A fatty acid desaturase modulates the activation of defense signaling pathways in plants

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Communicated by Clarence A. Ryan, Jr., Washington State University, Pullman, WA, May 23, 2001 (received for review November 6, 2000)

Salicylic acid (SA) plays an important role in activating various plant defense responses, including expression of the pathogenesis-related (*PR*) genes and systemic acquired resistance. A critical positive regulator of the SA signaling pathway in *Arabidopsis* is encoded by the *NPR1* gene. However, there is growing evidence that NPR1-independent pathways can also activate *PR* expression and disease resistance. To elucidate the components associated with NPR1-independent defense signaling, we isolated a suppressor of the *npr1–5* allele, designated *ssi2*. The recessive *ssi2* mutation confers constitutive *PR* gene expression, spontaneous lesion formation, and enhanced resistance to *Peronospora parasitica*. In contrast, a subset of defense responses regulated by the jasmonic acid (JA) signaling pathway, including expression of the defensin gene *PDF1.2* and resistance to *Botrytis cinerea*, is impaired in *ssi2* plants. With the use of a map-based approach, the *SSI2* gene was cloned and shown to encode a stearoyl-ACP desaturase (S-ACP DES). S-ACP DES is an archetypical member of a family of soluble fatty acid (FA) desaturases; these enzymes play an important role in regulating the overall level of desaturated FAs in the cell. The activity of mutant S-ACP DES enzyme was reduced 10-fold, resulting in elevation of the 18:0 FA content in *ssi2* plants. Because reduced S-ACP DES activity leads to the induction of certain defense responses and the inhibition of others, we propose that a FA-derived signal modulates crosstalk between different defense signaling pathways.

Disease resistance in plants is sometimes associated with the development of a hypersensitive response, in which necrotic lesions form at the sites of pathogen entry and the pathogen is restricted to these regions (1). Concurrent with the hypersensitive response, salicylic acid (SA) levels in the inoculated leaves increase, and many defense-associated genes, including the pathogenesis-related (*PR*) genes, are induced. Subsequent to these events, increased levels of SA and *PR* gene transcripts are detected in the uninoculated leaves. Furthermore, the uninoculated portions of the plant develop a long-lasting resistance to a broad spectrum of pathogens, a phenomenon known as systemic acquired resistance (SAR). Because increases in *PR* gene expression are tightly correlated with both hypersensitive response and SAR development, these genes are excellent markers for the activation of resistance responses.

Many studies have demonstrated that SA is an important component of the signal transduction pathway leading to SAR (2–4). Genetic analyses have further revealed that the *NPR1* gene encodes a critical positive regulator of this pathway (5–8). Unlike wild-type (wt) plants, *npr1* mutants fail to develop SAR or express *PR* genes after treatment with SA or other SAR-inducing compounds. However, inoculation with pathogen triggers *PR* gene expression in *npr1* plants. This finding, along with studies of other *Arabidopsis* mutants and wt plants, suggests that an NPR1-independent pathway(s) also regulates *PR* gene expression and resistance to certain pathogens (7–11).

In addition to SA, the signaling molecules ethylene and jasmonic acid (JA) are involved in the induction of various defense responses. JA and/or ethylene is required for resistance to *Botrytis cinerea* and *Alternaria brassicicola* (12); they also signal induced systemic resistance triggered by *Pseudomonas fluorescens* (13, 14). Exogenously applied JA and/or ethylene also activates expression of the thionin (*THI2.1*) and defensin (*PDF1.2*) genes (15–17). Genetic analyses have revealed that all of these phenomena, with the exception of induced systemic resistance activation, are mediated by a pathway(s) that is independent of both SA and NPR1 (induced systemic resistance requires *NPR1*; 12–17).

The relationship between the SA and the JA/ethylene defense response pathways is not well understood (18). Some studies have demonstrated that these signals work synergistically to induce defense responses (19, 20). However, other evidence suggests that these pathways function antagonistically. For example, plants responding to a given pathogen usually do not activate both SA-associated and JA/ethylene-dependent defenses. Furthermore, SA and JA have been shown to antagonize the activation of each other's defense responses, and SA can inhibit JA biosynthesis (21, 22). The isolation of several *Arabidopsis* mutants that constitutively accumulate both SA-induced *PR* genes and the JA/ethylene-induced *PDF1.2* gene has led to the suggestion that these pathways share signaling components that are involved in the positive and/or negative cross-regulation of their activities (10, 23, 24).

To elucidate the pathway(s) through which NPR1-independent defense responses are activated, we conducted a suppressor screen in the *npr1–5* mutant background and scored for plants that constitutively expressed the *PR* genes. Through this process, the *ssi2* mutant was identified (25). Plants carrying the recessive *ssi2* mutation are severely stunted and exhibit constitutive activation of an *NPR1*-independent pathway, leading to spontaneous lesion formation, *PR* gene expression, and resistance to *Peronospora parasitica*. In contrast, the induction of some, but not all, JA-dependent defense responses is impaired in *ssi2* plants. Cloning of the *SSI2* gene revealed that it encodes a stearoyl-ACP desaturase (S-ACP DES). Consistent with this discovery, ssi2 protein exhibited reduced levels of S-ACP DES activity and the mutant plant accumulated elevated levels of its 18:0 substrate. Based on these findings, we propose a model in which a fatty acid (FA)-derived signal(s) modulates the crosstalk between different defense signaling pathways.

Abbreviations: SA, salicylic acid; PR, pathogenesis-related; SAR, systemic acquired resistance; TAC, transformation-competent artificial chromosome; GC-MS, gas chromatography–mass spectroscopy; GUS, β-glucuronidase; FA, fatty acid; S-ACP DES, stearoyl-ACP desaturase; wt, wild type; JA, jasmonic acid; MeJA, methyl JA; Nö, *Arabidopsis* ecotype Nössen; CAPS, cleaved amplified polymorphic sequence.

Data deposition: The sequence of SSI2 (FAB2) has been deposited in the GenBank database (accession no. AF395441).

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Materials and Methods

Plant Growth Conditions and Genetic Analysis. Plants were grown as described (10). A *ssi2*/*ssi2* plant derived from *Arabidopsis* ecotype Nössen (Nö) was crossed with a *SSI2*/*SSI2* (wt) plant from the Columbia (Col-0) ecotype. Cleaved amplified polymorphic sequence (CAPS) (26) and simple sequence length polymorphic (SSLP) (27) marker analyses were performed on 656 F_2 progeny that, based on their morphology and *PR-1* gene expression, were homozygous for the *ssi2* mutation. This analysis placed *ssi2* on chromosome 2, ≈0.2 cM from AthB102 on the centromeric side and 3.7 cM from GBF on the telomeric side. With the use of sequence information generated by the *Arabidopsis* genome project, 14 additional CAPS markers spanning this region were generated and used to further delimit the region containing *ssi2*.

Derived-CAPS Analysis. A 100-bp fragment was amplified with the use of PCR primers p1 (5′-AGAGAGGGCTAGAGAGCTCCCTG-3′) and p2 (5′-AGTGTTCAACATAGTTTGATAGGTCC*T*AA-3′) from the chromosomal DNA of wt, mutant, and T_1 or T_2 progeny of *ssi2*/*ssi2*::*SSI2* transgenic plants. The bases italicized in p2 were present as GG in the original sequence; this modification created a *Dde*I site in the PCR product amplified from wt DNA. Because the *ssi2* mutation alters the 3′ base flanking AA of p2, no *Dde*I site is present in the PCR product amplified from *ssi2* DNA.

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) following the manufacturer's instructions. Northern blot analysis and synthesis of random primed probes for *PR-1*, *BGL2* and *PR-5*, *PDF1.2*, and *THI2.1* were synthesized as described (9).

***Arabidopsis* Transformation.** Transformation-competent artificial chromosome (TAC), bacterial artificial chromosome, pBI121, pBin19, (28) or pVK18 (29) derived clones were moved into *Agrobacterium tumefaciens* strains GV3101 or MP90 by electroporation and were used to transform *Arabidopsis* via the floral dip method (30). Selection of transformants was carried out on media containing hygromycin or kanamycin.

Expression in *Escherichia coli, in Vitro* S-ACP Desaturase Assay, and Gas Chromatography–Mass Spectroscopy (GC-MS) Analysis. The putative signal peptide region of SSI2 was predicted by aligning it with the protein sequence from castor bean S-ACP DES. cDNAs from both wt and *ssi2* were amplified such that they lacked N-terminal 34 aa of the putative signal peptide, and the 35th aa was converted to a methionine. The cDNAs were isolated as a *Nco*I/*Eco*RI-linkered PCR products and cloned into pET-28a vector. Purification and determination of desaturase activity were carried out as described (31). Dimethyl disulfide adducts of fatty acid methyl esters were prepared as described (32). Methyl esters of unsaturated FA and their dimethyl disulfide derivatives were identified by MS analysis (32).

Results

Positional Cloning of *ssi2*. Through codominant cleaved amplified polymorphic sequence (CAPS) (26) and SSLP (27) marker analysis, the *ssi2* gene was mapped to a 41-kb region of chromosome 2 that is encompassed by the bacterial artificial chromosome clone F18O19 (Fig. 1*A*). To identify the *SSI2* gene, *npr1–5 ssi2* double-mutant plants were transformed with subclones of F18O19 that had been inserted into a binary bacterial artificial chromosome vector (33). Alternatively, these plants were transformed with overlapping clones from a transformation-competent artificial chromosome (TAC) library (34) that hybridized to a 2-kb PCR-generated probe corresponding to ORF 4 within the 41-kb region. Transformants were screened for restoration of the wt morphology and the absence of constitutive *PR-1* gene expression. Only TAC clone F23 complemented the *ssi2* mutation (Fig. 1 *B* and *C*). Furthermore, in 105 T_2 progeny from five independently derived F23-transformed T_1 lines, the presence or absence of the hygromycin-selectable marker correlated with the development of the *SSI2* or the *ssi2* phenotype, respectively (Fig. 1*C*).

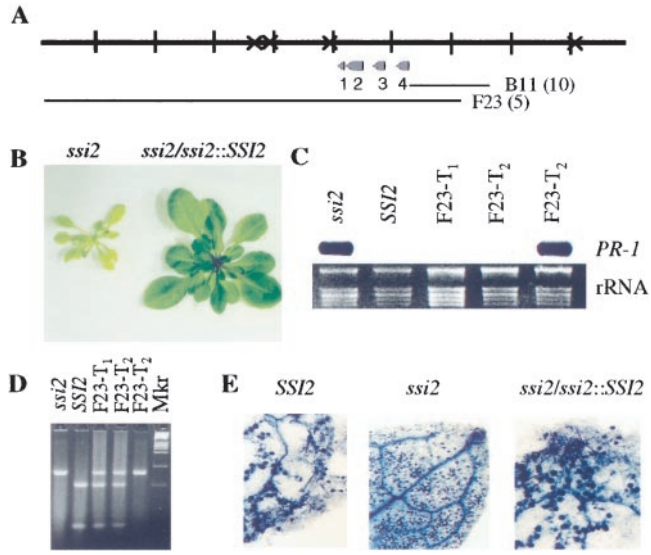

Fig. 1. Isolation of the *SSI2* gene. (*A*) The locations of several recombination break points identified by CAPS analysis are designated by X. Four ORFs in the 11.7-kb region are numbered and marked by arrowheads. The number of transformants obtained with B11 and F23 clones is shown in parentheses. (*B*) The morphological phenotype of T_2 transgenic plants complemented by the *SSI2* gene in comparison with that of the *ssi2* mutant. (*C*) Northern blot analysis showing *PR-1* gene expression in the *ssi2* mutant, *SSI2* (Nö), and T_1 and T_2 progeny of the F23 complemented transgenic *ssi2* plant. (*D*) Derived-CAPS analysis of same set of plants shown in *C*. (*E*) Approximately 50–60 plants of wt, *ssi2* and T_2 progeny of F23 transformed *ssi2* plants were spray inoculated with *P. parasitica* spores as described (57). Plants were sampled 7 days after inoculation and scored as susceptible if they developed 10 or more sporangiophores per cotyledon. Cotyledons of *SSI2* or *ssi2*/*ssi2*::*SSI2* plants showed an average of 30–40 sporagiophores per cotyledon, and 95% of these plants were susceptible. In contrast, only 5% of *ssi2* plants were susceptible, and they developed 2- to 4-fold fewer sporangiophores per cotyledon. Fungal structures and hypersensitive response-like cell death were visualized by Trypan blue staining (23). The dark-staining, round spots on *SSI2* and *ssi2*/*ssi2*::*SSI2* leaves are sporangiophores, and the dark-staining specks on *ssi2* are dead host cells.

Based on the complementation and recombination analyses, the *SSI2*-containing region of F23 was reduced to 11.7 kb. This region contains four ORFs, which were amplified by PCR and sequenced. Comparison with sequences from wt Nö plants revealed only one difference, a C-to-T transition detected in ORF2. Because this variation between wt and *ssi2* sequences could not be distinguished by restriction enzyme polymorphism, a derived-CAPS marker (35) was used to confirm the identity of the *ssi2* mutation. Analysis of 63 T_2 progeny from the *ssi2*/*ssi2*::*SSI2* complementing lines showed that stunted growth and constitutive *PR-1* gene expression cosegregated with the *ssi2*-specific band pattern (Fig. 1 *C* and *D*). The presence of the *SSI2* gene also correlated with a loss of *ssi2*-induced resistance to *P. parasitica* Emco5; those plants containing the hygromycin marker gene were as susceptible as the wt controls, whereas those lacking the marker were resistant (Fig. 1*E*). Final confirmation that the *SSI2* gene was isolated came from the demon-

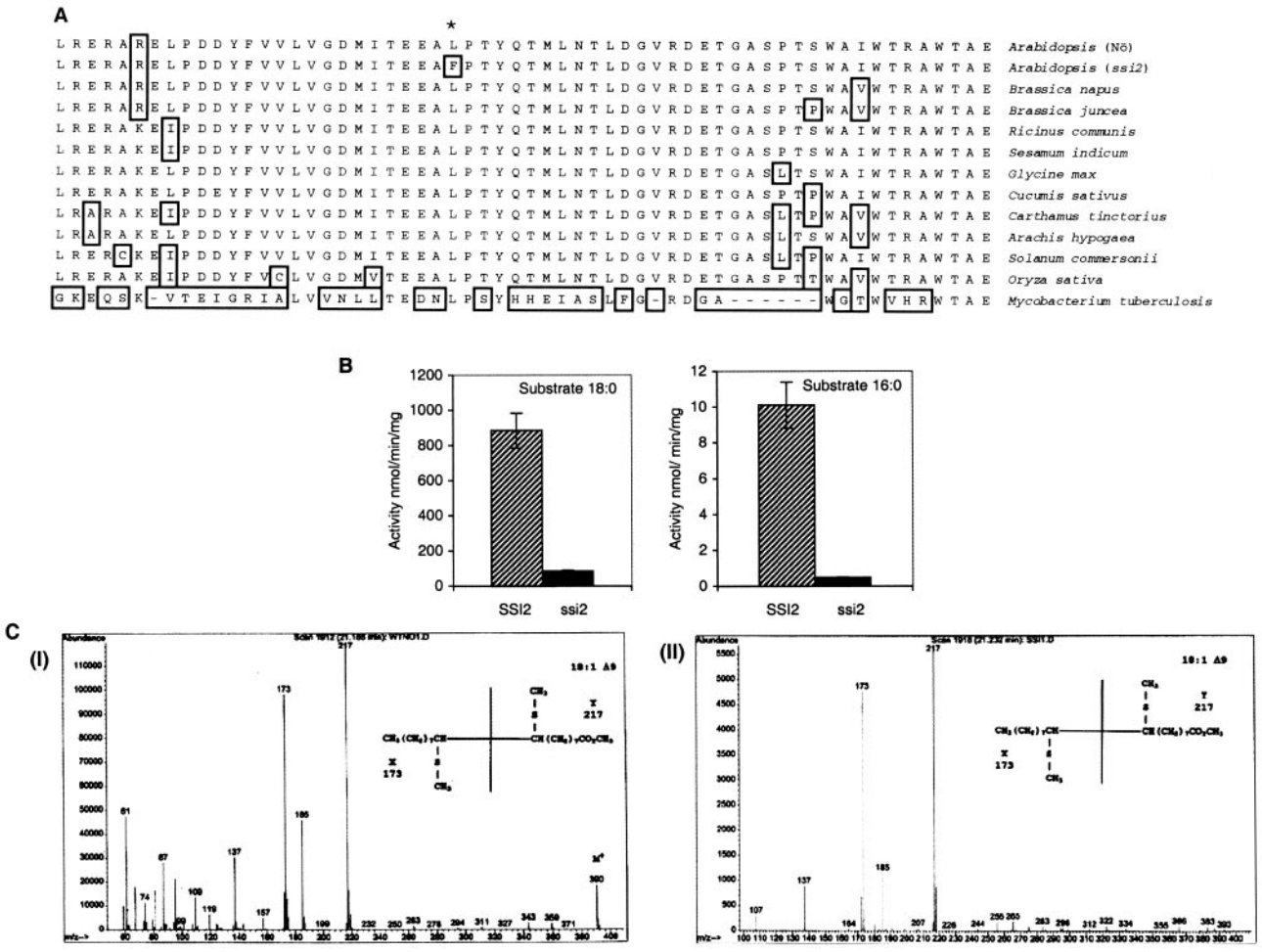

Fig. 2. SSI2 encodes an S-ACP DES with reduced activity. (*A*) A 60-aa region containing residues 121–180 was compared between S-ACP DES proteins from various plant and bacterial species. Variable amino acids are boxed; the mutated amino acids in *ssi2* is marked by an asterisk. (*B*) Enzymatic studies were carried out with a nearly homogeneous preparation of bacterial-expressed SSI2 (Nö) and mutant proteins. Desaturase activity was determined with the use of either 18:0 or 16:0 as a substrate (31). (*C*) GC-MS analysis of the double-bond position in the 18:1 FA methyl ester product generated by wt (I) and mutant (II) S-ACP DES. Although the scales are different for (I) and (II), the presence of 173 (X) and 217 (Y) ions is diagnostic for the two cleavage products of the derivatized 18:1 Δ^9 unsaturated FA formed by S-ACP DES.

stration that both a genomic clone and a cauliflower mosaic virus 35S promoter-driven cDNA clone of ORF2 restored wt morphology to *ssi2* plants (data not shown).

The *ssi2* Mutant Exhibits Reduced S-ACP DES Activity. Sequence analysis predicted that *SSI2* encodes an S-ACP DES, which is a prototypical member of the soluble FA desaturase enzyme family (36). These enzymes are key regulators of FA desaturation; S-ACP DES preferentially desaturates stearoyl-ACP (18:0-ACP) between carbons 9 and 10 to yield oleoyl-ACP (18:1$^{\Delta 9}$-ACP). The C-to-T mutation in *ssi2* changes the leucine (L) at amino acids position 146 to a phenylalanine (F). Comparison of 24 S-ACP DES proteins from various plants revealed that all, except ssi2, contain a leucine at this position (Fig. 2*A*). The high degree of conservation for L_{146}, combined with the recessive nature of the *ssi2* mutation, suggested that ssi2 might have reduced and/or altered S-ACP DES activity. To assess this possibility, we first determined whether bacterially expressed SSI2 exhibited S-ACP DES activity in an *in vitro* assay (31). Characteristic of S-ACP DES, the specific activity of wt SSI2 was ≈800 nm/min/mg, and its substrate preference for 18-carbon versus 16-carbon chain length FAs was 88:1 (Fig. 2*B*). GC-MS analysis confirmed the regiospecificity as Δ^9 (Fig. 2*C*). In contrast to the wt protein, ssi2 was ≈10-fold and 20-fold less active on both 18:0 and 16:0 substrates, respectively (Fig. 2*B*). However, its 18:16 substrate preference ratio and the Δ^9 regiospecificity were unaltered (Fig. 2*C*). Together, these results indicate that the wt and mutant enzymes display S-ACP DES activity, but that of ssi2 is substantially reduced.

The FA Composition in *ssi2* Plants Is Altered. To determine whether reduced S-ACP DES activity affects the FA composition in *ssi2* plants, the levels of various 16-carbon and 18-carbon FAs were monitored by GC-MS (Table 1). Leaves of the *ssi2* mutant contained considerably elevated levels of 18:0 compared with the wt and decreased levels of 16:3, 18:1, and 18:2 (Table 1). The levels of other FAs, including 18:3, were similar to or slightly reduced from those observed in wt plants. The presence of nearly wt levels of these FAs in *ssi2* plants is likely because of the activity of other S-ACP DES isoforms, several of which have been identified in *Arabidopsis* (37).

Activation of Some JA-Inducible Defense Responses Is Impaired in *ssi2* Plants. S-ACP DES catalyzes the first step in the pathway from stearic acid (18:0) to linolenic acid (18:3), and linolenic acid is a precursor for the defense signaling molecule JA (38). Because

Table 1. Fatty acid composition of total leaf lipids from wt and *ssi2*

Fatty acid	wt	*ssi2*
16:0	19.9 ± 1.0	18.1 ± 0.7
16:1-trans	2.7 ± 0.1	2.2 ± 0.3
16:1-cis	0.1 ± 0.1	0.2 ± 0.1
16:2	0.3 ± 0.0	0.2 ± 0.0
16:3	9.9 ± 0.7	6.3 ± 0.2
18:0	1.1 ± 0.1	13.4 ± 1.7
18:1	2.7 ± 0.1	0.9 ± 0.2
18:2	18.1 ± 0.4	14.9 ± 0.6
18:3	44.8 ± 1.0	43.5 ± 2.0

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

JA is required to activate the wounding response and defenses against insect pests (39) and certain microbial pathogens (40), we monitored *SSI2* gene expression after wounding, pathogen infection, or treatment with SA, JA, or ethylene. Analysis of transgenic plants expressing β-glucuronidase (*GUS*) driven by the *SSI2* promoter revealed that this promoter is active in all tissues studied, with the highest level of expression detected in flowers (Fig. 3*A*). Northern analysis further indicated that *SSI2*

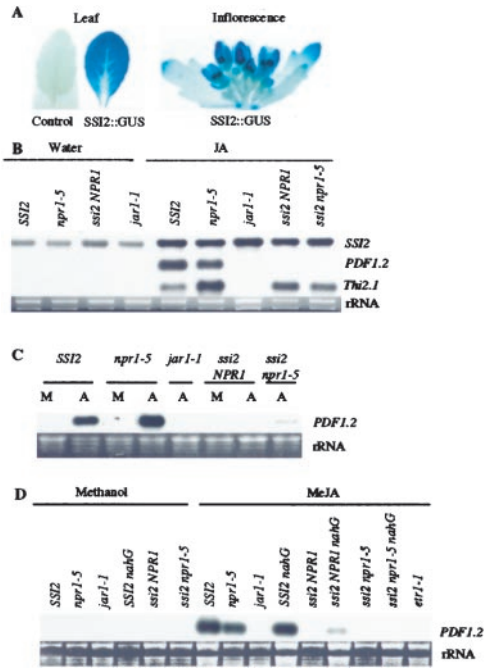

Fig. 3. Expression of the *SSI2* gene. (*A*) Histochemical staining of GUS activity in the leaves and inflorescence of transgenic plants expressing an *SSI2*::*GUS* reporter gene. A 1,631-bp fragment containing the *SSI2* promoter was transcriptionally fused upstream of *GUS* in pBI121, and three independent transgenic lines were analyzed in both T_1 and T_2 generations. The control is a stained leaf from a wt plant. (*B*) Northern blot analysis of *SSI2* (Nö), *npr1–5*, *jar1–1*, and *ssi2* plants treated with water or 50 μM JA. RNA was extracted 48 h after treatment, and the blot was sequentially probed with *SSI2*, *PDF1.2*, and *THI2.1*. Ethidium bromide-stained rRNA served as a control for gel loading. (*C*) Northern blot analysis of plants inoculated with spores of *A. brassicicola*. Mock (M) or fungal (A) inoculations were carried out as described (12). RNA was extracted 72 h after inoculation, and *PDF1.2* gene expression was monitored. (*D*) Northern blot analysis of *SSI2*, *npr1–5*, *jar1–1*, NahG, *etr1–1*, and *ssi2* plants treated with methanol or 50 μM MeJA. The plants were placed around a beaker containing methanol or MeJA diluted in methanol and covered with plastic wrap. RNA was prepared from leaves harvested 48 h after treatment and analyzed for *PDF1.2* gene expression.

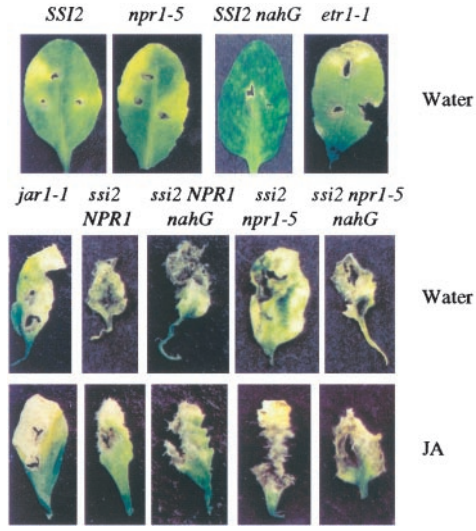

Fig. 4. Analysis of disease resistance to *B. cinerea*. Infections with *B. cinerea* were carried out by wounding the leaves by needle pricks and subsequently spot inoculating spores at the wounded site (12). The number of pricks made per leaf was based on the leaf size and ranged from three per leaf for *SSI2* (Nö) to one per leaf for the *ssi2* mutant. Plants were treated with either water or 50 μM JA for 48 h before and throughout the infection, and the inoculated leaves were photographed at 10 dpi.

gene expression was not affected by the *ssi2* or *npr1–5* mutations or the presence of the NahG transgene, which encodes salicylate hydroxylase (Fig. 3*B* and data not shown). It also did not increase substantially over basal levels at 12, 24, or 48 h after plants were treated with SA, JA, ethylene, wounding, or infection with turnip crinkle virus (data not shown; ref. 9).

The ability of *ssi2* plants to activate various JA-dependent defense responses was then assessed. Although JA treatment activated *PDF1.2* expression effectively in wt and *npr1–5* plants, it induced only low to undetectable levels of *PDF1.2* expression in *ssi2 NPR1*, *ssi2 npr1–5*, or JA-insensitive *jar1–1* mutant plants (Fig. 3*B*). In contrast, JA-induced activation of the *THI2.1* gene (15) and inhibition of root growth by JA or its derivative methyl JA (MeJA) (41) were unaffected in *ssi2* plants (Fig. 3*B* and data not shown). Inoculation with *A. brassicicola* induced strong expression of *PDF1.2* in wt and *npr1–5* plants, but only little to no expression in *ssi2* plants (Fig. 3*C*). Because loss of *PDF1.2* inducibility could be because of antagonism by the elevated SA levels found in *ssi2* mutants (25), we analyzed *PDF1.2* expression in *ssi2 nahG* plants. The presence of the NahG transgene did not restore wt levels of *PDF1.2* expression in MeJA-treated (Fig. 3*D*) or *A. brassicicola*-inoculated *ssi2 NPR1* or *ssi2 npr1–5* plants (data not shown). Thus, the reduction in *PDF1.2* inducibility in *ssi2 nahG* plants is not because of elevated SA levels. Because *PDF1.2* expression depends on concomitant activation of the ethylene and JA signaling pathways (17), we also attempted to determine whether ethylene signaling is altered in the *ssi2* mutant. A treatment of 10 or 20 parts per million of ethylene induced *PDF1.2* expression in wt plants, but not in *ssi2* plants (data not shown). However, *ssi2* plants were highly susceptible to infection by *A. brassicicola*, which is pathogenic on JA-insensitive but not ethylene-insensitive mutants (42). Based on this result, the mutation in S-ACP DES does not appear to perturb the ethylene signaling pathway.

In addition to *PDF1.2* expression, resistance to *B. cinerea*, which is mediated by JA- and ethylene-dependent pathways, was impaired in *ssi2 NPR1* and *ssi2 npr1–5* plants (Fig. 4). Exogenously applied JA or MeJA failed to restore *B. cinerea* resistance on *ssi2* or *ssi2 nahG* plants. Indeed, the symptoms exhib-

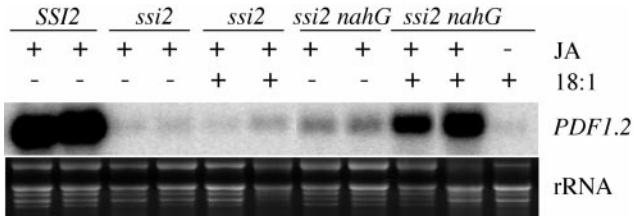

Fig. 5. Complementation of JA-dependent *PDF1.2* expression in 18:1-treated *ssi2 nahG* plants. Oleic acid (18:1; 0.5 mM; Sigma) or water was injected into the leaves of *SSI2* (Nö), *ssi2*, or *ssi2 nahG* plants followed by treatment with 50 μM JA or water. Eight to ten individual plants each of *SSI2*, *ssi2*, or *ssi2 nahG* were analyzed in two independent experiments. RNA was extracted 48 h after treatment, and *PDF1.2* gene expression was monitored by Northern blot analysis. Ethidium bromide-stained rRNA served as a control for gel loading.

ited by these plants were as severe as those displayed by *jar1–1* mutants. In contrast, ethylene-insensitive *etr1–1* plants displayed moderate symptoms, and wt, *npr1–5*, and NahG transgenic plants were resistant.

JA Plus 18:1 Induces *PDF1.2* Expression in *ssi2 nahG* Plants. A likely explanation for the failure of JA to activate *PDF1.2* and resistance to *B. cinerea* in *ssi2 nahG* plants is that certain JA-dependent responses require a second signal that is generated by S-ACP DES. *ssi2* or *ssi2 nahG* plants would lack or have reduced levels of this coactivating signal. Consistent with this hypothesis, treatment of *ssi2 nahG* plants with a combination of JA and 18:1 activated *PDF1.2* (Fig. 5). *ssi2* plants failed to respond to JA plus 18:1 (which is reduced 3-fold in *ssi2*), probably because of antagonistic effects of the high levels of endogenous SA.

Discussion

The recessive *ssi2* mutation was identified as a suppressor of the *npr1–5* allele. In this paper, we describe the cloning and characterization of the *SSI2* gene. Based on sequence analysis and biochemical assays, we demonstrate that *SSI2* encodes S-ACP DES. This enzyme, along with other soluble FA desaturases, is a key determinant of the overall level of unsaturated FAs. Analyses of the ssi2 protein revealed that its substrate preference and regiospecificity were unaltered; however, its activity was 10- to 20-fold lower than that of the wt enzyme. Consistent with this finding, the 18:0 FA content was elevated in *ssi2* plants, and the 16:3, 18:1, and 18:2 contents were reduced. The composition of 16:0, 16:1, 16:2, and 18:3 in *ssi2* plants was similar to or only slightly reduced from that of wt plants, presumably because of the activity of other S-ACP DES isoforms.

Previous studies have demonstrated that *Arabidopsis* carrying the *fab2* mutation has a stunted morphology and contains substantially elevated levels of 18:0 (43). Based on these results, it was proposed that *fab2* plants contain a defect in S-ACP DES, and that the *fab2* mutation causes stunted growth by increasing the saturation of membrane lipids, which reduces membrane fluidity and thereby inhibits cell expansion (44). Supporting this possibility, elevated temperatures were found to substantially correct the dwarf phenotype without lowering the 18:0 content. Because of the phenotypic similarities between the *fab2* and the *ssi2* mutants, we sequenced the *SSI2* gene from *fab2* plants and found it to be a null allele; a point mutation from G to A results in a truncated protein of 172 aa (data not shown).

Because S-ACP DES catalyzes a desaturation step that is required for JA biosynthesis, we attempted to determine whether the induction of JA-dependent defense responses is affected in *ssi2* plants. Both resistance to *B. cinerea* and induction of *PDF1.2* expression were found to be impaired. One explanation for this result is that the *ssi2* mutation impairs S-ACP DES activity, which reduces JA biosynthesis and thereby blocks the signaling pathway leading to these defense responses. However, this possibility seems unlikely because the level of JA precursor 18:3 in *ssi2* plants was comparable to that in wt plants. In addition, some JA-induced responses, including *THI2.1* expression and root growth inhibition, were unaffected in *ssi2* mutant plants. Moreover, exogenously supplied JA neither activated *PDF1.2* expression nor enhanced resistance to *B. cinerea*.

Another explanation for the loss of *PDF1.2* inducibility and *B. cinerea* resistance is that the elevated levels of SA found in *ssi2* plants (25) inhibit the JA signaling pathway. Previous studies have demonstrated that these signals are mutually antagonistic (18, 21, 22). This hypothesis could explain why JA treatment failed to activate *PDF1.2* expression or restore *B. cinerea* resistance. However, the presence of the NahG transgene did not restore wt levels of *PDF1.2* expression in MeJA- or pathogen-treated *ssi2* plants; it also did not restore resistance to *B. cinerea*. Furthermore, SA-mediated inhibition of JA signaling should have blocked all JA-dependent responses, rather than just a subset.

A more likely explanation for our results is that activation of certain JA-dependent responses requires a second signal that is generated by S-ACP DES. Because *ssi2* mutants would lack or have depressed levels of this coactivating signal, JA treatment would be insufficient to activate *PDF1.2* expression or restore resistance to *B. cinerea*. In contrast, activation of strictly JA-dependent responses, such as *THI2.1* and root growth inhibition, would remain unimpaired. Supporting this possibility is the discovery that injecting 18:1 into the leaves of *ssi2 nahG* plants restores JA-inducible *PDF1.2* expression. The inability of 18:1 to rescue *PDF1.2* expression in *ssi2* plants is likely because of the high endogenous SA levels, which could antagonize the action of JA. These results also suggest that 18:1 or an 18:1-derived signal works in conjunction with JA to induce JA-dependent defense gene expression and pathogen resistance.

In addition to lacking certain JA-induced defenses, *ssi2* plants exhibit constitutive expression of several SA-associated defense responses. Because pathogen infection of wt plants generally induces the expression of either *PDF1.2* or the *PR* genes, our results suggest that components of the FA desaturation pathway may cross-regulate the activation of these defenses. It is possible that the coactivating signal inhibits the *NPR1*-independent pathway; loss of this signal in *ssi2* plants would allow constitutive activation of the *NPR1*-independent responses. Alternatively, the ratio of saturated versus unsaturated FAs or changes in their subcellular distribution might regulate crosstalk between defense signaling pathways. For example, an increase in 18:0 content might lead to activation of lipid signaling, which could then induce the *PR* signal transduction pathway (45). Increases in unsaturated FAs also could stimulate (46) or inhibit (47) protein phosphatase(s) activity, which might then alter protein kinase- or mitogen-activated protein kinase-regulated pathway(s), respectively. Interestingly, an *Arabidopsis* mutant defective in the mitogen-activated protein kinase *mpk4* exhibits a phenotype similar to that of *ssi2*, including constitutive *PR* gene expression and suppressed *PDF1.2* expression (48). Perhaps reduced or altered unsaturated FA levels in the *ssi2* mutant relieve inhibition of phosphatase activity, which then results in inhibition of a mitogen-activated protein kinase (MPK4) pathway that negatively controls SA signaling and positively regulates JA signaling. The possibility that a decrease in S-ACP DES activity simply causes SA-mediated stress and *PR* gene expression is ruled out because the *ssi2* phenotypes were seen in *ssi2 nahG* plants (25). Likewise, the possibility that stress because of high FA levels induces constitutive *PR* gene expression seems unlikely because *fad2* mutants, which accumulate elevated levels of 18:1 (49) and *fad5* and *fab1* mutants, which contain high levels of 16:0 (50), do not show any of the phenotypes displayed by *ssi2* plants (data not shown). Furthermore, exogenous application of

18:0 does not induce *PR-1* gene expression in wt plants (data not shown).

Our results reveal intriguing parallels between the roles of FA signal signaling in mammals and plants. In mammals, FAs serve as an important energy source; they are also involved in various signal transduction pathways in different tissues, including those of the heart (51–53). In particular, altered stearoyl CoA desaturase activity has been implicated in the regulation of cell growth, differentiation, and signal transduction (53–54). Furthermore, altered activity of this enzyme is correlated with apoptosis (55) and neoplasia (56); these responses are similar to the cell death and altered defense signaling phenotypes associated with the *ssi2* mutation. Thus, although the mechanism(s) through which a mutation in *SSI2* affects defense response activation in plants is unclear, the discovery that a defect in FA desaturation can modulate these responses opens approaches to studying the pathways leading to disease resistance.

We thank Hugo Dooner for *fab2* seeds, the Kazusa DNA Research Institute for the TAC clones, and the Arabidopsis Biological Resource Centre for the bacterial artificial chromosome clones. We also thank Yumiko Shirano and Daisuke Shibata for providing the TAC library filters and Willem F. Broekaert and Bart P. H. J. Thomma for providing strains of *Alternaria* and *Botrytis*. We gratefully acknowledge Roy Navarre for useful comments and D'Maris Dempsey for critical reading of the manuscript. This work was funded by a grant from the National Science Foundation (MCB-9723952/0196168) to D.F.K. and the Office of Basic Energy Sciences of the U.S. Department of Energy for support of J.S. and E.J.W.

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