Activation of Plant Immune Responses by a Gain-of-Function Mutation in an Atypical Receptor-Like Kinase

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Arabidopsis (Arabidopsis thaliana) suppressor of npr1-1, constitutive1 (snc1) contains a gain-of-function mutation in a Toll/interleukin receptor-nucleotide binding site-leucine-rich repeat Resistance (R) protein and it has been a useful tool for dissecting R-protein-mediated immunity. Here we report the identification and characterization of snc4-1D, a semidominant mutant with snc1-like phenotypes. snc4-1D constitutively expresses defense marker genes PR1, PR2, and PDF1.2, and displays enhanced pathogen resistance. Map-based cloning of SNC4 revealed that it encodes an atypical receptor-like kinase with two predicted extracellular glycerophosphoryl diester phosphodiesterase domains. The snc4-1D mutation changes an alanine to threonine in the predicted cytoplasmic kinase domain. Wild-type plants transformed with the mutant snc4-1D gene displayed similar phenotypes as snc4-1D, suggesting that the mutation is a gain-of-function mutation. Epistasis analysis showed that NON-RACE-SPECIFIC DISEASE RESISTANCE1 is required for the snc4-1D mutant phenotypes. In addition, the snc4-1D mutant phenotypes are partially suppressed by blocking jasmonic acid synthesis, suggesting that jasmonic acid plays an important role in snc4-1D-mediated resistance. Identification of snc4-1D provides us a unique genetic system for analyzing the signal transduction pathways downstream of receptor-like kinases.

Receptor-like kinases (RLKs) are a large group of kinases with a variable extracellular domain and a cytoplasmic kinase domain linked by a single transmembrane motif. RLKs have been shown to play diverse roles in regulating plant innate immunity as well as growth and development (Morillo and Tax, 2006). The extracellular domains of RLKs are believed to bind directly to ligands to perceive extracellular signals, whereas the cytoplasmic kinase domains transduce these signals into the cell. There are over 600 RLKs (Shiu and Bleecker, 2001) in Arabidopsis (Arabidopsis thaliana). The biological functions of most RLKs are unknown.

Several RLKs have been identified to be receptors of microbe-associated molecular patterns (MAMPs). FLS2 and EFR are two well-characterized RLKs with extracellular Leu-rich repeats (LRRs) that recognize bacterial flagellin and translation elongation factor EF-Tu, respectively (Gomez-Gomez and Boller, 2000; Zipfel et al., 2006). BAK1 is also an RLK with extracellular LRRs. BAK1 seems to function as an adaptor protein for multiple RLKs including BR1L, FLS2, and BIR1 (Li et al., 2002; Nam and Li, 2002; Chinchilla et al., 2007; Heese et al., 2007; Gao et al., 2009). Interestingly, knocking out BIR1 activates cell death and defense responses mediated by another RLK, SOBIR1 (Gao et al., 2009). Recently, the rice (Oryza sativa) RLK Xa21 was also suggested to be a MAMP receptor. Xa21 recognizes a peptide derived from the secreted effector protein AvrXa21, which is conserved among different Xanthomonas species (Lee et al., 2009). Unlike FLS2, EFR, and Xa21, the putative receptor for chitin is an RLK with three extracellular LysM domains instead of LRRs that are required for the perception of chitin as well as resistance against bacterial pathogens (Miya et al., 2007; Wan et al., 2008; Gimenez-Ibanez et al., 2009).

Perception of MAMPs by receptors leads to the rapid activation of mitogen-activated protein (MAP) kinases including MAP KINASE3 (MPK3), MPK4, and MPK6 (Boller and Felix, 2009). MAP KINASE SUBSTRATE1 (MKS1) was identified as an MPK4-interacting protein that positively regulates defense responses (Andreasson et al., 2005). Silencing of MKS1...
compromises basal resistance to *Pseudomonas syringae pv tomato* DC3000, whereas overexpression of MKS1 leads to enhanced pathogen resistance. Activation of MAMP receptors also induces a number of responses such as oxidative burst, callose deposition, and increased salicylic acid synthesis (Boller and Felix, 2009). Defense responses induced by different MAMP’s seem similar, suggesting that they may share common signaling components. Identification of the signaling components downstream of RLK receptors remains a major task in understanding MAMP-triggered immunity.

Here we report the identification and characterization of *suppressor of npr1-1, constitutive4-1D (snc4-1D)*, a gain-of-function mutant of an atypical RLK that is autoactivated by a mutation in its kinase domain. The *snc4-1D* mutant plants constitutively express defense marker genes *PR1*, *PR2*, and *PDF1.2*, and display enhanced resistance to *Hyaloperonospora arabidopsidis* (*H. a.*) Noco2. Epistasis analysis showed that *snc4-1D*-mediated defense responses are dependent on multiple factors including NON-RACE-SPECIFIC DISEASE RESISTANCE1 (NDR1), MKS1, and OPR3.

**RESULTS**

**Identification and Characterization of *snc4-1D***

Our lab has been interested in identifying novel negative regulators of plant immunity (Gao et al., 2008). Loss-of-function mutations in these negative regulators would yield mutants with enhanced pathogen resistance and dwarfism that is usually associated with strong resistance phenotypes. Very rarely, gain-of-function mutations in positive regulators in the same pathways would yield mutants with similar phenotypes, such as in *snc1* (Li et al., 2001). When we screened an ethyl methanesulfonate (EMS)-mutagenized population in the Columbia-0 (Col-0) background for mutants with enhanced resistance against the oomycete pathogen *H. a.* Noco2, one of the mutants, designated as *snc4-1D*, exhibited snc1-like dwarfism (Fig. 1A) and strong resistance to the virulent pathogen *H. a.* Noco2 (Fig. 1B). Plants heterozygous for *snc4-1D* have intermediate sizes, suggesting the mutant is semidominant and the mutation is most likely a gain-of-function mutation in a positive regulator of defense. Real-time reverse transcription (RT)-PCR analysis showed *snc4-1D* constitutively expresses defense marker genes *PR1* (Fig. 1C), *PR2* (Fig. 1D), and *PDF1.2* (Fig. 1E). These data suggest that defense responses are constitutively activated in *snc4-1D*.

Mutants with enhanced disease resistance often accumulate high levels of hydrogen peroxide (H$_2$O$_2$) and exhibit spontaneous cell death. To test whether *snc4-1D* accumulates H$_2$O$_2$, 3, 3’-diaminobenzidine (DAB) staining was performed on the *snc4-1D* mutant seedlings. Strong staining was observed in *snc4-1D* mutant seedlings (Fig. 1F), indicating that *snc4-1D* accumulated high levels of H$_2$O$_2$ compared to wild type. In addition, microscopic cell death was also observed in the *snc4-1D* mutant from trypan blue staining (Fig. 1G).

**Map-Based Cloning of *snc4-1D***

To map the *snc4-1D* mutation, *snc4-1D* was crossed with Landsberg erecta (*Ler*). Plants with *snc4-1D* morphology were selected from the F2 progeny and used for crude mapping. The mutation was initially mapped to a region between F1O11 and F20P5 on chromosome 1 (Fig. 2A). Further analysis of approximately 1,100 F2 plants flanked the mutation to a 35-kb region between marker T4O24 and F1O19. The coding sequence of genes in the region were amplified by PCR from the genomic DNA of *snc4-1D* and sequenced. A single G to A mutation was found in one of the sequenced genes, *At1g66980* (Fig. 2B). Previously it was reported that a knockout mutant of *At1g66980* displayed wild-type morphology (Hayashi et al., 2008), suggesting that the mutant phenotypes observed in *snc4-1D* is not likely caused by loss of function of the protein encoded by *At1g66980*.

*At1g66980* encodes a predicted RLK (Fig. 2C). The extracellular region of this protein contains two putative glycerophosphoryl diester phosphodiesterase (GDPD) domains. The protein also has a predicted N-terminal signal peptide and a single transmembrane domain between the cytoplasmic kinase domain and the extracellular GDPD domains. Originally identified in *Escherichia coli*, GDPD catalyzes the hydrolysis of a glycerophosphodiester to an alcohol and glycerol 3-P (Larson et al., 1983). GDPD activity was also detected in cell wall fractions of carrot (*Daucus carota*; Van Der Rest et al., 2004). Blast analysis failed to identify any other RLK with extracellular GDPD domains in Arabidopsis and other plants, suggesting that *At1g66980* probably evolved recently through combining its GDPD and kinase domains. To confirm that the GDPD and kinase domains are encoded in the same gene, the size of the *SNC4* cDNA is determined by RT-PCR (Supplemental Fig. S1A). In addition, western-blot analysis showed that the SNC4-GFP fusion protein expressed in transgenic plants has a mass of about 150 kD, close to the predicted size of the fusion protein (Supplemental Fig. S1B).

The SNC4-GFP fusion protein localized to the plasma membrane (Supplemental Fig. S2), suggesting that SNC4 is a plasma membrane protein as predicted. The mutation in *snc4-1D* changes the Ala 850 to Thr in the kinase domain of the protein, which does not change the localization of the protein (Supplemental Fig. S2).

**Complementation**

Since T-DNA knockout mutants of *At1g66980* did not display *snc4-1D* phenotypes, we deduced that the Ala-850 to Thr mutation is most likely a gain-of-function mutation in *At1g66980*. If that is the case, transforming the mutant version of *At1g66980* in wild-
type background would yield plants with snc4-1D phenotypes. When we transformed constructs expressing the wild-type or mutant protein of At1g66980 under the control of its native promoter into wild-type plants, all transgenic lines obtained for the wild-type SNC4 construct displayed wild-type morphology. When we transformed constructs expressing the wild-type or mutant protein of At1g66980 under the control of its native promoter into wild-type plants, all transgenic lines obtained for the wild-type SNC4 construct displayed wild-type morphology. In contrast, about half of the transgenic lines with the mutant snc4-1D construct exhibit snc4-1D-like morphology (Fig. 3A). Real-time PCR analysis of defense marker genes PR1, PR2, and PDF1.2 showed that these marker genes were constitutively expressed in the transgenic plants carrying the mutant snc4-1D gene but not in the transgenic plants expressing the wild-type SNC4 gene (Fig. 3, B–D). In addition, transgenic plants expressing the mutant At1g66980 displayed enhanced resistance to H. a. Noco2 (Fig. 3E). Progeny from a representative line expressing the wild-type or mutant gene were further analyzed for the accumulation of H2O2 and cell death. As shown in Supplemental Figure S3, strong DAB and trypan blue staining was observed in the transgenic line carrying the mutant snc4-1D transgene but not in the line with the wild-type SNC4 transgene. Thus, all the snc4-1D mutant phenotypes can be recaptured by expressing the At1g66980 mutant gene in wild-type plants, indicating that SNC4 is At1g66980 and the mutation in snc4-1D is a gain-of-function mutation that activates downstream defense responses.

Analysis of Intragenic Suppressors of snc4-1D

To identify mutants that suppress the snc4-1D mutant phenotypes, we mutagenized snc4-1D seeds with EMS and screened for mutants with wild-type morphology in the M2 population. Twenty-one snc4-1D suppressor mutants were obtained from the screen. Because snc4-1D seems to be a gain-of-function mutation, we hypothesized that some of the mutants recovered may be intragenic suppressors of snc4-1D. Sequence analysis of the SNC4 locus identified six mutants containing mutations in snc4-1D (Fig. 4A). Among these mutations, one is a missense mutation in one of the GDPD domains, two are missense mutations in the kinase domain, and the rest are nonsense mutations that cause early truncations of the protein (Fig. 4B). Real-time RT-PCR analysis showed that suppression of the snc4-1D mutant morphology correlates with reduced expression of PR1, PR2, and PDF1.2 (Fig. 4, C–E). These data further confirm that At1g66980 is SNC4 and snc4-1D is a gain-of-function mutation.

NDR1 Is Required for the Mutant Phenotypes of snc4-1D

Arabidopsis NDR1 is generally required for resistance mediated by coiled-coil-nucleotide binding site (NB)-LRR Resistance (R) proteins, whereas EDS1 is required for resistance mediated by many Toll/inter-
leukin receptor-NB-LRR R proteins (Century et al., 1995; Parker et al., 1996; Aarts et al., 1998). To test whether snc4-1D activates NDR1 or EDS1-dependent signaling pathways, snc4-1D was crossed with ndr1-1 or eds1-2 (backcrossed into Col background). As shown in Figure 5A, the snc4-1D mutant morphology was largely suppressed by ndr1-1 but not by eds1-2. Analysis of PR1 and PR2 expression in the double mutants showed that the constitutive expression of both PR1 and PR2 in snc4-1D was largely blocked by ndr1-1. In contrast, PR1 and PR2 expression were only partially affected by the eds1-2 mutation (Fig. 5, B and C). The elevated PDF1.2 expression in snc4-1D is partially suppressed by ndr1-1 and to a lesser extent by eds1-2 (Fig. 5D). We further tested whether resistance to H. a. Noco2 in snc4-1D is affected in the double mutants. As shown in Figure 5E, resistance to H. a. Noco2 was reduced in both snc4-1D ndr1-1 and snc4-1D eds1-2.

**MKS1 Is Required for snc4-1D-Mediated Resistance Responses**

RLKs that serve as MAMP receptors, such as FLS2 and EFR, signal through MAP kinase cascades (Boller and Felix, 2009). If snc4-1D signals through a similar cascade, one would expect that mutation in a positive regulator of the MAP kinase pathway would block snc4-1D-mediated immunity. Arabidopsis MKS1 is a positive regulator of defense responses that interacts

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**Figure 2.** Map-based cloning of snc4-1D. A, Map of the snc4-1 locus. B, Exon/intron structure of SNC4. The coding regions are indicated with boxes. snc4-2, Salk_122292; snc4-3, WiscDsLox444C5. C, Predicted protein structure of SNC4. TM, Transmembrane motif; PTK, protein Tyr kinase. The locations of the mutations in SNC4 are as indicated. aa, Amino acids.

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**Figure 3.** Complementation analysis of snc4-1D. A, Morphology of wild-type (WT), snc4-1D, WT::SNC4 (wild type transformed with a SNC4 genomic clone), and WT::snc4-1D (wild type transformed with a snc4-1D genomic clone) transgenic lines. All plants were grown on soil and photographed when they were 3 weeks old. B to D, Expression of PR1 (B), PR2 (C), and PDF1.2 (D) in wild-type, snc4-1D, WT::SNC4, and WT::snc4-1D transgenic lines. Values were normalized to the expression of ACTIN1. Error bars represent ± so from averages of three measurements. E, Growth of H. a. Noco2 on wild-type, snc4-1D, WT::SNC4, and WT::snc4-1D transgenic lines. Infection was performed and scored as in Figure 1B. The values presented are averages of three replicates ± so. [See online article for color version of this figure.]
with MPK4 (Andreasson et al., 2005). When we made the snc4-1D mks1-1 double mutant, the morphological phenotypes of snc4-1D were largely suppressed by mks1-1 (Fig. 6A). Real-time RT-PCR analysis showed that the elevated expression of PR1 in the snc4-1D single mutant was blocked in the snc4-1D mks1-1 double mutant plants (Fig. 6B). In contrast, the expression of PR2 and PDF1.2 expression in snc4-1D is only modestly affected by mks1-1 (Fig. 6, C and D). Analysis of resistance to H. a. Noco2 showed that the enhanced resistance observed in snc4-1D was largely suppressed by mks1-1 (Fig. 6E).

JA Is Required for snc4-1D-Mediated Resistance Responses

Since snc4-1D constitutively expresses PDF1.2, a defense marker gene of the jasmonic acid (JA) pathway, we tested whether JA is required for the snc4-1D mutant phenotypes by crossing snc4-1D into opr3-2, a loss-of-function mutant of 12-oxo-phytodienoic acid reductase 3 that is required for jasmonate biosynthesis (Stintzi and Browse, 2000). As shown in Figure 7A, the snc4-1D opr3-2 double mutant has intermediate size compared to the wild-type and snc4-1D mutant plants, suggesting that opr3-2 partially suppresses the snc4-1D mutant phenotype. PDF1.2 expression in snc4-1D was dramatically lowered by opr3-2 (Fig. 7B). Surprisingly, the expression of PR1 (Fig. 7C) and PR2 (Fig. 7D) in snc4-1D is also largely suppressed by opr3-2. When snc4-1D opr3-2 plants were challenged by H. a. Noco2, the pathogen grew much better on the double mutant than on snc4-1D, but at a lower level than on wild-type plants and opr3-2, suggesting that JA is required for pathogen resistance activated by snc4-1D (Fig. 7E).

DISCUSSION

Previous studies have identified several gain-of-function mutations in NB-LRR R genes that lead to autoactivation of defense responses (Shirano et al., 2002; Zhang et al., 2003a; Noutoshi et al., 2005; Igari et al., 2008). One of the mutants, snc1, has been used to identify components involved in R-protein activation (Palma et al., 2005, 2007; Zhang et al., 2005; Zhang and Li, 2005; Goritschnig et al., 2007, 2008; Cheng et al., 2009). The snc4-1D mutation reported here results in activation of an atypical RLK with an unusual extracellular region consisting of two GDPD domains. SNC4 appears to be a unique RLK with no clear orthologs in other plant species. Its kinase domain is

Figure 4. Characterization of the intragenic suppressors of snc4-1D. A, Morphology of wild-type (WT), snc4-1D, and the intragenic suppressor mutant plants. Plants were grown on soil at 23°C and photographed 3 weeks after planting. B, Mutations identified in the intragenic suppressor mutant alleles. C to E, Expression of PR1 (C), PR2 (D), and PDF1.2 (E) in wild type, snc4-1D, and the suppressor mutants of snc4-1D. Values were normalized to the expression of ACTIN1. Error bars represent so from averages of three measurements. aa, Amino acid. [See online article for color version of this figure.]
most similar to RLKs in the LRK10L-2 subfamily (Shiu and Bleecker, 2001). Functions of RLKs in this subfamily are unknown. The mutation in snc4-1D is located in an unconserved region within subdomain III of the kinase. No similar gain-of-function mutations in other RLKs have been previously identified to constitutively activate downstream signaling pathways. The mechanism on how the mutation in snc4-1D activates defense responses remains to be determined.

In Arabidopsis, there are six GDPD domain-containing proteins that are closely related to the extracellular domain of SNC4 (Supplemental Fig. S4).
SNC4 either. It suggests that SHV3, SNC4, and related proteins may not have the same enzymatic activity as the GDPD in *E. coli*.

The GDPD domain of SNC4 could be involved in perception of lipid-derived molecules from microbial pathogens or damage responses. Interestingly, one of the intragenic suppressors of *snc4-1D (snc4-4)* contains a mutation in the GDPD domain. Western-blot analysis showed that this mutation does not affect the stability of the protein (Supplemental Fig. S5), suggesting that the GDPD domain may be important for the function of SNC4. It is unclear how the *snc4-4* mutation affects the function of the protein. Additional mutagenesis analysis of the GDPD domain of SNC4 will help us better understand its function.

To analyze downstream signaling pathways activated in *snc4-1D*, we carried out epistasis analysis between *snc4-1D* and several known mutants involved in pathogen resistance. One unexpected finding is that the mutant morphology as well as activation of defense gene expression and pathogen resistance in *snc4-1D* is suppressed by *ndr1-1*, a mutant previously known to be defective in resistance mediated by a number of coiled-coil-NB-LRR R proteins (Century et al., 1995). In contrast, the *eds1-2* mutation does not affect the mutant morphology and *PR2* expression in *snc4-1D*. However, resistance to *H. a.* Noco2 and expression of *PR1* are reduced in the *snc4-1D eds1-2* double mutant. Since EDS1 is also required for basal resistance (Parker et al., 1996), reduced resistance to *H. a.* Noco2 could be caused by lowered basal resistance by *eds1-2*.

Another mutation that partially suppresses the *snc4-1D* mutant phenotypes is *mks1*. MKS1 has previously been shown to be a positive regulator of defense responses, where it is phosphorylated in response to bacterial pathogen infections (Andreasson et al., 2005). Our results suggest that MKS1 functions downstream of the RLK SNC4, suggesting that MAP kinase signaling could participate in *snc4-1D*-mediated immunity. It will be interesting to determine whether *snc4-1D* activates MKS1 through a MAP kinase cascade that functions downstream of the known MAMP receptors.

Epistasis analysis showed that JA-dependent resistance pathways are activated in *snc4-1D*. Blocking JA synthesis suppresses the morphological phenotypes as well as constitutive expression of *PDF1.2*, *PR1*, and *PR2* in *snc4-1D*. While it is known that expression of *PDF1.2* is regulated by JA, activation of *PR1* and *PR2* are often associated with salicylic acid-induced defense response. How JA modulates the expression of *PR1* and *PR2* in *snc4-1D* remains to be determined.

Based on our data, a working model is proposed (Supplemental Fig. S6). SNC4 probably encodes a pathogen-associated molecular pattern or damage-associated molecular pattern receptor candidate. Perception of the pathogen-associated molecular pattern or damage-associated molecular pattern signal leads to activation of SNC4 and downstream defense pathways. Since mutations in either *NDR1* or *OPR3* par-
tially suppress the mutant phenotypes of \textit{snc4-1D} and these mutations have different effects on the expression of defense marker genes, NDR1 and OPR3 may function in parallel downstream of SNC4. The stronger effect of \textit{ndr1-1} on PR1 and PR2 expression than \textit{mks1-1} suggests that NDR1 may function in earlier events of the signaling pathways comparing to MKS1. We tentatively placed NDR1 upstream of the MAP kinase cascade. Additional studies are required to identify the upstream signal of SNC4 and to better define the relationships between NDR1, OPR3, MKS1, and MAP kinase cascades activated in \textit{snc4-1D}.

In conclusion, we have identified a unique gain-of-function mutant of an atypical RLK. Epistasis analysis showed that \textit{snc4-1D} activates multiple downstream resistance pathways. Our results suggest that \textit{snc4-1D} could be utilized as a powerful tool for genetic analysis of resistance pathways activated by RLKs. Further characterization and cloning of the \textit{snc4-1D} extragenic suppressors may lead to better understanding of signal transduction downstream of RLKs.

MATERIALS AND METHODS

Plant Growth Conditions and Mutant Screens

All plants were grown under 16 h light at 23°C and 8 h dark at 20°C. To screen for mutants with \textit{snc1}-like constitutive defense responses, wild-type Col-0 seeds were mutagenized with EMS and the M2 progeny were analyzed for enhanced resistance to \textit{H. a.} Noco2. \textit{snc4-1D} was one of mutants identified. To search for suppressors of \textit{snc4-1D}, seeds of \textit{snc4-1D} were obtained by growing the plants at 28°C and mutagenized with EMS. About 12,000 M2 plants were screened to identify mutants that can grow to maturity and set seeds at 22°C.

Mutant Characterization

Two-week-old seedlings grown on one-half-strength Murashige and Skoog medium were used for gene expression analysis, trypan blue, and DAB staining. For gene expression analysis, RNA was extracted using Takara RNAliso reagent (Takara) and RT was performed using the Takara AMV RTase cDNA synthesis kit. Real-time PCR was carried out with the SYBR Premix Ex (Takara). The primers used for amplification of \textit{Actin1} and \textit{PR2} were described previously (Zhang et al., 2007). The markers used to map \textit{snc4} previously described (Zhang et al., 2003b). The primers used for amplification of \textit{PDF1.2} are \textit{5'-gcttgaatctgtcgttgcttc-3'} and \textit{5'-cggggatccgagctcgaatctttagtgggccttgg-3'} and \textit{SNC4-GF} (\textit{5'-ggggatccgagctcgaatctttagtgggccttgg-3'}). Trypan blue and DAB staining were carried out as previously described (Parker et al., 1996). The \textit{eds1-2} line used was introgressed into Col by multiple backcrossing and was kindly provided by Dr. Jane Parker. \textit{opr3-2} was kindly provided by Dr. Jianmin Zhou, and it contains a mutation in \textit{OPR3} that creates an early stop codon in the cDNA. The \textit{mks1-1} mutant is in \textit{Ler} background and it is closely linked to the \textit{RPP5} locus. The \textit{snc4-1D} \textit{mks1-1} double mutants were therefore identified for the presence of the \textit{RPP5} locus (from \textit{Ler}) and two independent lines with \textit{RPP5} absent were used for \textit{H. a. Noco2} infection experiments.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number HM461758.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Analysis of the size of \textit{SNC4} cDNA and \textit{SNC4-GFP} fusion protein.

Supplemental Figure S2. Localization of \textit{SNC4-GFP} and \textit{snc4-1D-GFP} fusion protein.

Supplemental Figure S3. Trypan blue staining (top section) and DAB staining (bottom section) of true leaves of wild type, \textit{snc4-1D}, \textit{WT::SNC4} line 1, and \textit{WT::snc4-1D} line 1.

Supplemental Figure S4. Sequence alignment of the extracellular domain of \textit{SNC4} and six GDP domain-containing proteins in Arabidopsis.

Supplemental Figure S5. Western-blot analysis of \textit{SNC4} protein levels in wild type (WT), \textit{snc4-1D}, and \textit{snc4-4} using a polyclonal anti-\textit{SNC4} antibody generated against a fragment of the \textit{SNC4} kinase domain.

Supplemental Figure S6. A working model for \textit{SNC4}-mediated resistance.

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