Effector-Triggered and Pathogen-Associated Molecular Pattern-Triggered Immunity Differentially Contribute to Basal Resistance to *Pseudomonas syringae*

Jie Zhang,² Haibin Lu,^{1,2} Xinyan Li,^{2,5} Yan Li,² Haitao Cui,² Chi-Kuang Wen,³ Xiaoyan Tang,⁵ Zhen Su,⁴ and Jian-Min Zhou²

¹College of Life Sciences, Peking University, Beijing, China; ²National Institute of Biological Sciences, Beijing, China;

³Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, China; ⁴State Key Laboratory of Plant Physiology and Biochemistry (SKLPPB), College of Biological Sciences, China Agricultural University, China; ⁵Department of Plant Pathology, Kansas State University, Manhattan, KS, U.S.A.

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Pathogens induce pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants. PAMPs are microbial molecules recognized by host plants as nonself signals, whereas pathogen effectors are evolved to aid in parasitism but are sometimes recognized by specific intracellular resistance proteins. In the absence of detectable ETI determining classical incompatible interactions, basal resistance exists during compatible and nonhost interactions. What triggers the basal resistance has remained elusive. Here, we provide evidence that ETI contributes to basal resistance during both compatible and nonhost *Arabidopsis*–*Pseudomonas syringae* interactions. Mutations in *RAR1* and *NDRI*, two genes required for ETI, compromise basal resistance in both compatible and nonhost interactions. Complete nonhost resistance to *P. syringae* pv. *tabaci* required a functional type III secretion system. PTI appears to play a greater role in nonhost resistance than basal resistance during compatible interactions, because abrogation of PTI compromises basal resistance during nonhost but not compatible interactions. Strikingly, simultaneous abrogation of ETI and flagellin-induced PTI rendered plants completely susceptible to the nonadapted bacterium *P. syringae* pv. *tabaci*, indicating that ETI and PTI act synergistically during nonhost resistance. Thus, both nonhost resistance and basal resistance to virulent bacteria can be unified under PTI and ETI.

As an abundant nutrient reservoir, plants are naturally exploited by microbes in their surroundings. An essential element in the evolution of land plants is to develop effective defenses against a wide range of potentially harmful microbes. Some pathogenic microbes manage to overcome the initial defense barrier and succeed in the colonization and infection of their host plants. This applies a selection pressure on the plant to drive a new round of evolution for resistance that, in turn, spurs further evolution in the pathogen for new virulence (Jones and Dangl 2006). Therefore, disease resistance in a nor-

mal plant is likely composed of multiple layers of defenses laid down over a long history of plant–microbe association. Conversely, an adapted pathogen must effectively overcome host resistance to enable its infection of a given plant. Plant immunity to pathogens can be induced upon the perception of relatively conserved pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) through cell surface receptors (Ausubel 2005; Chisholm et al. 2006). Many pathogenic microbes are capable of delivering effector proteins into the host cell to enhance virulence, often by inhibiting PAMP-triggered immunity (PTI). Some of these effectors are recognized by corresponding plant disease resistance proteins in a cultivar-specific manner to activate effector-triggered immunity (ETI). ETI often, but not always, result in hypersensitive response (HR), characterized by cell death (Ausubel 2005; Jones and Dangl 2006). It is thought that PTI and ETI are sequentially evolved to counter the co-evolving pathogens (Kang et al. 2003; Nürnberger and Lipka 2005; Jones and Dangl 2006). Modern-day pathogens have acquired effectors to collectively overcome PTI and ETI in their host plants.

According to the outcome, interactions between plants and pathogenic bacteria can be classified as compatible, incompatible, and nonhost interactions. Incompatible interactions are cultivar-specific and determined by ETI. In contrast, compatible interactions are thought to lack ETI. Mutations in effector genes or cognate plant resistance genes or plant genes required for ETI convert the otherwise incompatible interactions into compatible ones. Nonhost interactions refer to those between a plant species and nonadapted pathogens (Thordal-Christensen 2003). Some effectors from nonadapted pathogens also trigger ETI and induce HR, typically seen in the incompatible interactions. For example, HopQ1-1 of the DC3000 strain of *Pseudomonas syringae* pv. *tomato* triggers HR in *Nicotiana benthamiana*, a nonhost plant (Wei et al. 2007). Deletion of *hopQ1-1* from DC3000 converts this nonadapted bacterium into a strain fully virulent on *N. benthamiana*, indicating a decisive role of ETI in this nonhost interaction. However, a clear role of ETI in overall resistance during other nonhost interactions has not been established. For instance, the PT23 strain of *P. syringae* pv. *tomato*, a nonadapted bacterium on soybean, carries four effector genes (*avrA*, *avrD*, *avrE*, and *avrPto*), each capable of inducing HR and disease resistance in soybean in a cultivar-dependent manner (Lorang et al. 1994). Simultaneous deletion of all four avirulence (*avr*) genes failed to alter the nonhost interaction either qualitatively or quantitatively.

J. Zhang and H. Lu contributed equally to this work.

Corresponding author: J.-M. Zhou; E-mail: zhoujianmin@nibs.ac.cn

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In the absence of recognizable ETI, a weak but detectable inducible resistance exists in both nonhost and compatible interactions. This resistance has been referred to as basal resistance. PTI is known to contribute to basal resistance (Boller and Felix 2009). As a result, PTI and basal resistance have been used interchangeably in the literature. However, it is fully possible that weak ETI could also contribute to basal resistance.

We and others have shown previously that PAMP-induced defenses play an important role in nonhost resistance to *P. syringae* (Li et al. 2005; Truman et al. 2006). The lack of the flagellin-coding gene *fliC* in the *P. syringae* pv. *tabaci* strain compromises *Arabidopsis* nonhost resistance to *P. syringae* pv. *tabaci* (Li et al. 2005). However, the flagellin-mediated resistance only accounts for partial resistance to *P. syringae* pv. *tabaci* (Li et al. 2005). Therefore, additional defense layers may exist for highly effective resistance against nonadapted bacteria. Alternatively, nonadapted bacteria may lack compatibility factors required to be a pathogen in nonhost plants. In the case of compatible interactions, PTI is largely ineffective, at least when bacteria are infiltrated into the apoplast (Zipfel et al. 2004; Li et al. 2005; Melotto et al. 2006). It remains to be determined to what extent PTI and ETI contribute to basal resistance to compatible and nonadapted bacteria.

Here, we provide evidence that PTI and ETI differentially contribute to basal resistance to multiple strains of *P. syringae* bacteria that are either virulent or nonpathogenic on *Arabidopsis*. *Arabidopsis* mutants *rar1* and *ndr1* are compromised in resistance to two strains of *P. syringae* pv. *tabaci* but not *P. syringae* pv. *phaseolicola*. The *rar1* mutant also exhibits enhanced disease susceptibility to DC3000. Consistent with a role of effector-triggered defenses, the RAR1-mediated resistance to *P. syringae* pv. *tabaci* and DC3000 required a type III secretion system (TTSS). The involvement of effector-triggered defenses in DC3000 resistance was further supported by microarray analysis. The expression of a significant portion of *Arabidopsis* defense-related genes are enhanced by TTSS in DC3000 and RAR1 in plants. Furthermore, the *P. syringae* pv. *tabaci* mutant lacking *fliC* is highly virulent on *rar1* or *ndr1* mutant plants, suggesting that PAMP-triggered defenses and effector-triggered defenses act synergistically to restrict this nonadapted bacterium. These results collectively support the idea that PTI and ETI together account for basal resistance to various *P. syringae* bacteria and suggest that the effectiveness of PTI and ETI determines nonhost and compatible interactions.

RESULTS

RAR1 and NDR1 are differentially required for basal resistance to various *P. syringae* strains.

To determine the contribution of PTI and ETI in *Arabidopsis* basal resistance to various compatible and nonadapted *P. syringae* bacteria, we systematically analyzed four *P. syringae* strains belonging to three pathovars: *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *tabaci* R1152, *P. syringae* pv. *tabaci* 6505, and *P. syringae* pv. *phaseolicola* NPS3121. *Arabidopsis* is a highly susceptible host to DC3000 but is a nonhost plant to the other three strains.

We first determined bacterial growth on *rar1* and *ndr1* mutant plants. Both the HSP90 co-chaperone RAR1 and the membrane protein NDR1 are known to be required for ETI during incompatible interactions (Jones and Dangl 2006). NDR1 is known to play a role in basal resistance to DC3000, because the *ndr1* mutant displays enhanced disease susceptibility whereas overexpression of NDR1 enhances resistance to this strain (Coppinger et al. 2004). However, the role of RAR1 in DC3000 basal resistance has been controversial, because

one report indicated normal susceptibility while another suggested enhanced disease susceptibility in the *rar1-20* mutant (Warren et al. 1999; Holt et al. 2005). A role of RAR1 and NDR1 in nonhost resistance to *P. syringae* has not been tested. We independently tested two *rar1* mutants, *rar1-20* and *rar1-29*, for basal resistance to DC3000. Mutant *rar1-29* is an ethyl methanesulphonate (EMS)-generated allele carrying a point mutation in the CHORD II domain that disrupts the interaction with SGT1 (Shang et al. 2006). In repeated experiments, DC3000 bacteria grew to a 20- to 50-fold higher population in the two mutants (Fig. 1A). Both *rar1* mutations increased the growth in *Arabidopsis* of *P. syringae* pv. *tabaci* R1152 by approximately 10-fold (Fig. 1B). The *rar1* mutations also increased the growth of *P. syringae* pv. *tabaci* 6505 in three independent experiments (Fig. 1C). However, the increase was relatively small (three- to fourfold), although the *rar1-29* mutation nearly abolished the *P. syringae* pv. *tabaci* 6505-induced HR (Table 1). The EMS mutant *rar1-29* showed similar or greater susceptibility than the deletion mutant *rar1-20*. Therefore, the *rar1-29* mutant was used in the rest of experiments described in this article. In contrast to the other three strains, *P. syringae* pv. *phaseolicola* NPS3121 did not grow and had indistinguishable bacterial populations on either wild-type (WT) or *rar1-29* plants (Fig. 1D). We also tested bacterial growth on *ndr1* mutant plants. The *ndr1* mutation compromised resistance to *P. syringae* pv. *tabaci* R1152 and *P. syringae* pv. *tabaci* 6505 but not *P. syringae* pv. *phaseolicola* NPS3121 (Fig. 1E). Together, these results indicate that RAR1 and NDR1 are differentially required for basal resistance to the four *P. syringae* strains, suggesting that the RAR1- and NDR1-dependent ETI contributes to basal resistance to DC3000, *P. syringae* pv. *tabaci* R1152, and *P. syringae* pv. *tabaci* 6505 but not *P. syringae* pv. *phaseolicola* NPS3121.

RAR1-dependent resistance is mediated by TTSS function.

To test whether TTSS effector-mediated defenses collectively contribute to resistance to *P. syringae* pv. *tabaci* 6505, we generated an *hrcC*⁻ strain by inserting a truncated *hrcC* gene into TOPO vector and introduced the construct into *P. syringae* pv. *tabaci* 6505 through homologous recombination. HrcC is an essential structural protein of the *P. syringae* TTSS. We tested the resulting mutant for its ability to induce HR. As expected, the *hrcC*⁻ strain was completely impaired in HR induction (Table 1; Supplementary Fig. S1), an observation that is consistent with the known function of HrcC. The *hrcC*⁻ strain was next compared with WT *P. syringae* pv. *tabaci* 6505 for the ability to multiply in WT plants. The mutant strain consistently grew more than WT 6505 (Fig. 2A), supporting the idea that TTSS effectors of this strain contributed to overall resistance in *Arabidopsis*. We also tested the WT *P. syringae* pv. *phaseolicola* NPS3121 and an NPS3121 *hrpA* mutant for growth on WT plants. Both the WT *P. syringae* pv. *phaseolicola* NPS3121 and the *hrpA* mutant had indistinguishable bacterial populations in plants (Fig. 2B), suggesting that NPS3121 TTSS effectors do not collectively contribute to virulence or resistance. However, it still is possible that these effectors may individually play a role in bacterial virulence or trigger ETI responses in *Arabidopsis*.

To further test whether the RAR1-dependent basal resistance to DC3000 and *P. syringae* pv. *tabaci* 6505 resulted from ETI, we determined the impact of a DC3000 *hrpL* mutation (Zwiesler-Vollick et al. 2002) and a 6505 *hrcC* mutation on RAR1-dependent resistance. HrpL is a transcription regulator required for the transcription of TTSS genes. The DC3000 *hrpL*⁻ strain had indistinguishable populations in WT and *rar1-29* plants (Fig. 2C). Similarly, the *P. syringae* pv. *tabaci* 6505 *hrcC*⁻ strain also had indistinguishable populations on WT and *rar1*

plants (Fig. 2D). The results indicate that RAR1 does not impact plant resistance to *P. syringae* TTSS mutants, supporting the proposal that the RAR1-dependent basal resistance to DC3000 and 6505 is triggered by TTSS-delivered effectors.

Role of *Arabidopsis* RAR1 and *P. syringae* pv. *tabaci* *hrcC* in defense gene induction.

ETI in *Arabidopsis* is associated with a strong induction of *PR1* gene expression. Although PTI also induces *PR1*, the

induction is significantly weaker. The WT *P. syringae* pv. *tabaci* 6505 induced a strong *PR1* expression in the WT plants (Fig. 3A). Mutations in either the bacterial *hrcC* gene or *Arabidopsis* *RAR1* gene greatly reduced *PR1* expression. We also tested the expression of three PAMP-induced genes, *FRK1*, *WRKY22*, and *NHO1* (Asai et al. 2002; Li et al. 2005). All three genes were significantly induced by *P. syringae* pv. *tabaci* 6505 inoculation. The *rar1-29* mutation in *Arabidopsis* and *hrcC* mutation in strain 6505 had no discernable effect on

Fig. 1. *RAR1* and *NDR1* are differentially required for basal resistance during compatible and nonhost interactions. **A**, Multiplication of DC3000 bacteria in wild-type (WT)* and *rar1* mutants. **B**, Multiplication of *Pseudomonas syringae* pv. *tabaci* R1152 in WT* and *rar1* mutants. **C**, Multiplication of *P. syringae* pv. *tabaci* 6505 in WT, WT*, *rar1-20*, and *rar1-29* mutants. **D**, Multiplication of *P. syringae* pv. *phaseolicola* NPS3121 in WT* and *rar1-29* mutant. **E**, Multiplication of *P. syringae* pv. *tabaci* R1152, *P. syringae* pv. *tabaci* 6505, and *P. syringae* pv. *phaseolicola* NPS3121 in WT and *ndr1* mutant plants. The WT* and *rar1-29* plants contain a *RAP2.6* reporter gene. Error bars indicate standard deviation. Student's *t* test was carried out to determine the significance of difference; * and ** indicate significant difference at *P* < 0.05 and 0.01, respectively. Experiments were repeated three times with similar results.

the three PAMP-induced genes tested (Fig. 3A). These results suggested that, whereas *RAR1* and TTSS function are required for effector-triggered defense gene expression, they are dispensable for PAMP-induced gene expression. To further test this possibility, we treated WT and *rar1-29* plants with flg22 and determined the expression of *FRK1*, *WRKY22*, and *NHO1* (Fig. 3B). As expected, both WT and *rar1-29* plants showed similar induction of the three tested genes.

Microarray analysis of PAMP-induced and effector-induced defenses in response to DC3000.

To investigate the role of TTSS function in PAMP- and effector-triggered defense gene expression in response to DC3000, we examined public microarray databases for genes regulated by WT and *hrcC*⁻ DC3000 and *P. syringae* pv. *phaseolicola* NPS3121 (included as a nonhost control). A two-fold increase or decrease at a *P* value of 0.05 was used as criteria for differentially expressed genes. Genes up- or downregulated by these strains were compared with 1,928 flg22-inducible genes identified in previous reports (Navarro et al. 2004; Qutob et al. 2006; Chen et al. 2009) (Supplementary Tables S1 and S2). At 2 h after bacterial inoculation, different *P. syringae* strains, either virulent or nonpathogenic, behaved similarly and induced approximately 250 to 440 genes. The vast major-

ity (80 to 89%) of these genes were flg22-inducible genes, more than 200 of which were induced by all three strains. This expression pattern remained steady over the course of 24 h for *P. syringae* pv. *phaseolicola* NPS3121 and the DC3000 *hrcC*⁻ strain. However, the WT DC3000-treated tissues showed dramatically distinct gene expression profiles at 6 and 24 h. WT DC3000 induced only 43 flg22-inducible genes at 6 h, down from 249 at 2 h. A comparison between the WT DC3000 and DC3000 *hrcC*⁻ strain showed that 416 genes were repressed by DC3000 TTSS at 6 h. Among these, 334 were flg22 inducible,

Table 1. Strain *Pseudomonas syringae* pv. *tabaci* 6505 induces hypersensitive response in an *hrcC*⁻ and *RAR1*-dependent manner^a

Plant, strain ^b	9 h	12 h	24 h
Wild type (WT)			
WT	9/18	16/18	16/18
<i>hrcC</i> ⁻	0/18	0/18	0/18
<i>fliC</i> ⁻	15/18	18/18	18/18
<i>rar1-29</i>			
WT	0/18	0/18	3/18
<i>hrcC</i> ⁻	0/18	0/18	0/18
<i>fliC</i> ⁻	0/18	3/18	10/18

^a Shown are numbers of collapsed leaves out of a total of 18 infiltrated leaves.

Fig. 2. Type III secretion system contributes to resistance to *Pseudomonas syringae* pv. *tabaci* 6505 but not *P. syringae* pv. *phaseolicola* NPS3121. **A**, Multiplication of *P. syringae* pv. *tabaci* 6505 wild type (WT) (*Ptab* 6505) and *hrcC*⁻ (*Ptab* 6505 *hrcC*⁻) in *Arabidopsis* plants. **B**, Multiplication of *P. syringae* pv. *phaseolicola* NPS3121 WT (*Pph* NPS3121) and *hrpA*⁻ (*Pph* NPS3121 *hrpA*⁻) in *Arabidopsis* plants. **C**, Multiplication of DC3000 *hrpL*⁻ in WT* and *rar1-29* plants. **D**, Multiplication of *P. syringae* pv. *tabaci* 6505 *hrcC*⁻ in WT* and *rar1-29* plants. Error bars indicate standard deviation. Student's *t* test was carried out to determine the significance of difference. Results shown are representative of three independent experiments.

confirming that a major function of DC3000 TTSS is to suppress PAMP signaling in plants.

A close examination indicated a complex pattern of gene expression in the DC3000-treated tissues at 24 h. As expected for the virulence function, DC3000 TTSS repressed 1,502 genes, including 284 flg22-inducible genes. Interestingly, DC3000 TTSS also induced 1,483 genes, including 328 flg22-inducible genes. Thus, it appears that some flg22-induced genes were suppressed while others were induced by the DC3000 TTSS effectors.

Some of the DC3000-induced genes at 24 h may have resulted from weak ETI. Alternatively, they may be indirectly induced by stress caused by disease-related physiological changes. To further determine the involvement of effector-triggered defenses at 24 h, we carried out additional microarray analysis by comparing gene expression in WT and *rar1-29* plants 24 h after WT DC3000 inoculation. The experiment consisted of three independent biological replicates. Correlation analysis showed that the three biological replicates were ≥95% similar. In total, 154 genes were upregulated when WT plants were compared with the *rar1-29* mutant (Supplementary Table S3). At least 43 of these genes encode proteins with apparent roles in disease resistance and included *NDRI*, *NHO1*, *NPR1/NIM1* interacting protein gene *NIMIN-1*, and genes encoding receptor-like kinases, nucleotide-binding site leucine-rich repeat resistance proteins, WRKYs, and pathogenesis-related (PR) proteins. Of the 154 upregulated genes, 95 (62%) were flg22 inducible, indicating that genes induced

downstream of RAR1 largely overlap with PAMP-induced genes. Consistent with the idea that some DC3000 effectors trigger RAR1-dependent defenses, 18 DC3000 TTSS-induced genes were induced in a RAR1-dependent manner. Even among the 284 flg22-inducible genes that were repressed by DC3000 TTSS, 22 were less repressed in WT plants compared with *rar1-29* plants. These results support the idea that some DC3000 effectors trigger or enhance the expression of defense-related genes, including flg22-inducible genes, in an RAR1-dependent manner.

Inhibition of PAMP-signaling pathway compromises nonhost resistance but not basal resistance to DC3000.

We previously showed that the lack of the flagellin-encoding gene *fliC* in *P. syringae* pv. *tabaci* 6505 resulted in partial loss of nonhost resistance in *Arabidopsis*, indicating that PAMP-induced defenses contribute to nonhost resistance to 6505 (Li et al. 2005). However, because the bacterium carries multiple PAMPs, the *fliC*[−] strain is likely to trigger a partial PTI response. Mitogen-activated protein kinases (MAPK) play a central role in immunity by integrating signals from various PAMPs (Zipfel 2009). We previously identified an effector, HopAII, from *P. syringae* pv. *tomato* that targets MAPK (Li et al. 2005; Zhang et al. 2007). Transgenic plants expressing HopAII are largely abolished in PTI defenses. Therefore, we further examined growth of *P. syringae* pv. *phaseolicola* NPS3121 and *P. syringae* pv. *tabaci* 6505 bacteria in *HopAII* transgenic plants. Transgenic expression of *HopAII* enhanced

Fig. 3. Bacterial type III secretion system and plant *RAR1* are required for *PR1* induction but not pathogen-associated molecular pattern (PAMP)-induced genes. **A**, Northern blot analysis of gene expression in response to bacterial inoculation. Wild-type (WT)* and *rar1-29* plants treated with *Pseudomonas syringae* pv. *tabaci* 6505 WT (*Ptab* 6505) or the *hrcC* mutant (*Ptab* 6505 *hrcC*[−]) bacteria were collected at the indicated times before RNA isolation. RNA blots were hybridized with the indicated probes. Equal loading of RNA was indicated by a representative ethidium-bromide-stained gel. **B**, Northern blot analysis of PAMP-induced genes in WT* and *rar1-29* plants. Plants infiltrated with 1 μM flg22 were collected at the indicated times, and RNA was isolated for Northern analysis.

bacterial populations of the two strains by 50- to 200-fold (Fig. 4A). Although a potential suppression of ETI by HopAII cannot be ruled out, these results are consistent with an important role of PAMP-mediated defenses in nonhost resistance to both *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *tabaci*.

Previous reports indicated that FLS2 signaling contributes to pre-penetration resistance to DC3000 (Zipfel et al. 2004; Melotto et al. 2006). We tested whether the inhibition of the MAPK pathway compromised basal resistance to DC3000. DC3000 grew similarly in WT plants and transgenic plants expressing *HopAII* (Fig. 4B). Because all of our inoculation experiments were done by syringe infiltration, the result suggested that PAMP-signaling does not contribute to post-penetration resistance to DC3000 in the apoplast. This is also consistent with our earlier finding that depletion of the *fliC* gene in DC3000 did not enhance its growth on *Arabidopsis* when the bacteria were infiltrated into leaves (Li et al. 2005).

ETI and PTI synergistically contribute to *Arabidopsis* nonhost resistance to *P. syringae* pv. *tabaci*.

Results described above indicate that abrogation of either PTI or ETI leads to a partial loss of resistance to *P. syringae* pv. *tabaci* 6505. To test the possibility that PTI and ETI act

synergistically in nonhost resistance to this bacterium, we examined the impact of the lack of flagellin-dependent defenses and RAR1- or NDR1-dependent defenses on overall resistance to strain 6505. The *fliC*⁻ strain grew by approximately 1,000-fold in either the *rar1-29* or *ndr1* mutant plants, a level similar to compatible interactions (Fig. 5A and B). These results indicate that ETI and PTI in combination account for most of the plants' resistance to this nonhost bacterium. We have previ-

Fig. 4. Inhibition of pathogen-associated molecular pattern (PAMP) signaling by *HopAII* transgene compromises nonhost resistance but not basal resistance during a compatible interaction. **A**, Multiplication of *Pseudomonas syringae* pv. *tabaci* 6505 or *P. syringae* pv. *phaseolicola* NPS3121 in wild-type (WT) and *HopAII* transgenic plants. **B**, Multiplication of DC3000 in WT and *HopAII* transgenic plants. Plants were treated with 40 μ M estradiol for 24 h before being inoculated with the indicated strains for bacterial growth assay. Error bars indicate standard deviation. Student's *t* test was carried out to determine the significance of difference; * and ** indicate significant difference at $P < 0.05$ and 0.01 , respectively. A lower inoculum (10^5 CFU/ml) was used in this experiment. Experiments were repeated twice with similar results.

Fig. 5. Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) synergistically contribute to nonhost resistance to *Pseudomonas syringae* pv. *tabaci* 6505 (*Ptab* 6505). The *fliC*⁻ strain is fully virulent on **A**, *rar1-29* plants; **B**, *ndr1* plants; and **C**, *nho1* plants. WT (*Ptab* 6505) or *fliC*⁻ (*Ptab* 6505 *fliC*⁻) bacteria were infiltrated into WT*, *rar1-29*, WT, or *nho1* plants, and leaf bacterial populations were determined at the indicated times. Error bars indicate standard deviation. Student's *t* test was carried out to determine the significance of difference; * and ** indicate significant difference at $P < 0.05$ and 0.01 , respectively. Experiments were repeated three times with similar results.

ously shown that *NHO1*, a gene acting downstream of both ETI and PTI, is required for resistance to a broad range of non-host, nonpathogenic, or incompatible *P. syringae* strains (Kang et al. 2003; Li et al. 2005). *nho1* is only partially susceptible to the WT *P. syringae* pv. *tabaci* 6505 but the *fliC*⁻ strain grew by nearly 2,000-fold on the *nho1* plants (Fig. 5C), further supporting the synergistic effect of PTI and ETI in resistance to this *P. syringae* strain.

DISCUSSION

In this study, we demonstrate that PTI and ETI differentially contribute to basal resistance during compatible and nonhost interactions. Our data showed that ETI quantitatively contributes to nonhost resistance to *P. syringae* pv. *tabaci* 6505 and *P. syringae* pv. *tabaci* R1152. Both *RAR1* and *NDR1* are required for resistance to these strains. Supporting the possibility that the *RAR1*-dependent resistance corresponded to ETI, the *hrcC* mutation of strain 6505 and *rar1* mutation in plants had virtually the same effects: a diminished *PR-1* induction, abolished HR, and a three- to fivefold increase of bacterial population in plants. Furthermore, 6505 *hrcC*⁻ had equal bacterial populations in *rar1* and WT plants. Ham and associates (2007) showed that inoculation of *Arabidopsis* with *P. syringae* pv. *phaseolicola* NPS3121 induced both PTI and ETI responses. In our study, neither *RAR1* nor *NDR1* is required for resistance to strain NPS3121. Although a role of ETI cannot be ruled out completely, the data are consistent with a lesser role of ETI in this particular nonhost interaction.

Our results also showed that basal resistance to DC3000, a compatible strain on *Arabidopsis*, is primarily caused by weak ETI. This conclusion is supported by the following findings. *RAR1* and *NDR1* are required for limiting the growth of DC3000, and *RAR1* is not required for resistance to DC3000 *hrpL*⁻. Effectors from DC3000 are known to suppress PTI and ETI (Espinosa et al. 2003; Li et al. 2005; Guo et al. 2009). Microarray analysis indicated that DC3000 TTSS also induce defenses, likely through a subset of effectors. At 6 h after inoculation, DC3000 TTSS predominantly suppresses genes normally induced by flg22. However, a small number of flg22-induced genes are induced by DC3000 TTSS. This becomes more apparent at 24 h after inoculation. Consistent with a role of *RAR1* in ETI, a significant number of defense-related genes, including many induced by flg22, are induced by DC3000 in a *RAR1*-dependent manner. Weak ETI may be common during compatible interactions, because virulent *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *syringae* strains are known to carry certain effectors capable of triggering defenses in their susceptible host plants (Jackson et al. 1999; Vinatzer et al. 2006). Indeed, some *Arabidopsis* resistance genes have been shown to specify weak recognition of *P. syringae* effectors (Eitas et al. 2008; Kim et al. 2009). The possibility that DC3000 triggers ETI in *Arabidopsis* is consistent with an early report showing that plants treated with compatible and incompatible bacteria show similar expression profiles, with the compatible interaction showing delayed defense gene induction (Tao et al. 2003).

Compatible and nonhost interactions in this study differ mainly in the effectiveness of PTI, which plays a greater role in the latter interaction. Although it is often assumed that basal resistance during compatible interactions is induced by PAMPs, critical analysis is scarce. It appears that PTI primarily contributes to pre-penetration resistance to compatible *P. syringae* but is largely ineffective after bacteria have gained access to the apoplast. *Arabidopsis fls2* mutant plants lacking the receptor for flg22 show increased susceptibility to DC3000 when spray inoculated but normal susceptibility when bacteria are infiltrated into leaves (Zipfel et al. 2004). This is because

FLS2 triggers stomata closure upon the recognition of flagellin, and the *fls2* mutant permits better penetration by the bacteria (Melotto et al. 2006). Introduction of the *fliC* mutation into DC3000 or abrogation of PTI by expressing the *HopAII* transgene in plants do not further enhance DC3000 growth when bacteria were infiltrated into leaves (Li et al. 2005) (Fig. 4B). This is likely because DC3000 effectors are highly effective in suppressing PAMP-induced defenses (Li et al. 2005). In contrast, PTI acts in both pre- and post-penetration resistance during nonhost interactions (Li et al. 2005; Melotto et al. 2006). First, the *fliC* mutation in *P. syringae* pv. *tabaci* 6505 significantly enhanced *Arabidopsis* susceptibility to strain 6505 (Li et al. 2005). Second, transgenic expression of *HopAII* compromised resistance to strain 6505 and *P. syringae* pv. *phaseolicola* NPS3121 but not DC3000 (Li et al. 2005) (Fig. 4). Although it is possible that *HopAII* also inhibits ETI, the differential effects of the *HopAII* transgene expression on basal resistance to DC3000 and the nonadapted strains are most consistent with a greater role of PTI in nonhost resistance. Strain 6505 effectors do not appear to collectively suppress PTI, as indicated by the identical PAMP-response gene expression in plants inoculated with WT and *hrcC*⁻ 6505 strains (Fig. 3). The differential roles of PTI during compatible and nonhost interactions are consistent with the notion that overcoming PTI plays a critical role in the adaptation of virulent pathogens to their host plants.

ETI alone does not explain the complete resistance in non-host interactions. The disruption of ETI by *rar1* and *ndr1* mutations converts an incompatible interaction into a compatible one; however, the same mutations only modestly increase the *P. syringae* pv. *tabaci* 6505 or R1152 bacterial populations in plants. This is consistent with the notion that effectors in non-adapted bacteria are largely unable to inhibit PTI in *Arabidopsis*. Indeed, the combination of *fliC* mutation in *P. syringae* pv. *tabaci* 6505 and *rar1-29*, *ndr1*, or *nho1* mutation in the plant resulted in a near-compatible interaction. These results suggest that, when combined, PTI and ETI constitute powerful layers of resistance in this nonhost interaction. In addition, the ability of this bacterium to grow to a high level in immune-compromised plants indicates that the lack of presumed “compatibility factors” in nonadapted bacteria can be compensated for by the abrogation of PTI and ETI. In fact, *P. syringae* mutants lacking functional TTSS are capable of multiplying to high levels in transgenic plants expressing effectors that suppress PTI (Hauck et al. 2003; Kim et al. 2005). It can be deduced that the so-called “compatibility factors” are virulence factors that suppress host defenses. The combined effect of multilayered defenses has been seen for *Arabidopsis* nonhost resistance to *Blumeria graminis* f. sp. *hordei*, an obligated fungal pathogen that causes powdery mildew on barley. The *pen2/eds1* double-mutant plants compromised in both pre-penetration and post-penetration defenses are highly susceptible to this normally nonhost fungal pathogen (Lipka et al. 2005). Although the EDS1-mediated resistance is likely triggered by fungal effectors, there is evidence suggesting that the PEN2-mediated resistance is induced by PAMPs (Jones and Dangl 2006).

Often, basal resistance and PTI are used interchangeably. The work described here clearly showed that both PTI and ETI can contribute to basal resistance. This is consistent with the proposal put forward by Jones and Dangl (2006): “the most accurate definition of basal defense would be PTI plus weak ETI minus ETS” (effector-triggered susceptibility). Basal resistance, by definition, is a descriptive term that implies weak resistance. In contrast, PTI and ETI, which can be strong or weak, are mechanistically defined terms. We suggest that the use of basal resistance be limited to minimal resistance that remains mechanistically defined.

MATERIALS AND METHODS

Plants and bacterial strains.

Five-week-old WT Col-0, *ndr1*, *rar1-20*, and *rar1-29* mutants and *HopAII* transgenic plants (all in Col-0 background) were used for all experiments (Li et al. 2005; Shang et al. 2006). Bacterial strains used included *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *tabaci* R1152, WT and *fliC*⁻ *P. syringae* pv. *tabaci* 6505, and the WT *P. syringae* pv. *phaseolicola* NPS3121 and its *hrpA*⁻ derivative (Lu et al. 2001; Li et al. 2005).

To knockout the *hrcC* gene in *P. syringae* pv. *tabaci* 6505, a TOPO:*hrcC* plasmid was constructed by cloning an *hrcC* partial open reading frame (ORF) into the TOPO TA cloning vector (Invitrogen). The *hrcC*-F primer (5'-TTG GTC AGG AAT ACC ACT TC-3') and the *hrcC*-R primer (5'-TGA TGA GTT TCT CGT ACA TCG-3') were used for amplifying the *hrcC* partial ORF. The TOPO:*hrcC* plasmid was then introduced into *P. syringae* pv. *tabaci* 6505 by electroporation. Colonies with kanamycin and ampicillin resistance were selected and verified by diagnostic polymerase chain reaction analysis with the *hrcC*-Diag primer (5'-ATT TCA GTG AGT TCG CTG AG-3') in combination with the T7 primer (5'-TAA TAC GAC TCA CTA TTG GG-3') or the SP6 primer (5'-TAT TTA GGT GAC ACT ATA G-3').

HR and bacterial growth assay.

Bacteria were syringe infiltrated into 5-week-old *Arabidopsis* plants at 10⁸ CFU/ml for HR assay. For bacterial growth assay, plants were syringe infiltrated with 10⁶ CFU/ml bacteria (or otherwise indicated).

Northern analysis.

Plants were infiltrated with bacteria at 10⁶ CFU/ml, H₂O, or 1 μM flg22 peptide, and leaves were collected at the indicated times for RNA isolation. Total RNA (10 μg) was loaded in each lane and the RNA blot was hybridized with the indicated radio-labeled probes.

Microarray analysis.

WT or *rar1-29* plants were treated with DC3000 for 24 h prior to RNA isolation. Each treatment contained three biological replicates. Affymetrix ATH1 arrays were used for hybridization. GCOS software and MAS5 algorithm were used for data collection and normalization. To analyze the differentially expressed genes between control and treatment, fold-change analysis and a one-way analysis of variance test were applied. *P* value ≤0.05 and fold change ≥2 between treatment and control samples were considered as cut-off, and the induced or repressed genes were selected.

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