Antagonistic Interaction between Systemic Acquired Resistance and the Abscisic Acid–Mediated Abiotic Stress Response in Arabidopsis

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Systemic acquired resistance (SAR) is a potent innate immunity system in plants that is effective against a broad range of pathogens. SAR development in dicotyledonous plants, such as tobacco (Nicotiana tabacum) and Arabidopsis thaliana, is mediated by salicylic acid (SA). Here, using two types of SAR-inducing chemicals, 1,2-benzisothiazol-3(2H)-one1,1-dioxide and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester, which act upstream and downstream of SA in the SAR signaling pathway, respectively, we show that treatment with abscisic acid (ABA) suppresses the induction of SAR in Arabidopsis. In an analysis using several mutants in combination with these chemicals, treatment with ABA suppressed SAR induction by inhibiting the pathway both upstream and downstream of SA, independently of the jasmonic acid/ethylene-mediated signaling pathway. Suppression of SAR induction by the NaCl-activated environmental stress response proved to be ABA dependent. Conversely, the activation of SAR suppressed the expression of ABA biosynthesis–related and ABA-responsive genes, in which the NPR1 protein or signaling downstream of NPR1 appears to contribute. Therefore, our data have revealed that antagonistic crosstalk occurs at multiple steps between the SA-mediated signaling of SAR induction and the ABA-mediated signaling of environmental stress responses.

INTRODUCTION

Plants suffer various types of inevitable exogenous stresses, such as pathogen attacks, insect herbivory, and abiotic environmental stress. To survive such unfavorable conditions, plants have evolved unique hormonally regulated self-protection systems. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) contribute to responses against biotic stresses by influencing various signaling pathways that have complex networks of synergistic and antagonistic interactions (Kunkel and Brooks, 2002). By contrast, abscisic acid (ABA) plays important roles in both plant development and adaptation to abiotic stresses, such as drought, salinity, and low temperature (Xiong et al., 2002; Shinozaki et al., 2003). In addition, recent studies have shown that ABA also plays important roles in disease susceptibility, resistance to pathogen infection, and interaction with other hormone-mediated biotic stress responses (Mauch-Mani and Mauch, 2005; Melotto et al., 2006; de Torres-Zabala et al., 2007).

At infection sites, plants protect themselves from invading microorganisms using pathogen-associated molecular pattern–activated basal resistance, which is effective against general microorganisms and by a resistance (R) gene–mediated defense system that is effective even against pathogenic microorganisms (McDowell and Dangl, 2000; Belkhadir et al., 2004; Pozo et al., 2004). In incompatible interactions between plants and pathogenic microorganisms, plants recognize the avirulence gene products of individual pathogens using specific receptors, the R gene products. This interaction causes, at the infection site, a burst of reactive oxygen species (ROS), the rapid induction of a hypersensitive response (HR) involving regulated cell death, and the expression of pathogenesis-related (PR) genes (Durrant and Dong, 2004). Subsequent to these events in the infected leaves, the uninoculated leaves exhibit an increased level of PR gene expression and usually develop long-lasting enhanced resistance to further attacks by pathogens, termed systemic acquired resistance.
resistance (SAR) (Chester, 1933; Durner et al., 1997). SAR is effective against a broad range of pathogens, including fungi, bacteria, and viruses. SA has been identified as a signaling molecule that acts during SAR development in dicotyledonous plants, such as tobacco (Nicotiana tabacum) and Arabidopsis thaliana, a phenomenon confirmed by the lack of SAR development in NahG transgenic plants, which express the bacterial SA-degrading enzyme salicylate hydroxylase (Gaffney et al., 1993; Delaney et al., 1994).

Genetic approaches to unraveling plant defense signaling pathways performed in Arabidopsis have identified various types of mutants defective in the signal transduction that leads to SAR induction. NPR1 has been identified and characterized as a key regulatory component that functions downstream of SA in the signal transduction cascade that mediates SAR induction. Recent studies have indicated that the reducing environment induced by SA signaling is required for the activation of NPR1 during SAR induction (Mou et al., 2003). Several mutants defective in SA production have been produced: enhanced disease susceptibility1 (eds1) (Parker et al., 1996) and eds5 (Nawrath et al., 2002; Wiemer et al., 2005), phytoalexin-deficient4 (Zhao et al., 1998), and SA induction-deficient2 (sid2) (Wildermuth et al., 2001). ED5 shows similarity to members of the MATE (multidrug and toxin extrusion) transporter family and is necessary for the accumulation of SA and the PR-1 transcript after pathogen inoculation, suggesting that ED5 acts upstream of SA in SAR signaling. SID2, which encodes ISOCHORISIMATE SYNTHASE1 (ICS1), is required for SA biosynthesis in the development of local and SAR. These studies have revealed the detailed mechanisms of SAR induction, but most of the other mechanisms that regulate SAR remain to be clarified.

Treat the with SA induces resistance to pathogens and the expression of a set of PR genes (Ward et al., 1991). In addition, some synthetic compounds elicit SAR-related events, such as the induction of broad disease resistance and the expression of SAR marker genes, but have very little or no antibiotic activity. Among these chemicals, 2,6-dichloroisonicotinic acid (Métraux et al., 1990; Uknes et al., 1992), benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996), N-cyanomethyl-2-chloroisonicotinamide (Yoshida et al., 1990; Nakashita et al., 2002a; Yasuda et al., 2003a), 3-chloro-1-methyl-1H-pyrazole-5-carboxylic acid (Nishioka et al., 2003; Yasuda et al., 2003b), and N-(3-chloro-4-methylphenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (Yasuda et al., 2004) induce SAR by acting at the point of SA accumulation or downstream in the SAR signal transduction pathway. By contrast, probenazole and its derivative, 1,2-benzisothiazol-3(2H)-one1,1-dioxide (BIT), induce SAR by stimulating the SAR signaling pathway upstream of SA (Watanabe et al., 1979; Yoshida et al., 2001; Nakashita et al., 2002b). Some of these chemicals have been used to manage diseases in crops. However, crops sometimes suffer heavy damage from diseases despite the application of these chemicals. This could be caused by both pathogen activity and the physiological condition of the plant, which is affected by environmental factors.

Several studies have indicated that plant responses to environmental stresses, such as drought and low temperature, have some effects on their responses to pathogens. In Arabidopsis, a short period of drought stress significantly increased the in planta growth of the avirulent bacterium Pseudomonas syringae pv tomato (Pst) 1065 relative to its growth in unstressed plants (Mohr and Cahill, 2003). In rice (Oryza sativa) plants, low temperature suppressed the resistance to infection by Magnaporthe grisea (Koga et al., 2005). However, very little is known about the molecular mechanisms underlying these phenomena (Mauch-Mani and Mauch, 2005). Similarly, the involvement of ABA in plant–pathogen interactions is ambiguous. Increased endogenous levels of ABA were observed in response to infection by viruses, bacteria, and fungi (Steadman and Sequeira, 1970; Whenham et al., 1986; Kettnner and Dorffling, 1995). The application of ABA induced callose deposition, resulting in resistance against necrotic fungi (Ton and Mauch-Mani, 2004; Mauch-Mani and Mauch, 2005). In addition, ABA was shown to function positively in basal resistance. Recognizing the invasion of a bacterial or fungal pathogen induced closure of stomata, restricting further invasion (Melotto et al., 2006). By contrast, the application of ABA was also reported to increase the susceptibility of plants to fungal pathogens (Henfling et al., 1980; Ward et al., 1989; MacDonald and Cahill, 1999). Unlike many studies focusing on plant–microbe interactions, the effects of environmental stresses on the SAR have not been examined in detail.

Thus, we analyzed the effects of environmental stresses on SAR using SAR-inducing chemicals in Arabidopsis. Arabidopsis is a good model plant with which to investigate the mechanisms underlying the regulation of SAR because various types of SAR-related mutants and genes are available. SAR-inducing chemicals are also useful for the analysis of SAR signal transduction because they allow the timing of the SAR induction to be controlled. We used two types of SAR inducers: BIT, which induces SAR by activating the pathway upstream of SA; and BTH, which induces SAR without SA accumulation by activating the pathway downstream of SA. Here, we demonstrate the suppression of SAR by ABA-mediated environmental stresses. In addition, we provide evidence of antagonistic interactions between SA-mediated signaling leading to SAR induction and ABA-mediated signaling leading to responses to environmental stressors.

RESULTS

Effect of Exogenous ABA on SAR Induction in Arabidopsis

To determine the effects of environmental stress on SAR, we analyzed the effects of ABA treatment on chemically induced SAR in Arabidopsis. We used a soil-drenching method for the pathogen infection assay to avoid the dilution of the chemicals that would have been caused by dipping the plants in bacterial solutions. Treatment of Arabidopsis ecotype Columbia (Col-0) plants with BIT or BTH induced resistance against the virulent bacterial pathogen Pst DC3000. By 3 d postinoculation, BIT- or BTH-treated plants contained 10-fold lower bacterial titers than did water-treated control plants (Figure 1A). By contrast, in ABA-pre-treated plants, treatment with BIT or BTH did not reduce bacterial growth; the bacterial levels were similar to those in control or ABA-treated plants (Figure 1A). This indicates that ABA suppressed the effects of BIT and BTH, suggesting that either
SAR induction or the disease resistance mechanism of SAR was inhibited by ABA-mediated signaling.

Some PR genes are coordinately expressed in Arabidopsis during the induction and maintenance of SAR and are also expressed during SAR induced by chemical plant activators such as BIT and BTH (Uknes et al., 1992; Lawton et al., 1996; Yoshioka et al., 2001). Real-time PCR analysis indicated that foliar treatment with BIT induced the expression of acidic PR-1 in plants (Figure 1B). However, pretreatment with ABA suppressed the BIT-induced expression of PR-1 in a dose-dependent manner, while at the same time, inducing the ABA-responsive gene RAB18 dose dependently (Figure 1B). The BIT-induced expression of PR-2 and PR-5 was also suppressed by pretreatment of ABA (see Supplemental Figure 1A online). This negative effect of ABA was also observed for BTH-induced expression of PR genes (Figure 1B; see Supplemental Figure 1A online).

We confirmed the effect of ABA on the expression of PR-1 under the pathogen infection assay conditions by applying the chemicals as a soil drench (see Supplemental Figure 1B online). Suppression of PR-1 gene expression by infiltration of leaves with BIT or BTH indicated that the actions, but not the uptake, of SAR inducers were inhibited by pretreatment with ABA (see Supplemental Figure 1C online). The effect of ABA on PR gene expression was also assessed by histological analysis using plants transformed with the PR-2:GUS (for β-glucuronidase) construct (Cao et al., 1994). PR-2:GUS plants treated with BIT or BTH by foliar spraying exhibited GUS activity in their leaves and petioles (Figure 1C). However, pretreatment with ABA clearly and dramatically suppressed the PR-2 expression induced by BIT or BTH (Figure 1C). This result indicates that ABA suppresses the induction of SAR by inhibiting signal transduction downstream of BTH in the SAR signaling pathway.

**Figure 1.** Pretreatment with ABA Suppressed Chemically Induced SAR in Arabidopsis.

(A) Growth of *Pst* DC3000 in Arabidopsis leaf tissues. Four-week-old plants were pretreated with water (Control) or ABA (0.1 mg/pot) and treated with BIT (1 mg/pot) or BTH (0.2 mg/pot) by soil drenching 5 d prior to inoculation of *Pst* DC3000 (2 × 10⁵ CFU/mL). Leaves were homogenized at 3 d postinoculation. The number of colony-forming units (CFUs) was estimated by their growth on nutrient broth agar plates. Values presented are the average ± SD from six sets of three leaves each. Different letters indicate statistically significant differences between treatments (Student–Newman–Kuels [SNK] test, P < 0.05). The experiment was repeated three times with similar results.

(B) Real-time PCR analysis of PR-1 and RAB18 expression in Arabidopsis treated with BIT or BTH in combination with ABA. Four-week-old plants were foliar sprayed with 0, 4, 40, or 400 μM ABA 12 h prior to treatment with 2 mM BIT or 0.5 mM BTH. Leaves were collected 2 d after BIT or BTH treatment. Transcript levels were normalized to the expression of *UBQ2* measured in the same samples. Relative mRNA levels between treatments in each gene are presented. Each value is shown as the average of three independent experiments ± SD. Different letters indicate statistically significant differences in the analysis of each gene (SNK test, P < 0.05).

(C) Histochemical analysis of defense-related gene expression. Four-week-old PR-2:GUS plants were sprayed with water (Control) or 400 μM ABA 12 h prior to foliar treatment with 2 mM BIT or 0.5 mM BTH. Histochemical GUS staining was performed 48 h after treatment with BIT or BTH. Each experiment was performed with 12 plants and repeated twice with similar results.
A biologically active ABA analog, 1-(3-carboxyl-5-methylphenyl)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexene (RCA-7a) (Asami et al., 1998), strongly induced the ABA-responsive gene RAB18 (see Supplemental Figure 2A online). Real-time PCR analysis indicated that the levels of BIT-induced PR-1 transcripts were lower in plants pretreated with RCA-7a than in nontreated plants (see Supplemental Figure 2A online). Pretreatment with RCA-7a also significantly suppressed BIT-induced SA accumulation (see Supplemental Figure 2B online). These results strongly suggest that the suppressive effect of ABA on SAR induction occurs via the hormonal action of ABA.

To confirm the mechanism of the suppression of SAR by ABA, we assessed the effect of ABA on the SAR signaling pathway by activating only the portion of the pathway downstream of SA. In the SA biosynthesis-deficient mutants sid2-1 (Wildermuth et al., 2001) and eds5-1 (Nawrath et al., 2002), the SAR signaling pathway downstream of SA can be activated by treatment with BTH without affecting the steps upstream of SA. ABA pretreatment suppressed BTH-induced PR-1 gene expression in both mutants, confirming that signal transduction downstream of SA is inhibited by ABA (Figure 2A). This finding suggests that some regulatory mechanism downstream of SA plays an important role in the crosstalk between the SA- and ABA-mediated signaling pathways.

In nature, the onset of SAR is associated with increased levels of SA both at the infection site and systemically (Malamy et al.,...

Figure 2. Effects of ABA Downstream and Upstream of SA in the SAR Signaling Pathway.
suggesting that SA biosynthesis is one of the essential regulating steps in SAR development. Thus, we examined the effect of ABA on SA accumulation in plants. Application of BIT, which activates the SAR signaling pathway upstream of SA, gradually increased the levels of free and total (free + glycoside-conjugated) SA in wild-type plants (Figure 2B; see Supplemental Figure 3 online). However, in ABA-pretreated plants, the accumulation of free and total SA was dramatically suppressed after BIT application (Figure 2B). We also examined the effect of ABA on expression of the SA biosynthesis-related gene *ICS1*. BIT, similarly to pathogen infection, induced the expression of *ICS1*, but the expression was suppressed by pretreatment with ABA in a dose-dependent manner (Figure 2C). These results indicate that the application of ABA suppressed the induction of SAR by inhibiting signal transduction both upstream and downstream of SA accumulation in the SAR signaling pathway. Therefore, the SAR signaling pathway contains more than two regulatory components, located upstream and downstream of SA, that are negatively regulated by ABA signaling.

**Suppression of SAR Development by ABA is Independent of the JA/ET Signaling Pathway**

ABA and JA are involved in responses to wounding (León et al., 2001). In ABA-deficient mutants of tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*), wound-inducible genes are not activated upon wounding but are induced by the treatment of undamaged tissues with ABA or JA (Peña-Cortés et al., 1995). This suggests that ABA and JA mediate the wound response and JA acts downstream of ABA in the signal transduction pathway. By contrast, antagonistic interactions occur between the SA and JA signaling pathways (Feyes and Parker, 2000; Spoel et al., 2003). Thus, it is possible that exogenous ABA activates the JA signaling pathway, resulting in the suppression of the SAR signaling pathway. To determine the involvement of other defense-related hormones, such as ET and JA, in the suppressive effect of ABA on SAR induction, the expression of *PR-1* following BIT or BTH treatment was analyzed in the JA-insensitive mutant *coi1-1* and the ET-insensitive mutant *ein2-1* (Guzmán and Ecker, 1990; Xie et al., 1998). Levels of the *PR-1* transcript similar to those in wild-type plants were detected in BIT- or BTH-treated *coi1-1* and *ein2-1*mutants (Figures 1B and 3). By contrast, pretreatment with ABA suppressed *PR-1* expression in both the mutants and the wild type (Figures 1B and 3). This result indicates that the suppressive effect of ABA on SAR is regulated by unknown mechanisms, independent of the JA/ET-mediated signaling pathway.

**The Suppression of SAR Induction by Environmental Stress Is Dependent upon ABA**

ABA is synthesized in response to stress, such as drought or high salinity (Xiong et al., 2002), and functions to induce physiological changes and the expression of various genes to protect plants. To determine whether environmental stresses do in fact provoke the suppression of SAR, we examined the effect of NaCl treatment on the induction of SAR. In wild-type plants, the addition of NaCl (10 mM in pots) had no effect on the numbers of *Pst DC3000 in the inoculated leaves (Figure 4A). However, the NaCl treatment significantly suppressed BIT- and BTH-induced disease resistance (Figure 4A). Further analysis revealed that the NaCl treatment suppressed both BIT- and BTH-induced *PR-1* expression, while *RAB18* expression was induced in NaCl-treated plants (Figure 4B). These results indicated that signal transduction downstream of SA was affected under these conditions. In addition, the accumulation of SA with BIT treatment was suppressed by the NaCl treatment (Figure 4C), indicating that signal transduction upstream of SA was also inhibited. Measurement of the levels of endogenous ABA revealed that NaCl-treated wild-type plants contained ~3.6-fold higher levels of ABA than the control plants (Table 1), confirming that NaCl treatment activated the ABA-mediated signaling pathway. Thus, the NaCl treatment suppressed the induction of SAR by...
Figure 4. Suppression of SAR Development by NaCl in Wild-Type Plants and CYP707A3ox.
which constitutively express CYP707A3ox treated the water-treated control plants, bacterial levels in BIT- and BTH-wild-type plants were 13 and 8%, respectively, of those found in though the levels of bacterial growth in BIT- and BTH-treated wild-type plants were 60% lower than those found in wild-type plants and our experimental conditions, the ABA levels in Measurement of endogenous ABA levels revealed that under normal and dehydrated conditions (Umezawa et al., 2006). 

The observed suppression of SAR development by ABA accumulation is required to elicit the suppressive effect of environmental stress responses on SAR. In CYP707A3ox plants, pretreatment with NaCl did not suppress the BIT- or BTH-induced disease resistance (Figure 4A), indicating the requirement for ABA for the suppressive effect on SAR. Further analysis revealed that NaCl pretreatment in CYP707A3ox plants failed to induce RAB18 expression or to suppress the PR-1 expression and SA accumulation triggered by SAR-activating chemicals (Figures 4B and 4C), indicating that the suppressive effects both upstream and downstream of SA were ABA dependent. These results strongly suggest that ABA is a crucial molecule in the suppressive effect of environmental stress responses on SAR induction.

Suppressive Effect of SAR on ABA Signaling

The observed suppression of SAR development by ABA-mediated signal transduction led us to ask whether the activation of SAR suppresses the ABA signaling pathway. We first analyzed the effect of the activation of SAR using the Arabidopsis lesion mimic (Continued).

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<tr>
<th>Plants and Treatment</th>
<th>ABA (ng/gFW)</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>5.05 ± 0.43*</td>
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<tr>
<td>Wild type +NaCl 12 h</td>
<td>18.36 ± 7.41**</td>
</tr>
<tr>
<td>CYP707A3ox</td>
<td>1.99 ± 0.26*</td>
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<tr>
<td>CYP707A3ox + NaCl 12 h</td>
<td>2.55 ± 0.40*</td>
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Values presented are the average of ABA contents ± SD, each from four or six sets of 25 to 30 plants. Asterisks indicate statistically significant differences (SNK test, P < 0.05). Similar results were obtained in repeated experiments.
mutant len3, which constitutively expresses PR genes and accumulates SA (Ishikawa et al., 2006). The len3 npr1 double mutant does not exhibit the cell death phenotype or PR-1 gene expression, whereas significant amounts of SA accumulation and the faint expression of the PR-2 and PR-5 genes are still observed (Ishikawa et al., 2006). Microarray experiments were performed as a screen to find potentially interesting effects on different classes of genes in these mutants and the wild type. The data suggested that some ABA signaling–related genes might be affected in the different genetic backgrounds.

Thus, we used real-time PCR to monitor the transcript levels of ABA biosynthesis–related genes (ABA1, ABA2, NCED3, and AAO3) and ABA–responsive genes (RAB18, COR15A, MYC2, and RD22) in the wild type and the npr1 mutant following NaCl treatment. The transcript abundance of all of the genes examined was higher in NaCl-treated plants than in control or BIT-treated plants, although ABA2 exhibited a low response (Figures 5A and 5B). By contrast, pretreatment with BIT strongly suppressed the effect of NaCl on the expression of these genes, except for ABA2 and AAO3. Pretreatment with BIT for 5 d induced the accumulation of PR-1 gene transcripts, the levels of which were unaltered 6 h after the NaCl treatment (Figure 5C). These results demonstrate a suppressive effect of SAR signaling on ABA–mediated signal transduction, conclusively indicating that antagonistic interactions occur between SAR and ABA–mediated environmental stress responses. All of the examined ABA biosynthesis–related genes were also induced by NaCl treatment in the npr1 mutant (Figures 5A and 5B). Whereas BIT pretreatment had significant suppressive effects on the NaCl–induced expression of ABA1, COR15A, and RD22 in wild-type plants, it did not in the npr1 mutant. By contrast, the NaCl–induced expression of NCED3, RAB18, and MYC2 was suppressed by pretreatment with BIT in both the npr1 mutant and the wild-type plant. Significant expression of PR-1 was not detected in the npr1 mutant under this experimental condition (Figure 5C).

These results suggest that the NPR1 protein, or signaling downstream of NPR1, partly contributes to the suppressive effect of SAR signaling on ABA–mediated signal transduction.

**DISCUSSION**

**Antagonistic Interaction between ABA- and SA-Mediated Signaling**

Our results demonstrate an antagonistic interaction between SA signaling leading to SAR induction and ABA signaling in the environmental stress response. Analyses using various mutants as well as chemical activators and inhibitors of signal transduction have revealed multiple antagonistic crosstalk points between these signaling pathways (summarized in Figure 6). The biosynthesis and accumulation of ABA are enhanced by salt, drought, and, to some extent, cold stress (Xiong et al., 2002), suggesting that drought, and probably cold stress, would fit this model. Several reports have indicated that ABA modulates SA–dependent defense responses to some pathogens (Audenaert et al., 2002; Mauch-Mani and Mauch, 2005; Mohr and Cahill, 2007); however, the influences of ABA on SAR induction remain to be determined. This study indicates that ABA plays an important role in the regulation of defense mechanisms not only at the site of pathogen infection but also in systemic signaling for the enhancement of the plant innate immunity system.

Previous reports have shown that exogenous ABA increases plant susceptibility to various pathogens, especially fungi, which generally activate JA/ET–mediated defense signaling. Treatment with ABA increased the susceptibility of potato tuber slices to the fungal pathogens Phytophthora infestans and Cladosporium cucumerinum (Henfling et al., 1980). The susceptibility of tobacco to blue mold disease caused by Peronospora tabacina was also increased by treatment with ABA (Salt et al., 1986). Similarly, ABA treatment reduced the resistance of plants to various pathogens, such as the rice blast fungus M. grisea in rice (Matsumoto et al., 1980; Koga et al., 2005) and Phytophthora megasperma f. sp glycinea in soybean (Glycine max) (Ward et al., 1989). Some of these phenomena have been explained with biochemical evidence. In soybean, inoculation with P. megasperma f. sp glycinea induced the gene expression and enzymatic activity of Phe ammonia lyase, which was suppressed by ABA treatment (Ward et al., 1989). In a tobacco cell culture, ABA treatment downregulated the accumulation of β-1,3-glucanase, an antifungal protein, but not chitinase (Rezzonico et al., 1998), probably because of the presence of the negative-acting ABA-responsive element TAACAAA in the promoter region of the β-1,3-glucanase gene (Giraudat et al., 1994). By contrast, there have been very few studies of the effects of ABA on bacterial infections that activate SA–mediated defense signaling. ABA treatment increased the susceptibility of Arabidopsis to the oomycete P. parasitica and the avirulent bacteria Pest DC3000 but not to the virulent bacteria Pest DC3000 (Mohr and Cahill, 2003). On the other hand, recently, it was reported that bacterial colonization of Pest DC3000 in Arabidopsis was enhanced by the activation of ABA signaling, whether the signaling was activated by the bacterial type III effector or by ABA treatment (de Torres-Zabala et al., 2007).

Despite these findings, the involvement of ABA in disease resistance seems to be complex and may depend on the type of pathogen. Little effort has been made to clarify the effect of ABA on SAR because it is difficult to investigate SA signaling by excluding the effects of other events, such as HR, when using pathogen infection. We successfully demonstrated the suppressive effect of ABA signaling on SA–mediated signaling in SAR induction using two SAR–inducing chemicals, BIT and BTH, in combination with various mutants. During SAR development, the positive regulator protein NPR1 and some TGA transcription factors need to be reduced via cellular redox changes caused by SA–mediated signaling (Després et al., 2003; Mou et al., 2003). Thus, ABA could have some effects on the redox change, an idea that is currently being studied.

The effects of SA on environmental stress responses have also been reported. In maize (Zea mays), treatment with SA suppressed drought tolerance (Németh et al., 2002). In Arabidopsis, the growth of wild-type plants was suppressed by SA accumulation below 5°C. However, NahG plants, which are unable to accumulate SA, can grow better than wild-type plants under these conditions (Scott et al., 2004). Similarly, NahG plants are more resistant to NaCl treatment (Borsani et al., 2001). These results suggest that SA plays a role in inhibiting environmental
Figure 5. Suppressive Effect of SAR Induction on the Expression of NaCl-Inducible Genes in Wild-Type Plants and npr1-1 Mutants.
**Figure 5.** (continued).

Real-time PCR analysis of expression of the ABA biosynthesis-related genes ABA1, ABA2, NCED3, and AAO3 (A). ABA-responsive genes RAB18, COR15A, MYC2, and RD22 (B), and SAR marker gene PR-1 (C). Plants were treated with NaCl (20 mM in pot) by soil drenching 5 d after pretreatment with water (Control) or 2 mM BIT by foliar spraying. Leaves were collected 6 h after NaCl treatment. The effect of BIT was confirmed by expression of the PR-1 gene. Transcript levels were normalized to the expression of UBQ2 measured in the same samples. Relative mRNA levels between treatments in each gene are presented. Each value is shown as the average of three independent experiments ± SD. Different letters indicate statistically significant differences in the analysis of each gene (SNK test, P < 0.05).

**Figure 6.** Proposed Model for the Crosstalk between Biotic and Abiotic Stress Responses.

SAR is induced through the SA-mediated signaling pathway, which is activated by the HR to avirulent biotrophic pathogens. The ABA-mediated signaling pathway is activated by environmental stressors, such as salinity, drought, and, to some extent, by cold. Crosstalk between these signaling pathways, shown as broken lines, was shown to antagonistically regulate each other. Arrows indicate positive regulation, and blunt ends denote negative regulation. White arrows indicate the activation site of SAR signaling pathway by the chemicals BIT and BTH. Some genes functioning in the signaling pathways are also shown.

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**Possible Mechanism of Crosstalk between ABA- and SA-Mediated Signaling**

Our analyses indicated that the suppressive effects of ABA on the SA signaling pathway did not require JA/ET-mediated signaling (Figure 3). Recently, it was reported that ABA has an antagonistic interaction at the gene expression level with the JA/ET-dependent signaling pathway, which plays an important role in response to pathogens and wounding (Anderson et al., 2004). These studies suggest that both the SA- and JA-dependent signaling pathways, which are activated in response to biotrophic and necrotrophic pathogens, respectively, can be suppressed by ABA-mediated signaling. On the other hand, in the complex network of regulatory interactions that occur during plant stress responses, an antagonistic relationship between the SA- and JA-mediated signaling pathways is evident (Doares et al., 1995; Niki et al., 1998; Petersen et al., 2000; Kunkel and Brooks, 2002). In conclusion, three-sided antagonistic interactions among SA-, JA-, and ABA-mediated signaling appear to function in the regulation of responses to exogenous biotic and abiotic stresses. In the SA-JA antagonism, NPR1, a key component of SAR signaling downstream of SA, likely plays a critical role in modulating the SA-mediated suppression of JA-responsive genes (Spoel et al., 2003), while this study has revealed that NPR1 also takes part in the suppressive effect of SAR signaling on ABA-mediated signaling.

How important are these antagonistic interactions for plants that experience various biotic and abiotic stresses in nature? Because the response reactions to both disease and environmental stresses require significant amounts of energy for gene expression and metabolic changes, plants need to effectively regulate the strength of these responses to survive (Heil et al., 2000; Tian et al., 2003; Dietrich et al., 2005). Since these antagonistic interactions occur in a complex manner in multiple steps, there should be several mechanisms functioning in this crosstalk. One possible mechanism is the regulation of the generation of ROS. ROS are speculated to act as a systemic signal for SAR induction (Alvarez et al., 1998). In addition, it has been reported in barley (*Hordeum vulgare*) that ABA treatment increases catalase activity, which plays an important role in scavenging H$_2$O$_2$ (Fath et al., 2001). However, further investigation is necessary to elucidate the involvement of ROS and to identify the molecules that function in the crosstalk between ABA and SA signaling. Unlike in SAR, treatment with non-protein amino acid β-aminobutyric acid, a known priming-effect inducer, simultaneously enhances disease resistance and salt and drought stress tolerance in *Arabidopsis* (Jakab et al., 2005; Ton et al., 2005), suggesting that not all disease resistance mechanisms are affected by ABA signaling. To date, several other induced disease resistance mechanisms have been identified. However, it remains to be determined whether ABA suppresses responses such as rhizobacteria-mediated induced disease resistance and brassinosteroid-mediated disease resistance induced by pathogen infection (Pieterse et al., 1998; Nakashita et al., 2003).

**The Function of Endogenous ABA in Plant Disease Resistance**

Several reports have indicated that endogenous ABA levels or signaling affect disease development. The ABA-deficient tomato *sitiens* mutant is more resistant to the necrotic fungus *Botrytis*
cinerea than is the wild type (Audenaert et al., 2002). In rice, the suppression of MAPK5, a MAP kinase that functions in ABA-mediated environmental stress responses, results in lowered tolerance to abiotic stress but enhanced disease resistance (Xiong and Yang, 2003). The Arabidopsis aba1-1 mutant, defective in ABA biosynthesis, is less susceptible to the biotrophic pathogen P. parasitica than is the wild type, although the ABA-insensitive mutant aba1-1 exhibits a wild-type level of susceptibility to the same pathogen (Mohr and Cahill, 2003). By contrast, both the ABA-deficient aba2-1 and ABA-insensitive myc2 mutants show enhanced resistance to the necrotic fungal pathogen Fusarium oxysporum (Anderson et al., 2004). Some saprophytic or parasitic fungal species, such as Cercospora rosicola and B. cinerea, synthesize ABA (Crocoll et al., 1991; Nambara and Marion-Poll, 2005). These phenomena suggest that endogenous ABA levels in plants are an important factor in disease development and that some pathogens may use ABA to invade plant tissues. Further investigation of the crosstalk between ABA and defense-related hormones may help to clarify these points.

**METHODS**

**Plant Materials and Treatments**

*Arabidopsis thaliana* Col-0 and eight different mutants and two trans-formants of the same ecotype were used: sid2-1 (Wildermuth et al., 2001), eds5-1 (Nawrath et al., 2002), coi1-1 (Xie et al., 1998), ein2-1 (Guzmán and Ecker, 1990), aox-3-4 (Seo et al., 2004), npr1-1 (Cao et al., 1994), len3 mutants, the len3 np1 double mutant (Ishikawa et al., 2006), and PR-2:GUS (Cao et al., 1994) and CYP707A3ox (Umezawa et al., 2006) trans-formants. Plants were grown in sterilized potting soil (Kureha) in plastic pots (5 cm × 5 cm × 5 cm) inside a growth chamber under a 16 h:8 h light:dark regimen at 22°C with 60% humidity. ABA ([+]-isomer; Sigma-Aldrich), BIT (Nakashita et al., 2002b), BTH (Syngenta Japan), abamine (Han et al., 2004), and NaCl were dissolved in sterilized distilled water. RCA-7a (Asami et al., 1998) solution was prepared by dilution of a stock solution (25 mM in acetone) with sterilized distilled water to a concentration of 500 μM. Four-week-old plants were treated with chemicals as indicated in the figures. Identification of homozygous *coi1-1* plants was performed as previously described (Abe et al., 2008).

**Pathogen Infection Assay**

*Pseudomonas syringae* pv tomato (Pst) DC3000 was cultured in nutrient broth medium (Eiken Chemical) for 24 h at 28°C, and a bacterial suspension was prepared in 10 mM MgCl₂ (2 × 10⁶ colony-forming units per mL). Challenge inoculation was performed by dipping the plants in bacterial solution. After incubation for 3 d at 22°C, the leaves were harvested from the inoculated plants. Three to four leaves from different plants were combined, weighed, and then homogenized in 10 mM MgCl₂; the homogenate was then plated on nutrient broth agar containing rifampicin (50 mg/L) at appropriate dilutions. After incubation for 2 d at 28°C, the number of rifampicin-resistant bacterial colonies was counted. More than six homogenized samples were prepared for each experiment.

**Measurement of SA Levels**

Leaf tissues (0.5 g) were homogenized and extracted with 10 mL of 90% methanol and then with 10 mL of 100% methanol. These two extracts were combined, and 2 mL of this extract was dried at 40°C. The dried residue was extracted with 4 mL of water at 80°C for 10 min, and the same aliquot (1 mL) amounts was used for free and total SA analyses. One aliquot was extracted with 2.5 mL ethyl acetate-cyclohexane (1:1) following the addition of 50 μL of concentrated HCl, and the upper layer was dried and dissolved in 1 mL of 20% methanol in 20 mM sodium acetate buffer, pH 5. This was subjected to HPLC analysis as the free SA sample. Then, a 1-mL β-glucosidase (Sigma-Aldrich) solution (3 units/mL) was added to the other aliquot and incubated for 6 h at 37°C. This was extracted with 2.5 mL ethyl acetate-cyclohexane (1:1) following the addition of 50 μL of concentrated HCl, and an HPLC sample was prepared following the method for free SA. SA analysis was performed using the 8000 series HPLC system (Jasco Spectroscopic) with a TSK-gel ODS 80 column (4.6 × 150 mm; Tosoh) run with 20% methanol in a 20 mM sodium acetate buffer, pH 5, at a flow rate of 1 mL/min. SA was detected and quantified fluorometrically (295-nm excitation and 370-nm emission).

**GUS Staining**

Four-week-old *PR-2:GUS* plants were foliar treated with 2 mM BIT or 0.5 mM BTH 12 h after pretreatment with 400 μM ABA by foliar spraying. Plants were harvested 3 d after treatment, and GUS staining was performed by incubating whole plants in a GUS staining buffer containing 50 mM sodium phosphate, pH 7.0, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 10 mM EDTA, 0.1% (v/v) Triton X-100, 2% (v/v) dimethyl sulfoxide, and 0.5 mM 5-bromo-4-chloro-3-indolyl-β-D-glucorodide (Nacalai Tesque) for 24 h at 37°C (Jefferson et al., 1987). Plants were then washed in a graded ethanol series (30, 50, 70, and 100% [v/v]) for ~30 min in each solution.

**Measurement of ABA Levels**

ABA was extracted and purified as previously described (Saika et al., 2007). ABA measurements were performed using the LC (ACQUITY UPLC system; Waters) MS/MS (Q-Tof premier; Micromass) system. The LC, equipped with an ACQUITY UPLC BEH C₁₈ column (2.1 × 50 mm, 1.7 μm; Waters), was used with a binary solvent system composed of acetonitrile containing water (A) and 0.05% acetic acid (B). Separation was performed at a flow rate of 0.2 mL/min with a linear gradient of solvent B from 3 to 98% in 10 min. The retention time of ABA and d₅-ABA was 4.18 min. MS/MS conditions were as follows: capillary (kV) = 2.8, source temperature (°C) = 80, desolvation temperature (°C) = 400, cone gas flow (L/h) = 500, collision energy = 8.0, and MS/MS transition (m/z): 263/153 for unlabeled ABA and 269/159 for D₅-ABA. The calibration curve was calculated using spectrometer software (MassLynx v. 4.1; Micromass) and the data from seven standard solutions, each containing 10, 20, 50, 100, 200, 500, or 1000 pg/μL of unlabeled ABA and 100 pg/μL of d₅-ABA.

**Real-Time PCR**

Total RNA was extracted using Sepasol-RNA I super reagent (Nacalai Tesque) and treated with DNaseI (Invitrogen) followed by phenol chloroform purification, according to the manufacturer’s instructions. Total RNA (1 μg) was converted into cDNA with the Omniscript reverse transcriptase kit (Qiagen) using oligo(dT)₁₂-₁₈ primers (GE Healthcare Bio-Sciences) according to the manufacturer’s instructions, yielding 20 μL of cDNA solution. Quantitative RT-PCR was performed using the GeneAmp SDS 7300 sequence detection system (Applied Biosystems). The amplification reaction mixture contained 2 μL of 10-fold diluted cDNA template, 2 μL of primer solution (containing 5 μM [5 pmol/μL] of each forward and reverse primer), 8 μL Milli Q water, and 12 μL 2 × SYBR Green PCR Master Mix (Applied Biosystems). The primer pairs used in the real-time PCR are listed in Supplemental Table 1 online. Thermal cycling conditions consisted of 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and...
1 min at 60°C. Real-time PCR results were captured and analyzed using the sequence detection software SDS version 1.2 (Applied Biosystems). Transcript levels were normalized to the expression of UBQ2 measured in the same samples (Maruyama-Nakashita et al., 2004).

Statistics

One-way analysis of variance was used to compare the averages of the different results, and the SNK test was used as a post-test for pairwise comparisons.

RNA Extraction and Gel Blot Analysis

Total RNA was extracted from frozen leaf samples using Sepasol-RNA I super reagent (Nacalai Tesque) according to the manufacturer’s instructions. DNA fragments of coding regions for PR-1, PR-2, PR-5, and RD29B genes were amplified by PCR from cDNA prepared from SA-treated Arabidopsis. The PCR products were cloned into plasmid pCR 2.1 (Invitrogen), and the nucleotide sequences of these were confirmed. 

$^{32}$P-labeled cDNA probes were synthesized by random priming of these DNA fragments using Ready-To-Go DNA labeling beads (GE Healthcare). Total RNA samples were subjected to 1.2% agarose–1.1% formaldehyde gel electrophoresis and then transferred to a nylon membrane (Hybond N+; GE Healthcare Biosciences). After the transfer, RNA was cross-linked to the membrane using a UV linker (GS Gene Linker; Bio-Rad). Prehybridization and hybridization in 0.5 M sodium-phosphate buffer, pH 7.2, containing 7% SDS, 1 mM EDTA, and 10 mg/mL BSA were performed at 68°C for 1 h or longer and for 8 h or longer, respectively. The membrane was washed twice with 2× SSC containing 0.1% SDS for 30 min at 68°C and then washed twice with 0.1× SSC containing 0.1% SDS for 15 min at 68°C. Detection was performed with a BAS2500 image analyzer (Fuji Photo Film).

Microarray Experiments

Wild-type, len3, and len3npr1 plants were grown on soil for 5 weeks under a 9:15 h light:dark regimen at 22°C with 60% humidity. Total RNA was isolated using Concert reagent (Invitrogen). mRNA was prepared using the MACS mRNA isolation kit (Mitenyi Biotec). Preparation of fluorescent probes, microarray hybridization, scanning, and data analysis were performed as previously described (Seiki et al., 2002; Sakuma et al., 2006). Cy3- and Cy5-labeled cDNA probes were prepared from mRNAs isolated from the len3 or len3 npr1 mutant and control wild-type plants, respectively, grown under unstrressed control conditions. The probes were hybridized with the cDNA microarray, and the expression profiles of the ~7000 genes were analyzed. To assess the reproducibility of the microarray, we repeated the experiment three times with the same mRNA samples. GeneSpring version 5.1 software was used for screening the genes exhibiting interesting expression patterns.

Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: ABA1 (At5g67030), ABA2 (At1g52340), NCED3 (At3g14440), AAO3 (At2g27150), MYC2 (At1g32640), RD22 (At5g25610), RAB18 (At5g66400), COR15A (At2g42540), RD29B (At5g52300), PR-1 (At2g14610), PR-2 (At3g57260), PR-5 (At1g57040), ICS1 (At1g74710), and UBQ2 (At2g36170). The complete set of microarray data has been deposited in the European Bioinformatics Institute ArrayExpress database under accession number E-MEXP-1084 (www.ebi.ac.uk/arrayexpress/).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Pretreatment with ABA Suppressed the Expression of SAR Marker Genes by BIT or BTH.

Supplemental Figure 2. The Active ABA Analog RCA-7a Suppressed SAR Induction.

Supplemental Figure 3. Accumulation of Free and Total SA in Wild-Type Plants.

Supplemental Figure 4. SAR induction in ABM-Treated Wild-Type Plants and ABA Biosynthesis-Deficient aao3.

Supplemental Table 1. Primer Pairs Used for Real-Time PCR.

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Antagonistic Interaction between Systemic Acquired Resistance and the Abscisic Acid–Mediated Abiotic Stress Response in Arabidopsis

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