Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2010) **11**(6), 783–793 DOI: 10.1111/J.1364-3703.2010.00642.X

Effector-triggered innate immunity contributes *Arabidopsis* resistance to *Xanthomonas campestris*

WEI RONG[1,2,3], FENG FENG[4], JIANMIN ZHOU[3] AND CHAOZU HE[1,*]

[1]*State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China*
[2]*Graduate School of Chinese Academy of Sciences, Beijing, 100039, China*
[3]*National Institute of Biological Sciences, Beijing, 102206, China*
[4]*School of Life Sciences and Graduate School at Shenzhen, Tsinghua University, Beijing, 100084, China*

SUMMARY

Xanthomonas campestris pv. *campestris*, the causal agent of black rot disease, depends on its type III secretion system (TTSS) to infect cruciferous plants, including *Brassica oleracea*, *B. napus* and *Arabidopsis*. Previous studies on the *Arabidopsis*–*Pseudomonas syringae* model pathosystem have indicated that a major function of TTSS from virulent bacteria is to suppress host defences triggered by pathogen-associated molecular patterns. Similar analyses have not been made for the *Arabidopsis*–*X. campestris* pv. *campestris* pathosystem. In this study, we report that *X. campestris* pv. *campestris* strain 8004, which is modestly pathogenic on *Arabidopsis*, induces strong defence responses in *Arabidopsis* in a TTSS-dependent manner. Furthermore, the induction of defence responses and disease resistance to *X. campestris* pv. *campestris* strain 8004 requires *NDR1* (NON-RACE-SPECIFIC DISEASE RESISTANCE1), *RAR1* (required for *Mla12* resistance) and *SGT1b* (suppressor of G2 allele of *skp1*), suggesting that effector-triggered immunity plays a large role in resistance to this strain. Consistent with this notion, AvrXccC, an *X. campestris* pv. *campestris* TTSS effector protein, induces *PR1* expression and confers resistance in *Arabidopsis* in a *RAR1*- and *SGT1b*-dependent manner. In *rar1* and *sgt1b* mutants, AvrXccC acts as a virulence factor, presumably because of impaired resistance gene function.

INTRODUCTION

Many plant pathogenic bacteria depend on the conserved type III secretion system (TTSS) to deliver effector proteins into plant cells and to promote parasitism (Buttner and Bonas, 2006; He *et al*., 2004). Some of the effector proteins were initially identified as products of avirulence (*Avr*) genes conditioning resistance (R) protein-mediated defences, termed effector-triggered innate immunity (ETI), in plants (Boller and He, 2009; Jones and Dangl, 2006; Zhou and Chai, 2008). ETI is often associated with the hypersensitive response (HR), a rapid, programmed host cell death at the infection site. However, some effector proteins elicit weak defence responses in the absence of HR, as exemplified by the recognition of the *Pseudomonas syringae* effector AvrB by the *Arabidopsis* R protein TAO1 (Eitas *et al*., 2008). In addition to ETI, plants are also equipped to perceive pathogen-associated molecular patterns (PAMPs) and to mount immune responses (Bittel and Robatzek, 2007; Boller and He, 2009; Zhou and Chai, 2008). PAMP-triggered innate immunity (PTI) is often accompanied by the activation of mitogen-activated protein kinases (MAPKs), the production of reactive oxygen species, callose deposition and induced defence gene expression. ETI and PTI activate a common set of gene expression (Navarro *et al*., 2004). Likewise, callose deposition occurs during both PTI and ETI (Ham *et al*., 2007), suggesting that the two pathways converge before the activation of downstream defence responses. It should be noted, however, that the defence responses are not necessarily responsible for disease resistance. Instead, they often correlate with disease resistance in plants.

Plant–pathogen interactions can be divided into compatible, incompatible and nonhost interactions. Unlike incompatible interactions, which are typically dictated by one or a few *R* genes specifying strong ETI, compatible interactions do not display visible ETI. However, plants possess a low level of resistance in compatible interactions. This low level of resistance is referred to as basal resistance. In the literature, basal resistance has been used interchangeably with PTI without careful experimental data. It should be cautioned, however, that basal resistance, by definition, is a descriptive term, whereas PTI is a mechanistic term. Recent evidence indicates that weak ETI can play a large role in *Arabidopsis* basal resistance to the virulent *P. syringae* strain DC3000 (Zhang *et al*., 2010). Nonhost interaction refers to plant resistance to non-adapted pathogens, and PTI and ETI collectively contribute to nonhost resistance (Li *et al*., 2005; Wei *et al*., 2007; Zhang *et al*., 2010).

**Correspondence*: Email: hecz@im.ac.cn

The largely overlapping defence responses hinder the distinction between PTI and ETI. However, because ETI is typically triggered by Gram-negative bacterial TTSS effectors, TTSS mutants are useful tools in the dissection of PTI and ETI. In addition, several host proteins are known to play crucial roles in ETI. For example, NDR1 (NON-RACE-SPECIFIC DISEASE RESISTANCE1), a glycophosphatidyl-inositol (GPI)-anchored plasma membrane protein, is required for ETI resistance conditioned by many, but not all, R proteins in *Arabidopsis* (Day *et al*., 2006; Tornero *et al*., 2002). Likewise, EDS1 (enhanced disease susceptibility1) is also required for ETI specified by some *R* genes (Aarts *et al*., 1998). Furthermore, HSP90 co-chaperones RAR1 (required for *Mla12* resistance) and SGT1 (suppressor of G2 allele of *skp1*) are required to stabilize some R proteins, and *rar1* and *sgt1* mutants are often compromised in ETI (Azevedo *et al*., 2002; Hammond-Kosack and Parker, 2003; Hubert *et al*., 2003, 2009; Muskett *et al*., 2002; Takahashi *et al*., 2003). *eds1*, *rar1* and *sgt1* mutants are not affected in flg22-induced disease resistance (Zipfel *et al*., 2004), suggesting that they can be used to differentiate between PTI and ETI.

It is well established that TTSS-deficient strains of *P. syringae* induce strong defence responses because of the presence of a collection of PAMPs (Hauck *et al*., 2003; Li *et al*., 2005). These defences are largely suppressed by TTSS effectors from the virulent *P. syringae* strain DC3000 (Hauck *et al*., 2003; Li *et al*., 2005). Many *P. syringae* TTSS effectors are also capable of inhibiting ETI (Guo *et al*., 2009). Artificial inhibition of host defences by transgenic expression of effector proteins often enables *P. syringae* TTSS mutants to multiply to high levels in plants (Hauck *et al*., 2003; Kim *et al*., 2005; Li *et al*., 2005). The TTSS-mediated suppression of callose deposition has also been reported for *Xanthomonas campestris* pv. *vesicatoria* (Brown *et al*., 1995). Therefore, suppression of host defences appears to be a major function of virulent *P. syringae* and *X. campestris* pv. *vesicatoria* TTSS effectors, at least in the early phase of infection. Whether this concept can be generally applied to other pathosystems remains to be determined.

Xanthomonas campestris pv. *campestris* (*Xcc*) is a xylem-colonizing systemic pathogen and causes black rot disease on large numbers of crucifers worldwide (Williams, 1980). *Xcc* multiplies in vascular tissues after entry into the plant via hydathodes (Hugouvieux *et al*., 1998; Sun *et al*., 2006), which is in contrast with *P. syringae* pv. *tomato*, which enters the plant through stomata and causes disease in leaves primarily as a result of mesophyll colonization. Thus, the two pathogens assume completely different life styles which probably involve distinct host responses. *Xcc* strain 8004 can infect and cause disease symptoms on *Arabidopsis* plants, making it a useful model for the study of *Xanthomonas* pathogens. Both syringe infiltration and wound inoculation have been used to study the *Arabidopsis–Xcc* interaction (Meyer *et al*., 2005). Syringe infiltration is an unnatural route of *Xcc* infection and is generally used to study the bacterial growth of *Xcc* and defence responses in plants. Wound inoculation mimics natural *Xcc* infection and is sensitive for monitoring disease symptoms.

In this study, we show that, unlike TTSS-deficient derivatives of *P. syringae* DC3000, *Xcc* strain 8004 TTSS mutants appear to induce only weak PTI defence responses in *Arabidopsis*. In contrast, wild-type *Xcc* strain 8004 induces strong defence responses, but grows to significantly higher levels than do TTSS mutants. *Xcc* strain 8004-induced defence responses require *RAR1*, *SGT1b* and *NDR1*, and the *rar1*, *sgt1b* and *ndr1* mutants are compromised in their resistance to *Xcc* strain 8004. Furthermore, the *Xcc* strain 8004 effector AvrXccC confers resistance in WT *Arabidopsis* plants, but enhances bacterial virulence on *rar1* and *sgt1b* mutant plants. These results collectively indicate that ETI plays an important role in *Arabidopsis* resistance to *Xcc* strain 8004, and that some *Xcc* strain 8004 TTSS effector proteins may contribute to virulence by modulating host processes independent of defence inhibition.

RESULTS

The TTSS of *Xcc* strain 8004 is required to induce defence responses in *Arabidopsis*

It is well established that PAMPs from *P. syringae* trigger strong defences in *Arabidopsis*, and these defences are largely suppressed by TTSS effectors from virulent *P. syringae* strains (Clay *et al*., 2009; DebRoy *et al*., 2004; Hauck *et al*., 2003). Some of the effectors from virulent *P. syringae*, however, appear to trigger weak ETI that contributes to basal resistance (Zhang *et al*., 2010). To determine the role of TTSS from *Xcc* in triggering *Arabidopsis* defences in response to this bacterium, wild-type *Xcc* strain 8004 and its TTSS structural gene *hrcV* (Δ*hrcV*) mutant, which lacks a conserved inner membrane protein of the core TTSS (Wang *et al*., 2007), were inoculated into *Arabidopsis* Col-0 leaves by syringe infiltration. Gene expression [*FRK1* (FLG22-INDUCED RECEPTOR-LIKE KINASE1), *PR1* (PATHOGENESIS-RELATED GENE1)] and callose deposition, which are defence responses activated during both PTI and ETI, were monitored. Wild-type *Xcc* strain 8004 strongly triggered callose deposition in leaves at 24 h after inoculation (Fig. 1A). Surprisingly, the Δ*hrcV* mutant strain failed to induce callose deposition above the background level, suggesting that *hrcV* is required for callose induction. Indeed, when the Δ*hrcV* mutant strain was complemented with a plasmid containing the wild-type *hrcV* gene, callose induction was restored (Fig. 1A). In addition, the expression of *PR1* and *FRK1* was induced more

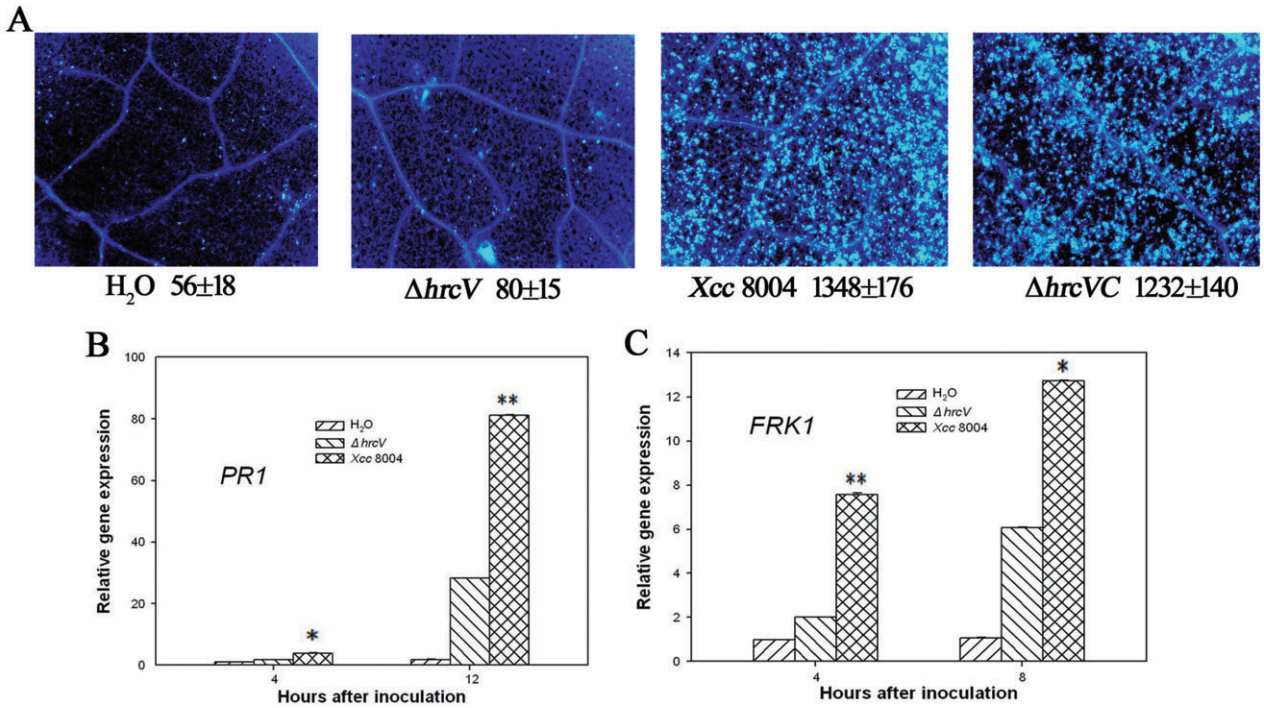

Fig. 1 *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004)-induced defence responses in *Arabidopsis* are type III secretion system (TTSS) dependent. (A) Callose deposition in wild-type (WT) *Arabidopsis* Col-0 leaves. Callose deposition was performed on 5-week-old *Arabidopsis* leaves at 24 h after syringe infiltration with H_2O or the indicated bacterial strains at 2×10^7 colony-forming units (CFU)/mL. Average numbers of callose deposits per microscopic field of 0.1 mm^2 are shown below each photograph. '±' represents the standard deviations from three leaves of each plant. Similar results were obtained in three independent experiments. The mRNA abundance of *PR1* (B) and *FRK1* (C) in leaves treated with H_2O, Δ*hrcV* and *Xcc* 8004 was determined at the indicated times by quantitative polymerase chain reaction (Q-PCR). Gene expression was monitored by Q-PCR after bacterial syringe infiltration at 2×10^7 CFU/mL at the indicated times. *(*t*-test, $P < 0.05$) and **(*t*-test, $P < 0.01$) indicate statistically significant differences between the *hrcV* mutant and WT *Xcc* 8004. Error bars indicate standard deviations. These experiments were repeated twice with similar results.

strongly by wild-type *Xcc* strain 8004 than by the Δ*hrcV* mutant (Fig. 1B,C). These findings indicate that TTSS is required for *Xcc*-induced defence responses in *Arabidopsis* and, in the absence of TTSS, *Xcc* PAMPs do not effectively trigger plant defences.

It has been reported that flagellin proteins isolated from different *Xcc* strains vary dramatically in their capability to elicit *FLS2* (FLAGELLIN-SENSITIVE2)-mediated responses in *Arabidopsis* (Abramovitch *et al*., 2006; Schwessinger and Zipfel, 2008; Sun *et al*., 2006). We investigated whether FLS2 is involved in defence responses and disease resistance to *Xcc* strain 8004. When infiltrated with *Xcc* strain 8004, the wild-type and *fls2* mutant, *Arabidopsis* leaves displayed similar callose deposition (Fig. 2A) and chlorosis symptoms (Fig. 2B), and supported similar bacterial growth in leaves (Fig. 2C). These results suggest that FLS2 does not contribute to *Arabidopsis* resistance to *Xcc* strain 8004. It has been shown that the *Xcc* flagellin amino acid valine-43 is essential for recognition by FLS2, and a Val43Asp substitution renders the flagellin noneliciting (Sun *et al*., 2006). We therefore aligned *Xcc* strain 8004, *Xcc* B186 (noneliciting) and *Xcc* B305 (eliciting) flagellin sequences corresponding to flg22. As expected, the *Xcc* 8004 flagellin 43 amino acid was identical to *Xcc* B186, but not *Xcc* B305 (Fig. 2D), suggesting that *Xcc* strain 8004 flagellin was not recognized by FLS2.

To further confirm the function of *Xcc* TTSS in triggering host defence responses, we determined callose deposition in response to two additional TTSS mutants Δ*hrpG* and Δ*hrpX* (Wang *et al*., 2007). HrpG, a two-component system response regulator belonging to the OmpR family, controls the expression of HrpX which is an AraC-type transcriptional activator and regulates the expression of *hrp* and effector genes. Figure S1 (see Supporting Information) showed that the Δ*hrpG* and Δ*hrpX* mutants triggered weak callose deposition relative to *Xcc* strain 8004. Although the TTSS mutant strains induced less callose deposition, they grew substantially less than wild-type bacteria at 3 days post-inoculation (Fig. S2, see Supporting Information), indicating that the defence responses induced by *Xcc* TTSS were not sufficient to restrict *Xcc* bacterial growth in *Arabidopsis*.

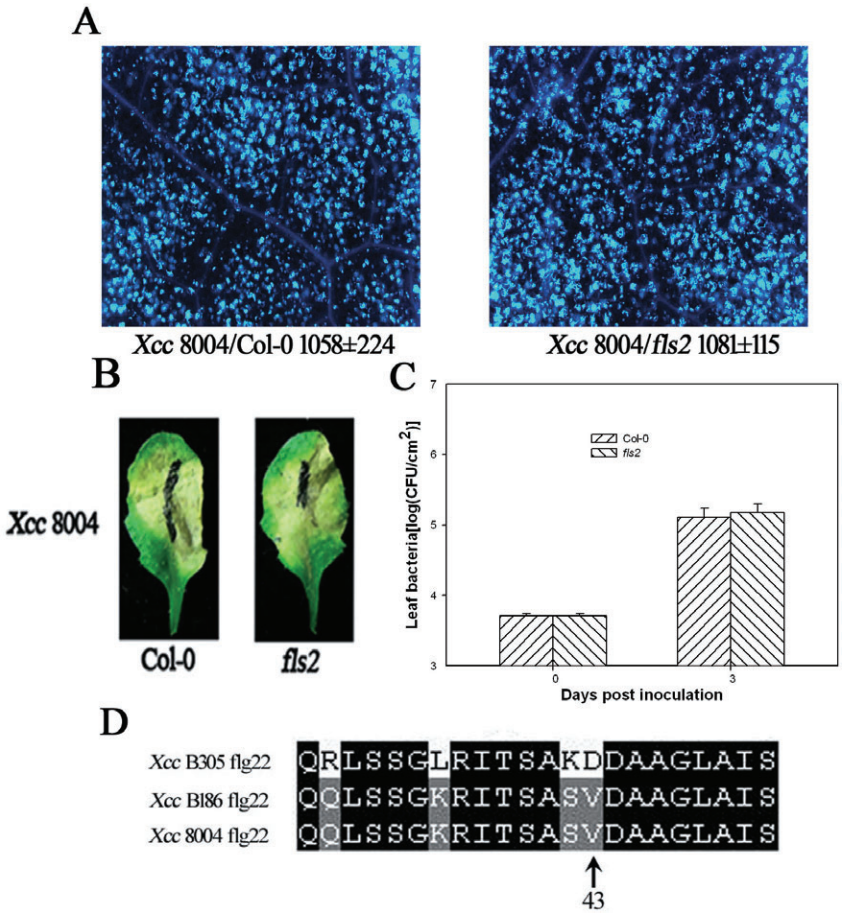

Fig. 2 *FLS2* is not required for *Arabidopsis* resistance to *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004). (A) Callose deposition in wild-type (WT) Col-0 and *fls2* mutant leaves was determined at 24 h following inoculation of *Xcc* 8004 at 2×10^7 colony-forming units (CFU)/mL. Average numbers of callose deposits per microscopic field of 0.1 mm^2 are shown below each photograph. '±' represents the standard deviations from three leaves of each plant, and similar phenotypes were observed in three independent experiments. (B) *Xcc* 8004 induces chlorosis in both Col-0 and *fls2* mutant. Symptoms were photographed 5 days after inoculation of *Xcc* 8004 at 1×10^7 CFU/mL. (C) Bacterial growth in Col-0 and *fls2* mutant. Five-week-old *Arabidopsis* plants were inoculated by *Xcc* 8004 at 5×10^5 CFU/mL, and the bacterial population in the leaf was determined at the indicated times. Each data point represents three replicates. Error bars indicate standard deviations. The experiments were repeated twice with similar results. (D) Alignment of FliC sequences corresponding to flg22 in *Xcc* B305 (eliciting), *Xcc* B186 (noneliciting) and *Xcc* 8004.

NDR1, RAR1 and SGT1 are involved in *Xcc*-induced defence responses in *Arabidopsis*, and the callose deposition induced by *Xcc* is dependent on salicylic acid (SA)

The TTSS-dependent defence responses induced by *Xcc* suggest an involvement of R proteins that probably recognize some of the TTSS effectors. To further test this possibility, we determined whether the NDR1-, RAR1- and SGT1-dependent defences contributed to resistance to *Xcc* strain 8004. We inoculated *ndr1*, *rar1* and *sgt1b* mutants with *Xcc* strain 8004 at 2×10^7 colony-forming units (CFU)/mL. As expected, callose deposition in *ndr1*, *rar1* and *sgt1b* mutants was substantially reduced relative to Col-0 at 24 h after inoculation (Fig. 3A). In addition, the *PR1* (Fig. 3B,C) and *FRK1* (Fig. 3D) mRNA abundance in Col-0 was significantly greater than that in the *ndr1*, *rar1* and *sgt1b* mutants at 12, 24 and 8 h, respectively, after *Xcc* strain 8004 inoculation. The results indicate that *NDR1*, *RAR1* and *SGT1b* are required for *Xcc* strain 8004-induced defence responses.

Bacterial growth assays indicated that *Xcc* strain 8004 bacteria multiplied approximately eight-fold more strongly in the *ndr1* mutant (Fig. 4A) and four- to five-fold more strongly in the *rar1* and *sgt1b* mutants relative to Col-0 at 3 days post-inoculation (Fig. 4B). These data demonstrate that *NDR1*, *RAR1* and *SGT1* are required for *Arabidopsis* resistance to *Xcc*, and suggest that ETI contributes significantly to the resistance.

Microbes can elicit callose formation in *Arabidopsis* leaves via the SA-dependent pathway (DebRoy *et al*., 2004). To determine whether *Xcc* strain 8004-induced callose formation was dependent on SA, we inoculated the leaves of the SA biosynthesis mutant *SALICYLIC ACID INDUCTION DEFICIENT2* (*sid2*) and wild-type *Arabidopsis* with *Xcc* strain 8004. The *sid2* mutant showed increased chlorosis following wild-type *Xcc* strain 8004 inoculation (Fig. 4C). Consistently, *sid2* leaves supported significantly greater bacterial growth (Fig. 4D). These results are consistent with a previous report (O'Donnell *et al*., 2003). Correlating with the increased susceptibility, *sid2* leaves showed a 10-fold lower callose deposition compared with Col-0 following *Xcc* strain 8004 inoculation (Fig. 3A).

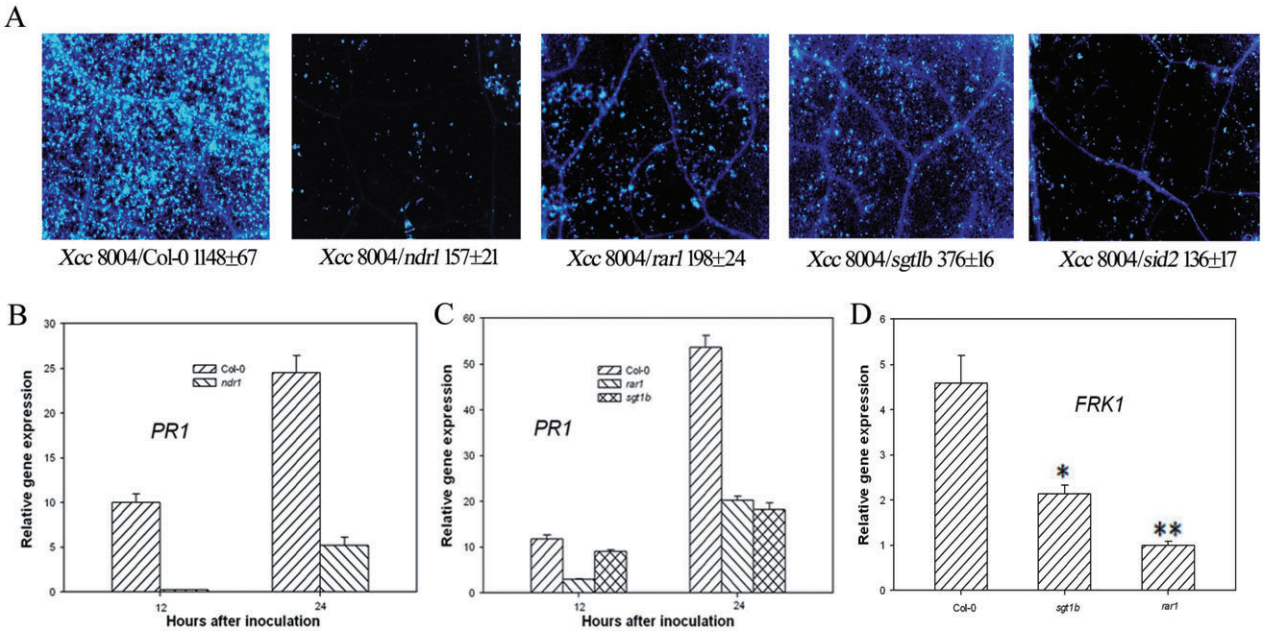

Fig. 3 *NDR1*, *RAR1*, *SGT1b* and *SID2* are required for *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004)-induced defence responses. (A) *ndr1*, *rar1*, *sgt1b* and *sid2* mutants are compromised in callose deposition in response to *Xcc* 8004. Leaves of the indicated genotypes were inoculated with *Xcc* 8004 at 2×10^7 colony-forming units (CFU)/mL, and callose deposits were determined 24 h later. Average numbers of callose deposits per microscopic field of 0.1 mm^2 are shown below each photograph. '±' represents the standard deviations from three leaves of each plant. Similar results were obtained in three independent experiments. (B) *Xcc* 8004-induced *PR1* expression requires *NDR1*. (C) *Xcc* 8004-induced *PR1* expression requires *RAR1* and *SGT1b*. (D) *Xcc* 8004-induced *FRK1* expression requires *RAR1* and *SGT1b*. *(*t*-test, $P < 0.05$) and **(*t*-test, $P < 0.01$) indicate statistically significant differences between *rar1*, *sgt1b* mutant and wild-type (WT) Col-0. Leaves of the indicated genotypes were infiltrated with *Xcc* 8004 at 2×10^7 CFU/mL, and RNA was extracted for quantitative reverse transcriptase-polymerase chain reaction analyses.

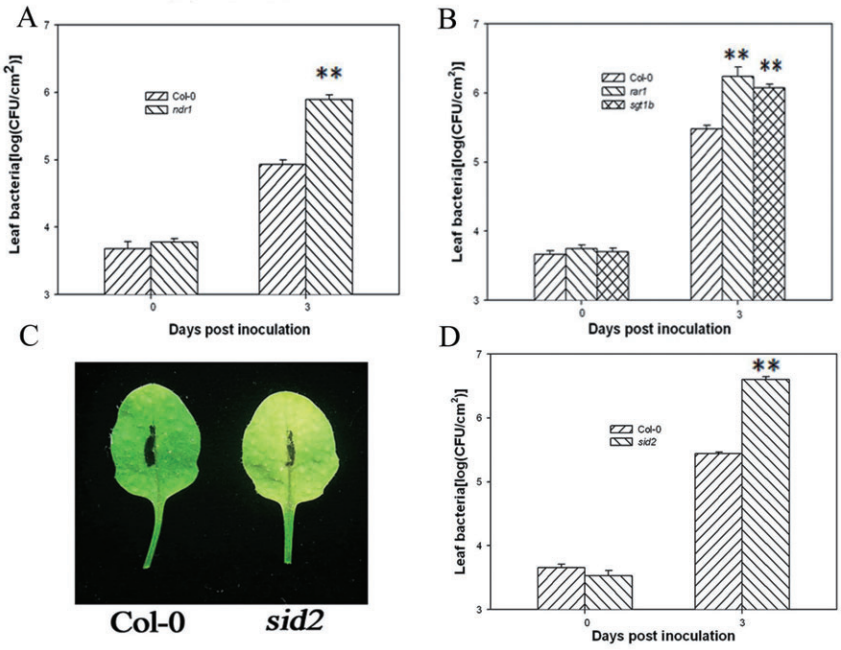

Fig. 4 *Arabidopsis* resistance to *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004) requires *NDR1*, *RAR1*, *SGT1b* and *SID2*. (A, B) *ndr1*, *rar1* and *sgt1b* show enhanced susceptibility to *Xcc* 8004. (C) *sid2* shows enhanced leaf chlorosis. (D) *sid2* shows enhanced susceptibility. Leaves of the indicated genotypes were infiltrated with *Xcc* 8004 at 5×10^5 colony-forming units (CFU)/mL for bacterial growth assay and 1×10^7 CFU/mL for leaf chlorosis assay. Each data point represents three replicates. Error bars indicate standard deviations. **(*t*-test, $P < 0.01$) indicates statistically significant differences between *ndr1*, *rar1*, *sgt1b*, *sid2* mutants and wild-type (WT) Col-0. The experiments were performed on 5-week-old *Arabidopsis* leaves and repeated twice with similar results.

RAR1 and SGT1 are required for *Xcc* effector protein AvrXccC-induced defence responses in *Arabidopsis*

It has been reported that the *Xcc* strain 8004 TTSS effector protein AvrXccC triggers ETI in *Brassica napiformis* (Wang *et al*., 2007). We therefore tested whether AvrXccC also triggers ETI in *Arabidopsis*. AvrXccC is homologous to the *P. syringae* effector AvrB, which is recognized by the R protein RPM1 and elicits strong HR (Desveaux *et al*., 2007). However, AvrXccC is not recognized by RPM1 (Desveaux *et al*., 2007). Wild-type *Xcc* strain 8004 and the Δ*avrXccC* mutant were similarly unable to induce electrolyte leakage in Col-0 leaves (Fig. S3, see Supporting Information), indicating that AvrXccC does not trigger a strong ETI in these *Arabidopsis* plants. To investigate the function of AvrXccC in *Arabidopsis*, we inoculated the leaves of wild-type Col-0, *rar1* and *sgt1b* mutants by infiltration with wild-type *Xcc* strain 8004, the Δ*avrXccC* mutant and the *avrXccC*-complemented (Δ*avrXccCC*) strain, respectively. The three strains showed indistinguishable disease symptoms and bacterial growth (data not shown). However, when inoculated by the piercing method, wild-type *Xcc* strain 8004 induced typical black rot disease symptoms on *rar1* and mild symptoms on *sgt1b* leaves, but was almost symptomless on wild-type Col-0 (Fig. 5A). The lack of symptoms in leaves pierced with wild-type *Xcc* strain 8004 was consistent with a previous report (Xu *et al*., 2008). In contrast, Δ*avrXccC* induced typical symptoms in both Col-0 and *rar1*, and mild symptoms in *sgt1b*, whereas the complemented strain was indistinguishable from the wild-type strain (Fig. 5A). Consistent with the increased disease symptoms in *rar1* and *sgt1b* leaves, the wild-type *Xcc* strain 8004 grew to higher levels on *rar1* and *sgt1b* leaves (Fig. 5B). These results are consistent with the notion that ETI contributes to *Arabidopsis* resistance to wild-type *Xcc* strain 8004. Interestingly, *rar1* and *sgt1b* leaves inoculated with wild-type *Xcc* strain 8004 always showed more severe disease symptoms and a greater bacterial population than those inoculated with the Δ*avrXccC* strain (Fig. 5A,B), indicating that *avrXccC* conferred resistance in Col-0 leaves, but virulence function in *rar1* and *sgt1b* leaves. Consistent with a role of *avrXccC* in ETI, the Δ*avrXccC* mutation abolished *Xcc* strain 8004-induced *PR1* expression during piercing inoculation (Fig. 5C), indicating that *avrXccC* is required for *PR1* induction. *PR1* induction also required *RAR1* and *SGT1b* in plants, further confirming that *avrXccC*-induced *PR1* expression is probably mediated by a single *R* gene or multiple *R* genes. However, *Xcc* strain 8004 failed to induce *FRK1* expression and callose deposition when inoculated by the piercing method (data not shown), indicating that *Xcc* strain 8004 only triggers a subset of defence responses when multiplying within vascular tissue.

To further confirm whether AvrXccC can induce defence responses in *Arabidopsis*, an oestrogen-inducible *AvrXccC* transgene was introduced into Col-0, *sgt1b* and *rar1*. Homozygous T2 transgenic lines accumulating similar levels of AvrXccC following oestradiol treatment were selected for the experiments (Fig. 5D). We examined the *FRK1* and *PR1* mRNA levels and callose deposition in these lines, 12 h after oestradiol treatment without bacterial inoculation. Quantitative polymerase chain reaction (PCR) showed that *PR1* gene expression was strongly induced in *AvrXccC*/Col-0 and mildly in *AvrXccC*/*sgt1b*, but not in *AvrXccC*/*rar1*, transgenic lines (Fig. 5E). The findings further support a role for AvrXccC in ETI induction. However, the expression of *AvrXccC* failed to induce *FRK1* expression and callose deposition in any genetic background tested (data not shown), suggesting that AvrXccC elicits only a subset of defence responses.

DISCUSSION

TTSS is critical for *Xcc* pathogenicity, and *Xcc* mutants lacking TTSS are unable to multiply or spread in plant tissues (Qian *et al*., 2005). In this study, we showed that, unlike *P. syringae* TTSS mutants which induce strong defences, the *Xcc* strain 8004 TTSS Δ*hrcV* mutant induced only weak defence responses when *FRK1* and *PR1* gene expression and callose deposition were examined. Surprisingly, wild-type *Xcc* strain 8004 strongly induced these defence responses in a *RAR1*- and *SGT1*-dependent manner, suggesting that ETI is responsible for *Xcc*-induced defence responses in *Arabidopsis*.

It has been reported that the *Xcc* strain 8004 TTSS effector protein AvrAC has an avirulence function in *Arabidopsis* ecotype Col-0 by piercing inoculation (Xu *et al*., 2008). This is consistent with our findings that *Xcc* strain 8004-induced defence responses require TTSS in the bacterium and *NDR1*, *RAR1* and *SGT1b* in the plant. We further showed that the *Xcc* strain 8004 effector protein AvrXccC, when delivered from the bacterium or expressed as a transgene, induces *PR1* expression and confers disease resistance in *Arabidopsis*. Wild-type *Xcc* strain 8004 and the Δ*avrXccC* strain were indistinguishable in the induction of *FRK1* expression and callose deposition, indicating that *avrXccC* is not required for these responses. It remains to be determined whether other effectors are responsible for the induction of these responses. Nonetheless, these results reinforce the notion that ETI plays an important role in *Arabidopsis* resistance to *Xcc* strain 8004.

It is interesting to note that *Xcc* strain 8004 bacteria induce callose deposition and *FRK1* expression only when inoculated through infiltration, but not through the piercing method. One explanation is that endogenous elicitors or damage-associated molecular patterns (DAMPs) released from affected cells (Boller and Felix, 2009) could act together with effectors to trigger defence responses. Alternatively, microscopic cell death may occur in infiltrated mesophyll cells, and this may indirectly induce callose deposition and *FRK1* expression.

Xcc strain 8004 appears to carry an inactive flagellin sequence for FLS2 recognition. This is consistent with our

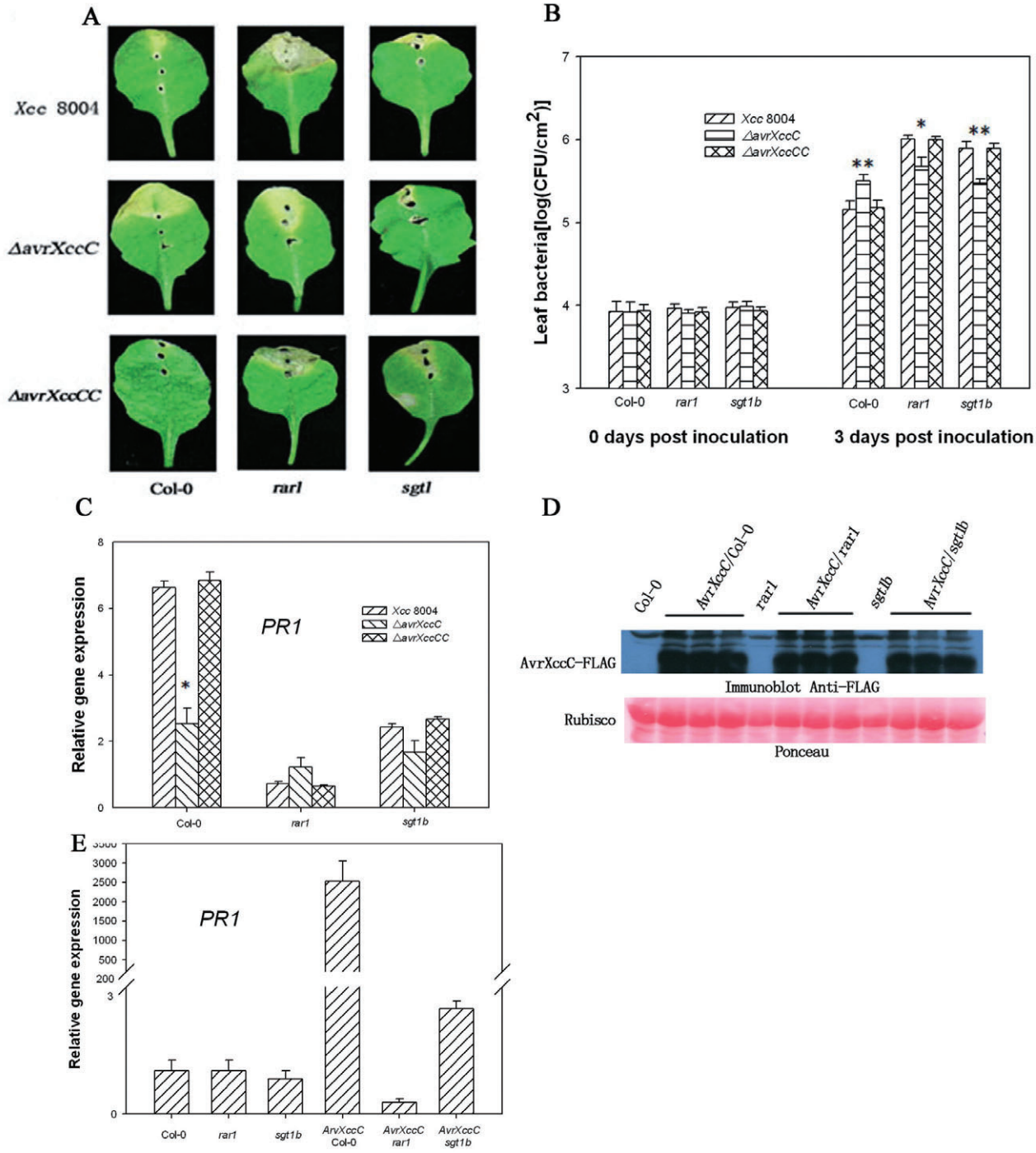

Fig. 5 AvrXccC has both avirulence and virulence functions in *Arabidopsis*. Leaves of the indicated genotypes were inoculated with *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004), the Δ*avrXccC* mutant and the *avrXccC*-complemented (Δ*avrXccCC*) strain at 5×10^8 colony-forming units (CFU)/mL using the piercing method. Disease symptoms (A) were photographed 5 days after inoculation. The bacterial population in leaves (B) was monitored at 0 and 3 days post-inoculation. *PR1* gene abundance (C) was determined at 24 h after inoculation. Five-week-old nontransgenic and *AvrXccC*-transgenic lines in the indicated genetic background were induced by oestradiol for 12 h, and the accumulation of AvrXccC was determined by immunoblot using anti-Flag antibody (D); *PR1* gene expression was determined by quantitative polymerase chain reaction (E). *(*t*-test, $P < 0.05$) and **(*t*-test, $P < 0.01$) indicate statistically significant differences between the *avrXccC* mutant and wild-type (WT) *Xcc* 8004. Each data point represents three replicates. Error bars indicate standard deviations. The experiments were repeated twice with similar results.

findings that the *fls2* mutant was not affected in terms of defence responses, disease symptoms and bacterial growth when inoculated with *Xcc* strain 8004. However, other PAMPs derived from *Xcc*, including lipopolysaccharides, harpins, cold shock proteins and flagellin, could induce defence responses in host and nonhost plants (Felix and Boller, 2003; Newman *et al*., 1995; Silipo *et al*., 2005; Xu *et al*., 2008). The *Xcc* strain 8004 Δ*hrcV* mutant, which carries a collection of PAMPs, induced only weak defence responses. This contrasts with the strong defence responses induced by *P. syringae* TTSS mutants and *X. campestris* pv. *vesicatoria* TTSS mutants. *Xcc* may have evolved multiple strategies to evade PTI in plants. For example, *Xcc* cyclic glucan and extracellular polysaccharide xanthan act as suppressors of host defences to promote bacterial growth (Silipo *et al*., 2009; Yun *et al*., 2006). Xanthan appears to be a major virulence determinant which acts by chelating extracellular calcium, thereby inhibiting PTI defences (Aslam *et al*., 2008).

Pathogen effectors are generally believed to assist pathogen infection or propagation in plants; it is often difficult to demonstrate a virulence function to many effectors. Our analyses using *rar1* and *sgt1b* plants revealed a previously unknown virulence function for AvrXccC in *Arabidopsis*. As other effectors may similarly possess both avirulence and virulence functions, the use of plant mutants compromised in ETI will allow a better assessment of effector virulence functions.

In conclusion, we have shown that ETI is primarily responsible for defence responses and disease resistance in the *Arabidopsis*–*Xcc* strain 8004 interaction. Although the *Xcc* TTSS mutants elicit much smaller defence responses than the wild-type bacterium, they do not grow or cause disease in *Arabidopsis* leaves. In contrast, wild-type *Xcc* strain 8004 grew to a significantly higher level in the presence of strong defence responses. A major role of virulent *P. syringae* TTSS effector proteins is to inhibit host defences. It remains to be determined to what extent *Xcc* strain 8004 effectors assist parasitism by inhibiting host defences. Because *Xcc* is a vascular pathogen which has a different life style, it is possible that some *Xcc* strain 8004 effector proteins may promote parasitism through mechanisms other than defence suppression.

EXPERIMENTAL PROCEDURES

Bacterial strains and culture conditions

The strains used in this study included wild-type *Xcc* strain 8004 (Turner *et al*., 1984), Δ*hrcV* (Tn5 insertion mutant), Δ*hrcVC* (*hrcV* mutant containing pHMVC), Δ*avrXccC* and Δ*avrXccCC* (Wang *et al*., 2007). *Xcc* strains were grown in peptone sucrose agar plates at 28 °C. Antibiotics were used at the following concentrations: 50 μg/mL kanamycin and 50 μg/mL spectinomycin for *Escherichia coli*; 100 μg/mL spectinomycin and 50 μg/mL rifampicin for *Xcc*.

Arabidopsis and mutants

Arabidopsis plants used in this study included the wild-type (Col-0) and the following mutants: *ndr1* (Zhang *et al*., 2010), *sid2-2* (Dewdney *et al*., 2000), *sgt1b* (formerly described as *edm1-1*; Tor *et al*., 2002), *rar1-20* (Tornero *et al*., *2002*) and *fls2*, salk_141277 (Xiang *et al*., 2008).

Callose deposition assay

Five-week-old *Arabidopsis* leaves were hand infiltrated with an *Xcc* bacterial suspension at 2×10^7 CFU/mL. Leaves were harvested 24 h after infiltration, cleared, stained with aniline blue (Hauck *et al*., 2003) and mounted in 50% glycerol. The leaves were examined with a fluorescence microscope under ultraviolet light. The number of callose deposits per microscopic field of 0.1 mm^2 was calculated from six leaves using Image J software (http://www.uhnresearch.ca/wcif).

RNA isolation and real-time reverse transcriptase (RT)-PCR

Arabidopsis leaves were infiltrated with an *Xcc* strain 8004 bacterial suspension at 2×10^7 CFU/mL for the indicated times before RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Five micrograms of RNA were used for cDNA synthesis. The gene expression level was determined by real-time RT-PCR using a SYBR Premix Ex Taq™ kit (TaKaRa, Changping, Beijing, China). Actin was used as a reference gene. Primers 5′-TACG CAGAACAACTAAGAGG-3′ and 5′-TCGTTCACATAATTCCCACG-3′ were used for *PR1*, and primers 5′-TGGTGGAAGCACA GAAGTTG-3′ and 5′-GATCCATGTTTGGCTCCTTC-3′ were used for actin. Primers 5′-TCTGAAGAATCAGCTCAAGGC-3′ and 5′-TGTTGGCTTCACATCTCTGTG-3′ were used for *FRK1*. The RT-PCR conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 25 s. The expression level was normalized to the actin control, and relative expression values were determined against buffer or wild-type Col-0 using the comparative C_t method.

Bacterial growth assay

Five-week-old *Arabidopsis* leaves were hand infiltrated with an *Xcc* strain 8004 bacterial suspension (5×10^5 CFU/mL), and the bacterial population in the leaves was counted at the indicated times. Alternatively, 5-week-old *Arabidopsis* leaves were inoculated by piercing three holes in the central vein with a

needle dipped in a bacterial suspension (5×10^8 CFU/mL), and the tips of the inoculated leaves were selected for the bacterial growth assay. At least four leaves were inoculated for each strain tested. Each data point consisted of at least six replicates.

Generation of *AvrXccC* transgenic plants

The *AvrXccC* fragment was PCR amplified from *Xcc* strain 8004 genome DNA using the following primers: 5′-CCGCTCGAG ATGGGTCTATGCGCTTCA-3′ and 5′-CCCATCGATAATTGGGGGG CGCTCAAA-3′. The *AvrXccC* fragment was ligated into pER8 (Shang *et al*., 2006) that had been digested with *Xho*I and *Csp*45I. The resulting clone containing *AvrXccC* under the control of the oestrogen-inducible promoter was transformed into *Arabidopsis* (wild-type Col-0 and mutants *rar1*, *sgt1b*) by *Agrobacterium*-mediated transformation. Transgenic plants were selected on Murashige and Skoog plates containing hygromycin. For *AvrXccC* induction, plants were sprayed with 50 μM oestradiol containing 0.01% silwet L-77. Three independent homozygous T2 transgenic lines were selected for the experiments.

Immunoblot analysis

Protein was extracted with a buffer containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES), pH 7.5, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 0.1% Triton, 1 mM dithiothreitol (DTT), 2 mM NaF and protease inhibitor cocktail (Roche, Indianapolis, IN, USA). Protein samples were electrophoresed through a 12% sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and protein was electrotransferred to an Immobilon P membrane (Millipore, Bedford, MA, USA). Immunodetection was performed with a 1 : 5000 dilution of an anti-FLAG monoclonal antibody (Sigma, Louis, MA, USA). The blot was then hybridized with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized with ECL Western blotting detection reagents (GE Healthcare, Amersham™, Buckinghamshire, UK).

Electrolyte leakage measurement

Five-week-old *Arabidopsis* leaves were inoculated with bacteria in water. Immediately after inoculation, 0.7-cm-diameter leaf discs were taken from injected leaves and washed with double-distilled H_2O three times for 15 min each time. Electrolyte leakage was measured using a Seveneasy S30 conductivity meter (Mettler Toledo, Schwerzenbach, Switzerland) according to Torres *et al*. (2005). Each treatment consisted of three replicates with four leaf discs per replicate.

ACKNOWLEDGEMENTS

This work was supported by the Programme for State Key Laboratories, Ministry of Science and Technology of China.

REFERENCES

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J. and Parker, J.E. (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **95**, 10306–10311.

Abramovitch, R.B., Anderson, J.C. and Martin, G.B. (2006) Bacterial elicitation and evasion of plant innate immunity. *Nat. Rev. Mol. Cell Biol.* **7**, 601–611.

Aslam, S.N., Newman, M.A., Erbs, G., Morrissey, K.L., Chinchilla, D., Boller, T., Jensen, T.T., De Castro, C., Ierano, T., Molinaro, A., Jackson, R.W., Knight, M.R. and Cooper, R.M. (2008) Bacterial polysaccharides suppress induced innate immunity by calcium chelation. *Curr. Biol.* **18**, 1078–1083.

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K. and Schulze-Lefert, P. (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science*, **295**, 2073–2076.

Bittel, P. and Robatzek, S. (2007) Microbe-associated molecular patterns (MAMPs) probe plant immunity. *Curr. Opin. Plant Biol.* **10**, 335–341.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406.

Boller, T. and He, S.Y. (2009) Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, **324**, 742–744.

Brown, I., Mansfield, J. and Bonas, U. (1995) *hrp* Genes in *Xanthomonas campestris* pv. *vesicatoria* determine ability to suppress papilla deposition in pepper mesophyll cells. *Mol. Plant Microbe Interact.* **8**, 825–836.

Buttner, D. and Bonas, U. (2006) Who comes first? How plant pathogenic bacteria orchestrate type III secretion. *Curr. Opin. Microbiol.* **9**, 193–200.

Clay, N.K., Adio, A.M., Denoux, C., Jander, G. and Ausubel, F.M. (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science*, **323**, 95–101.

Day, B., Dahlbeck, D. and Staskawicz, B.J. (2006) NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in *Arabidopsis*. *Plant Cell*, **18**, 2782–2791.

DebRoy, S., Thilmony, R., Kwack, Y.B., Nomura, K. and He, S.Y. (2004) A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants. *Proc. Natl. Acad. Sci. USA*, **101**, 9927–9932.

Desveaux, D., Singer, A.U., Wu, A.J., McNulty, B.C., Musselwhite, L., Nimchuk, Z., Sondek, J. and Dangl, J.L. (2007) Type III effector activation via nucleotide binding, phosphorylation, and host target interaction. *PLoS Pathog.* **3**, e48.

Dewdney, J., Reuber, T.L., Wildermuth, M.C., Devoto, A., Cui, J., Stutius, L.M., Drummond, E.P. and Ausubel, F.M. (2000) Three unique mutants of *Arabidopsis* identify eds loci required for limiting growth of a biotrophic fungal pathogen. *Plant J.* **24**, 205–218.

Eitas, T.K., Nimchuk, Z.L. and Dangl, J.L. (2008) *Arabidopsis* TAO1 is a TIR-NB-LRR protein that contributes to disease resistance induced by

the *Pseudomonas syringae* effector AvrB. *Proc. Natl. Acad. Sci. USA*, **105**, 6475–6480.

Felix, G. and Boller, T. (2003) Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. *J. Biol. Chem.* **278**, 6201–6208.

Guo, M., Tian, F., Wamboldt, Y. and Alfano, J.R. (2009) The majority of the type III effector inventory of *Pseudomonas syringae* pv. *tomato* DC3000 can suppress plant immunity. *Mol. Plant Microbe Interact.* **22**, 1069–1080.

Ham, J.H., Kim, M.G., Lee, S.Y. and Mackey, D. (2007) Layered basal defenses underlie non-host resistance of *Arabidopsis* to *Pseudomonas syringae* pv. *phaseolicola*. *Plant J.* **51**, 604–616.

Hammond-Kosack, K.E. and Parker, J.E. (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. *Curr. Opin. Biotechnol.* **14**, 177–193.

Hauck, P., Thilmony, R. and He, S.Y. (2003) A *Pseudomonas syringae* type III effector suppresses cell wall-based extracellular defense in susceptible *Arabidopsis* plants. *Proc. Natl. Acad. Sci. USA*, **100**, 8577–8582.

He, S.Y., Nomura, K. and Whittam, T.S. (2004) Type III protein secretion mechanism in mammalian and plant pathogens. *Biochim. Biophys. Acta*, **1694**, 181–206.

Hubert, D.A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K. and Dangl, J.L. (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J.* **22**, 5679–5689.

Hubert, D.A., He, Y., McNulty, B.C., Tornero, P. and Dangl, J.L. (2009) Specific *Arabidopsis* HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation. *Proc. Natl. Acad. Sci. USA*, **106**, 9556–9563.

Hugouvieux, V., Barber, C.E. and Daniels, M.J. (1998) Entry of *Xanthomonas campestris* pv. *campestris* into hydathodes of *Arabidopsis thaliana* leaves: a system for studying early infection events in bacterial pathogenesis. *Mol. Plant Microbe Interact.* **11**, 537–543.

Jones, J.D. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Kim, M.G., Cunha, L., McFall, A.J., Belkhadir, Y., DebRoy, S., Dangl, J.L. and Mackey, D. (2005) Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell*, **121**, 749–759.

Li, X.Y., Lin, H.Q., Zhang, W.G., Zou, Y., Zhang, J., Tang, X.Y. and Zhou, J.M. (2005) Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors. *Proc. Natl. Acad. Sci. USA*, **102**, 12990–12995.

Meyer, D., Lauber, E., Roby, D., Arlat, M. and Kroj, T. (2005) Optimization of pathogenicity assays to study the *Arabidopsis thaliana*–*Xanthomonas campestris* pv. *campestris* pathosystem. *Mol. Plant Pathol.* **6**, 327–333.

Muskett, P.R., Kahn, K., Austin, M.J., Moisan, L.J., Sadanandom, A., Shirasu, K., Jones, J.D. and Parker, J.E. (2002) *Arabidopsis* RAR1 exerts rate-limiting control of R gene-mediated defenses against multiple pathogens. *Plant Cell*, **14**, 979–992.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T. and Jones, J.D. (2004) The transcriptional innate immune response to flg22. Interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. *Plant Physiol.* **135**, 1113–1128.

Newman, M.A., Daniels, M.J. and Dow, J.M. (1995) Lipopolysaccharide from *Xanthomonas campestris* induces defense-related gene expression in *Brassica campestris*. *Mol. Plant Microbe Interact.* **8**, 778–780.

O'Donnell, P.J., Schmelz, E.A., Moussatche, P., Lund, S.T., Jones, J.B. and Klee, H.J. (2003) Susceptible to intolerance—a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. *Plant J.* **33**, 245–257.

Qian, W., Jia, Y., Ren, S.X., He, Y.Q., Feng, J.X., Lu, L.F., Sun, Q., Ying, G., Tang, D.J., Tang, H., Wu, W., Hao, P., Wang, L., Jiang, B.L., Zeng, S., Gu, W.Y., Lu, G., Rong, L., Tian, Y., Yao, Z., Fu, G., Chen, B., Fang, R., Qiang, B., Chen, Z., Zhao, G.P., Tang, J.L. and He, C. (2005) Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome Res.* **15**, 757–767.

Schwessinger, B. and Zipfel, C. (2008) News from the frontline: recent insights into PAMP-triggered immunity in plants. *Curr. Opin. Plant Biol.* **11**, 389–395.

Shang, Y., Li, X., Cui, H., He, P., Thilmony, R., Chintamanani, S., Zwiesler-Vollick, J., Gopalan, S., Tang, X. and Zhou, J.M. (2006) RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector AvrB. *Proc. Natl. Acad. Sci. USA*, **103**, 19200–19205.

Silipo, A., Molinaro, A., Sturiale, L., Dow, J.M., Erbs, G., Lanzetta, R., Newman, M.A. and Parrilli, M. (2005) The elicitation of plant innate immunity by lipooligosaccharide of *Xanthomonas campestris*. *J. Biol. Chem.* **280**, 33660–33668.

Silipo, A., Erbs, G., Shinya, T., Dow, J.M., Parrilli, M., Lanzetta, R., Shibuya, N., Newman, M.A. and Molinaro, A. (2009) Glycoconjugates as elicitors or suppressors of plant innate immunity. *Glycobiology*, **20**, 406–419.

Sun, W., Dunning, F.M., Pfund, C., Weingarten, R. and Bent, A.F. (2006) Within-species flagellin polymorphism in *Xanthomonas campestris* pv *campestris* and its impact on elicitation of *Arabidopsis* FLAGELLIN SENSING2-dependent defenses. *Plant Cell*, **18**, 764–779.

Takahashi, A., Casais, C., Ichimura, K. and Shirasu, K. (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **100**, 11777–11782.

Tor, M., Gordon, P., Cuzick, A., Eulgem, T., Sinapidou, E., Mert-Turk, F., Can, C., Dangl, J.L. and Holub, E.B. (2002) *Arabidopsis* SGT1b is required for defense signaling conferred by several downy mildew resistance genes. *Plant Cell*, **14**, 993–1003.

Tornero, P., Merritt, P., Sadanandom, A., Shirasu, K., Innes, R.W. and Dangl, J.L. (2002) RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the R gene assayed. *Plant Cell*, **14**, 1005–1015.

Torres, M.A., Jones, J.D. and Dangl, J.L. (2005) Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis* thaliana. *Nat. Genet.* **37**, 1130–1134.

Turner, P., Barber, C. and Daniels, M. (1984) Behavior of the transposons Tn5 and Tn7 in *Xanthomonas campestris* pv. *campestris*. *Mol. Gen. Genet.* **195**, 101–107.

Wang, L.F., Tang, X.Y. and He, C.Z. (2007) The bifunctional effector AvrXccC of *Xanthomonas campestris* pv. *campestris* requires plasma membrane-anchoring for host recognition. *Mol. Plant Pathol.* **8**, 491–501.

Wei, C.F., Kvitko, B.H., Shimizu, R., Crabill, E., Alfano, J.R., Lin, N.C., Martin, G.B., Huang, H.C. and Collmer, A. (2007) A *Pseudomonas*

syringae pv. *tomato* DC3000 mutant lacking the type III effector HopQ1-1 is able to cause disease in the model plant *Nicotiana benthamiana*. *Plant J.* **51**, 32–46.

Williams, P.H. (1980) Black rot: a continuing threat to world crucifers. *Plant Dis.* **64**, 736–742.

Xiang, T., Zong, N., Zou, Y., Wu, Y., Zhang, J., Xing, W., Li, Y., Tang, X., Zhu, L., Chai, J. and Zhou, J.M. (2008) *Pseudomonas syringae* effector AvrPto blocks innate immunity by targeting receptor kinases. *Curr. Biol.* **18**, 74–80.

Xu, R.Q., Blanvillain, S., Feng, J.X., Jiang, B.L., Li, X.Z., Wei, H.Y., Kroj, T., Lauber, E., Roby, D., Chen, B., He, Y.Q., Lu, G.T., Tang, D.J., Vasse, J., Arlat, M. and Tang, J.L. (2008) AvrAC(Xcc8004), a type III effector with a leucine-rich repeat domain from *Xanthomonas campestris* pathovar *campestris* confers avirulence in vascular tissues of *Arabidopsis thaliana* ecotype Col-0. *J. Bacteriol.* **190**, 343–355.

Yun, M.H., Torres, P.S., El Oirdi, M., Rigano, L.A., Gonzalez-Lamothe, R., Marano, M.R., Castagnaro, A.P., Dankert, M.A., Bouarab, K. and Vojnov, A.A. (2006) Xanthan induces plant susceptibility by suppressing callose deposition. *Plant Physiol.* **141**, 178–187.

Zhang, J., Lu, H.B., Li, X.Y., Li, Y., Cui, H.T., Wen, Q.G., Tang, X.Y., Su, Z. and Zhou, J.M. (2010) Effector-triggered and PAMP-triggered immunity differentially contribute to basal resistance to *Pseudomonas syringae*. *Mol. Plant Microbe Interact.* **23**, 940–948.

Zhou, J.M. and Chai, J. (2008) Plant pathogenic bacterial type III effectors subdue host responses. *Curr. Opin. Microbiol.* **11**, 179–185.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D., Felix, G. and Boller, T. (2004) Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature*, **428**, 764–767.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 The *Xanthomonas campestris* pv. *campestris* type III secretion system (*Xcc* TTSS) is required for callose induction. *Arabidopsis* leaves were infiltrated with the indicated bacterial strains at 2×10^7 colony-forming units (CFU)/mL, and callose deposition was examined 24 h later. Average numbers of callose deposits per microscopic field of 0.1 mm^2 are shown below each photograph. '$\pm$' represents the standard deviations from three replicates. Similar results were obtained in three independent experiments.

Fig. S2 Type III secretion system (TTSS) mutants Δ*hrcV*, Δ*hrpG* and Δ*hrpX* are nonpathogenic on *Arabidopsis*. Five-week-old Col-0 plants were inoculated with wild-type (WT) *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004), Δ*hrcV*, Δ*hrpG* and Δ*hrpX* mutants [bacterial suspension at a concentration of 5×10^5 colony-forming units (CFU)/mL]. The bacterial populations were determined at 0 and 3 days post-inoculation. Each data point represents three replicates. Error bars indicate standard deviations. The experiments were repeated twice with similar results.

Fig. S3 Electrolyte leakage assay on leaves infiltrated with wild-type (WT) *Xanthomonas campestris* pv. *campestris* strain 8004 (*Xcc* 8004) or Δ*hrcV* mutant. Five-week-old *Arabidopsis* leaves were inoculated with DC3000 (*avrB*), WT *Xcc* 8004 and Δ*hrcV* mutant [bacterial suspension at a concentration of 2×10^7 colony-forming units (CFU)/mL]. The electrolyte leakage was measured at the indicated times. Each data point represents three replicates. Error bars indicate standard deviations. The experiments were repeated twice with similar results.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

