Restoration of Defective Cross Talk in *ssi2* Mutants: Role of Salicylic Acid, Jasmonic Acid, and Fatty Acids in *SSI2*-Mediated Signaling

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The *Arabidopsis* mutants *ssi2* and *fab2* are defective in stearoyl ACP desaturase, which causes altered salicylic acid (SA)- and jasmonic acid (JA)-mediated defense signaling. Both *ssi2* and *fab2* plants show spontaneous cell death, express *PR* genes constitutively, accumulate high levels of SA, and exhibit enhanced resistance to bacterial and oomycete pathogens. In contrast to constitutive activation of the SA pathway, *ssi2* and *fab2* plants are repressed in JA-mediated induction of the *PDF1.2* gene, which suggests that the *SSI2*-mediated signaling pathway modulates cross talk between the SA and JA pathways. In this study, we have characterized two recessive nonallelic mutants in the *ssi2* background, designated as *rdc* (restorer of defective cross talk) 2 and *rdc8*. Both *ssi2 rdc* mutants are suppressed in constitutive SA signaling, show basal level expression of *PR-1* gene, and induce high levels of *PDF1.2* in response to exogenous application of JA. Interestingly, while the *rdc8* mutation completely abolishes spontaneous cell death in *ssi2 rdc8* plants, the *ssi2 rdc2* plants continue to show some albeit reduced cell death. Fatty acid (FA) analysis showed a reduction in 16:3 levels in *ssi2 rdc8* plants, which suggests that this mutation may limit the flux of FAs into the prokaryotic pathway of glycerolipid biosynthesis. Both *rdc2* and *rdc8* continue to accumulate high levels of 18:0, which suggests that 18:0 levels were responsible for neither constitutive SA signaling nor repression of JA-induced expression of the *PDF1.2* gene in *ssi2* plants. We also analyzed SA and JA responses of the *fab2*-derived *shs1* mutant, which accumulates levels of 18:0 over 50% lower than those in the *fab2* plants. Even though *fab2 shs1* plants were morphologically bigger than *fab2* plants, they expressed *PR* genes constitutively, showed HR-like cell death, and accumulated elevated levels of SA. However, unlike the *ssi2 rdc* plants, *fab2 shs1* plants were unable to induce high levels of *PDF1.2* expression in response to exogenous application of JA. Together, these results show that defective cross talk in *ssi2* can be restored by second site mutations and is independent of morphological size of the plants, cell death, and elevated levels of 18:0.

Plants respond in various different ways to the presence of pathogens. The ability of a plant to recognize an invading pathogen is governed by the direct or indirect interaction between the products of a plant resistance gene and a pathogen

avirulence gene (Flor 1971; Keen 1990). When both the plant and pathogen express the cognate R and Avr proteins, the plant rapidly activates many defense responses that ultimately prevent pathogen colonization. One of the more visible responses is the formation of necrotic lesions at the infection sites known as hypersensitive response (HR) (Dangl et al. 1996; Greenberg et al. 1994; Hammond-Kosack and Jones 1996). HR development restricts spread of the pathogen to the cells within and immediately surrounding the lesions. Concurrent with HR development, the inoculated leaves also exhibit increases in SA levels and the induction of various defense-associated genes (Hammond-Kosack and Jones 1996; Yang et al. 1997). Subsequent to these events in the inoculated leaves, the uninoculated portions of the plant develop a long-lasting, broad-based resistance to pathogen infection known as systemic acquired resistance (SAR).

A substantial body of evidence has demonstrated that SA is one of the key signaling hormones required for activation of *PR* gene expression and resistance to certain pathogens (Dempsey et al. 1999). Exogenous application of SA enhances disease resistance and induces the same families of *PR* genes as are expressed during SAR (Uknes 1993; Ward et al. 1991). In addition, the *in vivo* increase in endogenous SA upon pathogen recognition correlates well with *PR* gene induction and development of resistance. Furthermore, *Arabidopsis* plants that are unable to accumulate SA due to expression of the *nahG* transgene that encodes the SA-degrading enzyme salicylate hydroxylase fail to express *PR* genes and become hyper-susceptible to pathogen infection (Delaney et al. 1994; Gaffney et al. 1993; Vernooij et al. 1994). Conversely, studies on various SA-accumulating *Arabidopsis* mutants have also revealed a correlation between SA levels and disease resistance (Bowling et al. 1997; Clarke et al. 1998; Dietrich et al. 1994; Greenberg et al. 1994; Rate et al. 1999; Ryals et al. 1996; Shah et al. 1999, 2001; Shirano et al. 2002; Silva et al. 1999; Weymann et al. 1995). By contrast, mutants that are defective in the SA signaling pathway, such as the *sid2*, *eds5*, *pad4*, and *npr1/nim1* mutants, exhibit enhanced disease susceptibility (Cao et al. 1994; Delaney et al. 1995; Glazebrook et al. 1996; Nawrath and Metraux 1999; Reuber et al. 1998; Shah et al. 1997; Wildermuth et al. 2001).

To identify the components associated with NPR1-independent defense signaling, we isolated a suppressor of the *npr1-5* allele designated *ssi2* (Shah et al. 2001). The recessive *ssi2* mutation confers constitutive *PR* gene expression, spontaneous lesion formation, and enhanced resistance to both bacteria and oomycete pathogens (Shah et al. 2001; Kachroo et al. 2001). Interestingly, a subset of defense responses regulated by the jasmonic acid (JA) signaling pathway, including expression of the defensin

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gene *PDF1.2* and resistance to *Botrytis cinerea*, were impaired in *ssi2* plants (Kachroo et al. 2001). These results suggest that *ssi2* may be affected in cross talk between the SA and JA pathways. The *SSI2* gene encodes stearoyl ACP desaturase (S-ACP-DES), which is the archetype member of a family of soluble fatty acid (FA) desaturases that play an important role in regulating the overall levels of desaturated FAs in the cell. S-ACP-DES converts 18:0 to 18:1. The activity of the mutant S-ACP-DES enzyme is reduced 10-fold and results in elevation of the 18:0 content in *ssi2* plants (Kachroo et al. 2001). Since reduced S-ACP-DES activity leads to the induction of certain defense responses and the inhibition of others, we proposed that a FA-derived signal modulates cross talk between different defense signaling pathways (Kachroo et al. 2001).

The FA biosynthetic pathway is one of the primary metabolic pathways playing a fundamental role in the biosynthesis of basic cellular constituents (Ohlrogge and Browse 1995). Both *ssi2* and its allelic mutant *fab2*, show an imbalance in their 18:0 FA levels, which in turn produces a change in composition of various membrane lipids (Lightner et al. 1994). To understand relationship between FA-, SA-, and JA-dependent phenotypes of *ssi2*, we isolated two mutants that were restored in cross talk between SA and JA pathway and assessed these for various *ssi2*-triggered phenotypes and FA content. Our study shows that cross talk between SA and JA pathways can be restored by second site mutation in *ssi2* plants and that this cross talk is independent of morphological size, cell death, and high levels of 18:0.

RESULTS

Isolation and characterization of mutants restored in *ssi2*-mediated signaling.

A mutation in *ssi2* causes defects in both SA- and JA-mediated defense responses, which suggests that *ssi2* is an important component of a defense signaling cascade that modulates cross talk between SA and JA pathways. To further understand the basis of this cross talk and to assess if this defect can be rescued by a second site mutation, we screened 8,000 M₂ plants of mutagenized *ssi2* seeds and scored for plants that had wild-type (wt)-like appearance. Two putative mutant plants were identified that consistently showed wt-like morphology and absence of any visible lesions on their leaves (Fig. 1A). These mutants, designated as *rdc* (restorer of defective cross talk) 2 and *rdc8*, were backcrossed with the *ssi2* plants and were scored for segregation in the second backcross. The F₁ plants obtained from *ssi2 rdc* × *ssi2* backcrosses showed *ssi2*-like phenotype, suggesting that *rdc2* and *rdc8* were recessive mutations. In the F₂ generation, the *rdc2* mutant segregated as expected with approximately 25% of the plants being wt-like and the remaining 75% showing *ssi2*-like morphology. However, F₂ analysis of a *ssi2 rdc8* × *ssi2* backcross showed less than 10% wt-like plants and over 90% *ssi2*-like plants. This data indicates that either more than one allele was involved or that the mutation was linked to the *ssi2* gene. To distinguish between these possibilities, we crossed *ssi2 rdc8* with *fab2* (an *ssi2* allelic mutant in Col-0 background); plants with wt-like morphology segregating in the F₂ generation were chosen for mapping studies. The *rdc8* locus mapped within 500 kb centromeric to *ssi2*, thus confirming its linkage to the *ssi2* locus. To further establish that *rdc2* and *rdc8* were mutations in independent genes, we crossed *ssi2 rdc2* and *ssi2 rdc8* and scored ten F₁ plants for their phenotypes. All the F₁ plants showed typical *ssi2* morphology, suggesting that *rdc2* and *rdc8* were mutations in different genes.

To determine if *ssi2 rdc2* and *ssi2 rdc8* were restored in other *ssi2*-mediated phenotypes beside morphology, we first

used trypan blue staining of leaves to assess the spontaneous cell death phenotype. As shown previously, *ssi2* plants showed prominent cell death, while the wt plants showed normal cell morphology (Fig. 1B). Interestingly, while *ssi2 rdc8* plants showed complete absence of the cell death phenotype, *ssi2 rdc2* plants showed densely stained dead cells that were isolated from each other rather than organized into thick patches as in the *ssi2* leaves (Fig. 1B). Moreover, unlike the *ssi2* plants, *ssi2 rdc2* and *ssi2 rdc8* plants showed basal level expression of the *PR-1* gene (Fig. 1C). These results suggest that both *ssi2 rdc2* and *ssi2 rdc8* mutants are downregulated in constitutive SA signaling triggered by the *ssi2* mutation. In addition, analysis of the *ssi2 rdc2* mutant suggests that *PR-1* gene expression and cell death phenotypes are independent of each other. This is further supported by the observation that removal of endogenous SA in *ssi2 nahG* plants suppresses constitutive *PR-1* levels but does not compromise the cell death phenotype (Shah et al. 2001).

Analysis of a *fab2* suppressor reduced in stearate content.

In addition to *rdc* mutants, we studied a previously characterized dominant mutant isolated in the *fab2* background (Lightner et al. 1997). This mutant, designated as *shs1* (suppressor of high stearate content), was previously shown to be larger in morphology compared with its *fab2* parent and, therefore, was likely to provide additional insights into the relationship between FA content and its affect on SA and JA pathways. The *fab2 shs1* plants were smaller than the wt plants but 20-fold or more larger than *fab2* plants (Fig. 1A). However, a larger morphology of *fab2 shs1* plants did not abolish *fab2*-characteristic cell death; *fab2 shs1* plants showed pronounced spontaneous HR-like lesion formation on their leaves (Fig. 1A). This was further confirmed by trypan blue staining of *fab2 shs1* leaves, which, like *fab2* leaves, showed multiple patches of densely stained dead cells (Fig. 1B). Both *fab2* and *fab2 shs1* plants also showed constitutive expression of the *PR-1* gene. Interestingly, levels of *PR-1* transcript were higher in the *fab2 shs1* plants compared with *fab2* plants (Fig. 1C). These data suggest that the *shs1* mutation alters the stunted morphology of *fab2* plants without affecting the constitutively active SA signaling pathway.

Pathogen response and SA levels in *ssi2 rdc* and *fab2 shs1* mutants.

Both *ssi2* and *fab2* plants show enhanced resistance to a virulent biotype Emco5 of the oomycete pathogen *Peronospora parasitica*. We examined the response of the three suppressor mutations, namely, *ssi2 rdc2*, *ssi2 rdc8*, and *fab2 shs1* to this pathogen. Like the wt Nössen (Nö) ecotype and unlike *ssi2*, both *ssi2 rdc2* and *ssi2 rdc8* supported profuse growth and sporulation of the pathogen (Fig. 2A). By contrast, *fab2 shs1*, like its parent *fab2* and unlike the wt ecotype Col-0, showed resistance to Emco5, allowing less than 5% sporulation of this pathogen. These results suggest that *rdc* mutations are sufficient to revert the enhanced resistance phenotype conferred by the *ssi2* mutation while the *shs1* mutation does not show any visible effect.

To assess if levels of the *PR-1* transcript and response to Emco5 in *ssi2*, *fab2*, *ssi2 rdc2*, *ssi2 rdc8*, and *fab2 shs1* plants were associated with the endogenous levels of SA, we determined levels of SA and its glucoside (SAG) in these plants (Fig. 2B). As shown previously, *ssi2* plants accumulated high levels of SA and SAG in their leaves, compared with the basal levels of SA seen in wt plants. Although, both *rdc* mutants accumulated higher than basal levels of SA seen in wt plants, these levels were significantly lower than those present in the *ssi2* plants. Furthermore, both *ssi2 rdc2* and

ssi2 rdc8 mutants showed near basal levels of SAG, similar to wt plants, suggesting that these plants are downregulated in constitutive SA signaling triggered by the *ssi2* mutation. The levels of SA and SAG did not correlate with cell death phenotype, as *ssi2 rdc8* plants have slightly higher levels of SA compared with *ssi2 rdc2* plants but do not show any cell death. Surprisingly, *fab2*, which showed severely stunted phenotype, accumulated lower levels of SA and SAG than *ssi2* (Fig. 2B). Furthermore, the levels of SA and SAG in *fab2* and *shs1 fab2* are similar, despite their great difference in size. These results suggest that morphological phenotype in this group of mutants is not strictly governed by SA and SAG levels.

Jasmonate response in *rdc* and *shs1* mutants.

ssi2 plants are unable to respond to JA by activating expression of the *PDF1.2* gene. Therefore, we evaluated the response of *SSI2*, *ssi2*, *fab2*, *ssi2 rdc2*, *ssi2 rdc8*, and *fab2 shs1* mutants to exogenous application of JA (Fig. 3). The *fab2* mutation in Col-0 background, as well as the *ssi2* mutation in Nö background, resulted in loss of *PDF1.2* induction upon JA treatment. This suggests that the inability of S-ACP-DES-deficient mutants to induce *PDF1.2* gene expression is not influenced by ecotypic variations. Both *ssi2 rdc2* and *ssi2 rdc8* mutations restored JA-induced *PDF1.2* expression in *ssi2* plants. By contrast, JA responsiveness was not restored in *fab2 shs1* plants. These results indicate that *ssi2 rdc2* and *ssi2 rdc8* plants are

Fig. 1. Morphological and molecular phenotypes of *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants. **A**, Comparison of the morphological phenotypes displayed by the *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants. Plants were grown on soil and photographed after 4 weeks. **B**, Microscopy of trypan blue-stained leaves from *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants. Intensely stained areas of dead cells are marked by an arrow. **C**, Expression of the *PR-1* gene in *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants. Morphological size, trypan blue-stained leaf phenotype, and *PR-1* gene expression of wild-type Col-0 plants was similar to that of Nöessen plants. RNA gel blot analysis was performed on 5 µg of total RNA extracted from 4-week-old soil-grown plants. Ethidium bromide staining of rRNA was used as a loading control.

Fig. 2. Resistance response and levels of salicylic acid (SA) and its glucoside (SAG). **A**, Growth of *Peronospora parasitica* ecotype Emco5. Cotyledons of 7-day-old seedlings from the various plant genotypes listed at the left were inoculated by spraying conidiospores at a concentration of 10^6 spores per ml. There was no difference in the size of cotyledons between wild-type (wt) and mutant plants at the time of infection. Pathogen growth was assessed by counting the number of sporangiophores per cotyledon 8 days after inoculation. The shade of each box indicates the severity of infection based on the number of sporangiophores per cotyledon (see key at right). Numbers to the right of the sample boxes indicate the number of cotyledons assayed. The wt ecotype Landsberg (*Ler*) plants were used as resistant control. **B** and **C**, Endogenous SA and SAG levels in the leaves of 4-week-old soil-grown *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants. The wt plants from Col-0 and Nönnen ecotypes showed similar levels of SA and SAG. The values presented are averages of three replicates.

restored in a majority of *ssi2*-mediated defects, while *fab2 shs1* suppresses only the morphological phenotype of *fab2*.

FA levels in *rdc* mutants.

Since both *rdc2* and *rdc8* mutations restore wt-like phenotypes in the *ssi2* background, we determined the levels of various FAs in these mutants, to examine the possibility of any correlations between various FA levels and the various mutant phenotypes (Table 1). As expected, *ssi2* plants accumulated elevated levels of 18:0 and showed a reduction in levels of 16:3 and 18:1 while levels of most other FAs were similar to those of wt plants. Both *rdc* mutants showed different FA profiles and accumulated varying levels of 16:3. This difference between *ssi2 rdc2* and *ssi2 rdc8* is consistent with the fact that these are mutations in two independent genes. Interestingly, 18:0 levels in *ssi2 rdc2* as well as *ssi2 rdc8* plants were as high as in *ssi2* plants. In contrast, *fab2 shs1*, which is similar to *fab2* with respect to *PR-1* and *PDF1.2* gene expression, SA and SAG levels, and resistance to *P. parasitica*, exhibits almost a 60% reduction in 18:0 as compared with *fab2*. These results suggest that elevated levels of 18:0 may not act as a trigger for activation of the SA pathway or repression of JA responses.

To further investigate if 18:0 can induce SA or JA responses in wt plants, we injected wt (*SSI2*) Nö plants with water or 18:0 or exposed the plants to JA after 18:0 treatments and analyzed them for induction of *PR-1* and *PDF1.2* gene expression, respectively. Northern analysis showed basal level *PR-1* gene expression in Nö plants administered with 18:0 (Fig. 4A). Plants treated with both 18:0 and JA induced similar levels of *PDF1.2* as the untreated plants or the plants injected with water prior to JA application (Fig. 4B). These results strongly argue that increasing the endogenous levels of 18:0 may not cause induction of the SA signaling pathway or repression of JA-mediated responses.

Fig. 3. Jasmonic acid (JA)-responsiveness in the *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2* and *fab2 shs1* plants. Expression of the *PDF1.2* gene in *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* plants in response to 50 μ M JA treatment. Samples were harvested 48 h posttreatment and analyzed by RNA gel blot analysis performed on 5 μ g of total RNA. Ethidium bromide staining of rRNA was used as a loading control.

DISCUSSION

Several lines of evidence point to the existence of an intricate signaling network involving SA and JA that leads to the fine tuning of defense responses. Although SA and JA activate distinct signaling pathways, there is a growing body of literature showing that these pathways do not function independently. Rather, they are involved in a complex signaling network that influences the magnitude or amplitude of various signals derived from these pathways (Kunkel and Brooks 2002; Seo et al. 1997; Shah et al. 1999). One of the major roles postulated for JA is its antagonistic action on SA-dependent signaling pathways (Clarke et al. 2000; Creelman and Mullet 1997; Gupta et al. 2000; Petersen et al. 2000; Schaller and Oecking 1999; Seo et al. 1997; Spoel et al. 2003). However, some evidence also indicates that SA and JA can act either antagonistically or synergistically in controlling stress responses (Doares et al. 1995; Pena-Cortes et al. 1993). These studies clearly established that SA- and JA-dependent pathways do not act

Fig. 4. Injection of 18:0 into leaves of wild-type (wt) plants and its effect on *PR-1* and *PDF1.2* gene expression. Leaves from wt (ecotype Nössen) plants were **A**, injected with water or 50 μ M of 18:0 and were analyzed for *PR-1* gene expression 48 h posttreatment or **B**, were treated with jasmonic acid and *PDF1.2* gene expression was analyzed 48 h posttreatments. *ssi2* plants were used as positive control for *PR-1* gene expression and negative control for *PDF1.2* gene expression. RNA gel blot analysis was performed on 5 μ g of total RNA, and ethidium bromide staining of rRNA was used as a loading control.

Table 1. Fatty acid composition of total leaf lipids from *SSI2*, *ssi2*, *ssi2 rdc2*, *ssi2 rdc8*, *fab2*, and *fab2 shs1* mutants^a

Genotype	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
<i>SSI2</i>	16.6 ± 0.9	3.1 ± 0.7	0.8 ± 0.1	13.5 ± 0.5	1.2 ± 0.2	4.7 ± 1.0	14.4 ± 0.	45.7 ± 2.0
<i>ssi2</i>	16.5 ± 1.0	2.7 ± 0.3	0.7 ± 0.0	9.1 ± 0.5	10.1 ± 1.0	1.4 ± 0.2	16.0 ± 0.	43.5 ± 2.4
<i>ssi2 rdc2</i>	16.7 ± 1.2	3.2 ± 0.5	0.5 ± 0.0	3.4 ± 0.8	8.7 ± 1.1	4.3 ± 0.9	14.8 ± 0.7	48.4 ± 2.1
<i>ssi2 rdc8</i>	14.7 ± 1.0	1.8 ± 0.3	1.5 ± 0.7	11.3 ± 1.2	9.6 ± 1.2	2.0 ± 0.7	9.3 ± 0.8	49.8 ± 2.4
<i>fab2 shs1</i>	18.0 ± 0.7	1.8 ± 0.3	0.6 ± 0.1	15.3 ± 1.0	5.0 ± 0.9	3.3 ± 0.9	9.5 ± 1.0	46.4 ± 1.9
<i>fab2</i>	14.8 ± 0.3	1.8 ± 0.2	1.3 ± 0.5	8.3 ± 0.8	12.1 ± 1.1	2.1 ± 0.6	12.8 ± 1.1	46.8 ± 1.1

^a All measurements were made on plants grown at 22°C, and data are described as mol% ± standard error calculated for a sample size of six.

independently and that key regulatory components may control the cross talk between these signaling pathways. Recent studies have indicated that translocation of NPR1 between cytosol and nucleus may regulate cross talk between SA and JA pathways (Spoel et al. 2003). However, the mechanism of this cross talk between SA and JA signaling pathways in plant defense response remains to be elucidated.

The *ssi2* mutation is unique in that it not only confers constitutive *PR* gene expression, spontaneous lesion formation, and enhanced resistance to both bacteria and oomycete pathogens but also renders these plants impaired in a subset of defense responses regulated by JA (Kachroo et al. 2001). This suggests that *SSI2*-mediated signaling regulates the SA pathway negatively and the JA pathway positively. In this study, we have asked if the defective cross talk triggered by the *ssi2* mutation can be reversed by a second site mutation and have characterized two mutants that behave similar to wt plants in their SA- and JA-mediated responses.

Although several studies have shown a positive correlation between lesion formation and *PR-1* gene expression (Rate et al. 1999; Shah et al. 1999; Shirano et al. 2002; Yoshioka et al. 2001), *ssi2*-triggered cell death appears to be independent of constitutive *PR-1* expression. This is supported by the result that cell death in *ssi2 rdc2* plants does not induce any *PR-1* gene expression (Fig. 1C). Furthermore *ssi2 nahG* plants, which show basal levels of SA similar to wt plants, continue to display *ssi2*-characteristic cell death phenotype (Shah et al. 2001). Lesion formation in *ssi2 rdc2* plants also appears to be independent of endogenous levels of SA, since these plants continue to show cell death even though they accumulate lower levels of SA and SAG as compared with *ssi2 rdc8* plants (Fig. 2B). The levels of SA and SAG in *ssi2 rdc2* and *ssi2 rdc8* plants were significantly lower than in *ssi2* plants. Thus, we were able not only to isolate mutants downregulated in constitutive SA signaling triggered by the *ssi2* mutation but were also able to delineate SA levels, *PR* gene expression, and cell death phenotypes. This delineation of the different phenotypes can also be observed in the *fab2* suppressor *shs1*. Interestingly, as compared to *fab2* plants, *fab2 shs1* plants show increased expression of the *PR-1* gene, even though these two mutants accumulate similar levels of SA and SAG. It is possible that an increase in the size of *fab2 shs1* leaves facilitates spread of the HR-like lesions on these leaves, which could explain higher levels of *PR-1* expression in these plants. This is also supported by the observation that *fab2 shs1* plants show more pronounced visible lesions as compared with the *fab2* plants.

The morphological stunted phenotype of *fab2* plants is more pronounced as compared with *ssi2*. Increased stunting in *fab2* can be explained as a result of absence of S-ACP-DES activity in the truncated *fab2* protein as opposed to *ssi2*, which retains approximately 10% of the activity of the wt protein (Kachroo et al. 2001). However, compared with *ssi2*, other phenotypes, including SA levels and *PR-1* gene expression, are lower and weaker, respectively, in *fab2* plants and, therefore, are not consistent with a null function of the *fab2* protein. Since *ssi2* and *fab2* are derived from different ecotypic backgrounds, it is plausible that certain ecotype-specific factors influence the effects mediated by increased levels of SA, 18:0, or both in the *fab2* plants.

A marked reduction in 18:0 content in the *fab2 shs1* plant does not rescue constitutive SA signaling triggered by the *fab2* mutation. Furthermore, these plants do not induce expression of the *PDF1.2* gene on exogenous application of JA and therefore continue to remain defective in their regulation of both SA and JA pathways. FA profiles of wt, *ssi2*, and *ssi2 rdc* plants also confirmed that constitutive SA signaling in *ssi2* plants is independent of the 18:0 levels (Table 1); both *ssi2 rdc2* and

ssi2 rdc8 accumulate elevated levels of 18:0 but show basal levels of SAG and *PR-1* gene expression as in wt plants. Since, both *ssi2 rdc2* and *ssi2 rdc8* mutants differ in their levels of 16:3, it is likely that these mutations are affecting independent events leading to reversion of the various phenotypes in *ssi2* plants. The low level accumulation of 16:3 in *ssi2 rdc8* plants indicates that this mutation may limit the flux of FAs into the plastidal prokaryotic pathway. Interestingly, both *ssi2 rdc* mutants were slightly pale in color compared with the wt plants, suggesting that these mutants may be affected in their photosynthetic machinery. It is likely that the pale color of *ssi2 rdc* plants may be due to an uneven flow of FAs in the chloroplastic lipids that affects the photosynthetic ability of these plants. We have recently demonstrated that chloroplasts play an important role in *ssi2*-mediated signaling by showing that the NH₄-terminal chloroplast localizing putative signal peptide of *SSI2* is essential to rescue mutant phenotypes in *ssi2* plants (P. Kachroo and A. Kachroo, unpublished data).

The *rdc* mutations also overcome the defective JA responsiveness of *ssi2* plants (Fig. 3). Since *rdc* mutations suppressed a majority of the SA responses and restored JA responsiveness in *ssi2* plants, they are likely to represent genes that participate in mediating cross talk between SA and JA pathways. Interestingly, neither of the *rdc* mutations leads to any significant increase in the levels of 18:1, which can also mediate rescue of *ssi2*-conferred phenotypes (Kachroo et al. 2001, P. Kachroo and A. Kachroo, unpublished data). These results argue that reversion of the *ssi2* phenotypes can be brought about in several different ways and suggest that there may exist an intricate FA signaling network that regulates various steps of one or more defense signaling cascades. Further analyses of the function encoded by the *RDC* genes should help to elucidate molecular mechanisms underlying FA signaling and its role in defense.

MATERIALS AND METHODS

Plant growth conditions and genetic analysis.

Plants were grown as described previously (Shah et al. 1999). Mutagenesis and screening for the *rdc2* and *rdc8* mutants was conducted as described before (Shah et al. 1999). Backcrosses were performed by pollinating flowers of the *ssi2* parental line with pollen from the *rdc2* and *rdc8* mutants. The genotype at the *ssi2* locus in these plants was determined by derived-cleaved amplified polymorphic sequence (CAPS) analysis (Kachroo et al. 2001). The genotype at the *fab2* locus was determined by amplifying a 339-bp region spanning the mutant base, using the primers TTGGCAACCCCAGGA TTTCTT and CCAATCAAGTACTGAATGGTC and digesting the product with *Sau96AI*. The amplified product derived from wt DNA digested to produce two fragments of 222 and 117 bp, while a single bp change in the product derived from the *fab2* mutant resulted in the loss of the restriction enzyme site. Mapping of *rdc8* was carried out by crossing these plants to *fab2* plants, scoring for wt-like plants, and determining the map position of the mutant loci by carrying out CAPS (Konieczny and Ausubel 1993) and simple sequence length polymorphism (SSLP) analysis (Bell and Ecker 1994). A total of 1,200 F₂ plants were used for mapping analysis, and the *rdc2* was mapped 500 kb centromeric of *SSI2*.

RNA extraction and Northern analysis.

Small-scale extraction of RNA from one or two leaves was performed in the TRIzol reagent (Gibco-BRL, Gaithersburg, MD, U.S.A.), following the manufacturer's instructions. Northern blot analysis and synthesis of random primed probes for *PR-1* and *PDF1.2* were synthesized as described earlier (Kachroo et al. 2000).

Trypan blue staining.

Leaf samples were taken from 2-week-old plants grown on soil. Trypan blue staining was performed as described previously (Bowling et al. 1994).

SA and SAG measurement.

SA and SAG were extracted and measured from 0.25 g of fresh weight leaf tissue, as described previously (Bowling et al. 1994).

Pathogen infection.

Infection with *P. parasitica* Emco5 was carried out by spraying or applying a single drop of asexual inoculum suspension (10^6 conidiosporangia per ml) per cotyledon of 7-day-old seedlings. The seedlings were grown at 19°C and >90% relative humidity with an 8-h photoperiod. There was no difference in the size of cotyledons between wt and mutant plants at the time of infection. *P. parasitica* growth was assayed visually by counting the number of sporangiophores per cotyledon at 7 days postinfection.

FA treatment of plants and analysis.

Three-week-old plants were injected with 20 and 50 µM solutions of stearic acid (prepared in methanol and diluted in water) (Sigma Chemical Co., St. Louis), and leaves were harvested 24 and 48 h postinjection. The FA profile was determined from leaves of 4-week-old plants as described previously (Dahmer et al. 1989).

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