Schematic illustration of salicylic acid (SA)-abscisic acid (ABA), and ethylene (ET)-ABA antagonistic crosstalks in rice–*Magnaporthe grisea* interaction. In response to the pathogen infection (*M. grisea*), rice plants activate SA and ET signaling pathways, upon which ABA acts negatively. *M. grisea* may utilize this antagonism of signaling pathways as a means to slip through the rice defense mechanism, possibly by using its own ABA. Arrows and lines with a bar at the end indicate positive and negative regulation, respectively.

Taq mixture (Takara Bio) as described previously (Shimono et al. 2007). Primer sequences used for qRT-PCR are listed in Supplementary Table 1.

Pathogen culture and inoculations.

Culture and inoculations of the blast fungus *M. grisea* (compatible race 007.0 and incompatible races 102.0 and 002.0) were carried out essentially as described previously (Jiang et al. 2009), with slight modifications. Briefly, the fungus was grown on an oatmeal agar medium (oatmeal, 30 g/liter; sucrose, 5 g/liter; and agar, 16 g/liter) at 26°C for 10 to 12 days. After removing the aerial hyphae by washing with distilled water and a brush, conidia formation was induced by irradiation under continuous black blue light (FL15BLB; Toshiba, Osaka, Japan) at 24°C for 3 days. The conidia were suspended in 0.02% Silwet L-77 at a density of 10⁵/ml and sprayed onto rice plants at the four-leaf stage. After incubation in a dew chamber at 24°C for 24 h, the rice plants were moved back to the greenhouse.

Disease development was evaluated either by counting the number of blast lesions per 10-cm middle region of each fourth leaf or by determining *M. grisea* genomic 28S rDNA (Qi and Yang 2002) by qRT-PCR (Shimono et al. 2007) at 6 to 7 days after inoculation. At least 20 plants were used for each disease assay.

Measurement of ABA content.

For determination of ABA, *M. grisea* (race 007.0 and 102.0) was grown in 50 ml of 5× YEG medium (0.5% yeast extract, 1% glucose) at 25°C on a rotary shaker for 7 days. The culture was filtered through Miracloth (Calbiochem, La Jolla, CA, U.S.A.) and the mycelium was frozen in liquid nitrogen and homogenized. The culture filtrate was centrifuged at 2,000 × g for 10 min at room temperature to remove the mycelia completely, and the resulting supernatant was frozen in liquid nitrogen and lyophilized. Conidia were induced on an oatmeal agar medium and suspended in 0.02% Silwet L-77 as described above, and then, they were collected in a 2-ml Eppendorf tube by a brief centrifugation. The conidia were then frozen in liquid nitrogen and homogenized. The homogenates of mycelium and conidia and the culture filtrate were subjected to ABA determination according to the procedures described previously (Kojima et al. 2009).

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