MPMI Vol. 34, No. 3, 2021, pp. 297–308. https://doi.org/10.1094/MPMI-09-20-0257-R

e-Xtra*

A Novel Role of Salt- and Drought-Induced RING 1 Protein in Modulating Plant Defense Against Hemibiotrophic and Necrotrophic Pathogens

Vemanna S. Ramu,[1,2,†] Sunhee Oh,[1] Hee-Kyung Lee,[1] Raja Sekhar Nandety,[1] Youngjae Oh,[3] Seonghee Lee,[1,3] Jin Nakashima,[1] Yuhong Tang,[1] Muthappa Senthil-Kumar,[1] and Kirankumar S. Mysore[1,†]

[1] Noble Research Institute, LLC, Ardmore, OK 73401, U.S.A.
[2] Laboratory of Plant Functional Genomics, Regional Center for Biotechnology, Faridabad, India
[3] Gulf Coast Research and Education Center, Institute of Food and Agricultural Science, University of Florida, Wimauma, FL 33598, U.S.A.

Accepted 23 November 2020.

Many plant-encoded E3 ligases are known to be involved in plant defense. Here, we report a novel role of E3 ligase SALT-AND DROUGHT-INDUCED RING FINGER1 (SDIR1) in plant immunity. Even though SDIR1 is reasonably well-characterized, its role in biotic stress response is not known. The silencing of *SDIR1* in *Nicotiana benthamiana* reduced the multiplication of the virulent bacterial pathogen *Pseudomonas syringae* pv. *tabaci*. The *Arabidopsis sdir1* mutant is resistant to virulent pathogens, whereas *SDIR1* overexpression lines are susceptible to both host and nonhost hemibiotrophic bacterial pathogens. However, *sdir1* mutant and *SDIR1* overexpression lines showed hypersusceptibility and resistance, respectively, against the necrotrophic pathogen *Erwinia carotovora*. The mutant of SDIR1 target protein, i.e., SDIR-interacting protein 1 (SDIR1P1), also showed resistance to host and nonhost pathogens. In *SDIR1* overexpression plants, transcripts of *NAC* transcription factors were less accumulated and the levels of jasmonic acid (JA) and abscisic acid were increased. In the *sdir1* mutant, JA signaling genes *JAZ7* and *JAZ8* were down-regulated. These data suggest that SDIR1 is a susceptibility factor and its activation or overexpression enhances disease caused by *P. syringae* pv. *tomato* DC3000 in *Arabidopsis*. Our results show a novel role of SDIR1 in modulating plant defense gene expression and plant immunity.

Current address for Muthappa Senthil-Kumar: National Institute of Plant Genome Research, New Delhi, India.

[†]Corresponding authors: K. S. Mysore: ksmysore@noble.org and V. S. Ramu: ramu.vemanna@rcb.res.in

Funding: This work was supported by Noble Research Institute, LLC. The TCS SP2 Leica AOBS Confocal Laser Scanning Microscope used in this study was purchased by the National Science Foundation Major Research Instrumentation Program (MRI) (DBI 0400580). This work was partly funded by a Ramanujan Fellowship to V. S. Ramu, SERB, INDIA (SB/S2/RJN-046/2016).

*The *e*-Xtra logo stands for "electronic extra" and indicates there are supplementary materials published online.

The author(s) declare no conflict of interest.

Keywords: ABA, disease resistance, E3 Ligase, hormones, jasmonic acid, plant immunity, transcription factors

Numerous pathogens infect and cause many serious diseases in plants throughout the world. Several mechanisms have been identified by which pathogens cause disease on the host plant. In addition, there are several different defense strategies evolved by plants to defend against pathogen invasion. The plant basal defense response to a pathogen is weak but can still protect the plant by delaying the growth of pathogen and disease development (Liao et al. 2016; Senthil-Kumar and Mysore 2013). Some pathogens have the ability to cause disease on one or more plant species and are referred to as host pathogens. In contrast, most potential pathogens that can cause disease on a particular plant species but are unable to cause disease on another plant species are referred to as nonhost pathogens. Plants possess both host and nonhost disease resistance mechanisms against specific pathogens (Hadwiger and Tanaka 2017; Heath 2000; Lee et al. 2016; Senthil-Kumar and Mysore 2013). Understanding nonhost disease resistance mechanisms may provide a significant lead to identify candidate genes whose expression can be altered to confer durable disease resistance in crops (Fonseca and Mysore 2019).

The phytohormones jasmonic acid (JA) and salicylic acid (SA) are important components of plant immunity (De Vleesschauwer et al. 2014; Nahar et al. 2011). The JA pathway operates in association with a complex signaling network depending on specific exogenous stimuli such as stresses from wounding, pathogen infection, or herbivore (Wasternack and Hause 2013; Zhang et al. 2017). Protein modifications such as phosphorylation and ubiquitination are associated with the JA signaling mechanisms (Melotto et al. 2008; Thines et al. 2007). Under normal conditions, without any biotic or abiotic stimuli, the JA signaling pathway in plants is in a repressed state due to a family of jasmonate ZIM (zinc-finger inflorescence meristem)-domain (JAZ) proteins (Chini et al. 2007; Thines et al. 2007). Due to developmental or environmental signals, JA is rapidly synthesized and further converts into numerous conjugates, including a bioactive form, (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile) (Fonseca et al. 2009; Thines et al. 2007). The bioactive JA-Ile is recognized by CORONATINE INSENSITIVE1 (COI1), which associates with SKP/CUL/F-box complex proteins (SKP1-like 1 [ASK1/ASK2], Cullin 1

[CUL1], and RING Box 1 [RBX1] proteins] (Devoto et al. 2003; Sheard et al. 2010; Xu et al. 2002), to degrade the JAZ proteins via 26S proteasome system (Chini et al. 2007; Thines et al. 2007). This degradation leads to activation of JASMONATE-INSENSITIVE1 (JIN1)/MYC2 protein that triggers the expression of JA-responsive genes (Boter et al. 2004; Lorenzo et al. 2004). Several reports have suggested that pathogens target the plant ubiquitin system to suppress plant innate immunity (Anand et al. 2012; Bos et al. 2010; Ishikawa et al. 2014; Kaundal et al. 2017; Park et al. 2012). Ubiquitination is a posttranslational protein modification process in eukaryotic cells that involves ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) associated with the proteasome system (Berndsen and Wolberger 2014). Ubiquitination is involved in many biological processes in plants, such as the cell cycle, circadian rhythm control, hormone signaling, growth, and development (Duplan and Rivas 2014). In plant defense, E3 ligases act as both positive and negative regulators of defense and are activated by elicitor/avirulent factors (Dreher and Callis 2007). E3 ligases activate many downstream transcription factors (TFs) involved in upregulation of defense responsive genes. For example, the Ethylene Insensitive 3 (EIN3) type TF is regulated by ubiquitin SCF E3 ligase complexes containing the F-box subunits EIN3 binding F-box 1 (EBF1) or EBF2 (Delauré et al. 2008). The pathogen-induced JA signaling is also linked to the ubiquitination pathway through COI1. The *coi1* mutant shows pathogen insensitivity phenotype against hemibiotrophic bacterial pathogens (Xie et al. 1998). The RING DOMAIN LIGASE mutants *rglg3* and *rglg4* also show insensitive phenotypes against *Pseudomonas syringae* pv. *tomato* DC3000 (Zhang et al. 2012). The pepper E3 ubiquitin ligase *RING1* (*CaRING1*) gene–silenced plants showed enhanced susceptibility to avirulent *Xanthomonas campestris* pv. *vesicatoria* infection by reducing the *Pathogenesis Related 1* (*PR1*) gene expression and reducing SA (Lee et al. 2011). The E3 ligases also induce pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) (Ishikawa et al. 2014). In contrast, the *Arabidopsis* PLANT Ubiquitin-BOX PROTEIN22 (PUB22), PUB23, and PUB24 proteins act as negative regulators of PTI (Trujillo et al. 2008).

The JA and SA signaling pathways are known to act antagonistically during defense against biotrophic and necrotrophic pathogens (Caarls et al. 2015; Lorenzo et al. 2004; Melotto et al. 2006). As one of the PTI responses, plant stomata in *Arabidopsis* close upon perception of some bacterial pathogens (Lee et al. 2017; Melotto et al. 2006). Upon infection with certain bacterial pathogens such as *P. syringae* pv. *tomato* DC3000, stomata are reopened due to bacterially produced coronatine (COR) (a JA-Ile mimic) that induces JA production in plants, thus suppressing SA-mediated plant defense (Melotto et al. 2006; Zheng et al. 2012). The *coi1* mutant is defective in JA signaling, which enhances plant susceptibility to necrotrophic pathogens such as *Fusarium xysporum*, but it is resistant to biotrophic and hemibiotrophic pathogens (Berrocal-Lobo et al. 2002). It is well-established that some necrotrophic pathogens manipulate the SA signaling pathway that antagonizes the JA pathway to reduce plant resistance to pathogens. The expression of JA dependent proteinase inhibitor I and II is suppressed by interaction of SA induced NPR1 with TGA TF in necrotropic pathogens *Alternaria solani* and *Botrytis cinerea* (El Oirdi et al. 2011; Rahman et al. 2012).

Apart from their role in biotic stress response, E3 ligases are also important in abiotic stress responses of plants. SALT- AND DROUGHT-INDUCED RING FINGER1 (SDIR1) is a RING-type E3 ubiquitin ligase that is involved in drought-stress tolerance. Some RING-finger proteins are also involved in salt stress. The *Arabidopsis* SDIR1 and its homologous protein ZmRFP1 participate in regulation of drought stress. SDIR1 acts as an E3 ligase to ubiquitinate the SDIR-interacting protein 1 (SDIR1P1) and partially degrade it to regulate the key abscisic acid (ABA)-pathway gene *ABA-INSENSITIVE5 (ABI5)* (Zhang et al. 2015). *Oryza sativa* OsSDIR1 acts negatively in drought and salt stress (Park et al. 2010). Pepper CaRFP1 targets the basic PR-1 protein (CaBPR1) to modulate ABA signaling in salt stress (Hong et al. 2007). Genetic evidence suggests that SDIR1 is upstream of ABA signaling (Zhang et al. 2007). Even though SDIR1 is reasonably well-characterized, its role in biotic stress response is not known. In this study, we report the role of SDIR1 in bacterial disease resistance. Further, we show that transcripts of many genes involved in defense response is regulated by SDIR1.

RESULTS

Silencing of *NbME24D08* in *Nicotiana benthamiana* reduced multiplication of pathogens.

We have previously used virus-induced gene silencing (VIGS)-based forward genetic screens in *N. benthamiana* to identify plant genes that play a role in nonhost disease resistance against bacterial pathogens (Lee et al. 2017; Kaundal et al. 2017; Nagaraj et al. 2016; Rojas et al. 2012; Wang et al. 2012). Most of the genes identified in this screen showed compromised nonhost resistance when silenced. Interestingly, when one of the VIGS clones, *NbME24D08* (Senthil-Kumar et al. 2018), identified in the screen was silenced, the plants showed decreased growth of both host (*P. syringae* pv. *tabaci*) and nonhost (*P. syringae* pv. *tomato* DC3000) pathogens tested when compared with nonsilenced control plants (Fig. 1). *NbME24D08*-silenced plants had 75% transcripts of *24D08* compared with nonsilenced control plants and exhibited stunted growth and thin leaves with elongated branches (Supplementary Fig. S1A and B).

The nucleotide blast analysis of *NbME2408* with the *N. benthamiana* genome shows 93% homology with Niben101Scf03944g03002, a RING finger protein (zinc finger, RING/FYVE/PHD-type) (Supplementary Fig. S2A). Further, the tblast analysis of *Niben101Scf03944g03002* identified homologs in various species, including *Arabidopsis* SDIR1 (At3g55530; C3HC4-ZF-containing E3 ligase [Zhang et al. 2007; 2015]), with 75% identity and tobacco SDIR1 homolog Ring H2-Finger (RHF) with 100% identity. NtRHF1/SDIR1 is induced by drought and has also been shown to have E3 ligase activity similar to AtSDIR1 (Xia et al. 2013). SDIR1 is highly conserved among plants and homologs have been identified in many plant species (Supplementary Fig S2C and D). The amino acid sequence alignment of *N. benthamiana* Niben101Scf03944g03002 protein with *Arabidopsis thaliana* SDIR1, *Vitis vinifera* SDIR1 (CBI31650.3 [Tak and Mhatre 2013]), *Glycine max* SDIR1 (ACU20874), *Oryza sativa* SDIR1 (ABF95226.1 [Gao et al. 2011]), *Zea mays* SDIR1 (Np_001131833.1), *Sorghum bicolor* SDIR1 (Xp_002468109.1), and *Nictiana tabacum* SDIR1/RHF (Xia et al. 2013) is shown in Supplementary Figure S2C and D. SDIR1 possesses a conserved *trans* membrane domain in the N-terminal region and a RING domain in the C-terminal region, and these were clearly present in the Niben101Scf03944g03002 protein sequence, similar to AtSDIR1 (Supplementary Fig. S2F). These analyses clearly suggest that the identified *NbME24D08* is a *N. benthamiana* homolog of *AtSDIR1*.

Atsdir1 mutants are resistant to host and nonhost hemibiotrophic pathogens.

To determine the role of *AtSDIR1* in plant immunity, the expression of *AtSDIR1* in *Arabidopsis* during various biotic

stresses was assessed using publicly available gene expression data (Genevestigator). The transcript levels of *AtSDIR1* were not significantly altered when compared with wild type (Col-0) after treatment with various pathogens such as virulent *P. syringae* pv. *tomato* DC3000, avirulent *P. syringae* pv. *tomato* (AvrRPM1), *P. syringae* pv. *tomato* harpin (hrcC) mutant, and nonhost pathogen *P. syringae* pv. *phaseolicola* (Supplementary Fig. S3A). These results suggest that *SDIR1* is not transcriptionally regulated during biotic stress. However, posttranslational regulation of SDIR1 protein during biotic stress cannot be ruled out.

For functional characterization of *AtSDIR1*, two *Arabidopsis* T-DNA knockout mutants (*sdir1*) described by Zhang et al. (2007), Salk-114361 and Salk-052701, were identified and characterized (Supplementary Fig. S3C). In addition, *AtSDIR1* RNA interference (RNAi), overexpression, and complementation lines were developed in *Arabidopsis*. Quantitative reverse transcription PCR (RT-qPCR) analyses showed a high level of transcripts in *AtSDIR1* overexpression lines, no transcripts were detected in the mutants, and the RNAi plants had about 50% of *AtSDIR1* transcripts (Supplementary Fig. S3B). In the complemented line, *AtSDIR1* transcripts were twofold higher than the Col-0 plants (Supplementary Fig. S3B). *Arabidopsis sdir1* mutants, *AtSDIR1* overexpression lines (*SDIR1*-OE), RNAi lines, complemented lines, and wild-type Col-0 plants were challenged with nonhost pathogen *P. syringae* pv. *tabaci* (1×10^4 CFU ml^{-1}) and host pathogen *P. syringae* pv. *tomato* DC3000 (1×10^4 CFU ml^{-1}) by flood inoculation (Ishiga et al. 2011). Strikingly, the overexpression lines showed higher disease symptoms than Col-0 to both host (*P. syringae* pv. *tomato* DC3000) and nonhost (*P. syringae* pv. *tabaci*) pathogens tested. *sdir1* mutants and RNAi lines showed partial resistance against *P. syringae* pv. *tomato* DC3000 when compared with Col-0 and did not compromise resistance to the nonhost pathogen tested (Fig. 2A). Consistent with the disease symptoms, the overexpression lines supported higher bacterial multiplication of both host and nonhost pathogens when compared with Col-0 (Fig. 2B). The mutants and RNAi lines showed reduced bacterial multiplication compared with Col-0 (Fig. 2C). These data suggest that SDIR1 is a susceptibility factor, and its activation or expression enhances disease by *P. syringae* pv. *tomato* DC3000 in *Arabidopsis*.

Overexpression of *AtSDIR1* modulates phytohormones and associated responses in *Arabidopsis*.

SDIR1 is known to modulate levels of the phytohormone ABA under drought stress (Zhang et al. 2007). We tested whether other phytohormones, such as SA and JA, are also modulated by AtSDIR1, especially after pathogen inoculation. All the above-mentioned *Arabidopsis* lines were treated with host pathogen *P. syringae* pv. *tomato* DC3000 (1×10^4 CFU ml^{-1}), and phytohormones (SA, JA, and ABA) were quantified using liquid chromatography-mass spectrometry. SA and ABA showed slightly higher levels in *SDIR1*-OE plants with

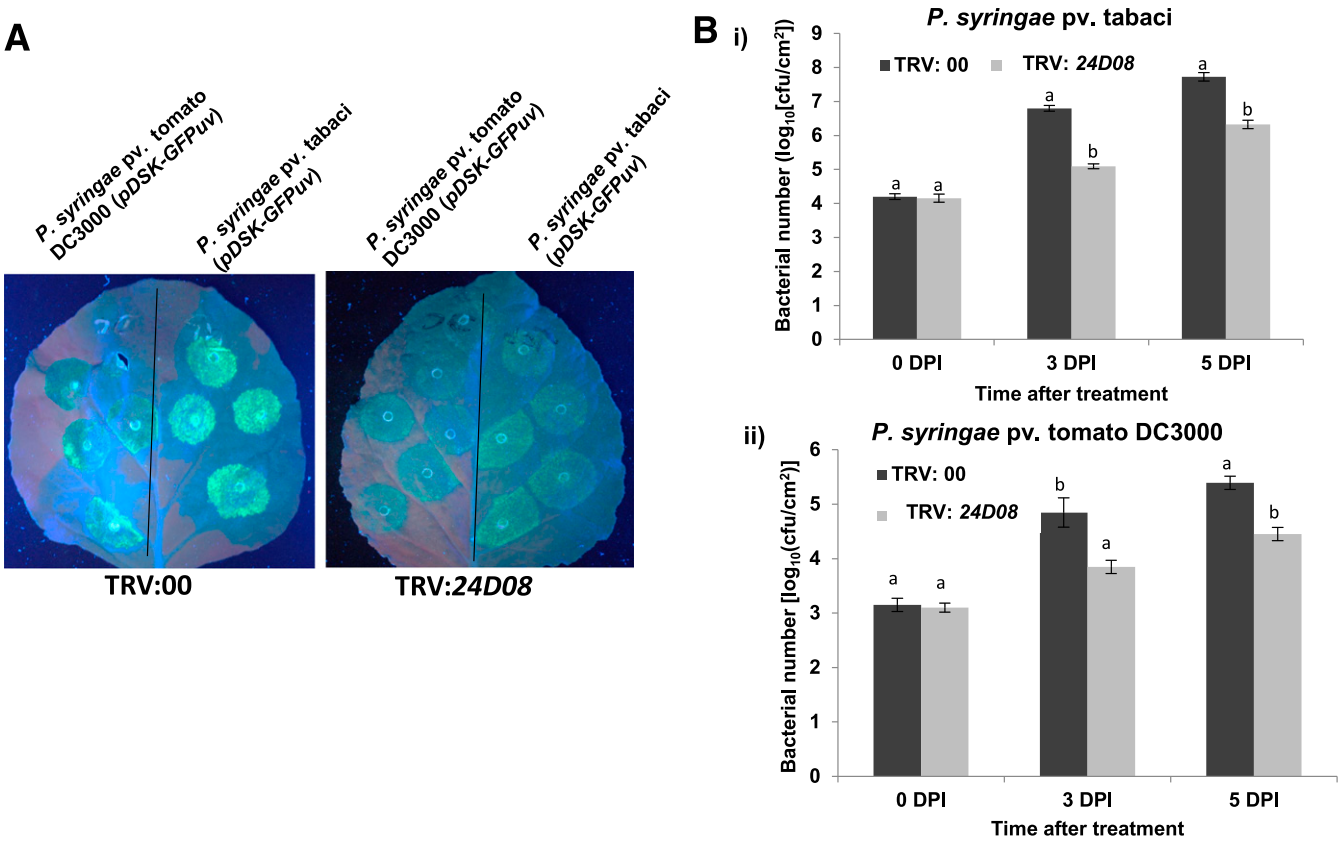

Fig. 1. Silencing the *24D08* cDNA clone in *Nicotiana benthamiana* reduces multiplication of both host and nonhost pathogens. **A,** Qualitative measurement of host and nonhost bacterial multiplication on TRV:*24D08* or TRV:00-inoculated *N. benthamiana* plants. Three weeks after TRV inoculation, *N. benthamiana* plants were syringe-infiltrated with *GFPuv*-expressing (*pDSK-GFPuv*) nonhost pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the host pathogen *P. syringae* pv. *tabaci*, at 1×10^4 CFU ml^{-1} concentration. Photographs were taken at 5 days postinoculation (dpi) under UV light. **B,** Quantification of host and nonhost bacterial multiplication in TRV:*24D08* and TRV:00. Leaves were syringe-inoculated with *GFPuv*-expressing host (*P. syringae* pv. *tabaci*) and nonhost (*P. syringae* pv. *tomato* DC3000) pathogens at 1×10^7 CFU ml^{-1} concentration. Average values of three biological replicates were used to generate bar graphs, and experiments were repeated three times with similar results. Error bars indicate standard error. Different letters above the bars indicate a significant difference from two-way analysis of variance at $P < 0.05$ with Tukey's honest significant difference means separation test ($\alpha = 0.05$) within a timepoint among respective TRV:00 and silenced plants.

response to host pathogen *P. syringae* pv. *tomato* DC3000 (Supplementary Fig. S4A and B). No significant variation was observed in JA levels upon pathogen infection. However, uninoculated *SDIR1*-OE plants had slightly higher JA levels when compared with wild-type Col-0 (Supplementary Fig. S4C). If the phytohormone quantity was to play a major role in conferring hypersusceptibiliy of *SDIR1*-OE lines to a hemibiotrophic pathogen, *P. syringae* pv. *tomato* DC3000, we would have expected less SA in *SDIR1*-OE lines. We therefore speculate that minor modulation of phytohormones is not the major contributor for alteration in disease resistance or susceptibility in *SDIR1*-OE and *sdir1* mutant lines.

The previous study using liquid nitrogen coupled scanning electron microscopy (Zhang et al. 2007) showed that *SDIR1*-OE lines had slightly smaller stomatal apertures and the *sdir1* mutant had fully open apertures under drought. Since SDIR1 is known to regulate ABA levels and stomata, the stomatal aperture in *SDIR1*-OE, *sdir1* mutant, and complemented lines was measured in response to ABA, host pathogen *P. syringae* pv. *tomato* (DC3000), and nonhost pathogen *P. syringae* pv. *tabaci*.

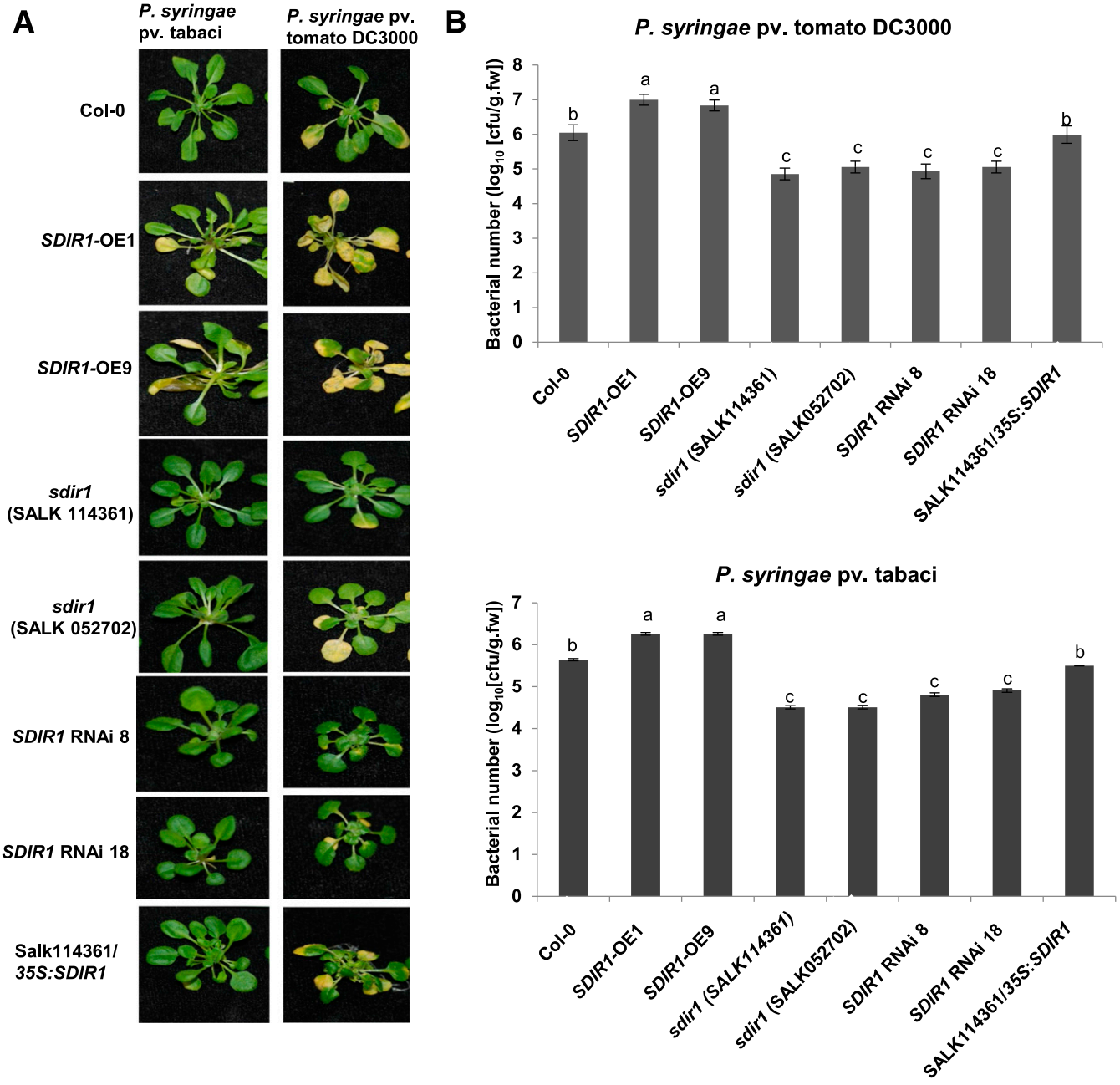

Fig. 2. Overexpression of *AtSDIR1* in *Arabidopsis* compromises nonhost disease resistance, and *sdir1* mutant lines show tolerance to host pathogen. **A,** Three-week-old *Arabidopsis SDIR1* overexpressor lines (*SDIR1*-OE1 and *SDIR1*-OE9), *sdir1* mutants (SALK-114361 and SALK-052702), *SDIR1*-RNAi lines (RNAi 8 and RNAi 18), complemented line (Salk114361/*35S:SDIR1*), and wild type (Col-0) were inoculated with either a nonhost pathogen, *Pseudomonas syringae* pv. *tabaci* (1×10^4 CFU ml^{-1}), or a host pathogen, *P. syringae* pv. *tomato* DC3000 (1×10^4 CFU ml^{-1}), by flooding. Photographs were taken 4 days postinoculation (dpi). **B,** Bacterial titer was assessed at 4 dpi from leaves of seedlings that were flood-inoculated with 1×10^4 CFU ml^{-1} of *P. syringae* pv. *tomato* DC3000 or *P. syringae* pv. *tabaci*. Error bars represent the standard error for three biological replicates in three independent experiments. Different letters above the bars indicate a significant difference from two-way analysis of variance at $P < 0.05$ with Tukey's honest significant difference means separation test ($\alpha = 0.05$) among respective genotypes.

The stomatal aperture sizes in *SDIR1*-OE lines were slightly smaller compared with wild-type Col-0 in response to all the tested stimuli (Supplementary Fig. S4D). In contrary, the stomatal aperture sizes of *sdir1* mutant lines were slightly larger than Col-0 (Supplementary Fig. S4D). These results did not correlate with bacterial susceptibility and resistance observed in *SDIR1*-OE and *sdir1* mutant lines, respectively. Therefore, *SDIR1*-mediated bacterial susceptibility is independent of stomata-mediated pathogen entry.

Arabidopsis SDIR1 overexpression lines are resistant to a necrotrophic pathogen.

To determine if *SDIR1* plays a role in plant immunity against a necrotrophic pathogen, the *Arabidopsis SDIR1*-OE, *sdir1* mutants, *AtSDIR1* RNAi, and complemented lines were challenged with necrotrophic pathogen *Erwinia carotovora* (1×10^5 CFU ml^{-1}) by syringe infiltration. Interestingly, the *SDIR1*-OE plants showed resistance with less bacterial multiplication, whereas the *sdir1* mutants showed a hypersusceptible phenotype with higher bacterial multiplication (Fig. 3A and B). These results suggest that SDIR1 plays an antagonistic role in conferring resistance or susceptibility against hemibiotrophic and necrotrophic bacterial pathogens.

SDIR1 and its interacting protein SDIRIP1 plays a role in JA signaling–mediated plant immunity.

Previously, it was shown that SDIR1 interacts with SDIRIP1 and the *AtSDIR1* overexpression plants and *sdirip1* RNAi plants are sensitive to ABA (Zhang et al. 2007, 2015). We sought to determine the role of *SDIR1* and *SDIRP1* along with JA- or COR-mediated defense pathway genes (*JAZ9* and *COI1*) in the plant response to JA and COR. Interestingly, the inhibitory effect of COR on root growth and elongation was not observed in *sdir1* and *sdirip1* mutants or silenced lines similar to *JAZ9* overexpression (*JAZ9*-OE) and *coi1* mutant lines (Fig. 4A; Supplementary Fig. S5A). In contrast, the root growth in *SDIR1*-OE lines was inhibited to a greater extent than was wild-type Col-0 in the presence of COR. Complemented lines of *sdir1* behaved like the Col-0 plants (Fig. 4A; Supplementary Fig. S5A). Similar responses to that of COR treatment were observed when all the above-mentioned lines were treated with MeJA (Fig. 4B; Supplementary Fig. S5B).

The response of *sdirip1* against host and nonhost pathogens was also tested, along with mutant or overexpression lines of COR- or JA-mediated defense pathway genes (*jaz9*, *coi1*, and *JAZ9*-OE), by flood inoculation. The *jaz9* mutant plants showed compromised nonhost resistance (Fig. 4C and D). The *JAZ9*-OE lines inhibited the growth of the nonhost pathogen, similarly to that of Col-0 and moderately resistant to the host pathogen tested (Fig. 4C and D). A similar response was observed for *coi1* and *sdirip1* mutant lines (Fig. 4C and D). These results suggest that the loss of either SDIR1 or its target protein SDIRP1 improves plant defense response.

SDIR1 modulates JA and ABA signaling gene expression in response to *P. syringae* pv. *tomato* DC3000 infection.

To further understand why *SDIR1*-OE lines are hypersusceptible to hemibiotrophic pathogens, the transcriptomic responses of Col-0, *SDIR1*-OE, and *sdir1* mutant lines were studied with or without *P. syringae* pv. *tomato* DC3000

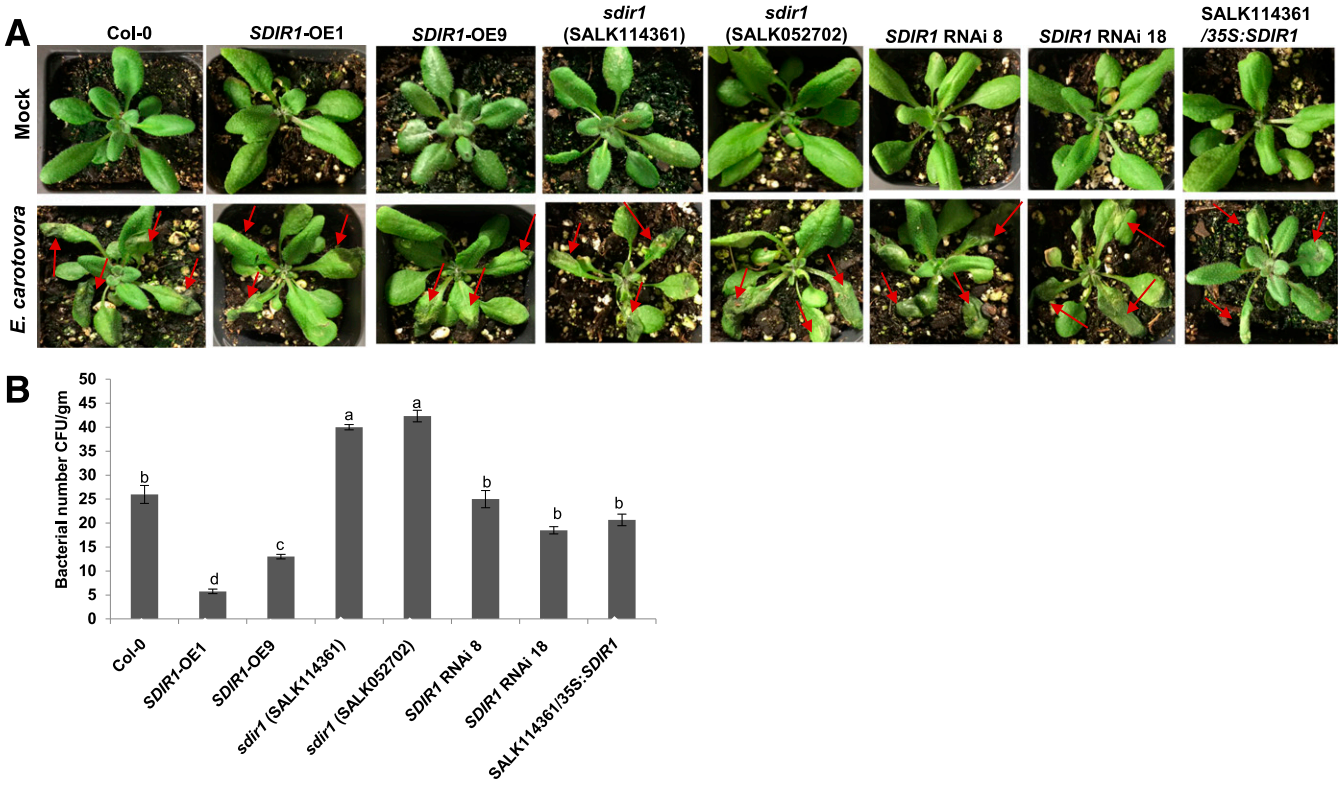

Fig. 3. Overexpression of *SDIR1* in *Arabidopsis* shows tolerance to necrotrophic pathogen *Erwinia carotovora*, whereas *sdir1* mutant and *SDIR1* RNAi plants show hypersusceptibility. **A,** Leaves of 4-week-old soil-grown *Arabidopsis SDIR1* overexpression lines (*SDIR1*-OE1 and *SDIR1*-OE9), *sdir1* mutants (SALK-114361 and SALK-052702), *SDIR1*-RNAi lines (RNAi 8 and RNAi 18), complemented line (Salk114361/*35S:SDIR1*), and wild type (Col-0) were inoculated with *E. carotovora* (1×10^5 CFU ml^{-1}) by a needleless syringe. Red arrows show disease symptoms in inoculated leaves. **B,** Bacterial titer was assessed at 4 days postinoculation (dpi) from leaves of seedlings that were inoculated with *E. carotovora.* Error bars represent the standard error for three biological replicates. Different letters above the bars indicate a significant difference from two-way analysis of variance at $P < 0.05$ with Tukey's honest significant difference means separation test ($\alpha = 0.05$) among different genotypes.

infection (3 days postinoculation [dpi]), using *Arabidopsis* Affymetrix microarrays. Microarray data analysis identified several differentially expressed genes (DEGs) with or without *P. syringae* pv. *tomato* DC3000 infection (Fig. 5A). The multiple experiment viewer (MeV) analysis clearly distinguished the different clusters of DEGs. Without DC3000 inoculation in the *SDIR1*-OE line, the transcript levels of 19 genes were highly abundant and 11 genes had low abundance when compared with Col-0 (Fig. 5B). In contrast, the *sdir1* mutant had more DEGs, wherein 122 genes were upregulated and 191 genes were downregulated when compared with Col-0 (Fig. 5B). Upon pathogen inoculation, thousands of DEGs were identified in both the *SDIR1*-OE and the *sdir1* mutant lines when compared with uninoculated controls (Fig. 5C). We focused on genes that were differentially expressed specifically in the *SDIR1*-OE or *sdir1* mutant line when compared with Col-0 after pathogen inoculation. In the *SDIR1*-OE line, transcript levels of 46 genes were higher and 11 genes were lower compared with wild-type Col-0 (Fig. 5D). In the *sdir1* mutant, transcripts of 10 genes were higher and 76 genes were lower when compared with Col-0 (Fig. 5D).

To determine the susceptibility of *SDIR1 OE* lines against the host pathogen *P. syringae* pv. *tomato* DC3000, the DEGs were mapped to different pathways especially involved in biotic stress response, using the MapMan tool (Supplementary Fig. S6A and B). In the *SDIR1*-OE line, several genes that are known to play a role in plant defense showed lower expression when compared with Col-0 (Supplementary Data Files S1 and S2). For example, genes encoding proteins such as JAZ7, JAZ8, three WRKY TFs, bHLH TF (At1g10585), lipoxygenase, R2R3 MYB TF, nine NAC TFs, six PR proteins, four defensins, six UDP-glucosyl transferases, a glycosyl transferase, eight glutathione transferases, and transcripts of several other defense-signaling genes were less abundant in the *SDIR1*-OE line when compared with the *sdir1* mutant treated with the pathogen (Supplementary Data file S1). In contrast, several genes encoding TFs, such as MYB30, MYB28, YAB5, and bZIP, were accumulated at higher levels. It is interesting to note that several abiotic-responsive genes such as *ABI5* and *ABF3*, which are downstream to SDIRIP1 in the ABA pathway, were differentially expressed in *sdir1* mutant plants. In *SDIR1*-OE plants, several genes encoding NAC TFs showed lower transcript levels compared with *sdir1* mutant plants with response to *P. syringae* pv. *tomato* DC3000 (Supplementary Tables S1 and S2), which caused stomatal opening by inhibiting SA biosynthesis, resulting in higher bacterial accumulation.

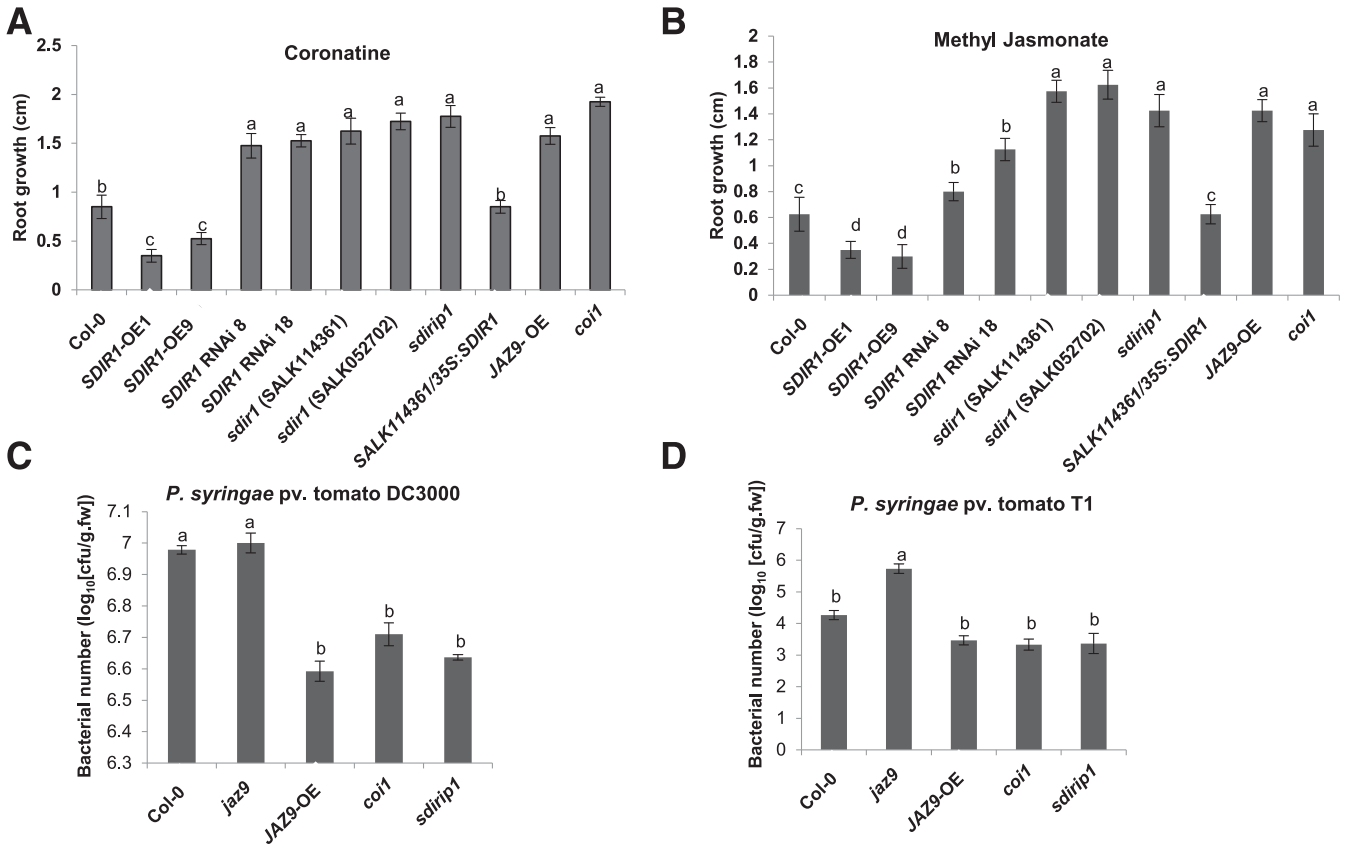

Fig. 4. Effect of coronatine (COR) or methyl jasmonate (MeJA) on jasmonic acid signaling pathway in *SDIR1*-OE, *sdir1*, *coi1*, *jaz9*, and *sdirip1*, using a root growth–inhibition assay. **A,** and **B,** Effect of COR or MeJA on root growth inhibition of *Arabidopsis* wild type (Col-0), *SDIR1* overexpressor lines (*SDIR1*-OE1 and *SDIR1*-OE9), *sdir1* mutants (SALK-114361 and SALK-052702), *SDIR1*-RNAi lines (RNAi 8 and RNAi 18), *sdirip1* mutant, complemented line (Salk114361-*35S*::*SDIR1*), *JAZ9* overexpression line (*JAZ9-OE*), and *coi1* mutant. The pregerminated seedlings were placed on square plates with half-strength Murashige and Skoog media containing 0.5 µm COR or 50 µm MeJA and were incubated vertically. The root growth was measured after 5 days. A minimum of five replications per line were used. Experiments were repeated three times with similar results. **C** and **D,** Response of *Arabidopsis JAZ9-OE*, *jaz9*, *coi1*, and *sdirip1* mutants to both host and nonhost pathogens. The *Arabidopsis* seedlings were flood-inoculated with nonhost pathogen *Pseudomonas syringae* pv. *tomato* T1 (1×10^4 CFU ml^{-1}) or host pathogen *P. syringae* pv. *tomato* DC3000 (1×10^4 CFU ml^{-1}). Bacterial multiplication was assessed at 4 days postinoculation (dpi) from leaves of seedlings that were flood-inoculated with host or nonhost pathogens. Error bars represent the standard error for three biological replicates in three independent experiments. Different letters above the bars indicate a significant difference from two-way analysis of variance at $P < 0.05$ with Tukey's honest significant difference means separation test ($\alpha = 0.05$) within a timepoint.

Transcript levels of genes encoding cell wall–associated proteins, such as expansins, hydroxy proline-rich proteins, cellulose synthase-like C5, arabinogalactan protein 4, and L-fucosidase 1 and a cytochrome P450 monooxygenase (PAD3) that are involved in the plant defense mechanism were lower in *SDIR1*-OE plants when compared with Col-0 upon pathogen infection (Supplementary Table S3). In contrast, in the *sdir1* mutant line, several defense-related gene transcripts were abundant when compared with Col-0 and this may contribute to the resistance against *P. syringae* pv. *tomato* DC3000.

DISCUSSION

This study characterized the role of intracellular membrane–localized ubiquitin E3 ligase SDIR1 in plant defense against pathogens. The role of SDIR1 in improving drought tolerance

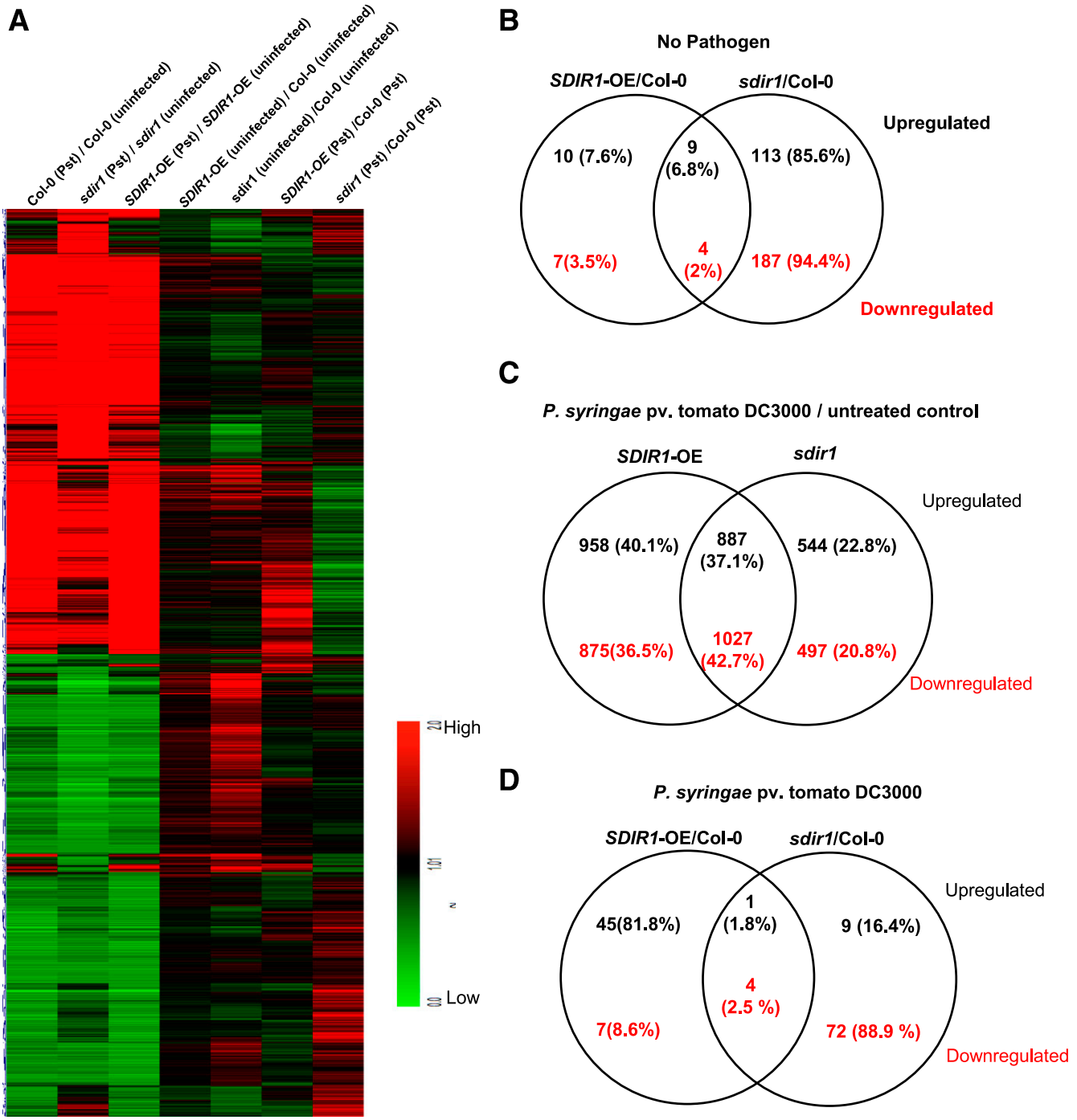

Fig. 5. Transcriptome analysis of *SDIR1* overexpressed (*SDIR1*-OE) and *sdir1* mutant plants after host pathogen inoculation. **A,** Heat map showing the differentially expressed genes (DEGs) in the samples treated with host pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Pst). Three-week-old *Arabidopsis* plants were flood-inoculated with *P. syringae* pv. *tomato* DC3000 (1×10^4 CFU ml^{-1}) and, after 3 days postinoculation (dpi), the tissue was collected and RNA was isolated for microarray experiment. The microarray data from Col-0, *SDIR1*-OE (*SDIR1*-OE1), and *sdir1* (salk-114361) mutant either untreated or treated with *P. syringae* pv. *tomato* (3 dpi) were analyzed. The expression data were visualized in the multiple experiment viewer (MeV) tool. **B,** DEGs in the *SDIR1*-OE and *sdir1* mutant in comparison with Col-0 plants without any pathogen infection. **C,** DEGs in *SDIR1*-OE and *sdir1* with response to *P. syringae* pv. *tomato* DC3000 compared with respective untreated samples. **D,** DEGs in *SDIR1*-OE and *sdir1* with response to *P. syringae* pv. *tomato* compared with wild-type Col-0 treated with *P. syringae* pv. *tomato*.

through ABA-mediated signaling to activate stress-responsive TFs has been shown in *Arabidopsis* and rice (Gao et al. 2011; Zhang et al. 2007, 2015). However, the function of SDIR1 in plant biotic stress is not known. A VIGS-based forward genetics screen in *N. benthamiana* identified a cDNA clone that encodes a putative SDIR1 when silenced led to reduced multiplication of the host pathogen *P. syringae* pv. *tabaci* (Fig. 1). Consistent with this data, *Arabidopsis sdir1* mutants and *SDIR1* RNAi lines also showed partial resistance against host pathogens tested (Fig. 2). Interestingly, overexpression of *SDIR1* in *Arabidopsis* led to increased susceptibility to biotrophic host pathogens and compromised resistance against nonhost pathogens when compared with wild-type Col-0 (Fig. 2). In contrast, *SDIR1* overexpression lines were slightly more resistant to the necrotrophic pathogen *E. carotovora* when compared with Col-0 (Fig. 3). This is probably due to the antagonism of JA- and SA-mediated signaling pathways and interplay of the ABA-mediated signaling pathway.

The *SDIR1*-OE plants showed slightly higher levels of ABA upon hemibiotropic pathogen treatment, but interestingly, no major changes in JA and SA levels were observed compared with Col-0. However, without any pathogen inoculation, the *SDIR1*-OE plants had twofold more JA when compared with Col-0 and this may have sufficiently primed the JA-mediated defense response, resulting in partial resistance against a necrotrophic pathogen. Consistent with this observation, the transcriptome data showed upregulation of TF *bHLH* (At4g01460), known to be involved in JA biosynthesis, in *SDIR1*-OE plants without pathogen infection. The *SDIR1*-OE lines showed a higher bacterial load, in spite of closed stomata, when inoculated with pathogen, whereas *sdir1* mutants showed lower bacterial load even though stomata was slightly more open compared with *SDIR1*-OE lines. Therefore, minor differences in stomatal aperture sizes among *SDIR1*-OE and *sdir1* lines does not contribute to disease phenotypes observed in these lines. Similar to *SDIR1*-OE, the *Arabidopsis* gain-of-function mutant for the *Constitutive disease susceptibility 2-1D* gene (*CDS2-1D*), which encodes 9-*cis*-epoxycarotenoid dioxygenase 5 (NCED5) involved in ABA synthesis, showed accumulation of JA and exhibited an antagonistic relationship with SA. Higher ABA accumulation in the *cds2-1D* mutant compared with wild type led to compromised resistance to the biotrophic oomycete *Hyaloperonospora arabidopsis*, whereas resistance to the necrotropic fungus *Alternaria brassicicola* was enhanced (Fan et al. 2009). In the *SDIR1*-OE plants, upon inoculation with host pathogen *P. syringae* pv. *tomato* DC3000, transcript levels of several genes encoding NAC family TFs were lower when compared with the *sdir1* mutant plants. Expression of many NAC TFs are known to be ABA-dependent. In *sdir1* mutant plants, ABA biosynthetic gene *NCED5* showed a low transcript number. During pathogen infection, NAC family TFs ANAC019, ANAC055, and ANAC072 have been shown to be activated by COR in a MYC2-dependent manner by inhibiting the genes associated with SA biosynthesis and metabolism and mediating stomatal reopening for bacterial entry (Xin and He 2013; Zhang et al. 2017; Zheng et al. 2012). Previous studies (Zhang et al. 2007, 2015) and our data suggest *SDIR1*-OE plants have higher levels of ABA. ABA affects pathogen-induced responses antagonistically by interfering with SA, JA, and ethylene-mediated defenses (Anderson et al. 2004; Asselbergh et al. 2008; Melotto et al. 2006). The suppression of SA-mediated defense by reducing callose deposition was observed during postinfection by virulent bacteria in ABA positive regulator *RCAR3* (*regulatory components of ABA receptor 3*) overexpressed *Arabidopsis* plants (Lim et al. 2014).

SDIR1 acts upstream of TFs ABF4 and ABI5 in the ABA pathway, and SDIRIP1 acts intermediately between these TFs (Zhang et al. 2007, 2015). Our data suggests that SDIR1 could be targeting JA or SA signaling pathways during biotic stress responses. The higher root growth in the *sdirip1* mutant in the presence of COR and MeJA is similar to that of *sdir1* mutant. The *sdir1* mutants are insensitive to necrotrophic pathogens or JA signaling. Similar responses were observed in *coi1* mutants (Xie et al. 1998) and in E3 ligase mutants *rglg3* and *rglg4* (Zhang et al. 2012). These results suggest that JA signaling events are mediating the root growth inhibition and disease phenotype in *SDIR1* overexpression lines through its previously identified substrate SDIRIP1 or through JAZ proteins. However, the disease phenotype of *Arabidopsis SDIRIP1* overexpression is required to study the clear mechanism. JAZ proteins are known to be the negative regulators of gene expression in JA-mediated responses (Withers et al. 2012; Zhang et al. 2015). The PAMP-mediated plant defense depends on JA signaling components of the SCF complex such as CUL1, JAZs, COI1, F-Box proteins, and JA for modulating downstream TFs such as MYC2 (Browse 2009; Burgess et al. 2012). It can be inferred that the ABA-mediated responses also could be operated by the same complex that is contributing to ABA-mediated signaling through SDIRIP1. The *coi1*, *sdirip1* mutants, and *JAZ9*-OE lines accumulated fewer host and nonhost hemibiotrophic pathogens when compared with Col-0. However, *SDIR1*-OE plants showed hypersusceptibility to these pathogens, while the *jaz9* mutant compromised nonhost resistance. This could be attributed to the role of SDIR1 in JA- and ABA-mediated or pathogen-mediated response to trigger the SCF^{COI1} complex. The degradation of target proteins mediated by SDIR1 may be leading to activation of MYC2 and other proteins, leading to susceptibility against hemibiotrophic pathogen *P. syringae* pv. *tomato* DC3000. The antagonistic effect of MYC2 activation showed JA-mediated disease resistance against necrotrophic pathogens (Lorenzo et al. 2004), which is consistent with *SDIR1*-OE lines showing resistance to *E. carotovora* (Fig. 3).

The transcriptome data from *SDIR1*-OE and mutant plants exposed to host pathogen *P. syringae* pv. *tomato* DC3000 showed several DEGs when compared with wild-type Col-0. TF-encoding genes such as the *WRKY* family, *R2R3MYB*, and *C2H2-ZF* showed low transcript levels in *SDIR1*-OE lines (Supplementary Table S1, part ii) that also showed compromised defense against hemibiotrophic pathogens. The role of these TFs in plant innate immunity in regulating the PTI, effector-triggered immunity, and hormone signaling has been reported (Seo and Choi 2015). The downregulation of defense-related TFs in the *sdir1* mutant could be due to downregulation of some protein kinases or *JAZ7*, *JAZ8*, and repression activity of JAZ- or SDIRIP-related proteins. In contrast, the *MYB30* and *MYB28* (Supplementary Table S1, part i) transcripts were higher in *SDIR1*-OE lines. JAZ9 is known to act as a repressor for the MYB TFs under cold- and pathogen-stress conditions (Lv et al. 2017; Qi et al. 2011). It has been shown that the interaction of JAZ9 with MYB30 TF that suppress *β-amylase (BMY)* genes reduces the cell membrane stability during cold stress (Lv et al. 2017). These results suggest that upregulation of *MYB30* and other genes is due to proteasome-mediated degradation of JAZ protein through SDIR1 during pathogen infection. Future studies are required to establish JAZ protein degradation by SDIR1.

In summary, our data clearly demonstrate that, when *SDIR1* is overexpressed, plants show compromised resistance to host and nonhost hemibiotrophic pathogens but results in increased resistance against a necrotrophic pathogen. SDIR1 plays a central role in ubiquitin-mediated degradation of SDIRIP1 protein and perhaps JAZ proteins to trigger ABA and JA signaling pathways, independent of stomata-mediated defense mechanism, to influence both biotic and abiotic stress responses.

MATERIALS AND METHODS

VIGS and bacterial growth assay in *N. benthamiana*.

Agrobacterium tumefaciens GV3101 containing TRV2: *24D08* (NbME24D08 [Noble Research Institute VIGS database]) (Senthil-Kumar et al. 2018) was grown at 28°C in Luria-Bertani medium supplemented with rifampicin (10 µg/ml) and kanamycin (50 µg/ml). The VIGS fragment with minimum off-targets was selected for silencing, using the pssRNAi web server (Ahmed et al. 2020). VIGS was carried out by mixing a 1:1 ratio of *Agrobacterium* strains containing tobacco rattle virus 1 (TRV1) and TRV2, as described (Senthil-Kumar and Mysore 2014). Three weeks after TRV inoculation, the silenced and nonsilenced plants were vacuum-infiltrated with host or nonhost pathogens. Bacterial strains were grown in King's B medium, at 30°C, supplemented with rifampicin (10 µg/ml), kanamycin (50 µg/ml), or streptomycin (50 µg/ml) when needed. To determine the bacterial titer, leaf samples from four biological replicates were collected at 0, 3, and 5 dpi, using a 1-cm core borer. Leaf samples were ground, were subjected to serial dilution, were plated on King's B agar medium supplemented with appropriate antibiotics, and were incubated at 28°C for 2 days, for bacterial colony counting. For visualization of bacterial multiplication using *GFPuv*-expressing strains, plants were syringe-infiltrated as described (Wang et al. 2007).

Development of *Arabidopsis* transgenic plants and disease assays in *Arabidopsis*.

Arabidopsis SDIR1 full-length coding sequence (CDS) (AT3g54540) was cloned in pMDC32 vector downstream of *2XCaMV 35S* promoter and was used for transformation. To generate *AtSDIR1*-RNAi lines, *pH7GWIW* G2 (II) vector (VIB-UGent Center for Plant Systems Biology) with two different approximately 300-bp fragments of AT3g55530 CDS was used. Floral dip inoculation (Clough and Bent 1998) was used to develop *Arabidopsis* transgenic lines that were screened on hygromycin (25 µg/ml) or Basta (25 µM/ml). The homozygous mutants of *sdir1* T-DNA insertion mutants SALK_114361 and SALK_052702 were obtained from the *Arabidopsis* Biological Resource Center.

For flood inoculation, 4-week-old plants grown in Murashige and Skoog (MS) plates were incubated for 1 min with 40 ml of bacterial suspension as described (Ishiga et al. 2011). The flood inoculation mimics a natural infection wherein the bacteria have to enter through natural openings such as stomata or hydathodes. Leaf inoculation or infiltration is an artificial process wherein the bacteria are forced into apoplast, bypassing stomatal entry. Unlike leaf infiltration, flood inoculation does not cause wounding of plant tissue that can induce a stress response in plants, which can mask the pathogen response. In addition to this, flood inoculation is more reliable with less variability and easy to perform (Ishiga et al. 2011). We therefore used flood inoculation and symptoms were observed after 5 days. For *E. carotovora* disease assay, 3-week-old *Arabidopsis* plants were infiltrated with a needleless syringe with 1×10^5 concentration of bacteria. To examine bacterial growth, entire rosettes from flood-inoculated plants or individual leaves from syringe-infiltrated plants were harvested, ground, and serially diluted as described (Ishiga et al. 2011). Leaf or rosette samples from three biological replicates were collected and the bacteria were quantified in a similar fashion as described above for *N. benthamiana*.

Phytohormone quantification.

A simplified phytohormone quantification protocol was followed (Almeida Trapp et al. 2014) to estimate ABA, JA, and free SA, using high-pressure liquid chromatography (HPLC) tandem mass spectrometry (Agilent Technologies), from the 3-week-old *Arabidopsis* plants treated with *Pseudomonas syringae* pv. *tomato* (DC3000) after 2 dpi. Frozen tissues (100 mg) were ground in liquid nitrogen and were extracted in 70% methanol and 30% water, and then, 50 pmol of labeled hormone standards of SA-d6, ABA-d6, and dihydrojasmonic acid were added and incubated, in the cold, on a rotating shaker for 1 h. The samples were centrifuged at 16,000 × *g* for 5 min at 4°C, and supernatant was transferred to a clean 2-ml glass vial and was dried. Further, the samples were dissolved in 100 µl of 100% methanol by vortexing and sonicating as needed, and 5 µl of processed samples was injected into an Agilent 1290 ultra-HPLC connected to an Agilent 6430 Triple Quad mass spectrometer (Agilent Technologies). Separation was carried out using a Waters BEH C18 column 1.76 µm, 2.1 × 150 mm (Waters Co.) with 0.05% formic acid and H_2O (solvent A) and acetonitrile and 0.05% formic acid (solvent B), starting with 5% solvent B, a gradient from 5 to 46% of solvent B over 19 min and a step to 90% B in 0.1 min, then, a hold at 90% B for 2 min and a step to 5% solvent B in 0.1 min at a flow rate of 0.4 ml min^{-1}. The temperature of the UPLC column was set to 40°C. The gas temperature was 300°C, gas flow 9 ml/min, and nebulizer was 25 psi. Fragmentor and collision energy were optimized for each compound individually. The selected reaction monitoring (SRM) analysis conditions for ABA and d6ABA (negative ion mode) were as follows: capillary = 4,000 V, fragmentor voltage = 100 V, collision energy = 4 V, dwell time = 200 ms, and SRM transition (*m*/*z*) = 263/153 for unlabeled ABA and 269/159 for d6ABA. The SRM analysis conditions for JA and H2JA (negative ion mode) were as follows: capillary = 4,000 V, fragmentor voltage = 100 V, collision energy = 6 V, dwell time = 200 ms, and SRM transition (*m*/*z*) = 209/59 for unlabeled JA and 211/59 for H2JA. The SRM analysis conditions for SA and [2H6]SA (negative ion mode) were as follows: capillary = 4,000 V, fragmentor voltage = 80 V, collision energy = 14 V, dwell time = 200 ms, and SRM transition (*m*/*z*) = 137/93 for unlabeled SA and 141/97 for [2H6]SA. Relative amounts of ABA, JA, and SA were based on comparisons to the labeled hormones. Final concentrations were expressed as nanograms of hormone per gram of sample fresh weight (FW).

Stomatal aperture measurement.

Arabidopsis leaves from 3-week-old plants were used for measurement of stomatal aperture. The epidermal peels were incubated in stomata opening buffer (5 mM KCl, 50 mM $CaCl_2$, and 10 mM MES-Tris, pH 6.1) or 10 µM ABA (Sigma) or with host and nonhost pathogens at room temperature under high light for 4 h. Photographs were taken after 4 h, using a NIKON optishot-2 microscope at 40× magnification and stomatal apertures were measured using ImageJ software (Kaundal et al. 2017).

RT-qPCR analysis.

To test the downregulation of *SDIR1* transcripts in *N. benthamiana*–silenced plants, tissue was collected 3 weeks after TRV inoculation. Total RNA was extracted, according to manufacturer instructions, using Qiagen total RNA extraction kit. The cDNA was synthesized by oligo (dT) primers using molony murine leukemia virus RT (Thermo Fisher Scientific) according to manufacturer instructions. The real-time RT-qPCR was performed with the Sigma KicQStart SYBR green kit. The conditions for the PCR were as follows: 95°C for 2 min, 25 cycles of denaturation at 94°C for 45 s, annealing for 30 s at 58°C, polymerization for 45 s (72°C), followed by plate reading at 72°C for 5 min, estimation of melting curve from 50 to 95°C, and incubation at 72°C for 4 min. *Actin* gene was used

as a reference to normalize RT-qPCR, and the transcript amount was quantified (Ruijter et al. 2009). The primers used in the study are given in Supplementary Table S4.

Root growth inhibition assay.

Seedlings were grown vertically on plates containing MS medium in the presence of different concentrations of MeJA or COR (Sigma Adlrich) for 5 days and were then photographed. Primary root length was measured from a minimum of five replications.

Transcriptome analysis of *SDIR1*-OE and *sdir1* mutant.

Arabidopsis seedlings were grown for 3 weeks on half-strength MS in controlled conditions with a 16-h-light and 8-h-dark cycle at 24°C. These seedlings were flood-inoculated (Ishiga et al. 2011, 2017) with host pathogen *P. syringae* pv. *tomato* DC3000 at 1×10^4 CFU ml^{-1} concentration. Three days after infection, tissue was frozen from both pathogen-treated and control samples. Total RNA in three biological replicates from *SDIR1*-OE (*SDIR1*-OE1), *sdir1* mutant (Salk_114361) and Col-0 leaves were isolated and purified using the RNeasy MinElute cleanup kit (Qiagen) and were used for two-channel microarray. RNA labeling and hybridization to Affymetrix ATH1 arrays were performed as described in the Affymetrix manual. Data normalization between chips was conducted using RMA (robust multichip average) (Irizarry et al. 2003). Gene selections based on associative T-test were made using MATLAB (MathWorks). The raw data are available at ArrayExpress accession E-MTAB-7937. Genes whose residuals between the compared sample pairs that were significantly higher than the measured background noise level were considered to be differentially expressed. A selection threshold of 2 for transcript accumulation (upregulated) and 1.5 times for downregulated genes were used for further analysis. Cluster analysis was performed using MeV, and pathways analysis was performed using MAPMAN tool.

Statistical analyses.

To analyze the data from different experimental results, two-way analysis of variance was carried out according to Fisher (1960). Lower case letters were used to indicate significant differences ($P < 0.05$) between samples, as determined by Tukey's honest significant difference test.

Data and materials availability.

The microarray data is available in the Array Express database under accession numbers E-MTAB-7937, NbME24D08, and AT3g54540.

ACKNOWLEDGMENTS

We thank S. Y. He (Michigan State University) for providing seeds of *JAZ9* overexpression and mutant lines, and J. Gallaway for assistance in plant care. V. S. Ramu acknowledges Fulbright-Nehru postdoctoral fellowship from USIEF, INDIA. K. S. Mysore acknowledges a Fulbright-Nehru Academic and Professional Excellence Award.

AUTHOR-RECOMMENDED INTERNET RESOURCES

ArrayExpress:
https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-7937
Genevestigator: https://genevestigator.com
MeV: http://mev.tm4.org
Noble Research Institute | VIGS Database: https://vigs.noble.org

LITERATURE CITED

Ahmed, F., Senthil-Kumar, M., Dai, X., Ramu, V. S., Lee, S., Mysore, K. S., and Zhao, P. X. 2020. *pssRNAit*: A web server for designing effective and specific plant siRNAs with genome-wide off-target assessment. Plant Physiol. 184:65-81.

Almeida Trapp, M., De Souza, G. D., Rodrigues-Filho, E., Boland, W., and Mithöfer, A. 2014. Validated method for phytohormone quantification in plants. Front. Plant Sci. 5:417.

Anand, A., Rojas, C. M., Tang, Y., and Mysore, K. S. 2012. Several components of SKP1/Cullin/F-box E3 ubiquitin ligase complex and associated factors play a role in *Agrobacterium*-mediated plant transformation. New Phytol. 195:203-216.

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R., and Kazan, K. 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16:3460-3479.

Asselbergh, B., De Vleesschauwer, D., and Höfte, M. 2008. Global switches and fine-tuning-ABA modulates plant pathogen defense. Mol. Plant-Microbe Interact. 21:709-719.

Berndsen, C. E., and Wolberger, C. 2014. New insights into ubiquitin E3 ligase mechanism. Nat. Struct. Mol. Biol. 21:301-307.

Berrocal-Lobo, M., Molina, A., and Solano, R. 2002. Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J. 29:23-32.

Bos, J. I., Armstrong, M. R., Gilroy, E. M., Boevink, P. C., Hein, I., Taylor, R. M., Zhendong, T., Engelhardt, S., Vetukuri, R. R., Harrower, B., Dixelius, C., Bryan, G., Sadanandom, A., Whisson, S. C., Kamoun, S., and Birch, P. R. 2010. *Phytophthora infestans* effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1. Proc. Natl. Acad. Sci. U.S.A. 107:9909-9914.

Boter, M., Ruíz-Rivero, O., Abdeen, A., and Prat, S. 2004. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. Genes Dev. 18:1577-1591.

Browse, J. 2009. Jasmonate passes muster: A receptor and targets for the defense hormone. Annu. Rev. Plant Biol. 60:183-205.

Burgess, R. J., Zhou, H., Han, J., Li, Q., and Zhang, Z. 2012. The SCFDia2 ubiquitin E3 ligase ubiquitylates Sir4 and functions in transcriptional silencing. PLoS Genet. 8:e1002846.

Caarls, L., Pieterse, C. M., and Van Wees, S. C. 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. Front. Plant Sci. 6:170.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L., and Solano, R. 2007. The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448:666-671.

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

De Vleesschauwer, D., Xu, J., and Höfte, M. 2014. Making sense of hormone-mediated defense networking: From rice to *Arabidopsis*. Front. Plant Sci. 5:611.

Delauré, S. L., Van Hemelrijck, W., De Bolle, M. F., Cammue, B. P., and De Coninck, B. M. 2008. Building up plant defenses by breaking down proteins. Plant Sci. 174:375-385.

Devoto, A., Muskett, P. R., and Shirasu, K. 2003. Role of ubiquitination in the regulation of plant defence against pathogens. Curr. Opin. Plant Biol. 6:307-311.

Dreher, K., and Callis, J. 2007. Ubiquitin, hormones and biotic stress in plants. Ann. Bot. 99:787-822.

Duplan, V., and Rivas, S. 2014. E3 ubiquitin-ligases and their target proteins during the regulation of plant innate immunity. Front. Plant Sci. 5:42.

El Oirdi, M., El Rahman, T. A., Rigano, L., El Hadrami, A., Rodriguez, M. C., Daayf, F., Vojnov, A., and Bouarab, K. 2011. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. Plant Cell 23:2405-2421.

Fan, J., Hill, L., Crooks, C., Doerner, P., and Lamb, C. 2009. Abscisic acid has a key role in modulating diverse plant-pathogen interactions. Plant Physiol. 150:1750-1761.

Fisher, R. A. 1960. The design of experiments. Hafner Publishing Company, Inc., New York.

Fonseca, J. P., and Mysore, K. S. 2019. Genes involved in nonhost disease resistance as a key to engineer durable resistance in crops. Plant Sci. 279: 108-116.

Fonseca, S., Chico, J. M., and Solano, R. 2009. The jasmonate pathway: The ligand, the receptor and the core signalling module. Curr. Opin. Plant Biol. 12:539-547.

Gao, T., Wu, Y., Zhang, Y., Liu, L., Ning, Y., Wang, D., Tong, H., Chen, S., Chu, C., and Xie, Q. 2011. *OsSDIR1* overexpression greatly improves drought tolerance in transgenic rice. Plant Mol. Biol. 76:145-156.

Hadwiger, L. A., and Tanaka, K. 2017. Non-host resistance: DNA damage is associated with SA signaling for induction of PR genes and contributes to the growth suppression of a pea pathogen on pea endocarp tissue. Front. Plant Sci. 8:446.

Heath, M. C. 2000. Nonhost resistance and nonspecific plant defenses. Curr. Opin. Plant Biol. 3:315-319.

Hong, J. K., Choi, H. W., Hwang, I. S., and Hwang, B. K. 2007. Role of a novel pathogen-induced pepper C3-H-C4 type RING-finger protein gene, *CaRFPI*, in disease susceptibility and osmotic stress tolerance. Plant Mol. Biol. 63:571-588.

Irizarry, R. A., Gautier, L., and Cope, L. M. 2003. An R package for analyses of Affymetrix oligonucleotide arrays. Pages 102-119 in: The Analysis of Gene Expression Data. Statistics for Biology and Health. G. Parmigiani, E. S. Garrett, R. A. Irizarry, S. L. Zeger, eds. Springer, New York.

Ishiga, Y., Ishiga, T., and Mysore, K. S. 2017. *Pseudomonas syringae* flood-inoculation method in *Arabidopsis*. Bio Protoc. 7:e2106.

Ishiga, Y., Ishiga, T., Uppalapati, S. R., and Mysore, K. S. 2011. *Arabidopsis* seedling flood-inoculation technique: A rapid and reliable assay for studying plant-bacterial interactions. Plant Methods 7:32.

Ishikawa, K., Yamaguchi, K., Sakamoto, K., Yoshimura, S., Inoue, K., Tsuge, S., Kojima, C., and Kawasaki, T. 2014. Bacterial effector modulation of host E3 ligase activity suppresses PAMP-triggered immunity in rice. Nat. Commun. 5:5430.

Kaundal, A., Ramu, V. S., Oh, S., Lee, S., Pant, B., Lee, H. K., Rojas, C. M., Senthil-Kumar, M., and Mysore, K. S. 2017. GENERAL CONTROL NON-REPRESSIBLE4 degrades 14-3-3 and the RIN4 complex to regulate stomatal aperture with implications on nonhost disease resistance and drought tolerance. Plant Cell 29:2233-2248.

Lee, D. H., Choi, H. W., and Hwang, B. K. 2011. The pepper E3 ubiquitin ligase RING1 gene, *CaRING1*, is required for cell death and the salicylic acid-dependent defense response. Plant Physiol. 156:2011-2025.

Lee, S., Senthil-Kumar, M., Kang, M., Rojas, C. M., Tang, Y., Oh, S., Choudhury, S. R., Lee, H. K., Ishiga, Y., Allen, R. D., Pandey, S., and Mysore, K. S. 2017. The small GTPase, nucleolar GTP-binding protein 1 (NOG1), has a novel role in plant innate immunity. Sci. Rep. 7:9260.

Lee, S., Whitaker, V. M., and Hutton, S. F. 2016. Mini review: Potential applications of non-host resistance for crop improvement. Front. Plant Sci. 7:997.

Liao, J., Huang, H., Meusnier, I., Adreit, H., Ducasse, A., Bonnot, F., Pan, L., He, X., Kroj, T., Fournier, E., Tharreau, D., Gladieux, P., and Morel, J. B. 2016. Pathogen effectors and plant immunity determine specialization of the blast fungus to rice subspecies. eLife 5:e19377.

Lim, C. W., Luan, S., and Lee, S. C. 2014. A prominent role for RCAR3-mediated ABA signaling in response to *Pseudomonas syringae* pv. *tomato* DC3000 infection in *Arabidopsis*. Plant Cell Physiol. 55: 1691-1703.

Lorenzo, O., Chico, J. M., Sánchez-Serrano, J. J., and Solano, R. 2004. *JASMONATE-INSENSITIVE1* encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell 16:1938-1950.

Lv, Y., Yang, M., Hu, D., Yang, Z., Ma, S., Li, X. and Xiong, L. 2017. The OsMYB30 transcription factor suppresses cold tolerance by interacting with a JAZ protein and suppressing β-amylase expression. Plant Phys. 173:1475-1491.

Melotto, M., Mecey, C., Niu, Y., Chung, H. S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., Howe, G. A., and He, S. Y. 2008. A critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. Plant J. 55:979-988.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S. Y. 2006. Plant stomata function in innate immunity against bacterial invasion. Cell 126:969-980.

Nagaraj, S., Senthil-Kumar, M., Ramu, V. S., Wang, K., and Mysore, K. S. 2016. Plant ribosomal proteins, RPL12 and RPL19, play a role in nonhost disease resistance against bacterial pathogens. Front. Plant Sci. 6:1192.

Nahar, K., Kyndt, T., De Vleesschauwer, D., Höfte, M., and Gheysen, G. 2011. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiol. 157:305-316.

Park, C. H., Chen, S., Shirsekar, G., Zhou, B., Khang, C. H., Songkumarn, P., Afzal, A. J., Ning, Y., Wang, R., Bellizzi, M., Valent, B., and Wang, G. L. 2012. The *Magnaporthe oryzae* effector AvrPiz-t targets the RING E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice. Plant Cell 24:4748-4762.

Park, G. G., Park, J. J., Yoon, J., Yu, S. N., and An, G. 2010. A RING finger E3 ligase gene, *Oryza sativa* Delayed Seed Germination 1 (OsDSG1), controls seed germination and stress responses in rice. Plant Mol. Biol. 74:467-478.

Qi, T., Song, S., Ren, Q., Wu, D., Huang, H., Chen, Y., Fan, M., Peng, W., Ren, C., and Xie, D. 2011. The jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. Plant Cell 23:1795-1814.

Rahman, T. A., Oirdi, M. E., Gonzalez-Lamothe, R., and Bouarab, K. 2012. Necrotrophic pathogens use the salicylic acid signaling pathway to promote disease development in tomato. Mol. Plant-Microbe Interact. 25:1584-1593.

Rojas, C. M., Senthil-Kumar, M., Wang, K., Ryu, C. M., Kaundal, A., and Mysore, K. S. 2012. Glycolate oxidase modulates reactive oxygen species-mediated signal transduction during nonhost resistance in *Nicotiana benthamiana* and *Arabidopsis*. Plant Cell 24:336-352.

Ruijter, J. M., Ramakers, C., Hoogaars, W. M., Karlen, Y., Bakker, O., van den Hoff, M. J., and Moorman, A. F. 2009. Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37:e45.

Senthil-Kumar, M., and Mysore, K. S. 2013. Nonhost resistance against bacterial pathogens: Retrospectives and prospects. Annu. Rev. Phytopathol. 51:407-427.

Senthil-Kumar, M., and Mysore, K. S. 2014. Tobacco rattle virus-based virus-induced gene silencing in *Nicotiana benthamiana*. Nat. Protoc. 9: 1549-1562.

Senthil-Kumar, M., Wang, M., Chang, J., Ramegowda, V., Del Pozo, O., Liu, Y., Doraiswamy, V., Lee, H. K., Ryu, C. M., Wang, K., Xu, P., Van Eck, J., Chakravarthy, S., Dinesh-Kumar, S. P., Martin, G. B., and Mysore, K. S. 2018. Virus-induced gene silencing database for phenomics and functional genomics in *Nicotiana benthamiana*. Plant Direct 2:e00055.

Seo, E., and Choi, D. 2015. Functional studies of transcription factors involved in plant defenses in the genomics era. Brief. Funct. Genomics 14:260-267.

Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., Kobayashi, Y., Hsu, F. F., Sharon, M., Browse, J., He, S. Y., Rizo, J., Howe, G. A., and Zheng, N. 2010. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400-405.

Tak, H., and Mhatre, M. 2013. Molecular characterization of *VvSDIR1* from *Vitis vinifera* and its functional analysis by heterologous expression in *Nicotiana tabacum*. Protoplasma 250:565-576.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S. Y., Howe, G. A., and Browse, J. 2007. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. Nature 448:661-665.

Trujillo, M., Ichimura, K., Casais, C., and Shirasu, K. 2008. Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. Curr. Biol. 18:1396-1401.

Wang, K., Kang, L., Anand, A., Lazarovits, G., and Mysore, K. S. 2007. Monitoring in planta bacterial infection at both cellular and whole-plant levels using the green fluorescent protein variant GFPuv. New Phytol. 174:212-223.

Wang, K., Senthil-Kumar, M., Ryu, C. M., Kang, L., and Mysore, K. S. 2012. Phytosterols play a key role in plant innate immunity against bacterial pathogens by regulating nutrient efflux into the apoplast. Plant Physiol. 158:1789-1802.

Wasternack, C., and Hause, B. 2013. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany.* Ann. Bot. 111:1021-1058.

Withers, J., Yao, J., Mecey, C., Howe, G. A., Melotto, M., and He, S. Y. 2012. Transcription factor-dependent nuclear localization of a transcriptional repressor in jasmonate hormone signaling. Proc. Natl. Acad. Sci. U.S.A. 109:20148-20153.

Xia, Z., Su, X., Liu, J., and Wang, M. 2013. The RING-H2 finger gene 1 (*RHF1*) encodes an E3 ubiquitin ligase and participates in drought stress response in *Nicotiana tabacum*. Genetica 141:11-21.

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

Xin, X. F., and He, S. Y. 2013. *Pseudomonas syringae* pv. *tomato* DC3000: A model pathogen for probing disease susceptibility and hormone signaling in plants. Annu. Rev. Phytopathol. 51:473-498.

Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W. L., Ma, H., Peng, W., Huang, D., and Xie, D. 2002. The SCF^{COI1} ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell 14:1919-1935.

Zhang, H., Cui, F., Wu, Y., Lou, L., Liu, L., Tian, M., Ning, Y., Shu, K., Tang, S., and Xie, Q. 2015. The RING finger ubiquitin E3 ligase SDIR1

targets SDIR1-INTERACTING PROTEIN1 for degradation to modulate the salt stress response and ABA signaling in *Arabidopsis*. Plant Cell 27: 214-227.

Zhang, L., Zhang, F., Melotto, M., Yao, J., and He, S. Y. 2017. Jasmonate signaling and manipulation by pathogens and insects. J. Exp. Bot. 68:1371-1385.

Zhang, X., Wu, Q., Ren, J., Qian, W., He, S., Huang, K., Yu, X., Gao, Y., Huang, P., and An, C. 2012. Two novel RING-type ubiquitin ligases, RGLG3 and RGLG4, are essential for jasmonate-mediated responses in Arabidopsis. Plant Physiol. 160:808-822.

Zhang, Y., Yang, C., Li, Y., Zheng, N., Chen, H., Zhao, Q., Gao, T., Guo, H., and Xie, Q. 2007. SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in *Arabidopsis*. Plant Cell 19:1912-1929.

Zheng, X. Y., Spivey, N. W., Zeng, W., Liu, P. P., Fu, Z. Q., Klessig, D. F., He, S. Y., and Dong, X. 2012. Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 11:587-596.