A critical role of STAYGREEN/Mendel’s I locus in controlling disease symptom development during Pseudomonas syringae pv. tomato infection of Arabidopsis

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ABSTRACT
Production of disease symptoms represents the final phase of infectious diseases and is a main cause of crop loss and/or marketability. However, little is known about the molecular basis of disease symptom development. In this study, a genetic screening was conducted to identify Arabidopsis mutants that are impaired specifically in the development of disease symptoms (leaf chlorosis and/or necrosis) after infection with the bacterial pathogen *Pseudomonas syringae pv. tomato* (*Pst*) DC3000. An ethane-methanesulfonate (EMS)-induced Arabidopsis mutant (*noc1*; for no chlorosis 1) was identified. In wild-type plants, the abundance of chlorophylls decreased markedly after *Pst* DC3000 infection, whereas the total amount of chlorophylls remained relatively unchanged in the *noc1* mutant. Interestingly, *noc1* mutant plants also exhibited reduced disease symptoms in response to the fungal pathogen *Alternaria brassicicola*. Genetic and molecular analyses showed that the nuclear gene *STAYGREEN* (*SGR* or Mendel’s *I* locus) is mutated (resulting in the aspartate to tyrosine substitution at amino acid position 88) in *noc1* plants. Transforming wild-type *SGR* cDNA into the *noc1* mutant rescued the chlorosis phenotype in response to *Pst* DC3000 infection. The *SGR* transcript was highly induced by *Pst* DC3000, *A. brassicicola*, or coronatine, a bacterial phytotoxin that promotes chlorosis. The induction of *SGR* expression by coronatine is dependent on COI1, a principal component of the jasmonate receptor complex. These results suggest that pathogen/coronatine-induced expression of *SGR* is a critical step underlying the development of plant disease chlorosis.
INTRODUCTION

Tissue chlorosis and necrosis are among the most frequently observed disease symptoms associated with infection by diverse plant pathogens. These disease symptoms generally occur at the late stages of disease, but are highly relevant to agriculture because they cause actual damage to plant tissues, resulting in yield loss and/or poor marketability of crops. Symptomless infection is often associated with benign or symbiotic plant-microbe associations. Despite its importance in disease, the molecular basis of disease chlorosis and necrosis remain poorly understood.

*Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000, the bacterial pathogen used in this study, is well known for causing localized necrosis and diffuse chlorosis in its hosts tomato and Arabidopsis (Ma and Cuppels 1991, Whalen et al. 1991, Katagiri et al. 2002). Many virulence genes are needed for *Pst* DC3000 to cause disease, among which two virulence systems have been studied extensively: the type III secretion system (T3SS) and the phytotoxin coronatine (COR). The T3SS delivers dozens of effector proteins into plant cells (Alfano and Collmer 2004, He et al. 2004, Buttner and He 2009). Many of these effectors suppress host immune responses (Boller and He 2009, Cui et al. 2009, Lewis et al. 2009) and some are also linked to production of disease necroses (Badel et al. 2003, DebRoy et al. 2004, Cohn and Martin 2005). COR, a molecular mimic of the plant hormone jasmonate, is not only involved in suppression of host immune responses, but also is important for the development of chlorosis symptoms (Feys et al. 1994, Mittal and Davis 1995, Bender et al. 1999, Kloek et al. 2001, Brooks et al. 2004, Block et al. 2005, Brooks et al. 2005, Melotto et al. 2008b). However, because effector- and COR-deficient bacterial mutants are generally affected in multiple steps of pathogenesis, it is often difficult to determine whether these virulence factors contribute to symptom development directly or indirectly through promoting bacterial colonization and/or multiplication.

An alternative approach to elucidate the molecular control of disease symptom production would be to isolate plant mutants that exhibit reduced disease necroses and/or
chlorosis in response to pathogen infection. Indeed, numerous Arabidopsis constitutive defense mutants that show no or reduced disease symptoms to \textit{P. syringae} infection have been isolated since the early 1990s (Bowling et al. 1994). However, similar to the situation with effector- or COR-defective \textit{Pst} DC3000 mutants, bacterial populations in such plant mutants are often reduced compared to those in susceptible plants, making it difficult to conclude whether corresponding plant genes have a direct role in mediating symptom development or indirectly through affecting bacterial multiplication. There are a few exceptions: The ethylene-insensitive Arabidopsis, \textit{ein2}, mutant, and the Arabidopsis mutant \textit{sgt1b} (suppressor of G2 allele of skp1 b; affected in jasmonate signaling) exhibit reduced disease symptoms to \textit{Pst} DC3000 infection without significantly affecting bacterial growth (Bent et al. 1992, Uppalapati et al. 2011), implicating the involvement of ethylene and jasmonate signaling in the production of \textit{Pst} DC3000-elicited disease symptoms. How ethylene or jasmonate signaling lead to downstream disease symptom is not understood.

To increase our understanding of the molecular basis of disease symptom development during \textit{Pst} DC3000 infection of Arabidopsis, we conducted a genetic screen for Arabidopsis mutants that are reduced in disease symptom development, but not \textit{Pst} DC3000 multiplication. In this paper, we report the identification and characterization of such an Arabidopsis mutant and cloning of the corresponding gene. Our results suggest that pathogen-responsive \texttt{STAYGREEN (SGR)}/\texttt{NON-YELLOWING (NYE1)}/Mendel’s \textit{I} locus plays a critical role in controlling disease chlorosis induced by \textit{Pst} DC3000 and, interestingly, also by a fungal pathogen, \textit{Alternaria brassicicola}.

**RESULTS**

**Identification of the noc1 mutant**

Approximately 10,000 EMS-mutagenized \textit{A. thaliana} ecotype Columbia (Col-0) \textit{gll} plants were screened for altered symptom development after the plants were dipped in a suspension containing $1 \times 10^8$ CFU/ml \textit{Pst} DC3000 bacteria. One mutant isolated from this
screen, *noc1* (*no chlorosis 1*), was found to be defective in symptom development. In all experiments, *noc1* leaves remained green whereas wild-type leaves began to show chlorosis between 48 and 72 hours after inoculation (Fig. 1A). In most experiments reduced severity of necrosis symptom was also observed in *noc1* plants, although this phenotype was not as obvious as the lack-of-chlorosis phenotype. There were no noticeable differences between wild-type and *noc1* plants in size, morphology, growth or development in the absence of pathogen inoculation. Most importantly, the reduction in disease symptoms in *noc1* plants was not caused by reduced bacterial multiplication because *Pst* DC3000 populations in Col-0 *glt1* and *noc1* plants were similar at 1 and 3 days post-infection (dpi) (Fig. 1B). Thus, *noc1* is a *bono fide* disease symptom mutant.

In addition to the *noc1* mutant, we also isolated several other mutants that exhibited reduced disease symptom development during this study. However, these other mutants were dwarf and/or necrotic, suggestive of constitutive activation of non-specific disease resistance (Bowling et al. 1994). We did not conduct further characterization of such mutants.

**Maintenance of the chlorophyll level in *noc1* plants after infection with *Pst* DC3000**

To quantify the chlorotic response to *Pst* DC3000, we conducted a chlorophyll abundance assay using leaf tissue infiltrated with 2x10^6 CFU/mL of *Pst* DC3000 and collected at 0, 24, 48, 72 and 96 hours post-inoculation (hpi). The results from one representative experiment are shown in Fig. 1C. Prior to inoculation with *Pst* DC3000, *noc1* and wild-type plants had approximately equal amounts of total chlorophyll (25.2 mg/cm^2 and 28.6 mg/cm^2, respectively). Wild-type plants began to lose chlorophyll by 24 hpi, with levels decreasing through 96 hpi. At 72 hpi, *noc1* plants contained almost three times more chlorophyll than wild-type plants (27.0 mg/cm^2 in *noc1* plants vs. 9.5 mg/cm^2 in wild-type plants). This experiment demonstrates that wild-type plants lose chlorophyll much faster than *noc1* plants after *Pst* DC3000 infection.

**The *noc1* phenotype results from a single nucleotide change in *AtSGR1* (At4G22920)**
To identify the NOC1 gene, noc1 plants were crossed with Ler plants and the F1 progeny were selfed to create an F2 population for mapping. The noc1 mutation shows normal Mendelian genetics and is recessive. We initially used bulk segregant analysis to analyze a pool of approximately 100 F2 individuals that exhibited the mutant phenotype (homozygous for the noc1 mutation). One marker, NGA107, located on the long arm of chromosome 4, showed linkage to the mutation. We tested a larger population of F2 individuals using additional chromosome 4-specific insertion and deletion (INDEL) markers identified from the Monsanto Arabidopsis Polymorphism, Ler Sequence Collection (St. Louis, MO). This delimited the region of the noc1 mutation to the area between two INDEL markers (T12H17-13C and F16G20-22) located on chromosome 4 at 11.96 Mb and 12.25 Mb, respectively (Fig. 2A).

Based on the impaired chlorosis phenotype of the noc1 mutant, we reasoned that the mutation might lie in a gene coding for a chloroplast-targeted protein. The mapped region for the noc1 mutation includes 6 genes encoding predicted chloroplast localization signals. The cDNA clones of candidate genes from noc1 and Col-0 gl1 plants were subjected to sequencing and revealed a single guanine to thymidine nucleotide mutation, resulting in an aspartate (D) to tyrosine (Y) amino acid substitution at position 88 of At4g22920/AtSGR/NON-YELLOWING/Mendel’s I locus (AtSGR hereinafter; Fig. 2B). Orthologs of AtSGR (Ren et al. 2007) in monocot and dicot species are tightly conserved overall with no variation occurring at this particular residue (Fig. 2C).

To determine whether the D88Y mutation in AtSGR is responsible for the noc1 phenotypes, we transformed noc1 plants with the full-length AtSGR cDNA from Col-0 gl1 cloned in pBAR-35S, which contains the CaMV 35S promoter and Basta (glufosinate)-resistance gene. Ten independent T2 lines were confirmed to exhibit Basta resistance and to harbor the AtSGR transgene. Three homozygous T3 lines were chosen for disease symptom observations after infection by Pst DC3000. In preliminary tests, all
three were restored in chlorosis symptom development. We then focused on line #1 for
further disease symptom and bacterial growth analyses. Results from this line are shown
in Fig. 3. By 72-96 hpi, *Pst* DC3000-infected *noc1/35S:AtSGR* plants showed disease
symptoms, including chlorosis, that were more pronounced than *Pst* DC3000-infected
Col-0 *gl1* plants (Fig. 3A-C). Interestingly, in these experiments we noticed that
*noc1/35S:AtSGR* plants did not support bacterial multiplication to the level observed in
either *noc1* or Col-0 *gl1* plants at day 3 (Fig. 3E). This observation raised the possibility
that accelerated disease symptoms may negatively affect *Pst* DC3000 growth and,
accordingly, the *noc1* mutant may allow better bacterial growth. However, we did not
observe an enhanced *Pst* DC3000 growth at day 3 in *noc1* plants (Figs. 1B and 4E). We
commonly use a 3-day period for assessing *Pst* DC3000 multiplication in the laboratory;
however, bacterial infection in the field involves longer durations. We therefore
extended our multiplication assay to 6 days, which led to an interesting finding. *Pst*
DC3000 populations declined in Col-0 *gl1* and *noc1/35S:AtSGR* plants, as infected
tissues senesced, whereas *Pst* DC3000 maintained a high population in the *noc1* plants
(Fig. 3E). These results suggest that disease symptom development restricts *Pst* DC3000
persistence in infected tissues.

**Effect of the *noc1* mutation on *A. brassicicola*-induced chlorosis**

To determine whether *noc1* plants are also affected in disease symptoms caused by a
fungal pathogen, Col-0 *gl1* and *noc1* plants were inoculated with spores of the
necrotrophic fungus *A. brassicicola*. A necrotic lesion developed at the site of inoculation
in Col-0 *gl1*, *noc1*, and complemented *noc1/35S:AtSGR* plants at 5 to 10 dpi. In some
experiments, a chlorotic halo, surrounding the necrotic lesion, may also develop within 5-
10 dpi in only Col-0 gl1 and noc1/35S:AtSGR plants (Fig. 4A). However, the chlorosis phenotype induced by A. brassicicola was variable between experiments and could not be quantified reproducibly. To quantify disease symptoms, we therefore measured necrotic lesion areas using Image J software. The area of necrotic lesion development was smaller in noc1 plants infected with A. brassicicola than that in Col-0 gl1 plants infected in the same manner (Fig. 4A and 4B). Fungus-induced disease necroses were restored in noc1/35S:AtSGR plants infected with A. brassicicola (Fig. 4A and 4B). Plants inoculated with buffer control (0.1% Tween 20) alone showed no signs of chlorosis or necroses (Fig. 4A). These results demonstrate that AtSGR1 is required for the development of disease symptoms caused by a necrotrophic pathogen.

*AtSGR is highly induced during Pst DC3000 and A. brassicicola infection*

Previous studies have shown that SGR expression is critical for initiation of developmentally regulated chlorophyll degradation in a number of plant species (Armstead et al. 2006; Armstead et al. 2007; Park et al. 2007; Ren et al., 2007). The requirement of AtSGR for disease chlorosis suggests that the expression of AtSGR might be induced during pathogen infection. To examine this possibility, we collected total RNA from H2O- and Pst DC3000-inoculated noc1 and Col-0 gl1 plants at 36, 48 and 60 hpi and performed northern blot analyses using an AtSGR-specific probe. We found that the AtSGR expression is strongly induced by Pst DC3000, but not H2O, in both Col-0 gl1 and noc1 plants at all sampled time points (Fig. 5A). Thus, AtSGR expression is regulated during Pst DC3000 infection and the noc1 mutation does not significantly affect this expression. RT-PCR analysis was also performed with RNA from A. brassicicola-infected leaves, showing that AtSGR expression was induced by fungal infection (Figs. 5B and S1).

We next investigated whether specific virulence factors of Pst DC3000 could induce the expression of AtSGR. COR is a well-known bacterial virulence factor that promotes the development of disease chlorosis in plants (Uppalapati et al. 2005; Uppalapati et al. 2007;
Ishiga et al. 2009). Recent studies have demonstrated that COR mimics the active form of the plant hormone jasmonate and directly targets the jasmonate receptor complex in which the COI1 F-box protein is a principal component (Katsir et al., 2008; Melotto et al., 2008a; Fonseca et al., 2009; Sheard et al., 2010). To determine whether COR could induce expression of AtSGR, we treated 8-day-old, Col-0 gl1 seedlings with buffer or 10 µM COR. Seedlings were collected at 3 hours post-treatment (hpt) and total RNA was isolated for each group. RT-PCR analysis showed that, like JAZ9 (a known COR/jasmonate-responsive gene; Thines et al., 2007), AtSGR expression was induced by COR (Figs. 6A and S2A). We next examined whether COR-induced expression of AtSGR requires COI1-dependent jasmonate signaling. As shown in Fig. 6A, COR could not induce AtSGR expression in the coi1 mutant (Feys et al., 1994). These results suggest that COR induction of AtSGR expression requires an intact jasmonate signaling pathway.

Finally, we examined whether COR production is necessary for Pst DC3000 to induce the expression of AtSGR. Col-0 gl1 plants were infected with Pst DC3000 or a COR-deficient mutant strain, DB29 (Uppalapati, et al. 2007). RT-PCR analysis revealed that expression of AtSGR is strongly induced by 48 hpi, continuing through 72 hpi, in Col-0 gl1 plants infected with either DB29 or Pst DC3000 (Figs. 6B and S2B), although expression of AtSGR was significantly higher in plants infected with DC3000 as compared to those infected with DB29. These data indicate that COR is sufficient but not necessary for Pst DC3000 to induce AtSGR expression during infection.

DISCUSSION

Development of disease symptoms represents the final stage of a pathogenic infection and is particularly destructive to the plant because it damages plant tissues, affecting not only crop productivity, but also crop marketability. Reduced disease symptoms, in the form of “disease tolerant cultivars,” could be exploited as a method of disease management. Despite its importance in disease, the molecular control of disease symptom production remains one of the least understood aspects of plant diseases.
Although ethylene and jasmonate signaling pathways are known to be important for \textit{Pst} DC3000-induced disease chlorosis (Bent et al., 1992; Uppalapati et al., 2011), it is not understood how these pathways ultimately impact chlorophyll homeostasis in infected tissues. Our isolation of the \textit{noc1} mutant and subsequent identification of \textit{AtSGR} gene begin to provide molecular insight into how \textit{Pst} DC3000 infection perturbs chlorophyll homeostasis and causes tissue chlorosis. Previous research has suggested that SGR-family proteins, once produced during senescence, enter the chloroplast and destabilize photosystem complexes, which seems to be a prerequisite for regulated chlorophyll degradation (Park et al., 2007). We found that \textit{Pst} DC3000 infection induces the expression of \textit{AtSGR} and transgenic constitutive expression of \textit{AtSGR} accelerates disease symptom development caused by \textit{Pst} DC3000 infection. These results indicate that pathogen-induced disease chlorosis results from activation of a key regulator of an endogenous, senescence-associated chlorophyll degradation program.

The identification and characterization of the \textit{noc1} mutant also raises several important issues regarding the relationship between disease symptom development and bacterial pathogenesis. First, our data show that, in the case of \textit{Pst} DC3000 infection at the inoculums of \(1 \times 10^6\) CFU/ml, the bacterial population reaches a maximum level at 3 dpi in both wild-type and \textit{noc1} plants. However, whereas the \textit{Pst} DC3000 population declined in wild-type Col-0 \textit{gl1} plants, it persisted at a high level for a longer time in \textit{noc1} mutant plants (Fig. 3). This finding has significant ramifications in terms of the role of disease symptom in bacterial pathogenesis. Disease symptoms are commonly believed to be a consequence of collateral damages caused by infection. However, our result suggests that this may not be the case: disease symptom development can play an active role in negatively impacting the persistence of hemi-biotrophic pathogens like \textit{Pst} DC3000 at late stages of pathogenesis. Interestingly, a similar result was observed in the study of XopD, a type III effector of \textit{Xanthomonas campestris} pv. \textit{vesicatoria} (Xcv), which delays symptom development and tissue degeneration in tomato via modulation of host transcriptional programming, resulting in increased pathogen multiplication (Kim et al. 2008).
Second, previous reports show that bacterial phytoxin COR induces chlorosis in plants and that COR-deficient mutants have reduced ability to cause chlorosis (Ma and Cuppels, 1991; Whalen et al. 1991; Uppalapati et al. 2005; Uppalapati et al. 2007). Consistent with these observations, we found that COR was sufficient to activate \textit{AtSGR} expression, providing a molecular basis for the involvement of COR in disease chlorosis. Additionally, COR-treated \textit{coi1} mutants showed no induction of \textit{AtSGR} expression (Fig. 6), suggesting that the jasmonate receptor complex is required for COR-induced expression of \textit{AtSGR}. Interestingly, we found a putative MYC2-binding motif (ACGTG) (Boter et al. 2004; Yadav et al. 2005) in the promoter region of \textit{AtSGR} at nucleotide position -23 (data not shown). MYC2 is a major transcription factor involved in jasmonate/COR-induced gene expression (Laurie-Berry et al. 2006, Lorenzo et al. 2004), further suggesting an involvement of jasmonate signaling in COR-mediated induction of \textit{AtSGR} expression. It should be pointed out, however, that in Arabidopsis, purified COR was shown to induce purpling, instead of chlorosis (Bent et al., 1992). The exact reason for this phenomenon is not known. Because COR structurally and functionally mimics jasmonate, which is known to induce anthocyanin production in Arabidopsis (Ellis and Turner 2001), it is possible that the chlorosis induced by COR in Arabidopsis is masked by purpling associated with anthocyanin production. In any case, although COR is sufficient for induction of \textit{AtSGR}, we found that it is not required for \textit{AtSGR} induction during infection with \textit{Pst} DC3000 (Fig. 6). Thus, it is likely that COR is not the only virulence factor in \textit{Pst} DC3000 that is involved in the development of disease chlorosis, but that the action of additional virulence factors (possibly type III effectors) may also be involved in the induction of \textit{AtSGR} and contribute to the production of disease chlorosis. Indeed, disease chlorosis is often observed in Arabidopsis mutants that allow high multiplication of COR-deficient \textit{Pst} DC3000 (Melotto et al. 2006, Zeng and He 2010).

Third, in addition to affecting disease chlorosis, the \textit{noc1} mutation also reduced disease necrosis caused by \textit{Pst} DC3000 and \textit{A. brassicicola} infection, although this effect is less obvious compared to that of disease chlorosis (Figs. 1 and 4). This is an interesting finding in light of a recent study that investigated the effect of \textit{AtSGR} overexpression and
RNAi-mediated suppression on the hypersensitive response (HR) elicited by *Pst* DC3000 (avrRpm1) in Arabidopsis (Mur et al. 2010). It was found that increased and decreased *AtSGR* expression, respectively, accelerated and suppressed the kinetics of HR-associated cell death in resistant Arabidopsis plants. Mur and colleagues postulate that some phototoxic chlorophyll catabolites contribute to HR cell death in resistant plants (Mur et al. 2010). If so, we speculate that such chlorophyll catabolites could also contribute to the formation of disease necrosis in susceptible Arabidopsis plants infected by *Pst* DC3000 or *A. brassicicola*, as observed in our study (Figs. 1 and 4).

The stay-green phenotype was first described in 1866 by Gregor Mendel in differentiating yellow and green cotyledon color in segregating populations of pea (Mendel 1866). *SGR* orthologues have been cloned from a wide range of dicot and monocot species (Armstead et al. 2006; Armstead et al. 2007; Jiang et al. 2007; Park et al. 2007; Sato et al. 2007; Aubry et al. 2008; Barry et al. 2008). Consequently, we hypothesize that pathogen-induction of *SGR* genes may be a common mechanism underlying disease chlorosis across a wide spectrum of plant-pathogen interactions. As such, further study of *SGR* genes and their regulation could lead to transgenic plants with not only controlled senescence, but also disease symptom expression, thereby benefiting agriculture.
MATERIALS AND METHODS

Plant material, mutagenesis and growth conditions

Approximately 1g of Arabidopsis thaliana ecotype Columbia-0 gl1 seeds was mixed with 100 ml of distilled water and 250 µl of ethylmethanesulfonate (EMS). The mixture was incubated overnight at room temperature in the dark with gentle agitation. The seeds were washed six times with 500 ml of distilled water, resuspended in 300 ml of 0.1% agarose and sown onto a soil mixture (equal portions of Baccto high-porosity professional plant mix, perlite and vermiculite, covered with a thin layer of fine vermiculite). The flats were covered with lids and incubated in the dark at 4°C for three days. The flats were then transferred to a growth chamber [20°C with 12 hours of fluorescent light (100 µEinsteins/m²/sec) and 12 hours of darkness]. The plants were self-fertilized to create a population of M2 plants.

Screening and isolation of Arabidopsis mutants

Four to six-week-old M2 plants were dipped in a 1x 10⁸ CFU/ml suspension of Pst DC3000 and 0.05% Silwet L-77 (Lehle Seeds, www.arabidopsis.com) for 2-3 seconds. The inoculated plants were incubated in high (80-90%) humidity conditions for 96 hours and screened for a lack of symptom development.

Bacteria enumeration in inoculated leaves of noc1 mutants and wild-type Col-0 gl1 plants

Four to five week-old plants were used for bacteria enumeration. Pst DC3000 was grown in low-salt Luria-Bertani broth to the mid-to-late logarithmic phase at 28°C. Bacterial cultures were pelleted and resuspended in sterile water to a final OD₆₀₀ of 0.2 [equivalent to 1 x 10⁸ colony-forming units (CFU)/ml] for dip-inoculation. Fully expanded leaves were dip-inoculated with bacterial suspensions. Plants were placed in trays with standing
water and covered with plastic wrap to maintain high humidity. During the experimental period there was no obvious tissue desiccation in inoculated plants. Bacteria enumeration followed the protocol described by Katagiri et al. (2002). P values were derived from multiplication data utilizing Microsoft Excel software for t-test statistical analysis.

**Alternaria brassicicola infection**

We inoculated 10 leaves of each plant with 10 µl of *A. brassicicola* spores at a concentration of 6.4 x 10^5 spores/ml suspended in 0.1% Tween 20. For control inoculations we used 0.1% Tween 20. Plants were covered with humidity domes and kept at high humidity throughout the infection process.

For quantification of disease symptoms caused by fungal infection, the areas of necrotic lesions caused by *Alternaria* inoculation were measured on 30-infected plant leaves. The average lesion area was calculated from 30-50 lesions at 5 and 8 dpi using Image J software. Older leaves were excluded from the sample set to avoid senescence-associated chlorosis and necrosis.

*AtSGR* expression was measured in *A. brassicicola*-infected tissue at 0 and 5 days post-inoculation (dpi). RNA was isolated from leaf tissue using an RNAeasy Kit (Qiagen, www.qiagen.com). Semi-quantitative RT-PCR was performed as described by the manufacturer (Takara, www.takara-bio.com).

**Chlorophyll extraction and quantification**

For chlorophyll abundance assays we infiltrated leaf tissue with 2 x 10^6 CFU/ml *Pst DC3000 and collected samples at 0, 24, 48, 72 and 96 hours post-inoculation. All chlorophyll extraction steps were conducted in near darkness. Leaf disks from four separate leaves at each time point were frozen in liquid nitrogen and stored at 80˚C. The frozen tissue was homogenized in 600 µl of 80% acetone, the homogenates were
centrifuged at 500 xg for three minutes at 4°C, and the supernatant was transferred to a new tube and kept on ice. The absorbance of four dilutions (1:10, 1:5, 1:3 and 1:2.5) of each sample was determined using a spectrophotometer. The amount of chlorophyll was calculated as previously described (Arnon 1949).

**Gene mapping**

Mapping of the *noc1* mutation was conducted as described by Lukowitz and colleagues (Lukowitz et al. 2000). Initial genome-wide screening was conducted using an array of primers (Invitrogen, www.invitrogen.com) to detect simple sequence length polymorphisms (SSLPs) from each chromosome. Additional insertions and deletions (INDELs), identified in the Monsanto Arabidopsis polymorphism and Ler sequence collection (www.arabidopsis.org/browse/Cereon/index.jsp), were used to further define the region containing the *noc1* mutation (Jander et al. 2002).

Amino acid sequences of *AtSGR* and its orthologues were aligned using ClustalW (www.ddbj.nig.ac.jp/search/clustalw-j.html) and were then adjusted manually. Complementation of the *noc1* phenotype was accomplished by transforming *noc1* plants with 35S:*AtSGR* (At4G22920) in binary vector pBAR-35S (provided by J. Dangl, University of North Carolina, Chapel Hill). The cDNA was amplified from a pool of total Col-0 *gl1* RNA using LA *Taq™* Polymerase (Takara) and *AtSGR*-specific primers carrying *XhoI* (5’) and *SpeI* (3’) restriction sites (F:ggctcgagatgtgtagtttgtcggc and R: ggactagtctagagtttctccggatt) to facilitate eventual cloning into pBAR-35S. The PCR product was first cloned into pTOPO 2.1 (Invitrogen) and the *AtSGR* insert sequenced. The sequence-verified *AtSGR* insert was then transferred into the *XhoI* and *SpeI* sites of the binary vector pBAR-35S. Plant transformation was conducted as described (Clough and Bent 1998). Transformants were screened in the T1 and T2 generations for Basta herbicide resistance and in T2 and T3 generations for the presence of the *AtSGR* transgene using RT-PCR with primers specific to the 5’ and 3’ UTRs (pBAR1 vector sequence) of the transgene: F-ggattgtgtgtatctccac and R-caagagcgcaacagatcc.
Northern blotting

For northern blotting analysis, total RNA was isolated from Arabidopsis leaves using the RNAgents total RNA isolation system (Promega, www.promega.com). The RNA concentration was determined based on absorbance at 260 nm, and RNA was separated on 2% formaldehyde agarose gels. The RNA was blotted onto Hybond N+ nylon membranes (Amersham, www.gelifesciences.com) using 10 x SSC, and UV-crosslinked using a Stratalinker (Stratagene, www.genomics.agilent.com) with the auto-crosslink setting. Approximately 100 ng of a 300-bp exon-coding fragment, from AtSGR cDNA, was amplified by PCR using the following primers: F:atgtgtagttgtcggcgat and R:cccggtatagcctatttgcc. The resulting fragment was purified using the Qiagen Gel Extraction Kit (Qiagen) and labeled with 32P-CTP using random hexamers. Blotted membranes were hybridized with labeled probes using PerfectHyb Plus hybridization buffer (Sigma-Aldrich, www.sigmaaldrich.com), following the manufacturer's protocol, and then washed in 0.5 x SSC and exposed to X-ray film for ~16 hours.

Quantitative Real-Time PCR (qRT-PCR)

Total RNAs were isolated from Arabidopsis leaves using the RNAgents total RNA isolation system (Promega, www.promega.com). Poly d(T) complementary DNA was prepared from 500 ng total RNA using M-MLV reverse transcriptase (Invitrogen) and quantified on an ABI7500 Fast Real-Time PCR system (Applied Biosystems) with the Fast SYBR Green Master kit (Applied Biosystems) according to Manufacture’s protocols. Expression levels were normalized to AtPP2AA3 (PROTEIN PHOSPHATASE 2A SUBUNIT A, At1g13320) (Czechowski et al. 2005). The primers used for RT-PCR were: AtSGR, 5’- ACTACCTGTGGTGTTGAAGG-3’ and 5’- CGACTTTGTGTTAACTCATTGAC-3’, AtPP2AA3 5’- GGTACAAGACAAGGTTCACCTTC-3 and 5’- CATTCAGGACCAACTCTTCAG-3’,
and AtJAZ9 (At1g70700) 5’-ATGAGGTAAACGATGATGCTG-3 and 5’-CTTAGCCTCTGGAAAATCTG-3’.

**Induction of AtSGR expression by coronatine**

Arabidopsis seeds (Col-0 gl1 and coi1) were germinated and grown on Murashige and Skoog (MS) agar plates for 4 days with coi1 seedlings being selected on 10 μM methyl jasmonate (Sigma-Aldrich). Seedlings were transferred to fresh MS plates and grown for another 4 days and were treated with 10 μM COR (Sigma-Aldrich) or 0.1% EtOH by spraying. Seedlings were harvested at 3 hours post-treatment and total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen) per manufacturer’s instructions. cDNA synthesis of AtSGR, JAZ9, and UBC was completed using avian reverse transcriptase and oligo-DT primers followed by RT-PCR using gene-specific primers (AtSGR: F: 5’atgtgtagtttgtcggcgat3’ and R: 5’ctagagtttctccggatttg3’; JAZ9: F: 5’atggaaagagattttctgggtttg3’ and R: 5’tatataggagaagtagaagagta; UBC21: F: 5’tcaaatggacgctcttatc3’ and R: 5’cacagactgaagcgtccaa3’).

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FIGURE LEGENDS

**Figure 1.** The phenotypes of the *noc1* mutant following *Pst* DC3000 infection. (A) *Arabidopsis thaliana* Col-0 *gl1* plant exhibiting typical chlorosis and water-soaking phenotype associated with *Pst* DC300 infection at 72 hpi (left); *noc1* mutants display water soaking but little chlorosis following *Pst* DC3000 infection (right). (B) Col-0 *gl1* and *noc1* plants were dip-inoculated with *Pst* DC3000 at $10^8$ cfu/ml. Bacterial populations were determined at 1 and 3 days post-inoculation (dpi). (C) Chlorophyll amounts in the *noc1* mutant and wild-type Col-0 *gl1* subsequent to *Pst* DC3000 infection at 0, 24, 48, 72 and 96 hpi.

**Figure 2.** Map-based identification of the *noc1* mutation. (A) A portion of the long arm of Arabidopsis chromosome 4 where the *noc1* mutation is located between the two INDEL markers (T12H17-13C and F16G20-22; indicated by black arrowheads) at chromosomal position (cp) 11963745 and 12254730, respectively. (B) Gene structure of *AtSGR* (At4g22920; cp: 12016525-12018492), showing the location of the *noc1* mutation, an aspartate (D) to tyrosine (Y) substitution at position 88 resulting from a guanine to thymidine nucleotide mutation. (C) The residue D$^{88}$ is conserved among *SGR* orthologues in diverse plants, including *Zea mays* (gi 58866285), *Sorghum bicolor* (gi 59506606), *Oryza sativa* (gi 58866281), *Horde vulgur* (gi 58866283), and *Glycine max* (gi 58866291). Orthologous sequences were arranged using CLUSTALW software.

**Figure 3.** Complementation of the *noc1* mutation by 35S:*AtSGR*. Disease symptoms on Col-0 *gl1* (A), *noc1* (B), and *noc1/35S:*AtSGR line #1 (C) leaves 4 days after dip-
inoculation with \( Pst \) DC3000 at \( 1 \times 10^8 \) CFU/ml. (D) RT-PCR using \( AtSGR \)-specific primers showing native expression of endogenous \( AtSGR \) in \textit{Col-0 gl1} and \textit{noc1} plants and increased expression in \textit{noc1} lines complemented with \( 35S:AtSGR \) transgene (top). RT-PCR using primers utilizing transgene-specific primers (see Experimental Procedures) showing expression of \( 35S:AtSGR \) only in complemented \textit{noc1} lines (middle). RT-PCR of \( ACTIN8 \) as a loading reference (bottom). (E) \( Pst \) DC3000 multiplication assay. \textit{Col-0 gl1}, \textit{noc1}, and \textit{noc1/AtSGR} plants (line #1) were dip-inoculated with \( Pst \) DC3000 at \( 10^8 \) cfu/ml. Bacterial populations were determined at 1, 3 and 6 days post-inoculation (dpi). \( t \)-tests were performed in Microsoft Excel using a two-sample, equal variance formula. Comparisons are between \textit{Col-0 gl1} and \textit{noc1} and between \textit{Col-0 gl1} and \textit{noc1/AtSGR} at each time point. \( P \) values of <0.05 are indicated with a single “*”.  

\textbf{Figure 4.} Effects of the \textit{noc1} mutation on \textit{Alternaria brassicicola}-induced disease symptoms. (A) Upper panels: Chlorosis and/or necrosis caused by \textit{A. brassicicola} infection in \textit{Col-0 gl1} (left), the \textit{noc1} mutant (center), and \textit{noc1/35S:AtSGR} (right) leaves 8 days post-infiltration (dpi). Lower panels: Mock inoculations with 0.1% Tween 20 are shown. (B) Necrotic lesion areas were measured at 5 and 8 dpi in \textit{A. brassicicola}-infected \textit{Col-0 gl1}, \textit{noc1} and \textit{noc1/AtSGR} plants using ImageJ software. \( t \)-tests were performed in Microsoft Excel using a two-sample, equal variance formula. Comparisons are between \textit{Col-0 gl1} and the \textit{noc1} mutant and between \textit{Col-0 gl1} and \textit{noc1/35S:SGR} at each time point. \( P \) values of <0.05 are indicated with a single “*”, \( P \) values <0.005 are indicated by “**”.
**Figure 5.** Induction of $AtSGR$ expression during infection. (A) Northern blot analysis of $AtSGR$ expression in $Pst$ DC3000-infected Col-0 $gl1$ and $noc1$ mutant plants. 26S ribosomal RNA visualized by ethidium bromide staining was used as a loading reference. (B) qRT-PCR analysis of $AtSGR$ expression in mock- and *Alternaria*-infected Col-0 $gl1$ plants at 0 dpi and 5 dpi. Error bars represent standard deviations from three biological replicates. The experiment was repeated once and similar results were obtained. Asterisks indicate significant difference (P<0.05) between mock- and *Alternaria*-infected Col-0 $gl1$ plants using the Student’s t test.

**Figure 6.** Role of COR in the induction $AtSGR$ expression. (A) qRT-PCR showing $AtSGR$ and $AtJAZ9$ expression in mock and COR treated WT and $coi1$ background plants. Error bars represent standard deviation from three biological replicates. (B) $AtSGR$ expression is induced by 48 hpi in Col-0 $gl1$ plants infected with $Pst$ DC3000 (COR+) or DB29 (COR-). qRT-PCR shows $AtSGR$ and $AtJAZ9$ expression in mock and *P. syringae* infected Col-$gl1$ plants. Error bars represent standard deviations from three biological replicates. Experiments were repeated once and similar results were obtained. Asterisks indicate significant difference (P<0.05) when comparing bacterial infections to mock inoculations using the Student’s t test.
SUPPLEMENTARY FIGURE LEGENDS

Figure S1. RT-PCR analysis of AtSGR expression in Alternaria-infected Col-0 gl1, the noc1 mutant and noc1/AtSGR plants at 0 dpi and 5 dpi. RT-PCR of constitutively expressed UBC was used as a control.

Figure S2. (A) Semi-quantitative RT-PCR showing levels of expression for AtSGR and JAZ9 in Col-0 seedlings 3 hours after treatment with 10 µM COR (+) or buffer (-). COR induced expression of both AtSGR and JAZ9. In contrast, no induction of AtSGR or JAZ9 expression was observed in the coi1 mutant. RT-PCR of constitutively expressed UBC was used as a loading reference. (B) Northern blot showing that AtSGR expression is induced by 48 hpi in Col-0 gl1 plants infected with Pst DC3000 (COR+) or DB29 (COR-). ACTIN8 transcript was used as a loading reference.
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