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RESEARCH ARTICLE

Arabidopsis SON1 Is an F-Box Protein That Regulates a Novel Induced Defense Response Independent of Both Salicylic Acid and Systemic Acquired Resistance

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One of several induced defense responses in plants is systemic acquired resistance (SAR), which is regulated by salicylic acid and in Arabidopsis by the NIM1/NPR1 protein. To identify additional components of the SAR pathway or other genes that regulate SAR-independent resistance, we performed genetic suppressor screens of mutagenized *nim1-1* seedlings, which are highly susceptible to infection by *Peronospora parasitica*. We isolated the *son1* (*suppressor of nim1-1*) mutant, which shows full restoration of pathogen resistance without the induction of SAR-associated genes and expresses resistance when combined with a salicylate hydroxylase (*nahG*) transgene. These features indicate that *son1*-mediated resistance is distinct from SAR. Resistance is effective against both the virulent oomycete Peronospora and the bacterial pathogen *Pseudomonas syringae* pv *tomato* strain DC3000. We cloned *SON1* and found it to encode a novel protein containing an F-box motif, an element found within the specificity determinant in the E3 ubiquitin-ligase complex. We propose the existence of a novel defense response that is independent of SAR and negatively regulated in Arabidopsis by SON1 through the ubiquitin-proteosome pathway.

INTRODUCTION

Plants use a number of pathogen-induced defense pathways to prevent or control disease, which together constitute the general or innate immune system. Identification of several key regulators of plant immunity has revealed a complex network of interacting defense pathways (Feys and Parker, 2000). However, the means by which these pathways are coordinated or how they intersect are largely unknown. The most intensively studied pathogen-induced defense response is systemic acquired resistance (SAR), which provides to the plant broad-spectrum resistance against not only an initial pathogen but also against subsequent infection by a variety of other viral, fungal, and bacterial pathogens (Ryals et al., 1996; Delaney, 1997; Sticher et al., 1997).

The naturally occurring compound salicylic acid (SA) is a crucial signaling compound for the induction of SAR. Application to plants of SA or its functional analog 2,6-dichloroisonicotinic acid (INA) effectively induces SAR (Kessmann et al., 1994), enabling the facile manipulation of the SAR pathway for molecular and genetic studies. SA accumulation was shown to be essential for the induction of SAR in studies of transgenic tobacco plants that express *nahG*, a bacterial salicylate hydroxylase gene whose product catalyzes the conversion of SA into the inactive compound catechol; NahG plants showed neither SA accumulation nor induction of SAR (Gaffney et al., 1993). NahG plants also are hypersusceptible to a wide range of pathogens and are significantly compromised in their expression of several examples of resistance gene–mediated defense (Delaney et al., 1994).

Exogenous application of SA or INA to plants triggers the onset of disease resistance coincident with the induction of a large number of defense response genes, including many pathogenesis-related (PR) genes, which are used commonly as molecular markers to monitor SAR activation (Ward et al., 1991; Yalpani et al., 1991; Uknes et al., 1992). Despite the tight correlation between SAR activation and the high-level expression of many PR genes, transgenic plants that express these PR genes display only modest resistance against pathogens (Alexander et al., 1993), indicating that the concerted expression of several PR genes may be required to produce robust resistance or that other important resistance effectors have yet to be discovered.

Using SA or its functional analogs, several groups performed mutant screens to identify additional components in the

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SAR pathway. Two of these groups used transgenic plants that harbored PR gene promoter–reporter fusions and isolated mutants unable to induce the reporter after treatment with INA or SA (Cao et al., 1994; Shah et al., 1997). In our screen, we focused on the induced resistance phenotype and identified mutants unable to express INA- or SA-induced pathogen resistance (Delaney et al., 1995). The rationale for seeking loss of pathogen resistance as the mutant phenotype in our screen was based on the belief that this could provide access to a wider range of mutants than had we monitored PR gene expression as the terminal trait.

Despite these differences in design, the approximately 11 mutants isolated in these screens were allelic, having mutations in the same gene called *NIM1*/*NPR1*/*SAI1* (Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997). Plants carrying mutations in the *NIM1*/*NPR1*/*SAI1* gene (henceforth, *NIM1*) are nonresponsive to SA accumulation and therefore are impaired in downstream transduction of the SA signal, resulting in a lack of PR gene expression, SAR, and other NIM1-dependent responses. A clue to the mechanism by which NIM1 functions was provided by the cloning of the gene by two groups, which showed the predicted protein product to contain ankyrin repeat domains and sequence similarity to the IκB family of mammalian transcriptional regulators (Cao et al., 1997; Ryals et al., 1997).

In addition to regulating SAR, NIM1 has been shown to be required for the activation of a SAR-independent defense pathway called induced systemic resistance (ISR) (Pieterse et al., 1996, 1998) that is induced upon exposure of Arabidopsis roots to certain nonpathogenic *Pseudomonas fluorescens* strains. Because the response does not require SA accumulation and is not associated with SAR-linked PR gene expression, ISR is distinct from SAR. Also unlike SAR, induction of ISR depends on jasmonic acid and ethylene signaling (Knoester et al., 1999; van Wees et al., 1999; Pieterse et al., 2000). How NIM1 modulates the expression of both SAR and ISR is unclear, but it is becoming apparent that *NIM1* plays a central role in multiple defense signaling pathways.

To reveal additional regulators of the plant immune response associated with *NIM1*, we screened for genetic suppressor mutations that ameliorate the highly pathogen-susceptible *nim1-1* mutant phenotype. We screened for plants resistant to a highly virulent strain of *Peronospora parasitica*, which gave us access to a range of types of mutations that could produce resistance in a *nim1-1* mutant background. We identified five *son* (*suppressor of nim1-1*) mutants based on their pathogen-resistant phenotype. One of these, *son1*, exhibited heightened resistance without the attendant induction of SAR-associated *PR* genes or *PDF1.2*, a marker for a jasmonic acid–dependent inducible defense response (Epple et al., 1997; Manners et al., 1998). Resistance in *son1* also was expressed in the presence of *nahG*, indicating that it does not require SA accumulation.

Because *son1* mutants exhibit resistance in a *nim1-1* background, do not accumulate *PR* or *PDF1.2* gene transcripts, and are resistant independent of SA accumulation, pathogen resistance in *son1* plants is unlike other previously characterized defense mechanisms. The *son1* mutation is recessive, indicating that *SON1* negatively regulates a novel SAR- and NIM1-independent defense response. Cloning of *SON1* revealed that it encodes a novel protein containing an F-box, an element found within the specificity-determining component of the E3 ubiquitin-ligase complex (Bai et al., 1996), implicating the ubiquitin-proteosome pathway in SON1-regulated disease resistance.

RESULTS

nim1-1 Suppressor Mutant Screen and Genetic Analysis of *son1*

Homozygous Wassilewskija (Ws) *nim1-1* kanamycin-resistant seeds were mutagenized using ethyl methanesulfonate and grown to mature plants, which were harvested to yield M2 seeds. Two-week-old M2 seedlings were screened for resistance against the highly virulent Peronospora isolate Emwa1. Approximately 95,000 M2 plants were screened to identify individuals that exhibited resistance to Peronospora infection, and five *son* mutants (*son1* to *son5*) were isolated. M3 seeds derived from each *son* mutant were grown on agar medium containing kanamycin to verify that the drug resistance trait was fixed, ensuring that the mutants were not false positives resulting from contamination with wild-type seeds or pollen (data not shown).

The *son1* mutant showed a high level of resistance to Peronospora, with little evidence of downy mildew disease or hyphal development (Figure 1A). Quantification of conidiophore production showed that *son1* plants had one-quarter the number of these reproductive structures compared with *nim1-1*, and ~40% fewer than Ws-0 wild type (Figure 1B).

Resistance of *son1* to *Pseudomonas syringae* pv *tomato*

To determine whether disease resistance in *son1* plants also was effective against a pathogen distinct from Peronospora, *son1* mutants were infected with the virulent bacterial pathogen *Pseudomonas syringae* pv *tomato* strain DC3000 (*Pst* DC3000). Three days after infection, *son1* mutants displayed less chlorosis and fewer disease symptoms than either wild-type or *nim1-1* plants (Figure 1C). Bacterial growth also was restricted in *son1* mutants compared with wild-type and *nim1-1* plants (Figure 1D), indicating that *son1*-mediated resistance was active against *Pst* DC3000.

Genetic Analysis of the *son1* Mutant

To determine the pattern of inheritance for the *son1* mutation, *son1 nim1-1* plants were backcrossed to *SON1 nim1-1*,

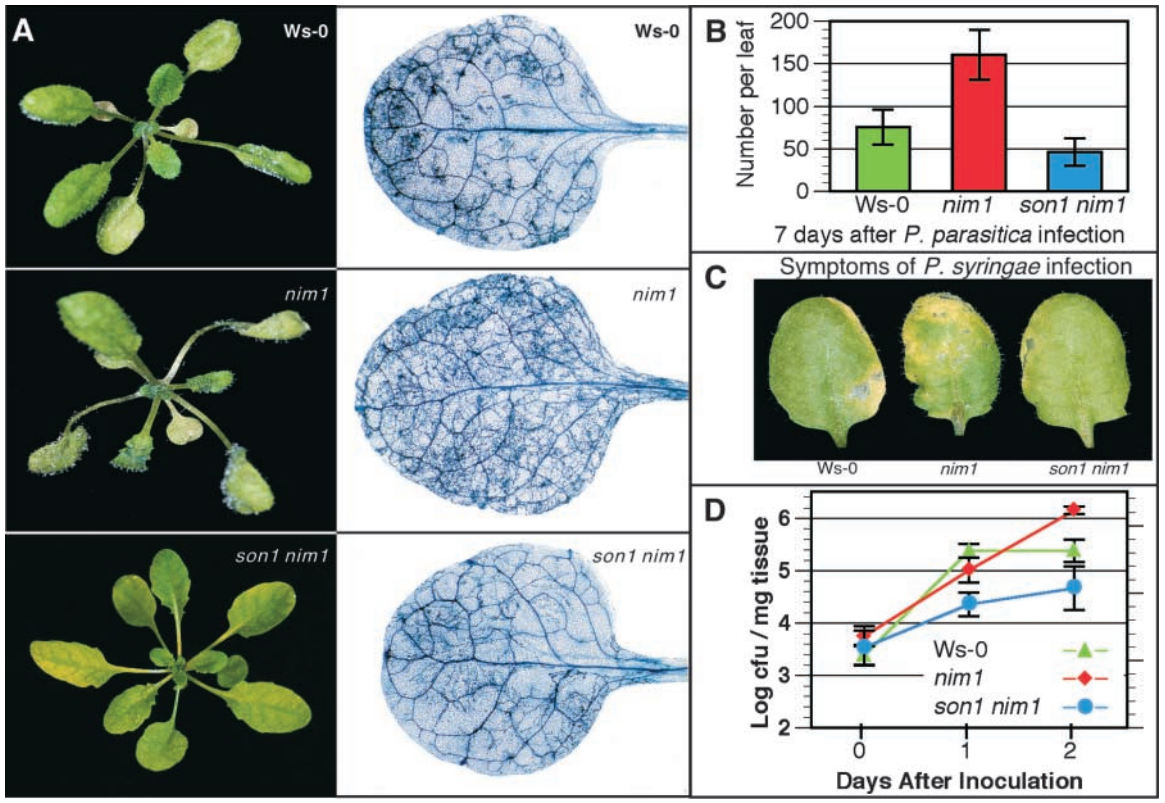

Figure 1. Resistance of *son1 nim1-1* Plants to Peronospora and *Pst* DC3000.

(A) The normally virulent Peronospora isolate Emwa1 showed moderate to heavy sporulation on wild-type Ws-0 and *nim1-1* plants but failed to grow on *son1 nim1-1* plants. Photographs were taken 7 days after inoculation with 5×10^4 conidiospores/mL (left column). Examination by lactophenol trypan blue staining of infected leaves 7 days after inoculation revealed little hyphal development in *son1 nim1-1* leaves compared with the extensive growth seen in Ws-0 and *nim1-1* leaves (right column). Host genotypes are indicated at top right in each panel.

(B) To quantify resistance, production of conidiophores was examined 7 days after inoculation with Peronospora Emwa1. Plants carrying the *son1* mutation were resistant to this pathogen.

(C) The virulent *Pst* DC3000 failed to elicit disease symptoms in *son1 nim1-1* plants. Three days after dip inoculation with a suspension of *Pst* DC3000, leaves from *son1 nim1-1* mutants displayed fewer and less chlorotic lesions compared with leaves from infected wild-type Ws-0 and *nim1-1* plants.

(D) Measurements of bacterial growth 24 and 48 h after inoculation show less bacterial replication in *son1 nim1-1* plants than in wild-type or *nim1-1* plants. cfu, colony-forming units.

and the resulting F1 and F2 progeny were tested for susceptibility to Peronospora Emwa1. Because all F1 plants produced were susceptible to the test pathogen, we concluded that *son1*-mediated resistance was inherited as a recessive trait. Analysis of 120 2-week-old F2 seedlings showed that 29 were resistant to Peronospora infection ($\sim$3:1 susceptible:resistant plants; $\chi^2 = 0.044$, $P > 0.5$), supporting the conclusion that *son1* is recessive and indicating that a single locus is responsible for the *son1* phenotype. In crosses between *son1 nim1-1* and wild-type Ws-0 plants, $\sim$25% of the F2 progeny were resistant to Peronospora, indicating that resistance was expressed independent of the *nim1-1* mutation (data not shown).

To determine whether *son1*-mediated resistance was influenced by the presence of specific *nim1* alleles, *son1 nim1-1* plants were crossed to *nim1-2* and *nim1-4* plants. Allele-specific PCR was used to identify F2 seedlings homozygous for each of the introgressed *nim1* alleles, and at least five such lines advanced to produce F3 seeds. Because each F2 plant had a 75% probability of carrying at least one copy of the *son1* mutation, we were confident that, by analyzing five F3 populations, at least one would segregate *son1* homozygous F3 progeny. In both the *nim1-2* and *nim1-4* homozygous F3 lines, we found resistant *son1 nim1-2* and *son1 nim1-4* plants, showing that *son1*-mediated resistance is not allele specific, because it was expressed in the presence of two other *nim1* alleles (data not shown).

To determine whether *son1*-mediated resistance is dependent on SA accumulation, *son1 nim1-1* plants were crossed to NahG transgenic plants. F2 *son1 nim1-1* NahG homozygous lines were identified using PCR-based markers and the hygromycin marker linked to the NahG transgene. Mutant allele–specific primers were used to identify *nim1-1* homozygotes (Lo et al., 1992), whereas derived CAPS were used to detect *son1* homozygotes among F2 plants (Neff et al., 1998). F3 seeds from *son1 nim1-1* F2 plants then were plated on Murashige and Skoog (1962) medium containing hygromycin (20 μg/mL) to screen for NahG homozygous lines. Several *son1 nim1-1* NahG homozygous lines were identified and found to be resistant to Peronospora infection, showing that resistance is not dependent on SA (Figure 2). In some cases, inoculation triggered the formation of necrotic lesions on *son1 nim1-1* NahG leaves (Figure 2D).

Molecular Phenotype of *son1* Plants

To determine whether resistance in *son1* plants was accompanied by expression of PR or jasmonate-responsive genes, leaves from wild-type (Ws-0), *nim1*, *son1 nim1*, and *son1 NIM1* plants were collected before and 3, 5, and 7 days after Peronospora Emwa1 inoculation and used for RNA extraction. RNA gel blots were probed with radiolabeled cDNA probes corresponding to the Arabidopsis *PR-1*, *PR-2*, *PR-5*, and *PDF1.2* genes (Figure 3). The amount of *PR-1* mRNA accumulation in the *son1 nim1-1* double mutant after Peronospora infection was significantly less than the levels seen in wild type Ws-0 and slightly less than or equal to the amount observed in *nim1-1*. Similarly, *PR-2* and *PR-5* levels were reduced in *son1* relative to *nim1-1* and wild-type plants.

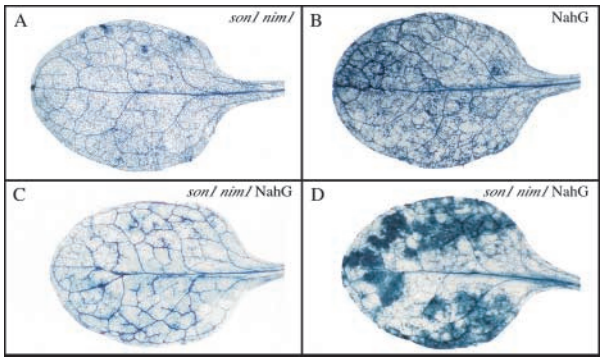

Figure 2. Resistance of *son1 nim1-1* NahG Plants to Peronospora.

Leaves were collected 7 days after inoculation with Peronospora isolate Emwa1 and stained with lactophenol trypan blue to visualize the extent of hyphal development. Leaves from *son1* NahG plants (**[C]** and **[D]**) were as resistant as leaves from *son1 nim1-1* **(A)** to pathogen infection and sometimes displayed extensive necrotic lesions **(D)**. Host genotypes are indicated at top right in each panel.

These data indicate that resistance in *son1 nim1-1* plants is not linked to the induction of SAR genes. Because *son1* mutants display heightened resistance to Peronospora infection but do not show induction of *PR* gene expression, we tested whether the expression of the jasmonic acid–responsive defensin gene *PDF1.2* was increased in *son1 nim1-1* plants after inoculation with Peronospora. We detected no increase in *PDF1.2* expression in *son1 nim1-1* mutants after this treatment (Figure 3). Examination of the molecular phenotype of *son1* plants that contain a functional *NIM1* gene revealed an interesting constitutive increase in *PR-1*, *PR-2*, and *PR-5* expression but not in *PDF1.2* expression (Figure 3). This finding suggests that the *son1* mutation activates both a SAR-independent defense response in *nim1-1* mutants and SAR in *NIM1* plants.

We also examined the accumulation of *PR* gene mRNAs after treatment with INA, an analog of SA. Wild-type plants showed the expected strong *PR* gene induction response to this treatment, whereas *nim1-1* plants showed a much reduced response, and *son1 nim1-1* plants were even less responsive than *nim1-1* (Figure 4).

Map-Based Cloning of *SON1*

The *son1* mutant was isolated in a screen of Ws *nim1-1* plants. Therefore, to map the *SON1* gene, we performed crosses between *son1-1 nim1-1* and Columbia (Col) *npr1-2* plants. The *son1* mutant phenotype was identified in F2 plants by their resistance to the Peronospora isolate Emco5, which is virulent in both Ws-0 and Col-0 accessions. The *npr1-2* allele was included in the cross to reduce the possibility that the segregation of *nim1-1* would complicate the identification of *son1* F2 progeny.

The F2 mapping population was phenotyped for Emco5 resistance on 2-week-old plants, and resistant individuals were examined for segregation of PCR-based molecular markers. For the initial mapping, 48 F2 plants resistant to Emco5 infection were tested with codominant cleaved amplified polymorphic sequence (CAPS) markers from each of the five chromosomes (Konieczny and Ausubel, 1993). The *THY1* marker on chromosome 2 showed cosegregation with the *son1* phenotype.

Further analysis of this region showed the *son1* mutation to be localized between *THY1* and *PHYB* markers, which are ~1030 kb apart (Figure 5A). To narrow the region in which *SON1* was located, the Cereon Genomics single nucleotide polymorphisms (SNP) database was used to design SNP markers between *THY1* and *PHYB* (Neff et al., 1998). Although the database is composed of SNPs between the Col-0 and Landsberg *erecta* accessions, we found that approximately one-third of the Col/Landsberg *erecta* polymorphisms tested also exist between Col-0 and Ws-0. After screening 948 F2 plants, *SON1* was localized to a 41-kb region that contained 10 open reading frames (ORFs) (Figure 5C). We obtained and compared the DNA sequences of

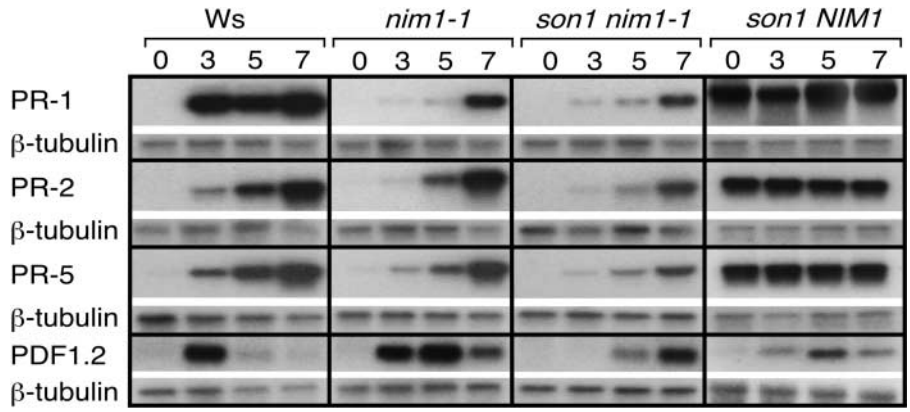

Figure 3. Molecular Phenotype of *son1 nim1-1* and *son1* Plants after Inoculation with Peronospora.

Wild-type (Ws-0), *nim1-1*, *son1 nim1-1*, and *son1 NIM1* plants were inoculated with the virulent Peronospora isolate Emwa1, and RNA accumulation was assessed 0, 3, 5, and 7 days later. RNA gel blots were probed with the SAR genes *PR-1*, *PR-2*, and *PR-5* or with the jasmonate-responsive gene *PDF1.2*. Replicate blots were probed with the β-tubulin gene as a loading control.

these 10 ORFs from *son1* and wild-type Ws-0 plants, which revealed a single base pair polymorphism in ORF4 that causes an Arg-to-Gln change in the amino acid sequence of the predicted gene.

To confirm that ORF4 corresponded to the *SON1* gene, *Agrobacterium tumefaciens*–mediated transformation was used to introduce into *son1* plants a cDNA that corresponds to ORF4 under the control of the 35S promoter of *Cauliflower mosaic virus*. Thirty-three primary transgenic lines were obtained and found to have lost resistance to Peronospora isolate Emwa1 and *Pst* DC3000, indicating that ORF4 is equivalent to *SON1* (Figure 6).

Analysis of *SON1* Structure

The *SON1* gene has an uninterrupted ORF of 1113 bp, which encodes a novel 370–amino acid protein (Figure 7A). The N-terminal region of SON1 contains an F-box domain, which is a conserved 40– to 50–amino acid motif found within F-box proteins that are part of the E3 ubiquitin-ligase complex and are involved in recognition of both the E2 and substrate for ubiquitination (Bai et al., 1996). Approximately half of the known F-box proteins contain a C-terminal protein–protein interaction domain, such as a Leu-rich repeat, WD-40, or Kelch region, that is believed to play a role in

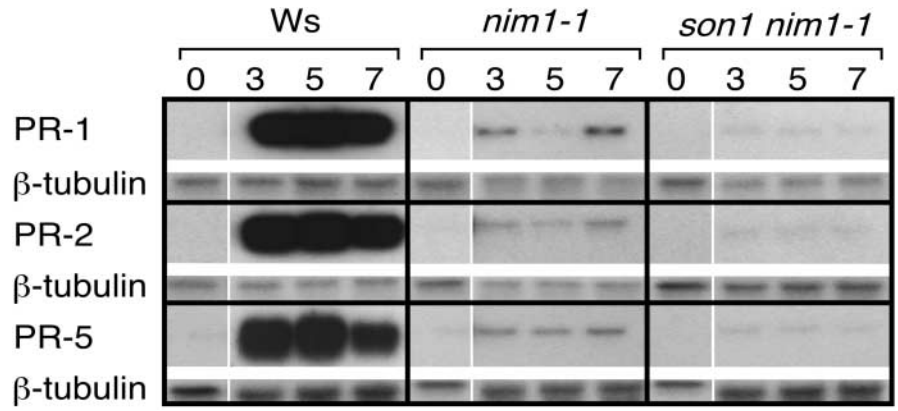

Figure 4. PR Gene Expression in *son1* Plants after INA Treatment.

Wild-type (Ws-0), *nim1-1*, and *son1 nim1-1* plants were treated with the SAR-inducing chemical INA and examined for RNA accumulation 3, 5, and 7 days later. Pretreatment (time 0) samples are the same as those in Figure 3, in that Emwa1 and INA experiments were run simultaneously. SAR gene probes *PR-1*, *PR-2*, and *PR-5* were tested on RNA gel blots. To assess equal loading, a β-tubulin gene probe was used on replicate blots.

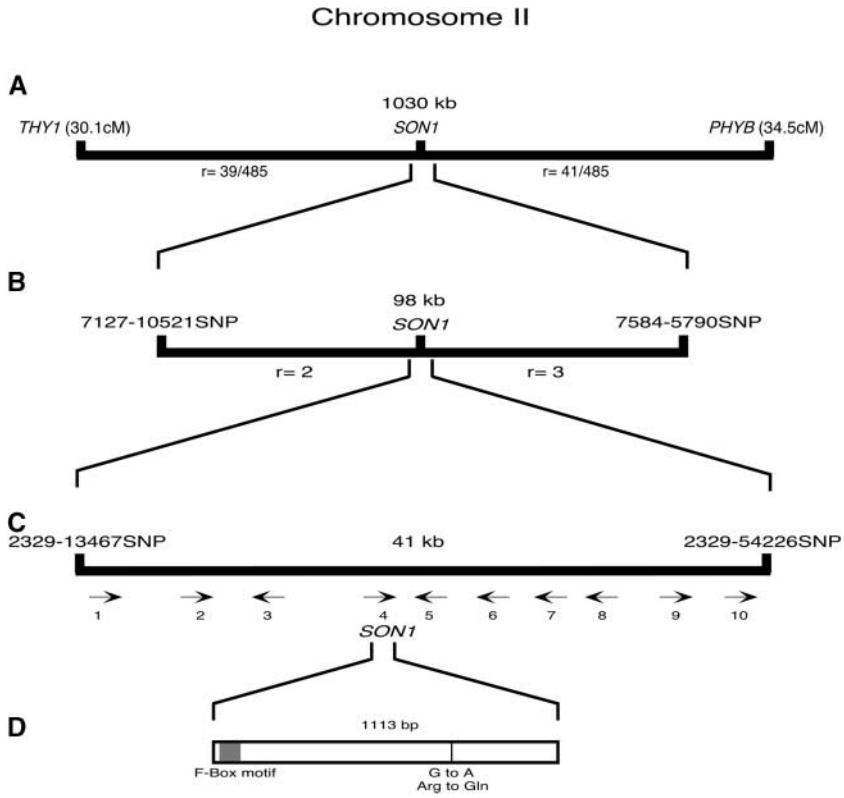

Figure 5. Map-Based Cloning of *SON1*.

The initial mapping of *SON1* using cleaved amplified polymorphic sequence markers located the gene in the interval between *THY1* and *PHYB* on chromosome 2 **(A)**. Additional PCR-based molecular markers (SNPs) were generated between *THY1* and *PHYB* and used to narrow the region in which *SON1* was located to 98 kb **(B)** and then 41 kb **(C)**, which contains 10 putative open reading frames (the numbered arrows in **[C]** correspond to the 10 ORFs). Sequence comparison of the 10 ORFs from wild-type Ws-0 and *son1* mutant plants revealed a single G-to-A transition in ORF4 (370 amino acids) that causes an Arg-to-Gln substitution at amino acid 257 in the predicted protein sequence **(D)**. In **(A)**, denominators indicate the number of F2 plants examined that showed the *son1* phenotype. In **(B)** and **(C)**, numbers ending in SNP indicate SNP molecular markers. cM, centimorgan; r, number of recombinant chromosomes identified in an F2 mapping population.

substrate recognition (Takahashi et al., 1985; van der Voorn and Ploegh, 1992; Neer et al., 1994; Adams et al., 2000; Andrade et al., 2001; Kobe and Kajava, 2001). By contrast, SON1 and the other half of the known F-box proteins do not contain recognizable interaction domains at their C-terminal ends.

However, the *son1-1* mutation occurs in the carboxyl third of the protein, a G-to-A transition that results in an Arg-to-Gln substitution at amino acid 257, suggesting that this region is important to SON1 function (Figure 7A). Although SON1 is unlike other described proteins, its F-box region is highly similar to those of other F-box proteins from Arabidopsis, yeast, and human (Figure 7B), suggesting that SON1 also functions in conjunction with the E3 ubiquitin-ligase complex. Analysis of SON1 using reverse position-specific BLAST (http://www.ncbi.nlm.nih.gov) indicates that the putative F-box in this protein is supported by an expected value of 1 e-06, as related to the conserved F-box domain Smart00256 (LPDEILEEILSKLPPKDLLRLRKVSRK-WRSLIDSHDFWFKL).

Accumulation of *SON1* mRNA in Wild-Type, *nim1-1*, and *son1 nim1* Plants

To determine if *SON1* was regulated transcriptionally in plants after exposure to a pathogen or treatment with INA, we examined wild-type, *nim1*, and *son1 nim1* plants after these treatments (Figure 8). We observed no significant change in *SON1* RNA accumulation in any of the genotypes after inoculation with Peronospora or INA treatment, but we did observe a low constitutive level of expression in all plants examined (Figure 8).

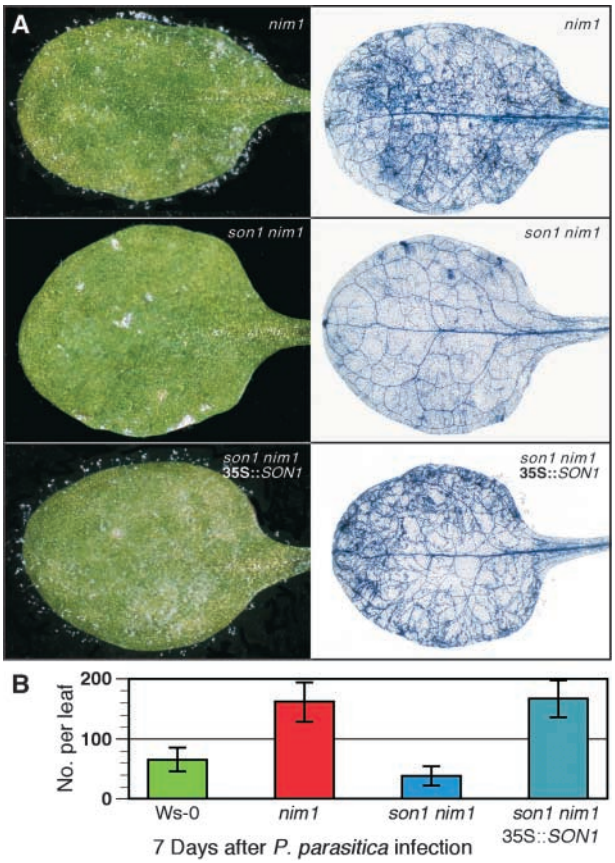

Figure 6. Introduction of Wild-Type *SON1* Complements the *son1* Mutant.

A cDNA corresponding to ORF4 was cloned under the control of the 35S promoter of *Cauliflower mosaic virus* in a binary vector for transformation into *son1 nim1-1* mutants. Thirty-three independent transgenic T1 lines tested showed complementation of the *son1* mutation after inoculation with Peronospora isolate Emwa1.

(A) Complementation was demonstrated by the heavy Peronospora sporulation observed on *son1 nim1-1* mutants that expressed ORF4, comparable to that observed on *nim1-1* plants. Rows are as follows: top, *nim1* is susceptible to Emwa1; middle, *son1 nim1* is resistant; bottom, complementation of *son1 nim1-1* with *SON1* makes the transformed plants again susceptible to Peronospora. Leaves in the right column were stained with lactophenol trypan blue and then cleared to show hyphal development.

(B) Quantification of conidiophore production (ordinate) confirms that resistant *son1 nim1* plants regained susceptibility after transformation with the wild-type *SON1* gene.

DISCUSSION

In a screen to identify genetic suppressor mutations of the *nim1-1* mutation and thereby reveal regulators of disease resistance, we isolated *son1*, a mutant that shows robust resistance to infection by both an oomycete and a bacterial pathogen and that appears to express a novel form of defense response. Resistance mediated by the recessive *son1* mutation is not attributable to SAR, because *son1 nim1-1* double mutants did not show significant accumulation of PR gene transcripts after pathogen exposure or in response to INA treatment, as would occur in wild-type plants or in *nim1-1* plants carrying suppressors that restore function to the NIM1 pathway. Furthermore, SA accumulation is not required for the expression of *son1*-mediated resistance, because *son1 nim1-1* NahG plants were as resistant to Peronospora and *Pst* DC3000 as *son1 nim1* plants.

The jasmonic acid–induced defensin gene *PDF1.2* also was not induced in *son1 nim1-1* plants or in *son1* plants, indicating that the mutation does not activate jasmonic acid signaling pathways. These unique characteristics distinguish *son1* from other known *nim1/npr1* suppressors, including *sni1*, *ssi1*, *ssi2*, *cpr5*, and *cpr6*. Plants carrying *sni1 npr1-1* mutations show strong inducibility of *PR* genes after INA treatment (Li et al., 1999), whereas *ssi1 npr1-5* and *ssi2 npr1-5* mutants are dwarfed, develop necrotic lesions, and show constitutive expression of *PR* genes in the absence of a functional *NIM1* gene (Shah et al., 1999, 2001). Unlike *son1*, *ssi1* is a dominant mutation and is accompanied by constitutive expression of *PDF1.2*.

In the presence or absence of *NIM1*, the *cpr5* and *cpr6* mutants also show constitutive expression of *PR* and *PDF1.2* genes and may express both SAR and SAR-independent defense responses (Bowling et al., 1997; Clarke et al., 1998). In *son1 nim1-1* plants, on the other hand, the lack of any known defense-related gene induction indicates that this mutant expresses a *NIM1*-independent disease resistance mechanism unlike that observed in *ssi* or *cpr* mutants. In addition to the distinguishing phenotypic characteristics of *son1* plants, the mapping and cloning of *SON1* shows it to be distinct from any other cloned *nim1/npr1* suppressor.

The constitutive expression of *PR* genes observed in *son1 NIM1* plants was dependent on a functional *NIM1*, suggesting that in the absence of elicitation, SON1 acts to repress NIM1-dependent *PR* gene expression. Furthermore, the expression in *son1 nim1-1* plants of SA- and *PR* gene–independent resistance indicates that SON1 acts as a negative regulator of a SAR-independent resistance (SIR) mechanism. Therefore, SON1 appears to have a dual role in repressing both SAR and SIR systems. Because constitutive expression of *PR* genes is not observed in *son1 nim1-1* plants with respect to SAR, *nim1* is epistatic to *son1*, indicating that the influence of SON1 on the SAR pathway occurs upstream of NIM1 or at NIM1 directly.

The dual role postulated for SON1 in SAR and SIR may be accomplished mechanistically by SON1 acting to repress the regulatory components required for both SAR and SIR or a regulator common to both defense systems. Alternatively, the pathogen-resistant phenotype of *son1* plants may be a secondary effect of the mutation rather than the result of a direct activation of a SON1-regulated defense pathway. Indirect activation of defenses has been observed in plant

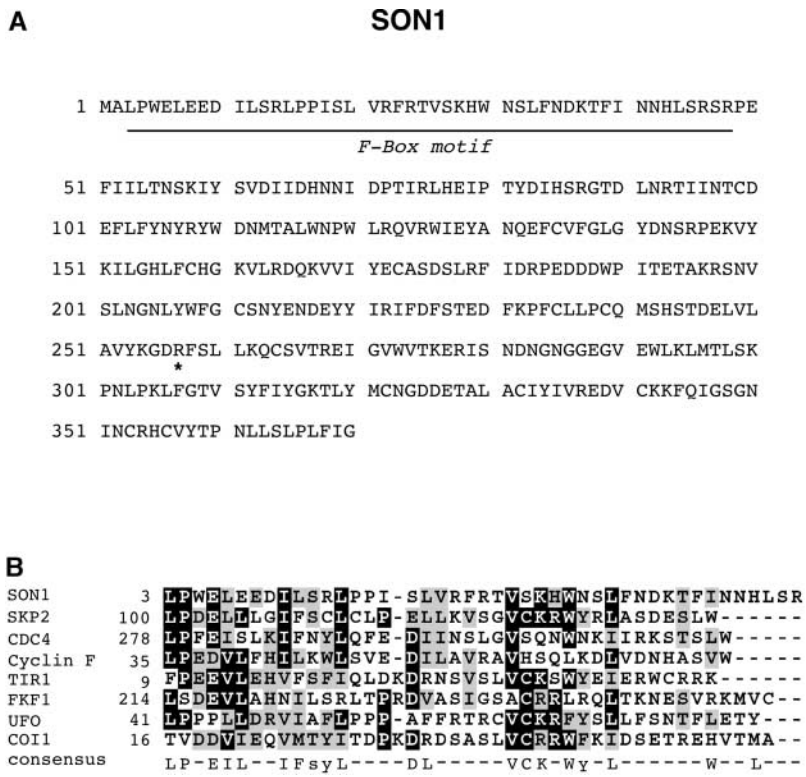

Figure 7. SON1 Primary Structure and F-Box Motif.

(A) *SON1* encodes a novel protein containing an F-box motif at its N-terminal end (underlined). The G-to-A missense mutation in *son1-1* plants causes an Arg-to-Gln substitution at amino acid 257 in the protein (asterisk).

(B) The 40 to 50 amino acids that constitute the conserved F-box motif from *SON1* are aligned with comparable regions from F-box–containing proteins from human (SKP2 and Cyclin F), yeast (CDC4), and Arabidopsis (TIR1, FKF1, UFO, and COI1); see text for literature citations. Black blocks indicate residues identical to the SON1 sequence, and gray blocks indicate similar amino acids. The consensus sequence at the bottom was generated using ClustalW on the aligned sequences.

mutants with defects in chlorophyll biosynthesis or catabolism, and a variety of other metabolic perturbations have been implicated in the induction of plant defense (Delaney, 1997; Glazebrook, 2001, and references therein).

To understand how *SON1* may regulate two distinct defense response pathways, we cloned *SON1* in a map-based approach using molecular markers and the published sequence of the Arabidopsis genome. *SON1* is a 1.1-kb intronless gene that encodes a novel 370–amino acid protein that contains an N-terminal F-box motif. SON1 is like approximately half of the known F-box proteins in that it lacks a recognizable C-terminal protein–protein interaction domain. This region in SON1, though, is likely to be important to its function, because the *son1-1* mutation causes a substitution in this part of the predicted protein.

Work with the yeast *Saccharomyces cerevisiae* has provided the greatest understanding of F-box protein function. In that species, these proteins have been shown to play a role as specificity factors in the SCF (Skp1, Cdc53/Cullin, F-box receptor) E3 ubiquitin-ligase complex that regulates the accumulation of specific cellular proteins through their polyubiquitination and proteolysis (Skowyra et al., 1997; Kaiser et al., 1998; Deshaies, 1999; Winston et al., 1999; Kipreos and Pagano, 2000). The F-box in these proteins has been shown to interact with Skp1, which helps form the E3 complex, whereas the C-terminal region of the F-box protein binds to specific substrate proteins, which consequently are targeted for polyubiquitination (for review, see Craig and Tyers, 1999).

The targeting specificity is conferred by the C-terminal end of the F-box protein, which often contains a recognizable protein–protein interaction region in the form of Leu-rich, WD-40, or kelch repeats (Craig and Tyers, 1999). Thus, F-box proteins have three roles: to interact with other components of the E3 complex; to mediate binding to E2; and to recruit specific substrates to the complex, leading to their ubiquitination and ultimate degradation (Patton et al., 1998; Xiao and Jang, 2000; Andrade et al., 2001). Because the

family of F-box proteins is large, this mechanism provides cells with a rapid and irreversible system for the targeted destruction of a wide range of proteins, including many with regulatory functions.

In Arabidopsis, SON1 is the seventh F-box protein revealed to have a known function, although ~337 F-box proteins are predicted based on genomic analysis of this plant (Arabidopsis Genome Initiative, 2000). The seven F-box proteins isolated in Arabidopsis to date have been found to regulate a diverse range of cellular functions, which include auxin responses by TIR1 (Ruegger et al., 1998), floral development by UFO (Samach et al., 1999), leaf senescence by ORE1 (Woo et al., 2001), circadian rhythms by ZTL1 and FKF1 (Nelson et al., 2000; Somers et al., 2000), wound- and jasmonate-regulated gene expression by COI1 (Xie et al., 1998), and regulation of SAR and a form of SIR by SON1 (this work). The large number of other F-box proteins encoded in the Arabidopsis genome suggests further diversification of F-box–regulated functions in plants.

Yeast two-hybrid studies also have shown that TIR1, UFO, ORE9, and COI1 are able to interact with AtASK1, the Arabidopsis homolog of the yeast SKP1 component of the SCF complex, supporting the involvement of ubiquitination-dependent proteolysis as an essential regulatory mechanism in plant signal transduction pathways (del Pozo and Estelle, 1999; Gray et al., 1999; Samach et al., 1999; Woo et al., 2001).

We suggest that *SON1* functions as a component of an SCF ubiquitin ligase complex and interacts specifically with one or more substrate proteins, thus controlling their stability through ubiquitination. Therefore, learning the identity of the target substrate for SON1 is of great interest, because it is likely to act as a positive regulator of both the SAR and SIR pathways. This assumption is based on the induction of these defense responses in *son1* plants and on the presumed role of SON1 in promoting the degradation of its substrate.

Based on the recognition that SON1 is an F-box protein, a possible mechanism for how the *son1* mutation activates two distinct defense responses is that SON1 targets for degradation different positive regulators of SAR and SIR or a single factor common to both pathways. Thus, in *son1* plants, the SON1 substrate(s) accumulates, activating SIR in *nim1-1* as well as SAR in *NIM1* plants. Candidates for such a regulatory factor would be proteins that displace negative transcriptional regulators of *PR* genes and genes whose expression may be associated with *son1*-mediated SIR. Alternatively, if NIM1 also regulates the SIR mechanism activated in *son1* plants, as it does in SAR, the target of SON1 may be NIM1 itself.

The mechanism by which SON1 activity is regulated is unknown, but regulation of F-box proteins may occur at a number of levels. Although we found no evidence for pathogen- or INA-induced changes in SON1 mRNA accumulation,

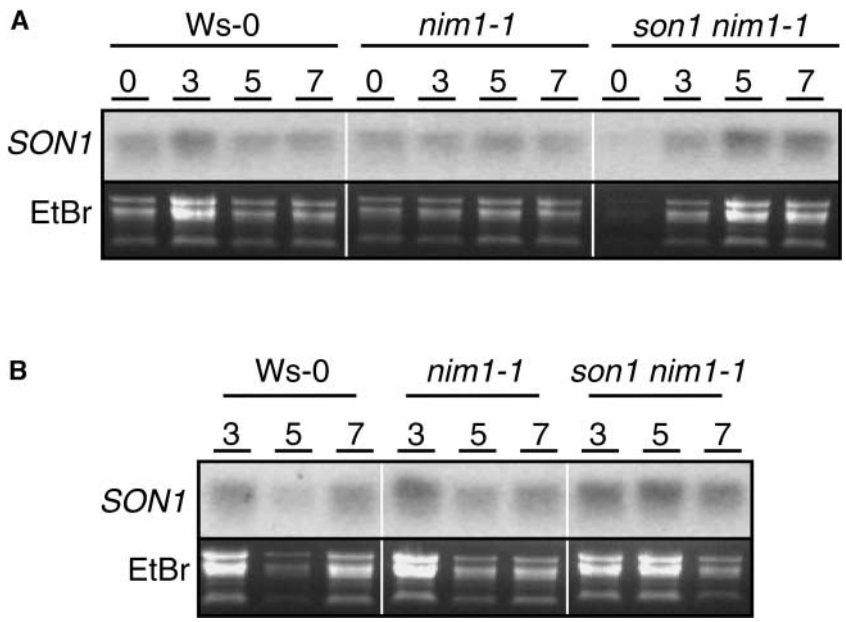

Figure 8. *SON1* mRNA Accumulation in Ws-0, *nim1-1*, and *son1 nim1-1* after Peronospora Infection or INA Treatment.

Wild-type (Ws-0), *nim1-1*, and *son1 nim1-1* plants were inoculated with Peronospora isolate Emwa1 **(A)** or treated with INA **(B)** and examined for SON1 mRNA accumulation 3, 5, and 7 days later using RNA gel blot hybridization. To assess loading, the ethidium bromide (EtBr)–stained RNA gel was photographed before being transferred to the membrane.

transcriptional and post-transcriptional regulation of F-box proteins has been observed in a few cases (Kornitzer and Ciechanover, 2000). However, a more important regulatory mechanism for F-box activity is in the phosphorylation of the F-box substrate protein, a modification that has been shown in many cases to be required for targeting by the SCF complex (Kornitzer and Ciechanover, 2000). Therefore, specific targeting mediated by substrate phosphorylation may be a general rule that governs this interaction. If this rule applies to SON1-controlled responses, then SON1 substrate protein(s) also may require phosphorylation before degradation.

The isolation and characterization of the *son1* mutant demonstrates the existence of an effective, broad-spectrum defense system independent from known defense responses that are mediated by SA or jasmonic acid accumulation. The phenotype of *son1* plants indicates that SON1 acts as a negative regulator of this defense response and, unexpectedly, also acts to negatively regulate SAR from a position upstream of or at NIM1. Cloning of the *SON1* gene showed that it encodes an F-box protein, suggesting that the gene product plays a role in the selective ubiquitination and degradation of one or more target proteins. Thus, the protein(s) targeted by SON1 may act as a positive regulator of SAR and SIR. Greater understanding of the mechanism of SON1 action, and elucidation of its targets, will contribute to the engineering of disease resistance in agricultural crop species.

METHODS

Plants and Growth Conditions

Arabidopsis thaliana accession Wassilewskija (Ws-0) was obtained from the ABRC (Ohio State University, Columbus). Ws *nim1-1* plants were described previously (Delaney et al., 1995), and the Ws NahG line (Molina et al., 1998) was provided by Syngenta (Research Triangle Park, NC). The Columbia (Col) *npr1-2* line used for mapping was generously provided by Jane Glazebrook (Torrey Mesa Research Institute, San Diego, CA; Glazebrook et al., 1996). Plants were grown at 22°C in 14 h of light (~150 μE provided by cool-white fluorescent lamps) at ~60% RH on Cornell soil mix (Boodley and Sheldrake, 1977).

Genetic Suppressor Screen

Kanamycin-resistant *nim1-1* seeds (Delaney et al., 1995) were mutagenized in a 0.3% solution of ethyl methanesulfonate in water for 17 h with gentle shaking. The seeds were washed extensively three times with 300 mL of sterilized water, vernalized overnight at 5°C, and sown on Cornell soil mix in 10 standard 21- × 11-inch plastic horticultural flats. The resulting M1 plants were partitioned into three groups per flat, and M2 seeds from each group were pooled upon harvest. To assess the efficiency of mutagenesis, slightly immature siliques from a sample of 100 M1 plants were dissected and scored for segregation of embryo-lethal (*emb*) mutations (Koncz et al., 1992). We observed ~33% of the plants to exhibit segregation of the *emb* mutant phenotype.

Pathogen Inoculations

Peronospora parasitica isolates Emwa1 and Emco5 (Holub et al., 1994) were inoculated as a suspension of conidia (~5 to 7 × 10^4 spores per mL of water) onto 2-week-old plants using a Preval spray mister (Precision Valve, Yonkers, NY). Inoculated plants were maintained at ~100% RH in a Percival (Des Moines, IA) growth chamber at 18°C and 12-h-day/12-h-night cycles, as described previously (Donofrio and Delaney, 2001).

Pseudomonas syringae pv *tomato* (*Pst*) strain DC3000 was grown on King's medium B (KB; King et al., 1954) plates for 48 h at 30°C to provide a source of inoculum. Plates were washed with 10 mL of 10 mM $MgCl_2$, which was transferred to a 250-mL flask containing 200 mL of 10 mM $MgCl_2$ and adjusted to an OD_{600} of 0.05 by dilution with the $MgCl_2$ solution. Two-week-old plants were dipped into the bacterial suspension containing 0.02% Silwet L-77 (OSI Specialties, Danbury, CT) according to Tornero and Dangl (2001). For each data point, four replicate samples consisting of pooled leaves from three identically treated plants (12 plants per treatment and time) were collected before and 24 and 48 h after inoculation to determine *Pst* DC3000 growth curves.

Collected leaf tissue from each sample was placed in preweighed 1.5-mL microcentrifuge tubes containing 1 mL of $MgCl_2$ plus 0.02% Silwet. The Eppendorf tubes were reweighed with sample before shaking at 250 rpm at 28°C for 1 h. Dilutions (10^{-1} to 10^{-6}) of each sample then were made using 10 mM $MgCl_2$ in 96-well microtiter plates. Using a 96-well pin stamp, the dilutions were plated onto KB plates and left to incubate at 30°C. Colonies were counted 48 h later to determine the number of colony-forming units per milligram of leaf tissue.

RNA Gel Blot Analysis

RNA was extracted from harvested leaf tissue frozen in liquid nitrogen using a hot phenol/chloroform method followed by lithium chloride precipitation (Verwoerd et al., 1989), and samples were adjusted to 0.05 mg/mL ethidium bromide for loading onto a formaldehyde agarose gel and electrophoresis as described (Uknes et al., 1993). Gels were photographed under UV light to assess equal loading of the samples and then blotted onto Hybond N^+ nylon membranes (Amersham Life Science, Arlington Heights, IL). Arabidopsis *PR-1*, *PR-2*, *PR-5*, *PDF1.2*, and *SON1* cDNA clones were labeled with ^{32}P by random priming using a commercial kit (Gibco Life Technologies/Invitrogen, Grand Island, NY). Hybridization of probe and subsequent washings were performed as described (Uknes et al., 1993).

2,6-Dichloroisonicotinic Acid Treatment

A 25% formulation of 2,6-dichloroisonicotinic acid (Syngenta, Basel, Switzerland) in wettable powder was dissolved in sterile water at 0.25 mg/mL (0.33 mM 2,6-dichloroisonicotinic acid) and spray misted onto 2-week-old plants. Leaf tissue from each plant was collected for RNA preparation and gel blot analysis at 0, 3, 5, and 7 days after treatment.

Fixation of Leaf Samples

Leaf samples were harvested and fixed in lactophenol trypan blue for 1 h before clearing with chloral hydrate (Uknes et al., 1993). Leaf samples were mounted on glass slides and photographed using a Leica MZ8 stereomicroscope (Wetzlar, Germany).

Map-Based Cloning of *son1*

To map *SON1*, the *son1 nim1-1* mutant (derived from the Ws-0 accession) was crossed to *npr1-2* in the Col-0 accession. Peronospora isolate Emco5, which is virulent on both Ws-0 and Col-0, was used to screen F2 progeny for the *son1* resistant phenotype. DNA was extracted (Dellaporta et al., 1983) from frozen leaves of 48 F2 plants that were scored as resistant to Emco5. PCR then was performed using DNA from the plants with cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993; http://www.arabidopsis.org/) chosen from each chromosome and separated by an average distance of 5 to 8 centimorgan.

The CAPS marker *THY1* on chromosome 2 was found to cosegregate with the *son1* resistant phenotype, with six recombination breakpoints found between these loci among the 48 F2 plants tested. Additional CAPS markers surrounding *THY1* were tested, and the *son1* mutation was localized between CAPS markers *THY1* and *PHYB*, 4.4 centimorgan or 1.02 Mb apart. DNA from an additional 485 F2 Emco5-resistant plants was tested with the *THY1* and *PHYB* markers, and 33 additional recombinations were found using *THY1* and 41 others found with *PHYB*.

To fine-map the location of *SON1*, single nucleotide polymorphism (SNP) markers were designed within the region flanked by *THY1* and *PHYB* using the Cereon Genomics SNP database and sequence information from the Arabidopsis genome sequencing project (http://mips.gsf.de/proj/thal/db/index.html). Using two SNPs, 7127-10521SNP (forward primer, 5′-TCCTTTGACGTCTTTATGTG-3′; reverse primer, 5′-GCCTCCTGTTTACTAATGAT-3′; T/C polymorphism in Col-0 versus Ws-0) and 7584-5790SNP (forward primer, 5′-TAATTCCATGTGATGCTTGTG-3′; reverse primer, 5′-AGTTGATACAACTCTCATGAAC-3′; G/A polymorphism in Ws-0 versus Col-0), we were able to localize *SON1* to a 98-kb region bounded by these markers.

The PCR protocol used for 7127-10521SNP and 7584-5790SNP was as follows: 95°C for 2 min, 95°C for 1 min, and 50°C for 2 min (for 30 cycles) followed by 72°C for 10 min. The 150-bp PCR product amplified using the 7127-10521SNP primers then was digested with the Fnu4HI restriction enzyme, which cleaves genomics DNA (gDNA) from Ws-0 plants but not Col-0 plants. The enzyme used to cut the 101-bp PCR product amplified with the 7584-5790SNP primers was AluI, which has a site in Col-0 gDNA but not in Ws-0. Both forward primers (7127-10521SNP and 7584-5790SNP) encompass the polymorphism and the resulting presence or absence of the restriction enzyme site. Of the 39 recombinations isolated using *THY1*, 2 remained at 7127-10521SNP, and of the 41 recombinations isolated using *PHYB*, 3 remained at 7584-5790SNP.

The interval between *THY1* and *PHYB* was examined using four additional SNP markers found in the Cereon SNP database, and the closest two were used to fine-map *SON1*. With one, 2329-13467SNP, we were able to reduce the number of recombinations remaining to one at the left side of *SON1*, whereas using the 2329-54226SNP marker, the number of recombinations remaining was reduced to two on the right border of *SON1*. The narrower interval defined by the two markers encompassed 41 kb of genomic sequence and contained 10 open reading frames (ORFs).

The 2329-54226SNP marker (forward primer, 5′-CCAATTCATTGTTTTTGAACC-3′; reverse primer, 5′-GATGGAGAGATCAACGAGC-3′) reveals in the 152-bp amplicon an A/T polymorphism between Ws-0 and Col-0 that produces an MfeI site in the Ws-0 product that is absent in Col-0. The 2329-13467SNP marker (forward primer, 5′-TTTGCTCTAAGTTTCAACAG-3′; reverse primer, 5′-GCCGACGTACGTTAATCATTTG-3′) exploits a length polymorphism that causes a 180-bp product to be produced from Ws-0, whereas the product from Col-0 is 150 bp. The PCR conditions for both markers were identical to those described above except that the annealing temperature for 2329-13467SNP was 55°C instead of 50°C.

Each of the 10 ORFs flanked by markers around *SON1* were examined by designing PCR primers to amplify each ORF from gDNA of the *son1* mutant and Ws-0 wild-type plants. Comparisons between the sequences of each of the ORFs from *son1* and wild-type plants revealed a single G-to-A base pair difference in ORF4 that produces an Arg-to-Gln substitution at amino acid 257 in the predicted protein sequence.

Complementation of *son1* with the Wild-Type Gene

A cDNA of ORF4 was cloned under the control of the 35S promoter of *Cauliflower mosaic virus* into a modified pCAMBIA-1302 binary vector using NcoI and SpeI sites and transformed into *son1 nim1* mutants using the floral dip method (Clough and Bent, 1998). T1 seeds were collected and plated onto Murashige and Skoog (1962) medium (Gibco Life Technologies/Invitrogen) containing 20 μg/mL hygromycin to select for primary transformants. Thirty-three independent lines were found to be resistant to hygromycin and were transferred to soil 1 week after germination. These 33 lines were tested in pathogen assays to assess the complementation of the *son1* mutation.

At 2 weeks of age, all 33 lines were inoculated with Peronospora and incubated in Percival growth chambers as described above. All 33 lines were as susceptible to Peronospora infection as *nim1-1* mutants and displayed increased sporulation compared with nontransformed *son1 nim1* plants.

Upon request, all novel materials described in this article will be made available in a timely manner for noncommercial research purposes. No restrictions or conditions will be placed on the use of any materials described in this article that would limit their use for noncommercial research purposes.

Accession Numbers

The GenBank accession number for the *SON1* sequence is AF472589. The GenBank accession numbers for the sequences shown in Figure 7 are NP116026 (human SKP2), P41002 (human Cyclin F), CAA86341 (yeast CDC4), NP567135 (Arabidopsis TIR1), NP564919 (Arabidopsis FKF1), NP564368 (Arabidopsis UFO), and NP565919 (Arabidopsis COI1).

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REFERENCES

Adams, J., Kelso, R., and Cooley, L. (2000). The kelch repeat superfamily of proteins: Propellers of cell function. Trends Cell Biol. **10,** 17–24.

Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl Goy, P., Luntz, T., Ward, E., and Ryals, J. (1993). Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. Proc. Natl. Acad. Sci. USA **90,** 7327–7331.

Andrade, M.A., Gonzalez-Guzman, M., Serrano, R., and Rodriguez, P.L. (2001). A combination of the F-box motif and kelch repeats defines a large Arabidopsis family of F-box proteins. Plant Mol. Biol. **46,** 603–614.

Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408,** 796–815.

Bai, C., Sen, P., Hofmann, K., Ma, L., Goebl, M., Harper, J.W., and Elledge, S.J. (1996). SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell **86,** 263–274.

Boodley, J.W., and Sheldrake, R., Jr. (1977). Cornell peat-lite mixes for commercial plant growing. NY State Coll. Agric. Life Sci. Inf. Bull. **43,** 1–8.

Bowling, S.A., Clarke, J.D., Liu, Y., Klessig, D.F., and Dong, X. (1997). The *cpr5* mutant of Arabidopsis expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell **9,** 1573–1584.

Cao, H., Bowling, S.A., Gordon, A.S., and Dong, X. (1994). Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell **6,** 1583–1592.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S., and Dong, X. (1997). The Arabidopsis *NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell **88,** 57–63.

Clarke, J.D., Liu, Y., Klessig, D.F., and Dong, X. (1998). Uncoupling PR gene expression from NPR1 and bacterial resistance: Characterization of the dominant Arabidopsis *cpr6-1* mutant. Plant Cell **10,** 557–569.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. **16,** 735–743.

Craig, K.L., and Tyers, M. (1999). The F-box: A new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. Prog. Biophys. Mol. Biol. **72,** 299–328.

Delaney, T.P. (1997). Genetic dissection of acquired resistance to disease. Plant Physiol. **106,** 5–12.

Delaney, T.P., Friedrich, L., and Ryals, J.A. (1995). Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA **92,** 6602–6606.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., and Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. Science **266,** 1247–1250.

Dellaporta, S.L., Wood, J., and Hicks, J.B. (1983). A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. **1,** 19–21.

del Pozo, J.C., and Estelle, M. (1999). Function of the ubiquitin-proteosome pathway in auxin response. Trends Plant Sci. **4,** 107–112.

Deshaies, R.J. (1999). SCF and Cullin/Ring H2-based ubiquitin ligases. Annu. Rev. Cell Dev. Biol. **15,** 435–467.

Donofrio, N.M., and Delaney, T.P. (2001). Abnormal callose response phenotype and hypersusceptibility to *Peronospora parasitica* in defense-compromised Arabidopsis *nim1-1* and salicylate hydroxylase-expressing plants. Mol. Plant-Microbe Interact. **14,** 439–450.

Epple, P., Apel, K., and Bohlmann, H. (1997). ESTs reveal a multigene family for plant defensins in *Arabidopsis thaliana*. FEBS Lett. **400,** 168–172.

Feys, B.J., and Parker, J.E. (2000). Interplay of signaling pathways in plant disease resistance. Trends Genet. **16,** 449–455.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science **261,** 754–756.

Glazebrook, J. (2001). Genes controlling expression of defense responses in Arabidopsis: 2001 status. Curr. Opin. Plant Biol. **4,** 301–308.

Glazebrook, J., Rogers, E.E., and Ausubel, F.M. (1996). Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. Genetics **143,** 973–982.

Gray, W.M., del Pozo, J.C., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W.L., Yang, M., Ma, H., and Estelle, M. (1999). Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. Genes Dev. **13,** 1678–1691.

Holub, E.B., Beynon, L.J., and Crute, I.R. (1994). Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. **7,** 223–239.

Kaiser, P., Sia, R.A., Bardes, E.G., Lew, D.J., and Reed, S.I. (1998). Cdc34 and the F-box protein Met30 are required for degradation of the Cdk-inhibitory kinase Swe1. Genes Dev. **12,** 2587–2597.

Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. (1994). Induction of systemic acquired resistance in plants by chemicals. Annu. Rev. Phytopathol. **32,** 439–459.

King, E.O., Ward, M.K., and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. **44,** 301–307.

Kipreos, E.T., and Pagano, M. (2000). The F-box protein family. Genome Biol. **1,** 3002.1–3002.7.

Knoester, M., Pieterse, C.M., Bol, J.F., and Van Loon, L.C. (1999). Systemic resistance in Arabidopsis induced by rhizobacteria requires ethylene-dependent signaling at the site of application. Mol. Plant-Microbe Interact. **12,** 720–727.

Kobe, B., and Kajava, A.V. (2001). The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. **11,** 725–732.

Koncz, C., Chua, N.-H., and Schell, J. (1992). Methods in Arabidopsis Research. (London: World Scientific Publishing).

Konieczny, A., and Ausubel, F.M. (1993). A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4,** 403–410.

Kornitzer, D., and Ciechanover, A. (2000). Modes of regulation of ubiquitin-mediated protein degradation. J. Cell Physiol. **182,** 1–11.

Li, X., Zhang, Y., Clarke, J.D., Li, Y., and Dong, X. (1999). Identification and cloning of a negative regulator of systemic acquired resistance, *SNI1*, through a screen for suppressors of *npr1-1*. Cell **98,** 329–339.

Lo, E.S., Lo, Y.M., Tse, C.H., and Fleming, K.A. (1992). Detection of hepatitis B pre-core mutant by allele specific polymerase chain reaction. J. Clin. Pathol. **45,** 689–692.

Manners, J.M., Penninckx, I.A., Vermaere, K., Kazan, K., Brown, R.L., Morgan, A., Maclean, D.J., Curtis, M.D., Cammue, B.P., and Broekaert, W.F. (1998). The promoter of the plant defensin gene *PDF1.2* from Arabidopsis is systemically activated by fungal pathogens and responds to methyl jasmonate but not to salicylic acid. Plant Mol. Biol. **38,** 1071–1080.

Molina, A., Hunt, M.D., and Ryals, J.A. (1998). Impaired fungicide activity in plants blocked in disease resistance signal transduction. Plant Cell **10,** 1903–1914.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15,** 473–497.

Neer, E.J., Schmidt, C.J., Nambudripad, R., and Smith, T.F. (1994). The ancient regulatory-protein family of WD-repeat proteins. Nature **371,** 297–300.

Neff, M.M., Neff, J.D., Chory, J., and Pepper, A.E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. Plant J. **14,** 387–392.

Nelson, D.C., Lasswell, J., Rogg, L.E., Cohen, M.A., and Bartel, B. (2000). *FKF1*, a clock-controlled gene that regulates the transition to flowering in Arabidopsis. Cell **101,** 331–340.

Patton, E.E., Willems, A.R., and Tyers, M. (1998). Combinatorial control in ubiquitin-dependent proteolysis: Don't Skp the F-box hypothesis. Trends Genet. **14,** 236–243.

Pieterse, C.M., van Wees, S.C., Hoffland, E., van Pelt, J.A., and van Loon, L.C. (1996). Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell **8,** 1225–1237.

Pieterse, C.M., van Wees, S.C., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., and van Loon, L.C. (1998). A novel signaling pathway controlling induced systemic resistance in Arabidopsis. Plant Cell **10,** 1571–1580.

Pieterse, C.M.J., Van Pelt, J.A., Ton, J., Parchmann, S., Mueller, M.J., Buchala, A.J., Metraux, J.P., and Van Loon, L.C. (2000). Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiol. Mol. Plant Pathol. **57,** 123–134.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., and Estelle, M. (1998). The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast Grr1p. Genes Dev. **12,** 198–207.

Ryals, J., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell **8,** 1809–1819.

Ryals, J., Weymann, K., Lawton, K.A., Friedrich, L., Ellis, D., Steiner, H.-Y., Johnson, J., Delaney, T.P., Jesse, T., Vos, P., and Uknes, S. (1997). The Arabidopsis NIM1 protein shows homology to the mammalian transcription factor inhibitor IκB. Plant Cell **9,** 425–439.

Samach, A., Klenz, J.E., Kohalmi, S.E., Risseeuw, E., Haughn, G.W., and Crosby, W.L. (1999). The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. Plant J. **20,** 433–445.

Shah, J., Kachroo, P., and Klessig, D.F. (1999). The Arabidopsis *ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. Plant Cell **11,** 191–206.

Shah, J., Kachroo, P., Nandi, A., and Klessig, D.F. (2001). A recessive mutation in the Arabidopsis *SSI2* gene confers SA- and NPR1-independent expression of PR genes and resistance against bacterial and oomycete pathogens. Plant J. **25,** 563–574.

Shah, J., Tsui, F., and Klessig, D.F. (1997). Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. Mol. Plant-Microbe Interact. **10,** 69–78.

Skowyra, D., Craig, K.L., Tyers, M., Elledge, S.J., and Harper, J.W. (1997). F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell **91,** 209–219.

Somers, D.E., Schultz, T.F., Milnamow, M., and Kay, S.A. (2000). *ZEITLUPE* encodes a novel clock-associated PAS protein from Arabidopsis. Cell **101,** 319–329.

Sticher, L., Mauch-Mani, B., and Metraux, J.P. (1997). Systemic acquired resistance. Annu. Rev. Phytopathol. **35,** 235–270.

Takahashi, N., Takahashi, Y., and Putnam, F.W. (1985). Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich alpha 2-glycoprotein of human serum. Proc. Natl. Acad. Sci. USA **82,** 1906–1910.

Tornero, P., and Dangl, J.L. (2001). A high-throughput method for quantifying growth of phytopathogenic bacteria in *Arabidopsis thaliana*. Plant J. **28,** 475–481.

Uknes, S., Mauch, M.B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. (1992). Acquired resistance in Arabidopsis. Plant Cell **4,** 645–656.

Uknes, S., Winter, A.M., Delaney, T., Vernooij, B., Morse, A., Friedrich, L., Nye, G., Potter, S., Ward, E., and Ryals, J. (1993). Biological induction of systemic acquired resistance in Arabidopsis. Mol. Plant-Microbe Interact. **6,** 692–698.

van der Voorn, L., and Ploegh, H.L. (1992). The WD-40 repeat. FEBS Lett. **307,** 131–134.

van Wees, S.C.M., Luijendijk, M., Smoorenburg, I., van Loon, L.C., and Pieterse, C.M.J. (1999). Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol. Biol. **41,** 537–549.

Verwoerd, B., Dekker, M., and Hoekema, A. (1989). A small-scale procedure for the rapid isolation of plant RNAs. Nucleic Acids Res. **17,** 2362.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl Goy, P., Métraux, J.P., and Ryals, J.A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell **3,** 1085–1094.

Winston, J.T., Koepp, D.M., Zhu, C., Elledge, S.J., and Harper, J.W. (1999). A family of mammalian F-box proteins. Curr. Biol. **9,** 1180–1182.

Woo, H.R., Chung, K.M., Park, J.H., Oh, S.A., Ahn, T., Hong, S.H., Jang, S.K., and Nam, H.G. (2001). ORE9, an F-box protein that regulates leaf senescence in Arabidopsis. Plant Cell **13,** 1779–1790.

Xiao, W., and Jang, J. (2000). F-box proteins in Arabidopsis. Trends Plant Sci. **5,** 454–457.

Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M., and Turner, J.G. (1998). *COI1*: An Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280,** 1091–1094.

Yalpani, N., Silverman, P., Wilson, T.M., Kleier, D.A., and Raskin, I. (1991). Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell **3,** 809–818.