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Arabidopsis *NHO1* Is Required for General Resistance against *Pseudomonas* Bacteria

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Nonhost interactions are prevalent between plants and specialized phytopathogens. Although it has great potential for providing crop plants with durable resistance, nonhost resistance is poorly understood. Here, we show that nonhost resistance is controlled, at least in part, by general resistance. Arabidopsis plants are resistant to the nonhost pathogen *Pseudomonas syringae* pv *phaseolicola* NPS3121 and completely arrest bacterial multiplication in the plant. Ten Arabidopsis mutants were isolated that were compromised in *nonhost* (*nho*) resistance to *P. s. phaseolicola*. Among these, *nho1* is caused by a single recessive mutation that defines a novel gene. *nho1* is defective in nonspecific resistance to *Pseudomonas* bacteria, because it also supported the growth of *P. s. tabaci* and *P. fluorescens* bacteria, both of which are nonpathogenic on Arabidopsis. In addition, the *nho1* mutation also compromised resistance mediated by *RPS2*, *RPS4*, *RPS5*, and *RPM1*. Interestingly, the *nho1* mutation had no effect on the growth of the virulent bacteria *P. s. maculicola* ES4326 and *P. s. tomato* DC3000, but it partially restored the in planta growth of the DC3000 *hrpS*$^-$ mutant bacteria. Thus, the virulent bacteria appear to evade or suppress *NHO1*-mediated resistance by means of an Hrp-dependent virulence mechanism.

INTRODUCTION

Plants interact with a wide array of microbes in the environment. However, a given plant species is resistant to most phytopathogens, and this phenomenon is referred to as nonhost resistance (Heath, 1987, 1996; Staskawicz et al., 1995). Nonhost resistance is pathovar/formae speciale specific or species specific, in contrast with the widely studied race- or cultivar-specific resistance that is mediated by resistance (*R*) gene–avirulence (*avr*) gene interactions. Considerable progress has been made in our understanding of *R*–*avr* interactions (Staskawicz et al., 1995; Martin, 1999). However, these advances do not readily explain why a pathogen fully virulent on one plant species is nonpathogenic on others. Nonhost resistance has been difficult to characterize due to the lack of a genetic system.

Pathogens possess basic compatibility even on a nonhost plant. For example, fungal pathogens are capable of forming an appressorium, penetrating nonhost plant tissues, and occasionally developing a haustorium (Heath, 1979, 1987). *Phytophthora megasperma* f sp *glycinea* (now renamed *P. sojae*) is a nonhost pathogen on parsley that normally causes a nonhost hypersensitive response (HR). However, parsley plants kept at 100% humidity after inoculation were completely susceptible to the otherwise incompatible fungus and allowed sporulation (Jahnen and Hahlbrock, 1988). Basic compatibility also is evident during the interaction of a bacterial pathogen and a nonhost plant. The expression of *hrp* genes (required for the HR on nonhost plants and for pathogenicity on host plants) that encode the type III secretion system and *avr* genes in bacterial pathogens often depends on signals from the host plant (Brito et al., 1999). This process apparently occurs normally when the bacterial pathogen is in contact with the nonhost plant cell. An *avr* gene in the bacterium can be recognized by a cognate *R* gene in the nonhost plant and can elicit the HR (Yu et al., 1993). This suggests that the bacterial pathogen is capable of sensing the contact with nonhost plant cells and delivering its effectors to the plant cell via the type III secretion system. The basic compatibility associated with the many potential pathogens reinforces the importance of plant defense mechanisms in nonhost interactions.

Heath (1987, 1996) proposed that nonhost resistance is a result of general resistance that is parasite nonspecific. The so-called general resistance assumes that plants are commonly equipped to recognize potential parasites in a nonspecific manner. This recognition leads to a defense reaction that limits the growth of pathogens. A successful parasite must suppress, avoid, or tolerate the general resistance by acquiring matching pathogenicity factors.

Consistent with the general defense hypothesis, plants possess highly sensitive receptors for bacterial and fungal elicitors (Boller, 1995). These elicitors are pathovar or formae

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speciale nonspecific and induce rapid plant defense responses at very low concentrations. The general elicitors characterized to date include oligosaccharides, peptides, and glycoproteins. A recent addition is the flagellin protein from a wide range of eubacteria (Felix et al., 1999). A 22–amino acid peptide from the conserved domain in flagellin proteins is a highly potent elicitor for the induction of oxidative burst, alkalinization of cultures, accumulation of callose, and expression of pathogenesis-related (PR) genes (Gomez-Gomez et al., 1999). Arabidopsis *flagellin sensing* (*fls*) mutants have been isolated. Map-based cloning enabled the isolation of a putative flagellin receptor gene with striking similarity to the rice resistance gene *Xa21* (Gomez-Gomez and Boller, 2000). However, it remains to be determined if flagellin sensing is required for resistance to any phytopathogens.

Early experiments suggested that the general defense is responsible, at least in part, for nonhost resistance. For example, treatment of nonhost plants with heat shock or protein synthesis inhibitors before the inoculation of several rust fungi enhanced hyphal growth and haustorium development (Heath, 1979). However, genetic evidence is scarce for a role of plant defense in limiting nonhost pathogen growth. Recent studies show that mutant plants defective in preformed or induced defense mechanisms can develop severe diseases upon infection by weak fungal pathogens or fungal pathogens that normally infect closely related plant species (Parker et al., 1996; Knoester et al., 1998; Multani et al., 1998; Vijayan et al., 1998; Papadopoulou et al., 1999; Thomma et al., 1999). Although the parasite–plant interactions in these examples are not always viewed as nonhost interactions, they do demonstrate the importance of race/cultivar-nonspecific resistance that is not determined by gene-for-gene interaction. Similar data do not exist for nonhost resistance to bacterial pathogens.

Here, we describe the Arabidopsis–*Pseudomonas syringae* pv *phaseolicola* interaction as a model genetics system to study plant defense mechanisms involved in nonhost interactions. We show that general resistance plays an important role in limiting the growth of *P. s. phaseolicola* on Arabidopsis and that nonhost resistance is amenable to genetic studies.

RESULTS

Reaction of Different Arabidopsis Ecotypes to *P. s. phaseolicola*

P. s. phaseolicola causes halo blight in bean but incites no disease on cruciferous plants, including Arabidopsis (Yu et al., 1993). To test the potential pathogenicity of *P. s. phaseolicola* strain NPS3121 on Arabidopsis, we inoculated NPS3121 at various concentrations on 17 accessions of Arabidopsis (see Methods). No accession showed any disease symptoms when inoculated with $\leqslant 10^6$ colony-forming units (cfu)/mL of bacteria. We also examined bacterial population in the plant. NPS3121 was unable to grow to a significant level in any of the Arabidopsis ecotypes tested. Four days after inoculation, bacterial populations either decreased onefold or increased less than fourfold. Similar results were obtained with repeated experiments. These findings support the idea that Arabidopsis is a nonhost species to *P. s. phaseolicola*.

Bacterial pathogens often induce the HR in nonhost plants. However, Arabidopsis ecotype Columbia (Col-0) plants do not react with an HR to NPS3121 (Yu et al., 1993). Similarly, we found that none of the 17 ecotypes tested developed an HR after NPS3121 inoculation (at 10^8 cfu/mL).

Active Defense in Arabidopsis Is Involved in Nonhost Resistance to *P. s. phaseolicola*

We next examined the expression of *PR1* and *GST1* in plants challenged with NPS3121 as an indication of active defense during a nonhost interaction. Inoculation of NPS3121 on Col-0 plants induced the expression of *PR1* and *GST1* (Figure 1A). The induction of *PR1* is abolished by the *nahG* transgene. Plants carrying *nahG*, a bacterial gene that encodes salicylic acid hydroxylase, are unable to accumulate salicylic acid in response to pathogen infection (Ryals et al., 1996). These results indicated that active defense was activated during the nonhost interaction.

We hypothesize that plant defense mechanisms are responsible, at least in part, for the absence of *P. s. phaseolicola* multiplication in Arabidopsis. To test this notion further, we challenged Arabidopsis plants carrying the *nahG* transgene with NPS3121 and measured bacterial growth (Figure 1B). Four days after inoculation, the bacterial number decreased slightly in the wild-type Col-0 plants. In contrast, the bacterial number increased $\sim$50-fold in Col-0 (*nahG*) plants. In addition, leaves of the *nahG* plants showed water-soaked lesions 5 days after inoculation. The results indicate that the salicylate-mediated defense pathway plays a profound role in the nonhost resistance against *P. s. phaseolicola*. More importantly, they indicate that screening for Arabidopsis mutants with compromised resistance to *P. s. phaseolicola* is feasible.

Isolation of *nho* Mutants

An initial screen based on the appearance of lesions or chlorosis upon *P. s. phaseolicola* inoculation failed because the observed symptoms did not correlate with the bacterial number in planta. We revised the strategy by monitoring leaf bacterial populations. We introduced the *uidA* gene (β-glucuronidase [GUS]) under the control of the *lacZ* promoter into NPS3121 (see Methods). Four days after bacterial inoculation, leaf discs were excised, incubated with 4-methylumbelliferyl β-D-glucuronide, and visualized under UV light.

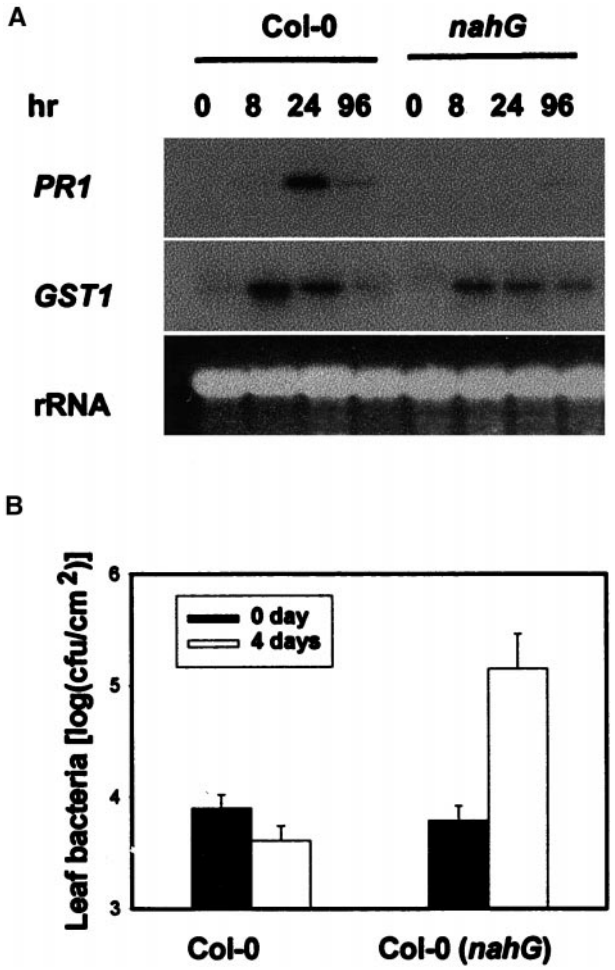

Figure 1. Active Defense Is Required for the Nonhost Resistance of Arabidopsis to *P. s. phaseolicola* NPS3121.

(A) Arabidopsis defense gene induction by *P. s. phaseolicola*. Arabidopsis plants were vacuum infiltrated with 10^6 cfu/mL *P. s. phaseolicola* bacteria and harvested at the times indicated for RNA extraction. RNA gel blots were hybridized with the *PR1* and *GST1* cDNA probes.

(B) Salicylic acid is required to limit the growth of *P. s. phaseolicola* in Arabidopsis plants. *P. s. phaseolicola* NPS3121 bacteria were syringe infiltrated into wild-type (Col-0) and *nahG* transgenic Arabidopsis plants at a concentration of 10^6 cfu/mL, and leaf bacterial numbers were measured at 0 and 4 days after inoculation. Error bars indicate ±SE.

Plants with strong fluorescence were selected as putative mutants that supported bacterial growth. Putative mutants then were verified by standard bacterial growth assays.

Approximately 18,000 ethyl methanesulfonate–mutagenized M2 plants (Col-0 background) constituting 12,000 families were screened. A total of 216 putative mutants were identified. Among these, 10 lines consistently supported more NPS3121 growth in the M3 and M4 generations. Four days after inoculation, bacterial populations in these mutants increased 10- to 50-fold compared with those in wild-type plants (Figure 2).

nho Mutations Do Not Enhance Disease Susceptibility to the Virulent Bacterium *P. s. maculicola* ES4326

Our mutant screening strategy was similar to that for the *eds* (for *enhanced disease susceptibility*) mutants (Glazebrook et al., 1996). Most *eds* mutants were isolated based on enhanced disease susceptibility to the virulent bacterial strain *P. s. maculicola* ES4326. However, these mutants do not exhibit compromised resistance to *P. s. phaseolicola* (Glazebrook et al., 1996; Rogers and Ausubel, 1997; Volko et al., 1998). We determined the growth of *P. s. maculicola* ES4326 on seven *nho* mutants. All seven *nho* mutants supported the same level of bacterial growth as did the wild-type plant in repeated experiments (Figure 3). These results indicate that the *nho* and *eds* mutant screens revealed distinct classes of mutants.

nho Mutants Support Hrp-Independent Bacterial Growth

We tested whether the virulence of NPS3121 on the *nho* mutants was Hrp dependent. The *P. s. phaseolicola* NPS4000 strain carries a Tn5 insertion in the *hrpA* gene that encodes a pilus structural protein essential for the type III secretion system (Lindgren et al., 1986; Wei et al., 2000). All *nho* mutants tested allowed more NPS4000 growth compared with Col-0 plants (Figure 4). The level of NPS4000 growth on *nho* plants was similar to that of the wild-type NPS3121 strain. These results indicate that the enhanced growth of NPS3121 on *nho* mutants was Hrp independent. They also suggest that the resistance defined by *nho* mutants did not require an Hrp-dependent signal.

nho1 Defines a Novel Gene for Disease Resistance

Among all mutants, *nho1409*, hereafter referred to as *nho1*, showed the strongest phenotype and thus was chosen for detailed analysis. The *nho1* mutant consistently supported more bacterial growth than any other mutants. Four days after infiltration with *P. s. phaseolicola* at 10^6 cfu/mL, necrosis was observed frequently in some leaves of *nho1*, but not wild-type plants. In the remaining experiments, a lower bacterial concentration (10^4 cfu/mL) was used because this simplified the bacterial growth assay. *nho1* mutant consistently showed 10- to 100-fold higher bacterial populations compared with wild-type plants when inoculated with either 10^4 or 10^6 cfu/mL bacteria. Genetic analysis of the backcrossed F1 and F2 plants indicated that *nho1* is a single recessive mutation (Table 1). The analysis of the backcrossed

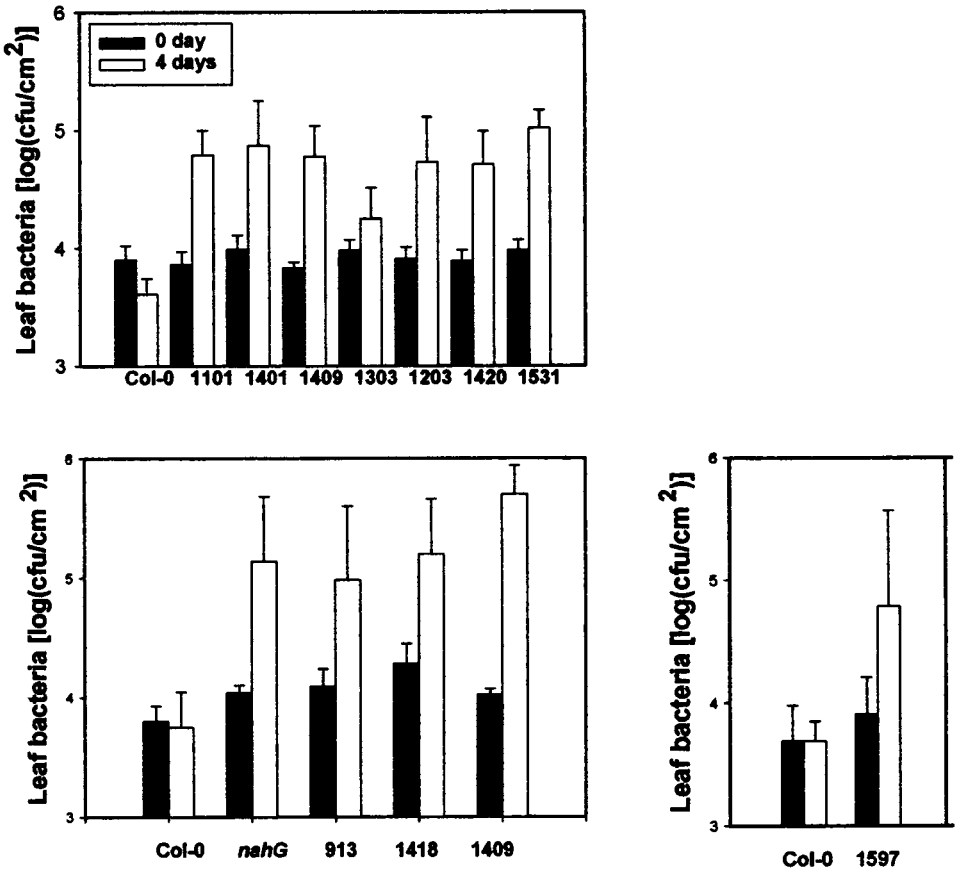

Figure 2. *nho* Mutants Support the Growth of *P. s. phaseolicola* NPS3121 Bacteria.

Bacteria were syringe infiltrated into leaves at a concentration of 10^6 cfu/mL. Bacterial numbers were measured at 0 and 4 days after inoculation. Numbers under the *x* axes denote different mutant lines. The three graphs represent three independent experiments. Error bars indicate ±SE.

F2 progenies also indicated that the *nho1* mutation was not associated with any morphological phenotype.

A mapping population was made by crossing the *nho1* mutant with the Landsberg *erecta* (L*er*) ecotype. F2 plants were infiltrated with NPS3121 bacteria (10^4 cfu/mL), and leaf bacteria were measured 4 days after inoculation. Initial mapping by using 109 F2 plants and 28 microsatellite markers (Bell and Ecker, 1994) that cover the five Arabidopsis chromosomes placed *NHO1* to the bottom of chromosome 1. Further mapping was performed by using four microsatellite markers spanning the last 2.5 Mb of chromosome 1. Analysis of 350 chromosomes identified 15 recombinant chromosomes between *nho1* and a marker derived from bacterial artificial chromosome (BAC) F22P28 (position 26,700 bp), five recombinant chromosomes for a marker derived from BAC T30F21 (position 28,500 kb), three recombinant chromosomes for a marker from BAC F18B13 (position 28,970 kb), and two recombinant chromosomes for a marker derived from BAC F23A5 (position 29,200 kb). F23A5 is the telomeric end of chromosome 1. Our current mapping data do not resolve whether *NHO1* is located on F23A5 or between F23A5 and F18B13. Nevertheless, the results suggested that *NHO1* was located on the telomeric side of the F18B13 marker that was only 270 kb from the telomere (chromosome 1 has a total of 29,240 kb).

nho1 Is Compromised in General Resistance

The susceptibility of *nho1* to *P. s. phaseolicola* bacteria was studied in greater detail. By using a strain carrying a green fluorescent protein (GFP) construct and confocal microscopy, we observed that the NPS3121 bacteria were able to form colonies in the intercellular spaces of *nho1* but not wild-type plants (data not shown). The GFP results qualitatively demonstrate that the *nho1* mutant permits the multiplication of NPS3121 bacteria.

We determined whether the defect in *nho1* also affected resistance to other bacteria, including *P. s. tabaci* and *P. fluorescens*. As shown in Figures 5A and 5B, leaf bacterial num-

bers for *P. s. tabaci* and *P. fluorescens* remained unchanged in the wild-type plant 4 days after inoculation. In contrast, bacterial numbers in *nho1* leaves increased at least 100-fold compared with those in the wild-type plant.

We also tested *nho1* for disease resistance mediated by gene-for-gene interactions. Arabidopsis Col-0 plants carry *RPS2*, *RPM1*, *RPS4*, and *RPS5* that confer resistance to *Pseudomonas* bacteria containing the avirulence genes *avrRpt2*, *avrB*, *avrRPS4*, and *avrPphB*, respectively. Although wild-type plants supported low levels of bacterial growth because of gene-for-gene resistance, *nho1* plants showed diminished resistance to these avirulent strains and gave rise to significantly higher (20- to 100-fold) bacterial numbers in the leaf compared with wild-type plants (Figures 5C to 5F). The results indicate that *nho1* has partially lost disease resistance mediated by multiple resistance genes. Thus, the *NHO1* gene is required for nonhost resistance to *Pseudomonas* bacteria and gene-for-gene resistance. The reduced resistance did not seem to be associated with an alteration of HR. Inoculation of *nho1* plants with high concentrations of *avrB* or *avrRpt2* strains produced strong HR similar to that observed in wild-type plants (data not shown).

P. s. tomato Evades the *NHO1*-Mediated Resistance by Using an Hrp-Dependent Virulence Mechanism

nho1 supports increased bacterial growth by *Pseudomonas* strains that are nonpathogenic on wild-type Arabidopsis plants, but it does not display enhanced susceptibility to the virulent bacterium *P. s. maculicola* ES4326. To further investigate the relationship between the virulence of the pathogen and the observed general resistance in *nho1*, we inoculated plants with the *P. s. tomato* DC3000 strain that is virulent on Arabidopsis. In repeated experiments, *nho1* and wild-type plants supported similar bacterial growth 4 days after inoculation (Figure 6A). Therefore, the resistance defined by *NHO1*, although effective at limiting growth by the nonhost bacteria, was completely ineffective against the virulent bacteria. We speculate that the virulent strains have evolved strategies to suppress or avoid the general resistance conditioned by *NHO1*. To determine whether these strategies are Hrp dependent, we conducted bacterial growth assays on *nho1* with a DC3000 *hrpS*$^-$ mutant (Wei et al., 2000). *hrpS* is a regulatory gene required for the expression of other *hrp* genes and many effector genes that are controlled by the *hrp* box in the promoter. *hrpS*$^-$ bacteria failed to grow in wild-type plants but grew 100-fold in *nho1* plants. Note that *nho1* did not completely restore the virulence of the *hrpS*$^-$ strain, because the growth of the latter in *nho1* plants was 10- to 100-fold lower than the wild-type DC3000, and the *hrpS*$^-$ strain only occasionally caused necrosis on *nho1* plants when high concentration (10^6 cfu/mL) of bacteria was used (data not shown).

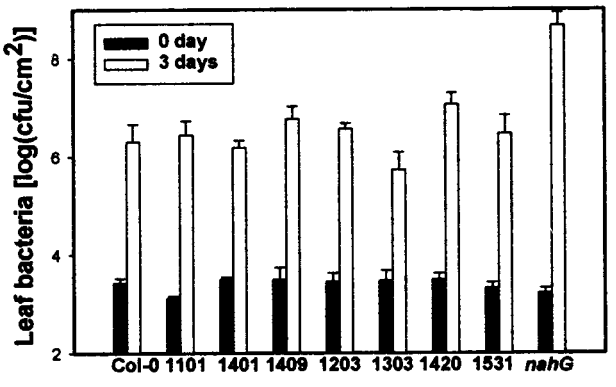

Figure 3. *nho* Mutations Do Not Enhance Susceptibility to the Virulent Bacterium *P. s. maculicola* ES4326.

P. s. maculicola ES4326 bacteria were syringe infiltrated into plants at a concentration of 10^5 cfu/mL. Leaf bacterial numbers were measured at 0 and 3 days after inoculation. Numbers under the *x* axis denote different mutant lines. Error bars indicate ±SE.

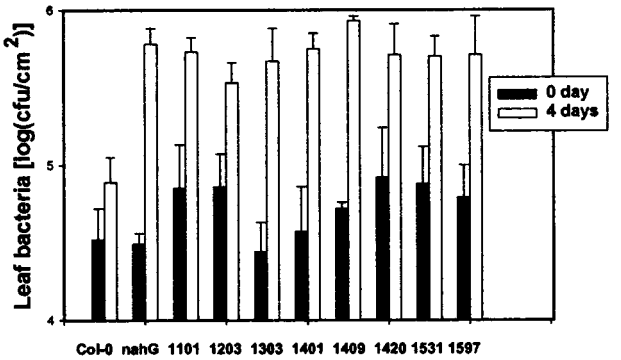

Figure 4. The Growth of *P. s. phaseolicola* in the *nho* Mutant Occurs without a Functional *hrpA* Gene.

P. s. phaseolicola NPS4000 bacteria were syringe infiltrated into Arabidopsis plants at a concentration of 10^6 cfu/mL, and leaf bacterial numbers were measured at 0 and 4 days after inoculation. Numbers under the *x* axis denote different mutant lines. Error bars indicate ±SE.

DISCUSSION

Nonhost disease resistance is a remaining challenge in the field of plant–microbe interactions. The work described here demonstrates that it is possible to systematically study the molecular mechanisms involved in nonhost disease resistance in plants. Nonhost resistance is thought to be more durable than *R* gene–mediated resistance and therefore is attractive for agricultural purposes. It has been suggested that nonhost resistance is a multigenic trait and thus difficult

Table 1. Genetic Analysis of *nho1*

	Total Plants Tested	Resistant[a]	Susceptible	χ^2[b]
Col-0	34	34	0	
nho1	35	0	35	
Col-0 × *nho1*				
F1	13	13	0	
F2	50	37	13	0.027
nho1 × Col-0				
F1	13	13	0	

[a] Plants were syringe infiltrated with NPS3121 at 10^4 cfu/mL, and bacterial numbers for individual plants were determined 4 days after inoculation. Plants with log(cfu/cm^2) lower than 2.8 were scored as resistant, and those with log(cfu/cm^2) higher than 3.2 were scored as susceptible.

[b] χ^2 for the expected ratio 3:1 ($P > 0.8$).

for genetic manipulation (Heath, 1996). Our success with the isolation of 10 *nho* mutants with compromised resistance to *P. s. phaseolicola* indicates that major genetic determinants do exist in plants for nonhost resistance. Therefore, the mechanisms involved in nonhost resistance are amenable to genetic studies. Note that Arabidopsis resistance to *P. s. phaseolicola* occurs in the absence of the HR. The isolation of *NHO* genes should provide novel tools for improving disease resistance in crop plants.

The *nho* mutants are different from the Arabidopsis *eds* mutants isolated to date in that they display distinct responses to the virulent pathogen *P. s. maculicola* and the nonhost pathogen *P. s. phaseolicola* (Glazebrook et al., 1996; Glazebrook, 1999). The *NHO1* gene may be a common component that also is shared by the *R* gene pathways. The Arabidopsis mutants *eds1*, *ndr1*, *pbs2*, and *pbs3* also are blocked in *R* gene functions (Century et al., 1995; Parker et al., 1996; Warren et al., 1998). Interestingly, the *eds1* mutant also supported sporulation by several *Peronospora* isolates and one *Albugo candida* isolate from the *Brassica* species, suggesting an effect on nonhost resistance (Parker et al., 1996). However, none of these mutants supported nonhost bacterial growth (Glazebrook, 1999). *NHO1* was mapped to the bottom of chromosome 1. This region does not contain any nucleotide binding site–leucine-rich repeat sequence, nor does it carry a disease-related locus defined by known mutants. Therefore, *nho1* defines a novel gene required for disease resistance. Together, these results demonstrate that novel disease resistance genes can be identified through genetic analysis of *nho* mutants.

It is well known that cloned *avr* genes from bacterial pathogens are recognized by previously unidentified *R* genes in nonhost plants (Whalen et al., 1988; Kobayashi et al., 1990). This has led to the hypothesis that nonhost resistance is determined by gene-for-gene interactions. In fact, the Arabidopsis *R* genes *RPS4* and *RPS5* recognize *avrRps4* from *P. s. pisi* and *avrPphB* from *P. s. phaseolicola*, respectively (Warren et al., 1998; Gassmann et al., 1999). Both bacteria are nonhost pathogens on Arabidopsis. However, it remains to be shown whether the *R* genes and *avr* genes determine the host range of different pathovars. For example, the PT23 strain of *P. s. tomato*, a nonhost pathogen on soybean, carries four *avr* genes (*avrA*, *avrD*, *avrE*, and *avrPto*). When transformed individually into *P. s. glycinea*, each *avr* gene can induce the HR and disease resistance in soybean in a cultivar-dependent manner (Lorang et al., 1994). However, simultaneous mutation of all four *avr* genes by deletions or Tn insertion fails to alter the nonhost interaction either qualitatively or quantitatively (Lorang et al., 1994). Conversely, the absence of known *R* genes in plants does not result in susceptibility to nonhost pathogens. Although *R* gene–mediated resistance certainly contributes to resistance in nonhost interactions, other mechanisms in addition to gene-for-gene recognition are likely to be involved as well.

Some strains of nonhost *Pseudomonas* pathogens do not induce the HR in Arabidopsis (Davis et al., 1991; Yu et al., 1993), suggesting that they may not carry *avr* genes recognizable to Arabidopsis. However, the lack of the HR does not permit these bacteria to grow in Arabidopsis. The Arabidopsis–*P. s. phaseolicola* NPS3121 interaction described in this study likely involves a general resistance rather than a specific resistance mediated by *R*–*avr* recognition. First, the NPS3121 strain does not elicit the HR on Arabidopsis plants, suggesting a lack of *avr* genes that can be recognized by Arabidopsis. More importantly, *nho* mutants supported the growth of both the wild-type strain and the *hrpA*$^-$ mutant of *P. s. phaseolicola*, whereas the wild-type plant did not. Thus, resistance defined by the *nho* mutants function in the absence of an Hrp-dependent signal. This suggests that recognition of an *avr* gene is not required for the *nho*-defined resistance, because almost all bacterial *avr* genes require the Hrp system to function. Furthermore, the *nho1* mutant showed compromised resistance to multiple bacterial strains. These findings collectively indicate that general resistance plays an important role in the nonhost interaction between Arabidopsis and *P. s. phaseolicola*.

The *nho1* mutant supported the growth of two *hrp* mutants and one nonpathogenic *Pseudomonas* bacterium, suggesting that *nho1* plants are affected in their immunity to saprophytes. One possibility is that the mutant plants are leaking nutrients into the apoplast. However, *nho1* plants did not support the growth of *Escherichia coli* when the latter is infiltrated into the intercellular spaces (B. Yang and J.-M. Zhou, unpublished results), arguing against the nutrient leakage hypothesis. The precise biochemical lesion awaits the isolation and characterization of the *NHO1* gene.

Results with *hrp*$^-$ mutants and virulent strains of *Pseudomonas* indicate a strong interaction between *NHO1*-mediated general resistance and the pathogenicity of virulent bacteria. The *nho1* mutation did not enhance susceptibility to the virulent bacterial strains ES4326 and DC3000. This is not because of spatial and nutrient constraints, because

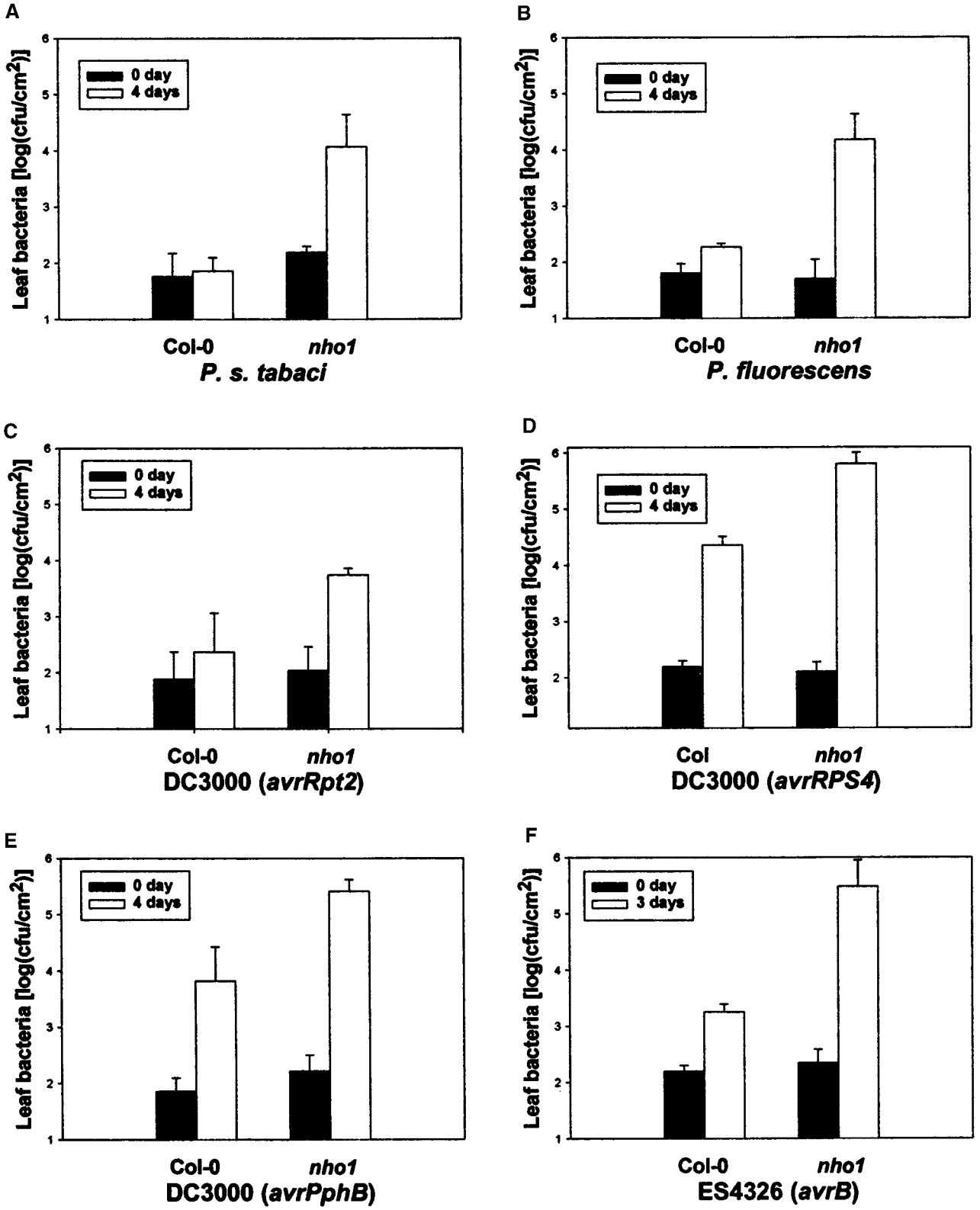

Figure 5. *nho1* Is Compromised in Nonspecific Resistance to *Pseudomonas* Bacteria.

In planta growth of *P. s. tabaci* R1152 race 0 **(A)**, *P. fluorescens* **(B)**, *P. s. tomato* DC3000 (*avrRpt2*) **(C)**, *P. s. tomato* DC3000 (*avrRPS4*) **(D)**, *P. s. tomato* DC3000 (*avrPphB*) **(E)**, and *P. s. maculicola* ES4326 (*avrB*) **(F)**. Bacteria were syringe infiltrated at a concentration of 10^4 cfu/mL, and bacterial numbers were measured at the times indicated. Error bars indicate ±SE.

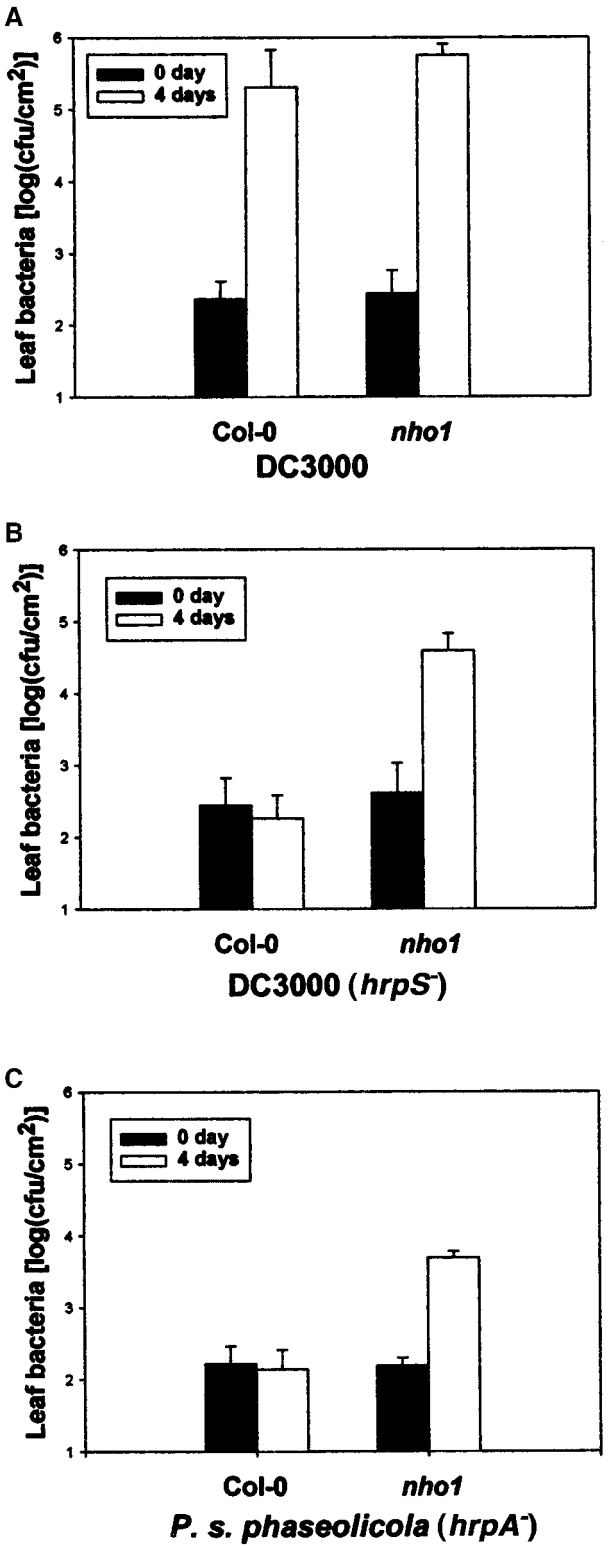

Figure 6. *nho1* Mutation Enhances the Growth of *P. syringae hrp* Mutants but Not of Virulent *P. s. tomato* DC3000.

ES4326 was able to grow 100-fold more in *nahG* plants compared with Col-0 and *nho1* plants. Therefore, these results indicate that the *NHO1*-mediated general resistance has no effect against virulent bacteria. The DC3000 *hrpS*$^-$ mutant was completely unable to grow in the wild-type Arabidopsis plant. Surprisingly, the *nho1* mutation in this plant largely restored the virulence of the DC3000 *hrpS*$^-$ mutant. One plausible explanation for this finding is that the virulent bacteria are capable of evading or suppressing *NHO1*-mediated general resistance by an Hrp-dependent mechanism. This is similar to the recently discovered type III–dependent virulence function of *Salmonella* (Vazquez-Torres et al., 2000). The *Salmonella* pathogenicity island 2 (SPI2) encodes the type III secretion apparatus required for virulence. One of the virulence functions mediated by SPI2 was interference with the deposition of phagocyte NADPH oxidase into bacteria-containing vacuoles. Mutants defective in the SPI2-encoded secretion system are diminished in virulence. However, the virulence can be restored when the host cell is deficient in NADPH oxidase. The isolation of the *NHO1* gene and understanding of its biochemical function should provide unique opportunities for understanding virulence mechanisms of plant bacterial pathogens.

According to the current model, the key function of the type III system is to secrete pathogenicity factors, including effector proteins that are targeted directly into host cells (Galan and Collmer, 1999). Although it is not clear if different pathovars of bacterial pathogens secrete distinct sets of effectors, it is possible that these effectors play an important role in the adaptation of bacteria to their respective host plant species. Increasing evidence from animal and plant bacterial pathogens indicates that at least some effectors secreted by the type III system interfere with host defense mechanisms. The YopJ protein secreted by the human pathogen *Yersinia* and the AvrBst protein secreted by the plant pathogen *Xanthomonas* are cysteine proteases that interfere with host defense signaling pathways by blocking ubiquitin conjugation and inhibiting mitogen-activated protein kinase kinases (Orth et al., 1999, 2000). Interestingly, YopJ homologs have been found in *Salmonella*, *Erwinia*, *Pseudomonas*, and *Rhizobium* (White et al., 2000). *X. c. vesicatoria hrp* mutants are unable to suppress papilla formation in the host plant cell, suggesting that pathogenicity

(A) Growth of *P. s. tomato* DC3000 bacteria in Col-0 and *nho1* plants.

(B) Growth of *P. s. tomato* DC3000 (*hrpS*$^-$) bacteria in Col-0 and *nho1* plants.

(C) Growth of *P. s. phaseolicola* NPS4000 (*hrpA*$^-$) bacteria in Col-0 and *nho1* plants.

Bacteria were syringe infiltrated at a concentration of 10^4 cfu/mL, and bacterial numbers were measured at 0 and 4 days after inoculation. Error bars indicate ±SE.

factors secreted by the Hrp system are able to suppress defense responses in the host plant cell (Brown et al., 1995). Recently, Kunkel's group showed that the AvrRpt2 protein suppressed PR gene expression in Arabidopsis plants lacking the cognate *RPS2* gene (Kloek et al., 2000). Furthermore, suppression of *R–avr* interactions in bean by a virulence gene has been reported for *P. s. phaseolicola* (Jackson et al., 1999; Tsiamis et al., 2000). Together with our results, these findings support the idea that the suppression of general resistance by bacterial effectors may play a major role in the adaptation of bacterial pathogens to their host plant species.

METHODS

Bacterial Strains and Plants

Bacterial strains used in this study include *Pseudomonas syringae* pv *phaseolicola* NPS3121 and NPS4000 (Lindgren et al., 1986), *P. s. tabaci* R1152 race 0 (Willis et al., 1988), *P. s. maculicola* ES4326 (Davis et al., 1991), *P. s. tomato* DC3000 (Innes et al., 1993), *P. s. tomato* DC3000 *hrpS*$^-$ mutant (Wei et al., 2000), *P. s. tomato* DC3000 (*avrRpt2*; Kunkel et al., 1993), *P. s. tomato* DC3000 (*avrRPS4*; Gassmann et al., 1999), *P. s. tomato* DC3000 (*avrPphB*; Warren et al., 1998), and *P. fluorescens* 2-79 (Thara et al., 1999). NPS3121 (β-glucuronidase [GUS]), NPS3121 (green fluorescent protein [GFP]), and ES4326 (*avrB*) were constructed by transforming the bacteria with the pHM1::pBS-GUS (obtained from Frank White, Kansas State University, Manhattan, KS), pCPP3069 (carrying a red-shift S65T GFP under the control of the trp promoter; obtained from Alan Collmer, Cornell University, Ithaca, NY; Gage et al., 1996), and pVSP61::*avrB* (Innes et al., 1993) plasmids, respectively.

All *Arabidopsis thaliana* ecotypes (Columbia [Col-0], Nd-0, Landsberg *erecta* [L*er*], Bensheim, RLD, Ws, No-0, Dijon G, S96, RLD1, Mh-0, Gre-0, C24, Kendalville, Aua/Rhon, Cvi-0, and Turk Lake) and ethyl methanesulfonate–mutagenized M2 seed (Col-0 background) were obtained from Lehle Seeds (Tucson, AZ). Col-0 (*nahG*) seeds (Gaffney et al., 1993) were supplied by Novartis Agricultural Business (Research Triangle Park, NC). All plants were grown in growth chambers at 20°C at night and 22°C during the day with a 10-hr/day photoperiod.

Bacterial Growth Assay

Bacterial culture was grown in King's medium B (King et al., 1954) with appropriate antibiotics, and inoculum was prepared as described (Thara et al., 1999). In earlier experiments, 5- to 6-week-old plants were syringe infiltrated at the 10^6 colony-forming units (cfu)/mL for bacterial growth assay. A lower concentration (10^4 cfu/mL) was used in the remaining experiments because it simplified the bacterial growth assay. Leaf bacterial numbers were measured by plating bacteria on King's medium B agar plates containing appropriate antibiotics. Each data point represents four to five replicates, two discs per replicate. All bacterial growth assays were repeated, and only results that were observed consistently are shown.

For confocal microscopy, plants were syringe infiltrated with NPS3121 (GFP) bacteria at a concentration of 10^6 cfu/mL. Leaves were examined with a confocal microscope and photographed 4 days after inoculation.

Mutant Screening and Genetic Analysis

M2 plants (18,000) were hand inoculated with NPS3121 (GUS) bacteria at a concentration of 10^6 cfu/mL. Four days after inoculation, leaf discs were removed from plants and incubated in a buffer containing 50 mM Na_2HPO_4, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% sarkosyl, 0.7 μL/mL β-mercaptoethanol, and 0.7 mg/mL 4-methylumbelliferyl β-D-glucuronide. Twelve hours after incubation, the leaf discs were visualized under UV light, and plants showing strong fluorescence were selected for bacterial growth assay. Plants that showed significant bacterial growth were verified further in the M3 and M4 generations. All results for *nho1* were verified with backcrossed progeny.

Reciprocal crosses were performed between *nho1* and wild-type Col-0 plants. F1 and F2 plants were hand inoculated with NPS3121 bacteria at a concentration of 10^4 cfu/mL. Plants were scored susceptible or resistant by using log(cfu/cm^2) values >3.2 or <2.8, respectively. For mapping, L*er* was used as a recipient for pollen grains from the *nho1* plants. F2 plants were syringe infiltrated with NPS3121 bacteria at 10^4 cfu/mL and scored by bacterial growth assay. Genomic DNA was isolated from the susceptible F2 plants and analyzed with microsatellite markers covering the five Arabidopsis chromosomes (Bell and Ecker, 1994). Primers for additional microsatellite markers are given below: bacterial artificial chromosome (BAC) F28P22, 5′-TGAGCAAAAGAAACTCCAGTTG-3′ and 5′-TTGAAGACAAGCTTTGCTCAGAG-3′; BAC T30F21, 5′-GGAACAATAACGGTCTCAAGAATG-3′ and 5′-TATGGCTTTTCGAAAGGATCCTAG-3′; BAC F18B13, 5′-TTTCGTTCTGCTTCCGAGCTTAG-3′ and 5′-ACCTGAAGCATCGTCACATTTATG-3′; and BAC F23A5, 5′-GTAAGAACCAACCTATTTCAATCAG-3′ and 5′-GCTTTAACAGTTATTGTAATCAGTCG-3′.

RNA Gel Blot Analysis

For RNA expression, plants were vacuum infiltrated with bacterial suspension, and leaves were collected at the times indicated for RNA isolation. Total RNA (10 μg/lane) was fractionated with formaldehyde gel, transferred to a nitrocellulose membrane, and hybridized to radiolabeled *PR1* cDNA (Uknes et al., 1992) and an expressed sequence tag for *GST1* (Reuber et al., 1998; GenBank accession number AA713231).

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