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Suppression and Restoration of Lesion Formation in Arabidopsis *lsd* Mutants

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Systemic acquired resistance (SAR) is a broad-spectrum, systemic defense response that is activated in many plant species after pathogen infection. We have previously described Arabidopsis mutants that constitutively express SAR and concomitantly develop lesions simulating disease (lsd). Here, we describe two new mutants, *lsd6* and *lsd7*, that develop spontaneous necrotic lesions and possess elevated levels of salicylic acid (SA) as well as heightened disease resistance, similar to the previously characterized *lsd* and accelerated cell death (*acd2*) mutants. Genetic analysis of *lsd6* and *lsd7* showed that the mutant phenotypes segregated as simple dominant traits. When crossed with transgenic Arabidopsis plants containing the SA-degrading enzyme salicylate hydroxylase, the F_1 progeny showed suppression of both SAR gene expression and resistance. In addition, salicylate hydroxylase suppressed lesion formation in the F_1 progeny, suggesting that SA or some SA-dependent process may have a role in pathogen-associated cell death. Surprisingly, lesions were restored in the *lsd6* F_1 progeny after the application of either 2,6-dichloroisonicotinic acid or SA. Lesions were not restored by treatment with either compound in the *lsd7* F_1 plants. Our findings demonstrate that steps early in the signal transduction pathway leading to SAR and disease resistance are potentiated by later events, suggesting feedback control of lesion formation.

INTRODUCTION

Host defenses against pathogens consist of both noninducible and inducible systems (Ward et al., 1994). One inducible resistance mechanism is systemic acquired resistance (SAR). It is distinguished from other resistance responses in that the protection against pathogens is broad spectrum and induced systemically (Ross, 1961; Kuc, 1982; Kessmann et al., 1994). In tobacco and Arabidopsis, the establishment of SAR is correlated with the expression of a coordinately regulated set of genes called SAR genes (Ward et al., 1991; Uknes et al., 1992, 1993). Nine SAR gene families in tobacco have been characterized (Ward et al., 1991), whereas in Arabidopsis the SAR genes compose at least the pathogenesis-related protein PR-1, PR-2, and PR-5 gene families (Uknes et al., 1992). In transgenic tobacco, constitutive expression of PR-1a confers significant protection against oomycete fungal pathogens, supporting a direct role for these genes in resistance (Alexander et al., 1993).

Salicylic acid (SA) has been implicated as a signal required for the establishment of SAR because it accumulates after pathogen infection and, when applied exogenously, induces gene expression and resistance similar to that seen after pathogen infection (White, 1979; Malamy et al., 1990; Métraux et al., 1990; Ward et al., 1991; Uknes et al., 1993). Furthermore, elimination of the SA accumulation in transgenic plants expressing salicylate hydroxylase (NahG), an SA-degrading enzyme, was shown to prevent both SAR gene expression and resistance (Gaffney et al., 1993; Delaney et al., 1994). However, relatively little is known about other components in the signal transduction pathway leading to SAR.

Recently, Arabidopsis has been established as a model system for SAR (Uknes et al., 1992, 1993; Cameron et al., 1994; Mauch-Mani and Slusarenko, 1994). To understand better the events leading to the establishment of SAR, we identified mutations involved in the signal transduction pathway. The first mutants found to be altered in the SAR response are the so-called *lsd* (for lesions simulating disease) and *acd2* (for accelerated cell death) mutants (Lawton et al., 1993; Dietrich et al., 1994; Greenberg et al., 1994). Spontaneous lesions on the leaves of these plants were observed concomitant with SAR gene expression and pathogen resistance. Because the mutations result in lesion formation, we suspect that they may affect steps early in the pathway leading to SAR.

To identify other steps in SAR signal transduction, mutagenized Arabidopsis plants were screened for constitutive SAR gene expression using RNA gel blot analysis. Two mutants that were isolated showed constitutive expression of SAR genes, elevated levels of SA, pathogen resistance, and spontaneous lesion formation. The mutations (*lsd6* and *lsd7*) segregated

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as single dominant genes. When either *lsd6* or *lsd7* was crossed to homozygous *nahG*-expressing plants, the F_1 progeny displayed suppressed SAR gene expression, disease resistance, and, interestingly, lesion formation. Treatment of these plants with 2,6-dichloroisonicotinic acid (INA), a compound that induces SAR, restored the spontaneous lesion phenotype, suggesting that SA or some SA-dependent process may have a modulating effect on pathogen-associated cell death.

RESULTS

lsd6 and *lsd7* Exhibit Spontaneous Lesion Formation

To isolate mutants in the SAR pathway, we screened ethyl methanesulfonate–mutagenized Arabidopsis plants in the Columbia ecotype (Col-0) background for elevated expression of SAR genes. Two of the mutants identified in this screen were found to develop necrotic lesions spontaneously; they are presented in Figure 1. *lsd6* possessed punctate necrotic lesions that were present on all true leaves from the time of emergence. Lesion formation appeared similar under both long-day (LD; see Methods) and short-day (SD) growth conditions. No obvious pattern to lesion location was observed. After treatment with trypan blue stain, many of the lesions appeared as symmetric areas of cells stained densely and surrounded radially by living cells. Other lesions appeared as patches of stained and unstained cells. The *lsd6* plants were dwarfed and possessed distorted, curled leaves. Interestingly, *lsd6* homozygotes exhibited a more extreme dwarfed and curled-leaf phenotype than did the heterozygote. The presence of lesions always cosegregated with the phenotype in backcrosses. *lsd6* failed to develop lesions if grown on agar plates but did so immediately after being transplanted to soil. Lesions then developed on the existing leaves that had formed while the seedling was on the agar plate. Plants grown in soil under high humidity also failed to develop lesions, suggesting that the phenotype can be affected by this environmental condition. This conditionality has been observed with other *lsd* mutants (M. Hunt and K. Weymann, unpublished data).

lsd7 possessed small, necrotic lesions that became evident when stained with trypan blue. These lesions developed along

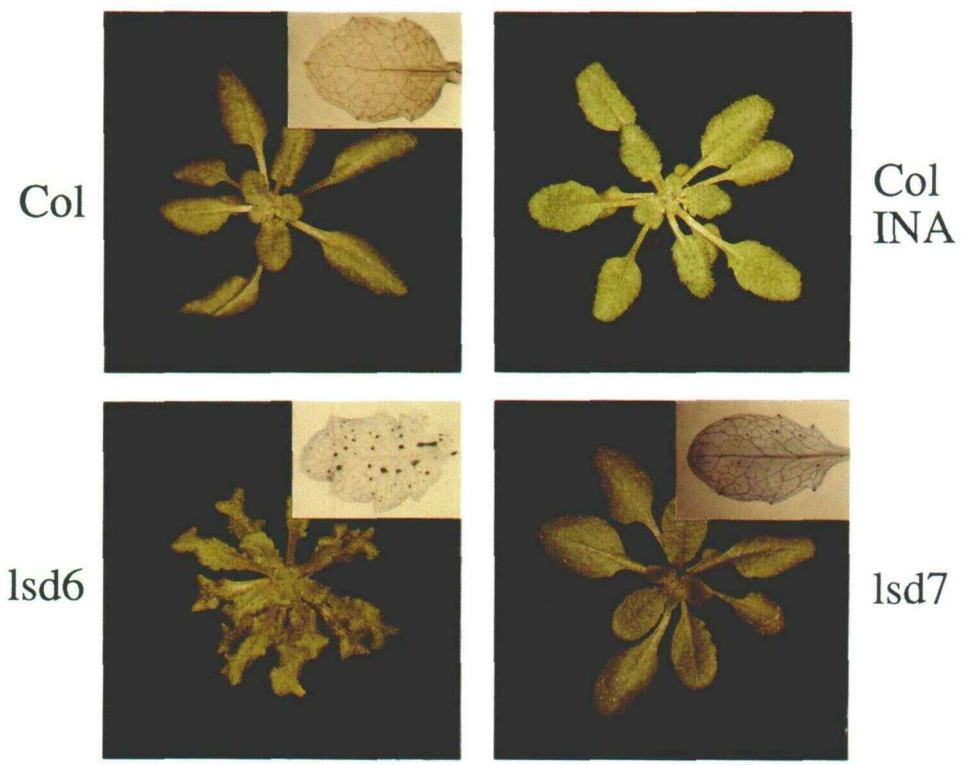

Figure 1. Visual Phenotypes of *lsd* Mutants.

The plants were photographed when ~4 weeks old. Columbia (Col) with and without INA treatment are included for comparison. The insets show leaves stained with trypan blue for better lesion visualization. *lsd6* is enlarged twofold relative to the other plants. Leaf insets of *Col* and *lsd7* are magnified ×30, and *lsd6* is magnified ×60.

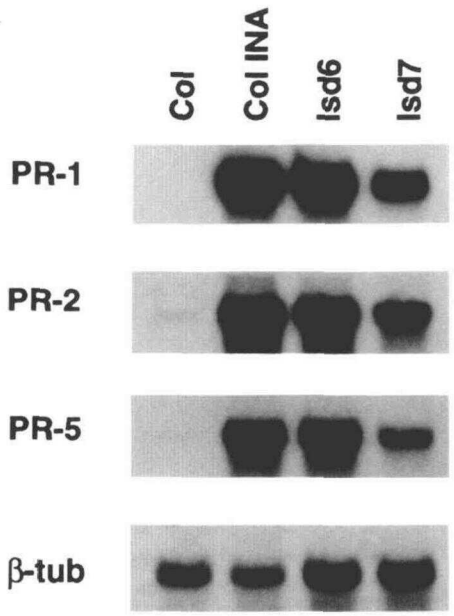

Figure 2. SAR Gene Expression in *lsd6* and *lsd7* Mutants.

The SAR cDNAs indicated (PR-1, PR-2, and PR-5) were used as probes for RNA blot analysis of the indicated plants. Col-0 (Col) and Col-0 treated with 325 μM of INA 2 days before sampling are presented for comparison. β-tubulin (β-tub) is provided as a loading standard.

the margins of a few of the oldest mature leaves. Because the lesioned phenotype occurred in mature leaves, lesions were often difficult to distinguish from senescence. This was especially true under SD growth conditions because the margins of the leaves were quite chlorotic (data not shown). The lesions were more distinct under LD conditions because there was little or no associated chlorosis. Lesion formation was the only apparent phenotype of *lsd7* under both LD and SD growth conditions.

lsd6 and *lsd7* Possess Constitutive PR Gene Expression

There is considerable evidence that SAR is tightly associated with the expression of SAR genes (Ward et al., 1991; Uknes et al., 1992). To examine SAR gene expression, gel blots of whole-plant RNA samples from *lsd6*, *lsd7*, wild-type Col-0, and Col-0 treated with INA (Ward et al., 1991; Vernooij et al., 1995) were probed with each of three cDNAs: PR-1 (unknown function), PR-2 (β-1,3-glucanase), and PR-5 (unknown function) (Uknes et al., 1992). Figure 2 shows the relative expression of PR-1, PR-2, and PR-5 in whole-plant samples. PR-1 expression was the most dependable molecular marker for SAR because its background levels were consistently low. Expression of these SAR genes in *lsd6* was comparable with the level of expression induced by INA, whereas *lsd7* was induced to a lesser degree. However, subsequent analysis revealed that lesion-positive *lsd7* leaves showed PR gene expression comparable to Col-0/INA controls, whereas lesion-negative leaves showed minimal expression (data not shown).

Genetic Characterization

The genetic segregation of *lsd6* and *lsd7* was examined after backcrossing mutant plants to the glabrous wild type. For both mutants, F_1 progeny displayed both lesion development and PR-1 expression, indicating that both *lsd6* and *lsd7* are dominant traits. In the *lsd6* F_2 population, PR-1 expression and the lesion phenotype were present in 94 of 125 seedlings. The F_2 segregation ratio was ~3:1 ($\chi^2 = 0.003$; $P > 0.95$), which is consistent with a single dominant mutation. Similarly, the *lsd7* F_2 population exhibited 116 of 142 PR-1 positive plants, giving an F_2 segregation ratio close to 3:1 ($\chi^2 = 3.4$; $0.1 > P > 0.05$), also indicative of a single dominant trait.

To facilitate genetic mapping, *lsd6* and *lsd7* mutants were used as pollen donors in crosses to Landsberg *erecta* (L*er*). The F_1 plants were identified by the absence of the recessive *erecta* phenotype as well as the presence of lesions and associated PR-1 gene expression. For genetic mapping, F_2 plants were scored for cosegregation of both PR-1 gene expression and lesion formation with simple sequence–length polymorphisms (SSLPs; Bell and Ecker, 1994) and cleaved amplified polymorphic sequence markers (Konieczny and Ausubel, 1993; Jarvis et al., 1994). Anaysis of 42 L*er* × *lsd6* F_2 progeny mapped *lsd6* to the bottom arm of chromosome 1, ~9.6 centimorgans from the SSLP marker nga111 and 15 centimorgans from the SSLP marker nga128. Analysis of 90 L*er* × *lsd7* F_2 progeny showed no linkage to either nga111 or nga128, indicating that *lsd6* and *lsd7* are not allelic.

lsd6 and *lsd7* Display Elevated SA Levels

SA has been shown previously to increase after pathogen-induced necrosis (Uknes et al., 1993) and to be required for SAR signal transduction (Gaffney et al., 1993; Vernooij et al., 1994). We measured SA concentrations in *lsd6* and *lsd7* lesioned leaves to determine whether endogenous SA levels were elevated. Figure 3 shows the levels of both free and total SA in 4-week-old *lsd6*, *lsd7*, and wild-type Col-0 plants. Total SA includes the concentration of SA and its sugar conjugate SA β-glucoside (Enyedi et al., 1992; Malamy et al., 1992). Free SA was found to be threefold higher in *lsd6* (195 ng/g fresh weight) as compared with Col-0 (69 ng/g fresh weight), whereas the level of total SA was 20-fold higher (6808 ng/g fresh weight versus 344 ng/g fresh weight). Basal levels of free SA in lesion-positive *lsd7* (308 ng/g fresh weight) leaves were fivefold higher than in Col-0, whereas total SA was elevated 15-fold (5232 ng/g fresh weight versus 344 ng/g fresh weight). The increased

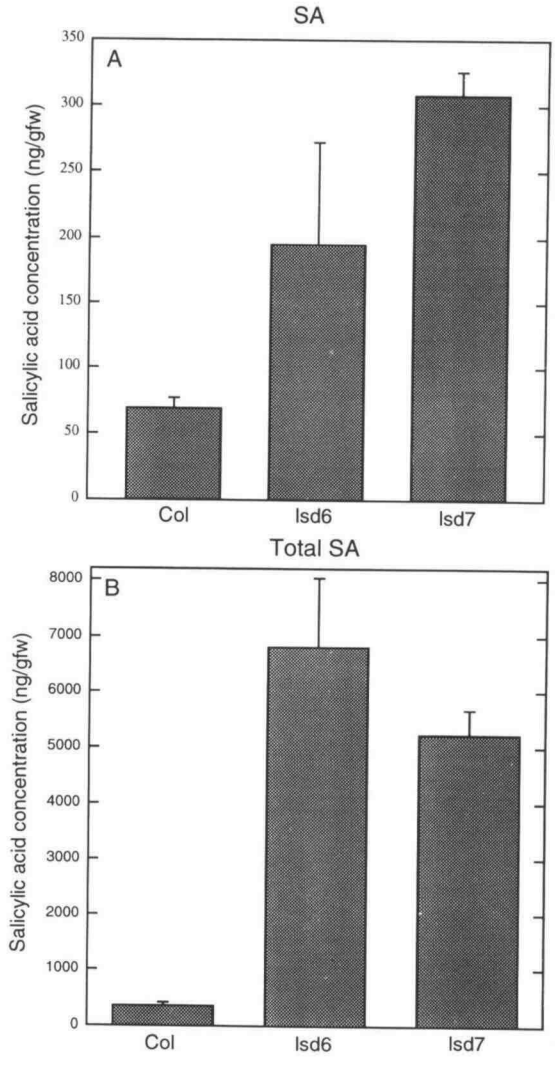

Figure 3. Free and Total SA Levels Present in *lsd6* and *lsd7* Leaves.

(A) Free SA.

(B) Total SA.

Free and total SA were quantified by HPLC from lesion-positive leaves. The levels of SA are shown as the average ± SD (nanograms of SA per gram leaf fresh weight [gfw]). Col, Col-0.

levels of SA in *lsd6* and *lsd7* are comparable with levels reported for leaves infected with a necrogenic pathogen (Uknes et al., 1993) and in a *cpr1* mutant showing constitutive PR gene expression (Bowling et al., 1994). The upper leaves of *lsd7* lacking lesions were found to have free and total SA levels twofold higher than those of the wild type (data not shown), which is comparable with the level of SA detected in uninfected leaves of pathogen-infected Arabidopsis (Delaney et al., 1995).

Removal of SA Suppresses PR-1 Gene Expression and Disease Resistance in *lsd6* and *lsd7*

To evaluate whether SA was required for SAR in *lsd6* and *lsd7*, each mutant was crossed to transgenic Arabidopsis plants harboring the enzyme salicylate hydroxylase, encoded by the *nahG* gene from *Pseudomonas putida* (Gaffney et al., 1993; Delaney et al., 1994). Because both the mutant and NahG Arabidopsis phenotypes are dominant, the effects of *nahG* gene expression on the mutant phenotypes were analyzed in the F_1 generation. To determine whether SAR gene expression was altered by *nahG* expression, we analyzed 58 *lsd6* × NahG and 36 *lsd7* × NahG F_1 individuals. A representative comparison is presented in Figure 4, showing that PR-1 gene expression was eliminated in both *lsd6* and *lsd7* F_1 individuals expressing *nahG*.

Disease susceptibility of the F_1 plants relative to controls was determined by inoculation with *Peronospora parasitica* isolate NOCO (Dangl et al., 1992). Wild-type Col-0 plants supported the growth of hyphae, conidia, and oospores. This infection was visualized with trypan blue stain, and results are presented in Figure 5. Both *lsd6* and *lsd7* were resistant to infection. However, resistance was suppressed in both *lsd6* and *lsd7* F_1 progeny (Figure 5). In comparison with susceptible wild-type Col-0, the F_1 progeny were hypersusceptible to *P. parasitica* isolate NOCO infection, a phenomenon that has been documented previously in plants expressing the *nahG* gene (Delaney et al., 1994). Disease ratings for the various

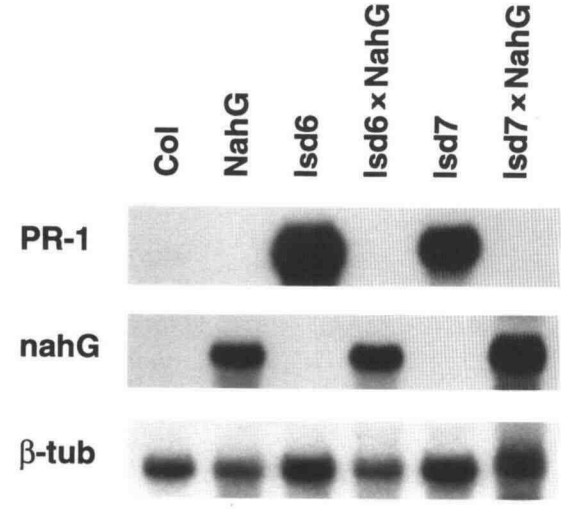

Figure 4. Gene Expression in *lsd6*, *lsd7*, and Respective NahG Crosses.

PR-1 and nahG were used as probes for a comparative RNA gel blot analysis of control plants (Col-0 [Col] and NahG), mutants (*lsd6* and *lsd7*), and F_1 progeny (*lsd6* × NahG and *lsd7* × NahG). β-tubulin (β-tub) is provided as a loading standard.

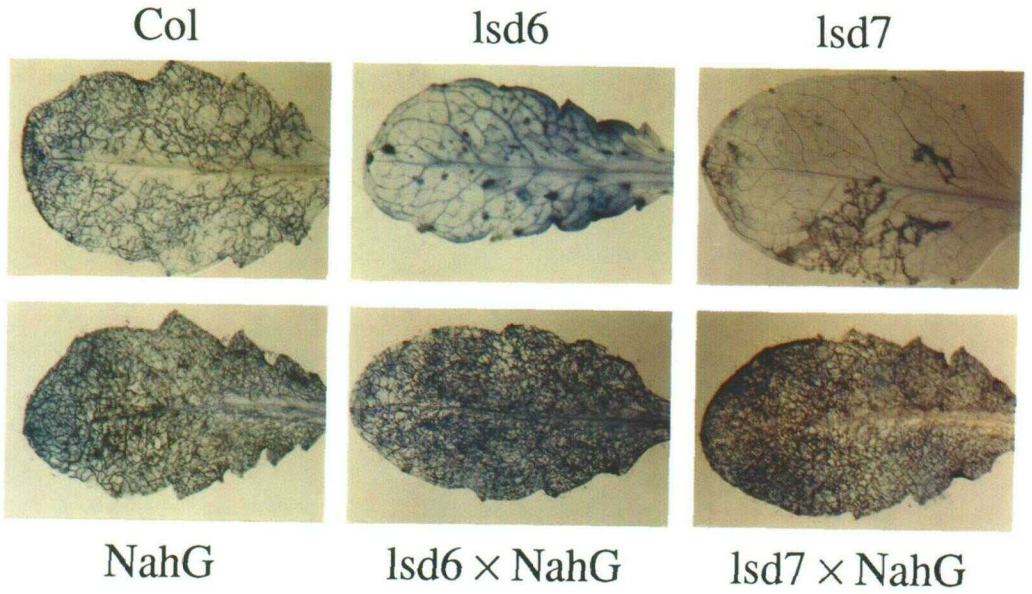

Figure 5. *nahG* Expression Renders *lsd6* and *lsd7* Hypersusceptible to *P. parasitica* Isolate NOCO Infection.

Growth of the compatible fungal isolate NOCO on wild-type Col-0 (Col) shows extensive growth of the fungus. *lsd6* and *lsd7* show enhanced resistance to NOCO infection. However, the NahG crosses of these mutants show hypersusceptibility to NOCO infection.

treatments are presented in Table 1. Taken together, the data indicate that SAR gene expression and disease resistance in *lsd6* and *lsd7* are dependent on SA accumulation.

Removal of SA Suppresses Lesion Formation

Both *lsd6* and *lsd7* exhibit spontaneous lesion formation in addition to the molecular markers associated with SAR. However, visual examination of 62 *lsd6* × NahG and 242 *lsd7* × NahG individual F_1 progeny revealed that the lesioned phenotype of both mutants was suppressed. Furthermore, the curled leaf phenotype of *lsd6* was also suppressed. To determine whether microscopic lesions were present, a single leaf from 48 *lsd6* × NahG and 135 *lsd7* × NahG F_1 individuals was stained with trypan blue. Representative samples of *lsd6*, *lsd7*, and the respective NahG F_1 individuals are presented in Figure 6. None of the F_1 progeny showed microscopic lesions. This contrasts with the lesions visible on *lsd6* and *lsd7*, which are shown at approximately ×400 magnification in the insets of Figure 6. F_1 plants did indeed carry the respective mutations, because seeds from F_2 progeny were planted and lesioned individuals were recovered (data not shown). These results contrast with those obtained with *lsd2*, *lsd4*, and *lsd5* in which lesion formation was unaffected by *nahG* expression (M. Hunt, T. Delaney, and K. Weymann, unpublished data).

INA and SA Treatment Restores the Lesion Phenotype in *lsd6* × NahG Progeny

INA has previously been shown to induce both SAR gene expression and resistance in *nahG*-expressing transgenic tobacco and Arabidopsis (Vernooij et al., 1995), strongly suggesting that INA induces SAR signaling downstream of SA accumulation. Based on this observation, *lsd6* × NahG F_1 plants were treated with INA to see if this would circumvent the block imposed by *nahG* expression. Examination of 29 *lsd6* × NahG F_1 progeny treated with INA showed recovery of the

Table 1. *P. parasitica* Race NOCO Disease Ratings

	Col-0	NahG	*lsd6*	*lsd6* × NahG	*lsd7*	*lsd7* × NahG
Disease rating[a]	3	5	0	5	0.7	5
Number of plants	6	11	6	15	6	32

[a] The scale is as follows: 0, no conidiophores on the plant; 1, 1 to 20% of leaves with >10 conidiophores; 2, 21 to 40% of leaves with >10 conidiophores; 3, 41 to 60% of leaves with >10 conidiophores; 4, 61 to 80% of leaves with >10 conidiophores; 5, 81 to 100% of leaves with >10 conidiophores. Values are averages of compiled disease ratings per treatment.

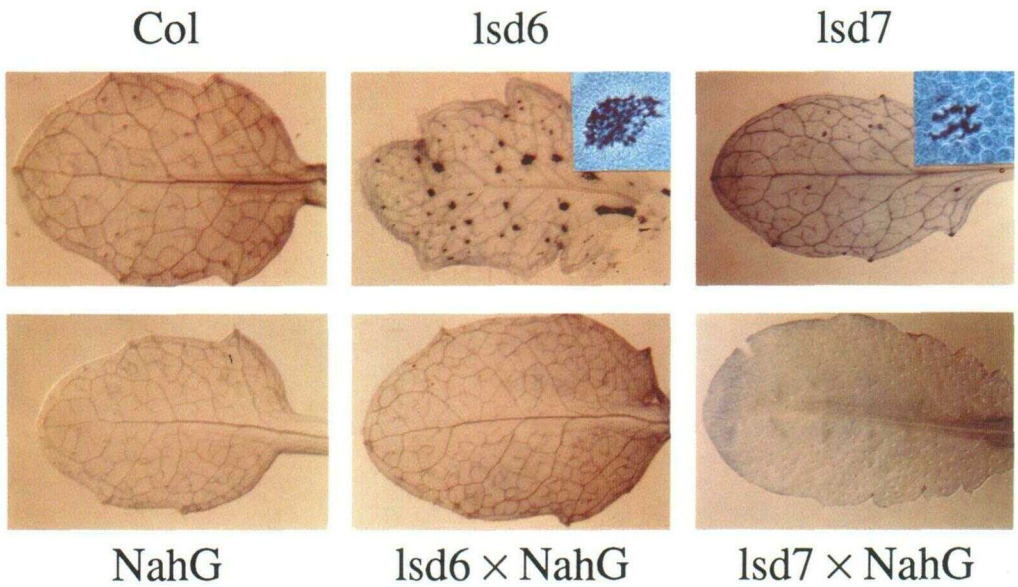

Figure 6. *nahG* Expression Suppresses Lesion Formation in *lsd6* and *lsd7*.

Trypan blue–stained leaves of *lsd6* and *lsd7* show the presence of lesions. However, lesion formation in *lsd6* × NahG and *lsd7* × NahG F_1 progeny is suppressed by *nahG* expression. Insets for *lsd6* and *lsd7* show areas exhibiting lesions and have been magnified ×400.

lesioned and curled leaf phenotype in all individuals, of which a representative is shown in Figures 7B, 7E, and 7H. Surprisingly, treatment of 13 *lsd6* × NahG F_1 individuals with SA also restored the mutant phenotype (Figures 7C, 7F, and 7I). However, the magnitude of phenotypic recovery was not as pronounced as that of *lsd6* × NahG F_1 progeny treated with INA. Two days after INA or SA application, newly emerging leaves showed the lesioned and curled phenotype. Lesions appeared on preexisting leaves after 4 days with no associated leaf curling. Lesion appearance on mature leaves differed from that on newly emerging leaves. After staining, lesions that formed on existing leaves appeared to be composed of patches of stained cells and unstained cells without defined borders (data not shown). In contrast, lesions that developed on new growth after inducer application were heavily stained and were bordered by radially arranged living cells, identical to the *lsd6* lesions described previously (Figure 6, inset). Lesions never appeared on 43 water-treated F_1 progeny or on the Columbia wild-type plants treated with INA or SA. Interestingly, lesion formation was not restored in INA- or SA-treated *lsd7* × NahG F_1 progeny.

DISCUSSION

Here, we report the isolation of two new Arabidopsis mutants based on their strong constitutive expression of the SAR-associated gene PR-1. We found that along with PR-1, these mutants constitutively express PR-2 and PR-5, accumulate high levels of SA, and, importantly, are resistant to fungal infection. Taken together, these data indicate that the mutations identify genes involved in a signal transduction pathway leading to SAR. Along with a constitutively elevated SAR signal transduction pathway, the two mutants described exhibit spontaneous lesion formation. Arabidopsis mutants that exhibit spontaneous lesion formation, elevated SA accumulation, SAR gene expression, and heightened disease resistance have been described previously (Dietrich et al., 1994; Greenberg et al., 1994). Based on these criteria, both mutants have been classified as new *lsd* loci. *lsd6* possesses punctate lesions on all true leaves from the time of emergence, whereas lesions on *lsd7* form conditionally only on mature leaves. The mutations in *lsd6* and *lsd7* are dominant, and their F_2 segregation ratios are consistent with single-gene traits. *lsd6* maps between nga111 and nga128 on the lower arm of chromosome 1. Although the map position of *lsd7* has not yet been determined conclusively, it clearly does not map to chromosome 1, indicating that it is an independent locus.

To test the relationship of lesion formation, SA accumulation, SAR gene expression, and fungal resistance, we crossed both *lsd6* and *lsd7* to *nahG*-expressing plants. Because both the *lsd* and NahG phenotypes are dominant, we were able to analyze epistasis in the F_1 plants. It was clear from the results that the presence of *nahG* suppressed SAR gene expression and fungal resistance, which would place both of these responses downstream of the accumulation of SA. This result

is consistent with our previous findings that *nahG*-expressing plants suppress SAR gene expression and enhance susceptibility to pathogens (Gaffney et al., 1993; Delaney et al., 1994). Interestingly, lesion formation was also suppressed in the *lsd6*- and *lsd7*-derived F_1 plants. This finding was unexpected, because in previous experiments we found that lesion formation was unaffected when either *lsd2*, *lsd4*, or *lsd5* expressed *nahG* (M. Hunt, T. Delaney, R. Dietrich, K. Weymann, J. Dangl, and J. Ryals, unpublished results). These conflicting results appear to place lesion formation both upstream and downstream of SA accumulation. However, considerable evidence places SA accumulation downstream of lesion formation. For example: (1) SA treatment of leaf tissue that leads to SAR gene expression does not induce lesion formation, although at very high concentrations it can result in phytotoxicity (Ward et al., 1991); (2) SA accumulates in infected leaves as a function of the number of lesions (Yalpani et al., 1991); (3) lesion size is not reduced in *nahG*-expressing plants (Gaffney et al., 1993); and (4) as discussed previously, in epistasis experiments between either *lsd2*, *lsd4*, or *lsd5* and NahG, lesions form normally, but SAR gene expression and resistance are suppressed. Taken together, these results argue that SA accumulation occurs as a result of lesion formation.

One possible explanation for the suppression of lesion formation in the *lsd6* × NahG F_1 plants is feedback regulation of lesion formation by SA or SA-dependent events. If that were true, lesion formation in *lsd6* would be dependent on SA. The suppression of lesions by the removal of SA and their restoration in the *lsd6* × NahG F_1 plants by INA application indicate a requirement for late SAR signaling events, which are SA dependent, in the regulation of early events such as lesion formation. This is solid evidence for a feedback loop in the SAR signal transduction pathway. Consequently, these mutants are disease lesion mimics that may function directly within the SAR signal transduction pathway, thereby illustrating their unique significance. Interestingly, *lsd7* × NahG F_1 plants showed suppressed lesion formation, but INA treatment did not restore lesion formation. Because of the conditional nature of *lsd7*, further characterization is necessary to determine the implications of this result.

An interesting issue is whether SA accumulation or the induction of an SA-dependent process is reponsible for lesion

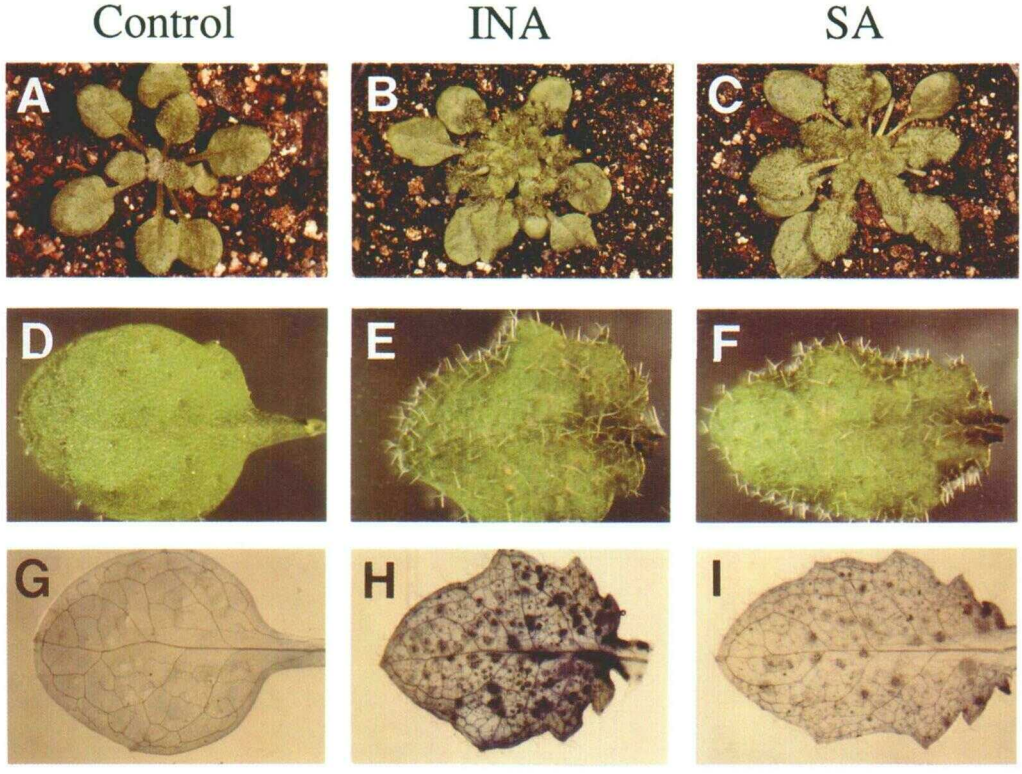

Figure 7. Recovery of the Lesioned Phenotype in *lsd6* × NahG F_1 Progeny after Inducer Treatment.

(A), **(D)**, and **(G)** Whole-plant, leaf, and trypan blue–stained leaf samples from an *lsd6* × NahG F_1 plant before treatment.
(B), **(E)**, and **(H)** Whole-plant, leaf, and trypan blue–stained leaf samples from an INA-restored F_1 plant.
(C), **(F)**, and **(I)** Whole-plant, leaf, and trypan blue–stained samples from an SA-restored F_1 plant.

restoration. This issue cannot be resolved at this point because INA cannot be excluded as a structural mimic for SA. The question may be answered as a result of epistasis experiments between *lsd6* and *nim1*, a recently characterized Arabidopsis mutant that is blocked in its ability to respond to SA and INA (Delaney et al., 1995).

The signal transduction pathway leading to the manifestation of SAR is clearly important for plant defense against pathogens as diverse as fungi, bacteria, and viruses (Delaney et al., 1994). This pathway is most likely complex, containing feedback regulation of early signaling events that function to regulate its expression finely. The ability to isolate and characterize novel mutants such as *lsd6* and *lsd7* that provide insight into the intricate regulation of SAR may lead to the isolation of plant factors that execute critical roles in plant defense.

METHODS

Mutant Isolation and RNA Methods

One or two leaves from 1100 individual M_2 ethyl methanesulfonate–mutagenized Arabidopsis plants were harvested, and total RNA was isolated using a rapid, RNA minipreparation (Verwoerd et al., 1989). Eighty putative mutants were identified by the presence of pathogenesis-related protein PR-1 and PR-2 gene expression. RNA gel blot analyses were performed, as described previously (Lagrimini et al., 1987; Ward et al., 1991). Probes for PR-1, PR-2, and PR-5 were as described by Uknes et al. (1992). A preliminary report on the isolation of these types of mutants has been made (Lawton et al., 1993). Details regarding the screen will be published elsewhere.

Plant Maintenance

Phytochamber growth conditions (short day; SD) were an 8-hr photoperiod, with 24°C day and 20°C night temperatures, 60% relative humidity, and a light intensity of 250 $\mu E\ m^{-2}\ sec^{-1}$. Greenhouse conditions (long day; LD) included a 16-hr photoperiod (sunlight supplemented to 16 hr with artificial lamps). Conditions of temperature and relative humidity in the greenhouse were variable.

Salicylic Acid Analysis

Salicylic acid (SA) and its glucose conjugate were analyzed, as described previously by Uknes et al. (1993). Leaf tissue was harvested from 4-week-old plants.

Genetic Analysis

Arabidopsis thaliana ecotype Columbia (Col-0) containing the recessive glabrous trait (*gl1*) was used as female recipients for backcrosses, and the absence of the *gl1* marker was used to identify F_1 progeny. The F_1 backcross of *lsd6* maintained the dwarf, curled leaf lesion phenotype. These F_1 plants had strong PR-1 expression and were resistant to infection by *Peronospora parasitica* isolate NOCO (isolated in Norwich, host ecotype Columbia). The subsequent F_2 population segregated 3:1 for the mutant phenotype and contained numerous glabrous plants with the documented *lsd6* phenotype. All plants without lesions lacked PR-1 expression and disease resistance. The F_1 backcross and the F_2 progeny of *lsd7* also maintained the lesion phenotype for all PR-1–expressing plants. F_2 analysis of the *lsd7* backcross resulted in an ~3:1 ratio of PR-1–positive plants.

For the mapping analyses, DNA was isolated from leaves of F_2 plants resulting from an Arabidopsis Landsberg *erecta* (Ler) × mutant F_1 using the method of Dellaporta et al. (1983). Cleaved, amplified polymorphic sequence markers and simple sequence–length polymorphism (SSLP) markers were amplified using the polymerase chain reaction and scored as described previously (Konieczny and Ausubel, 1993; Bell and Ecker, 1994). Genetic map distances were determined using MAPMAKER/EXP Version 3.0 software (Lander et al., 1987; Lincoln et al., 1992) running on a Sun SPARC workstation (Sun Microsystems, Inc., Mountain View, CA). Recombination frequencies were calculated using the MAPMAKER F_2 algorithm and converted to map distances in centimorgans using the Kosambi (1944) function.

Crosses with Arabidopsis Plants Expressing Salicylate Hydroxylase

Plants expressing salicylate hydroxylase (NahG) were made by transformation of the cauliflower mosaic virus 35S–driven *nahG* gene into Arabidopsis using Agrobacterium-mediated transformation (Huang and Ma, 1992; Gaffney et al., 1993; Delaney et al., 1994). Col–NahG Arabidopsis plants carry a dominant kanamycin resistance gene in addition to the dominant *nahG* gene, so Col–NahG was used as the pollen donor. F_1 seed were hydrated in water for 30 min and then surface sterilized in 10% Clorox, 0.05% Tween 20 for 5 min, and washed thoroughly in sterile water. Seeds were sown on germination media (GM; Murashige and Skoog medium containing 10 g/L sucrose buffered with 0.5 g/L 2-(*N*-morpholino)ethanesulfonic acid, pH 5.7, with KOH) containing 25 μg/mL kanamycin to select for F_1 plants (Valvekens et al., 1988).

Treatment with Chemicals Activating Systemic Acquired Resistance

2,6-Dichloroisonicotinic acid (INA), formulated as a 25% active ingredient in a wetable powder carrier, was suspended in distilled water at a concentration of 0.25 mg/mL (325 μM). SA was prepared in distilled water at a concentration of 5 mM. These solutions or distilled water alone was sprayed to the point of imminent runoff on the described plants.

P. parasitica Assays and Trypan Blue Staining

P. parasitica isolate NOCO was maintained and inoculated, as described previously by Uknes et al. (1992). Plants were 5 weeks old before inoculation. Trypan blue staining was performed as described previously (Keogh et al., 1980; Koch and Slusarenko, 1990).

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