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Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic *Pectobacterium carotovorum* bacteria

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SUMMARY

Boosted responsiveness of plant cells to stress at the onset of pathogen- or chemically induced resistance is called priming. The chemical β-aminobutyric acid (BABA) enhances *Arabidopsis thaliana* resistance to hemibiotrophic bacteria through the priming of the salicylic acid (SA) defence response. Whether BABA increases Arabidopsis resistance to the necrotrophic bacterium *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) is not clear. In this work, we show that treatment with BABA protects Arabidopsis against the soft-rot pathogen *Pcc*. BABA did not prime the expression of the jasmonate/ethylene-responsive gene *PLANT DEFENSIN 1.2* (*PDF1.2*), the up-regulation of which is usually associated with resistance to necrotrophic pathogens. Expression of the SA marker gene *PATHOGENESIS RELATED 1* (*PR1*) on *Pcc* infection was primed by BABA treatment, but SA-defective mutants demonstrated a wild-type level of BABA-induced resistance against *Pcc*. BABA primed the expression of the pattern-triggered immunity (PTI)-responsive genes *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*) and *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) after inoculation with *Pcc* or after treatment with purified bacterial microbe-associated molecular patterns, such as flg22 or elf26. PTI-mediated callose deposition was also potentiated in BABA-treated Arabidopsis, and BABA boosted Arabidopsis stomatal immunity to *Pcc*. BABA treatment primed the PTI response in the SA-defective mutants *SA induction deficient 2-1* (*sid2-1*) and *phytoalexin deficient 4-1* (*pad4-1*). In addition, BABA priming was associated with open chromatin configurations in the promoter region of PTI marker genes. Our data indicate that BABA primes the PTI response upon necrotrophic bacterial infection and suggest a role for the PTI response in BABA-induced resistance.

INTRODUCTION

Plants are equipped to sense evolutionarily conserved microbial molecular signatures, collectively called microbe-associated molecular patterns (MAMPs). Recognition of MAMPs activates immune responses (Ausubel, 2005; Boller and Felix, 2009). This general, early defence response is called pattern-triggered immunity (PTI) (Zhang and Zhou, 2010; Zipfel, 2009), and is characterized by the accumulation of reactive oxygen species, callose deposition and the expression of defence-related genes (Boudsocq *et al*., 2010; Gómez-Gómez *et al*., 1999; Zhang and Zhou, 2010). Bacterial-induced stomatal closure is also characteristic of the PTI response (Desclos-Theveniau *et al*., 2012; Melotto *et al*., 2006; Singh *et al*., 2012; Zeng *et al*., 2010). The virulent *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 reopens stomata in a coronatine (COR)-dependent manner (Melotto *et al*., 2006; Zeng *et al*., 2010).

In Arabidopsis, salicylic acid (SA)-dependent defence responses are considered to be effective mainly against biotrophic pathogens, such as the oomycete *Hyaloperonospora arabidopsidis*, the fungus *Erysiphe orontii* and the hemibiotrophic bacterium *P. syringae* (Glazebrook, 2005). Accordingly, impaired SA production leads to increased susceptibility to these pathogens. For example, reduction of SA production in *SA induction deficient* (*sid*) mutant plants results in increased susceptibility to both virulent and avirulent forms of *P. syringae* and *H. arabidopsidis* (Nawrath and Métraux, 1999). Activation of SA signalling can, however, also be effective against necrotrophic pathogens, such as the fungus *Botrytis cinerea* (Ferrari *et al*., 2003; Zimmerli *et al*., 2001). Jasmonic acid (JA)-dependent signalling mostly mediates resistance to insects and necrotrophic microbial pathogens (Creelman and Mullet, 1997; Norman-Setterblad *et al*., 2000; Overmyer *et al*., 2000; Reymond and Farmer, 1998). The *coronatine-insensitive 1* (*coi1*) mutant, which is impaired in the perception of JA, generally exhibits enhanced susceptibility to a variety of necrotrophic pathogens, including *Alternaria brassicicola* and *B. cinerea* fungi, oomycete *Pythium* sp. and the bacterium *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) (Norman-Setterblad *et al*., 2000; Thomma *et al*., 1998). It is noteworthy that some *coi1* alleles do not show enhanced susceptibility to *Pcc* (Kariola *et al*., 2003). The production of ethylene (ET) is one of the earliest plant responses to pathogens (Thomma *et al*., 1998). The necrotroph *Pcc* activates the ET-dependent defence response and the Arabidopsis *ethylene-insensitive 2* (*ein2*) mutant displays enhanced susceptibility to *Pcc* (Kazan and Manners, 2008; Norman-Setterblad *et al*., 2000). Defence pathways influence each other through a network

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of regulatory interactions, and thus plant responses to various stress stimuli are a result of this complex interplay (Bostock, 2005; Kunkel and Brooks, 2002). Several studies have described crosstalk among SA, JA and ET signalling pathways (Kunkel and Brooks, 2002; Pieterse *et al*., 2009). SA and JA signalling pathways interact on many levels and, in most cases, this relationship seems to be mutually antagonistic (Kunkel and Brooks, 2002).

On infection by pathogens or after treatment with various chemicals, plants can establish a unique physiological condition, called the 'primed' state (Conrath *et al*., 2002; Prime-A-Plant Group *et al*., 2006; Van der Ent *et al*., 2009). Priming is the phenomenon that enables cells to respond to much lower levels of a stimulus in a more rapid and robust manner than do nonprimed cells (Conrath *et al*., 2002; Conrath, 2011). Plants primed by treatments that induce resistance show a faster and/or stronger activation of defence responses when subsequently challenged by pathogens or abiotic stresses (Beckers *et al*., 2009; Conrath, 2011; Prime-A-Plant Group *et al*., 2006). Priming is observed in plants and also in animals (Beckers *et al*., 2009; Jung *et al*., 2009; Pham *et al*., 2007). Priming provides low-cost protection in relatively high disease pressure conditions (van Hulten *et al*., 2006). Many different organic and inorganic compounds have been shown to induce resistance in plants (Kuć, 2001). Among these, the nonprotein amino acid β-aminobutyric acid (BABA) has been shown to be a potent inducer of resistance against abiotic stress (Jakab *et al*., 2005; Zimmerli *et al*., 2008), nematodes (Oka *et al*., 1999), insects (Hodge *et al*., 2005) and microbial pathogens (Cohen, 2002; Jakab *et al*., 2001). BABA protects plants by priming pathogen-specific defence responses, notably BABA induces priming of *PATHOGENESIS RELATED 1* (*PR1*) expression on Arabidopsis infection by *Pst* DC3000 in an SA-dependent manner (Ton *et al*., 2005; Zimmerli *et al*., 2000).

BABA-induced resistance is efficient against plant microbial pathogens (Cohen, 2002; Jakab *et al*., 2001; Prime-A-Plant Group *et al*., 2006; Zimmerli *et al*., 2001), but whether BABA protects Arabidopsis plants against necrotrophic bacteria is not known. We report here that BABA provides Arabidopsis resistance against necrotrophic *Pcc* bacteria and that resistance is correlated with a primed Arabidopsis PTI response. In addition, we show that BABA treatment induces an open chromatin configuration at the promoter region of primed genes.

RESULTS

BABA-induced resistance to *Pcc* SCC1

The priming agent BABA protects Arabidopsis against hemibiotrophic bacteria (Ton *et al*., 2005; Tsai *et al*., 2011; Zimmerli *et al*., 2000), but the effect of this chemical in Arabidopsis against necrotrophic bacteria, such as *Pcc*, is not known. To further evaluate the spectrum of action of BABA, the resistance of BABA- and water-treated Arabidopsis to virulent *Pcc* SCC1 infections was compared. Pretreatment with BABA enhanced Arabidopsis resistance to *Pcc* SCC1 at both symptom and titre levels at 1 day post-inoculation (dpi) (Fig. 1A,B). BABA-treated Arabidopsis indeed demonstrated less *Pcc* SCC1-mediated water-soaked lesions (Fig. 1A) and *Pcc* SCC1 titres per leaf area were about 50 times lower in BABA-treated Arabidopsis when compared with water control plants (Fig. 1B). The protective effect of BABA lasted for at least 2 days (Fig. S1, see Supporting Information). These data indicate that BABA pretreatment protects Arabidopsis plants against the necrotrophic bacterium *Pcc*.

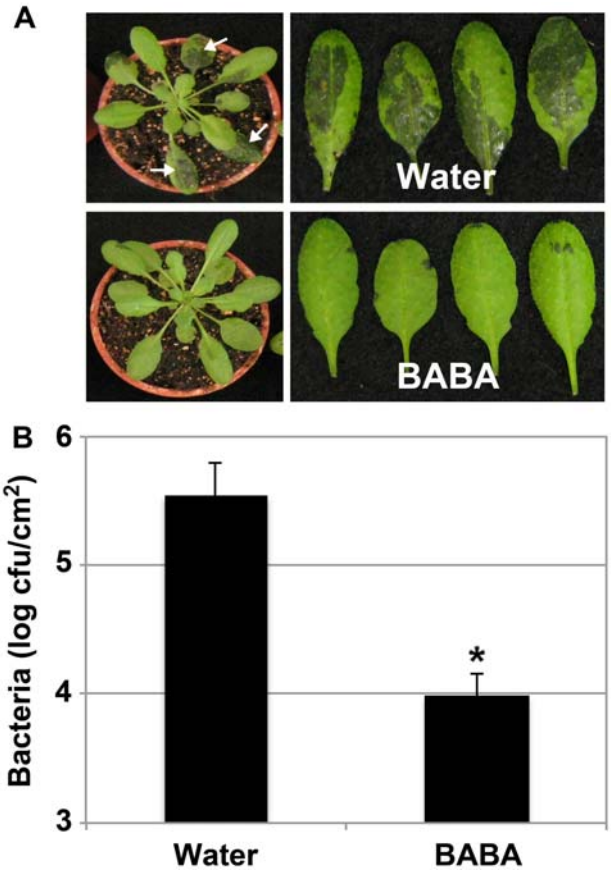

Fig. 1 Enhanced resistance of β-aminobutyric acid (BABA)-treated Arabidopsis to *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1 infection. (A) *Pcc* SCC1-mediated water-soaked lesions at 1 day post-inoculation (dpi). Five-week-old Arabidopsis plants were dipped in a bacterial solution of 5×10^6 colony-forming units (cfu)/mL. White arrows indicate *Pcc* SCC1-mediated water-soaked lesions. (B) Quantification of *Pcc* SCC1 infection in water- and BABA-treated Arabidopsis plants. Plants were dip inoculated as in (A) and bacterial titres were evaluated at 1 dpi. Data represent average values of three independent biological replicates ± standard deviation (SD) ($n = 9$). A statistically significant difference compared with water-treated Col-0 controls is indicated with an asterisk ($P < 0.05$) based on Student's *t*-test.

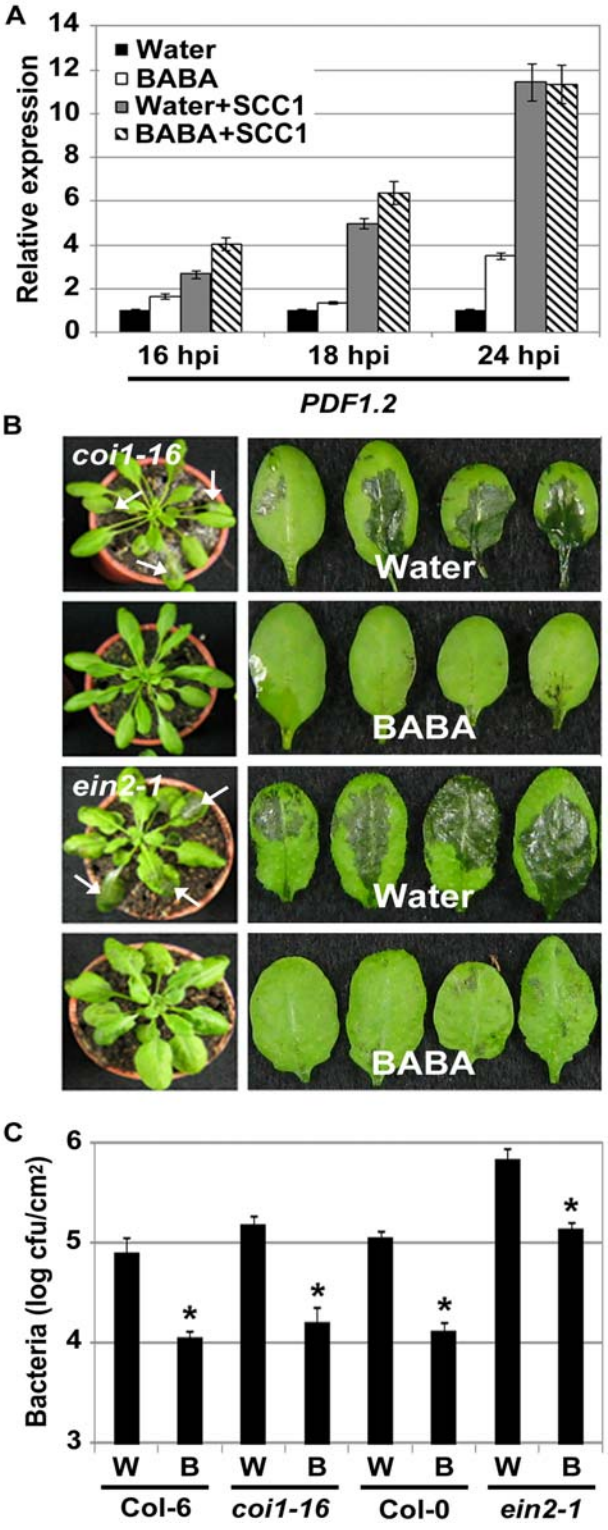

Fig. 2 Role of jasmonic acid/ethylene (JA/ET) defence signalling cascades in β-aminobutyric acid (BABA)-induced resistance to *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*). (A) Time course of *PLANT DEFENSIN 1.2* (*PDF1.2*) expression in Arabidopsis on *Pcc* SCC1 infection. Arabidopsis plants were treated with water or BABA and challenged 48 h later with virulent *Pcc* SCC1 at a concentration of 5×10^6 colony-forming units (cfu)/mL. Leaves were harvested at 16, 18 and 24 h post-inoculation (hpi). Transcript levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). Values of BABA-treated plants were not significantly different from those of water-treated controls based on Student's *t*-test ($P < 0.01$). (B) *Pcc* SCC1-mediated symptoms of water- and BABA-treated JA/ET signalling mutants. Five-week-old Arabidopsis plants were dip inoculated in 5×10^6 cfu/mL *Pcc* SCC1 and photographs were taken 16 h (*ethylene-insensitive 2-1*, *ein2-1*) or 1 day (*coronatine-insensitive 1-16*, *coi1-16*) later. White arrows indicate *Pcc* SCC1-mediated water-soaked lesions. (C) *Pcc* SCC1 titres in JA/ET signalling mutants. Water (W)- and BABA (B)-treated plants were dip inoculated as in (B) and bacterial titres were evaluated at 1 day post-inoculation (dpi). Average values ± SD from three independent experiments are presented ($n = 9$). Asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.05$).

Roles of JA and ET defence signalling pathways in BABA-induced resistance to *Pcc* SCC1 infection

The JA and ET defence responses are activated upon *Pcc* infection in Arabidopsis (Kariola *et al*., 2003; Norman-Setterblad *et al*., 2000), and defective JA/ET signalling generally leads to enhanced sensitivity to *Pcc* (Norman-Setterblad *et al*., 2000). JA/ET defence signalling is thus critical for Arabidopsis resistance to *Pcc*. We therefore evaluated the expression levels of the JA/ET marker gene *PLANT DEFENSIN 1.2* (*PDF1.2*) by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) after *Pcc* SCC1 infection. As expected (Kariola *et al*., 2003; Norman-Setterblad *et al*., 2000), the up-regulation of Arabidopsis *PDF1.2* was observed at 16, 18 and 24 h post-inoculation (hpi) with *Pcc* SCC1 (Fig. 2A). At these time points, water- and BABA-treated Arabidopsis demonstrated similar levels of *PDF1.2* mRNA accumulation, suggesting that BABA does not prime the JA/ET response upon *Pcc* infection. To further evaluate the role of JA/ET defence signalling cascades in BABA-induced resistance to *Pcc* SCC1, we analysed the effect of BABA in JA and ET signalling mutants. Both JA-insensitive *coi1-16* (Ellis and Turner, 2002) and ET-insensitive *ein2-1* (Guzman and Ecker, 1990) mutants were protected by BABA application. As shown in Fig. 2B, these mutants clearly developed less *Pcc* SCC1-mediated water-soaked lesions after BABA treatment. In addition, bacterial titres were about 10 times lower in *coi1-16* and *ein2-1* mutants pretreated with BABA (Fig. 2C). Therefore, *coi1-16* and *ein2-1* mutants displayed

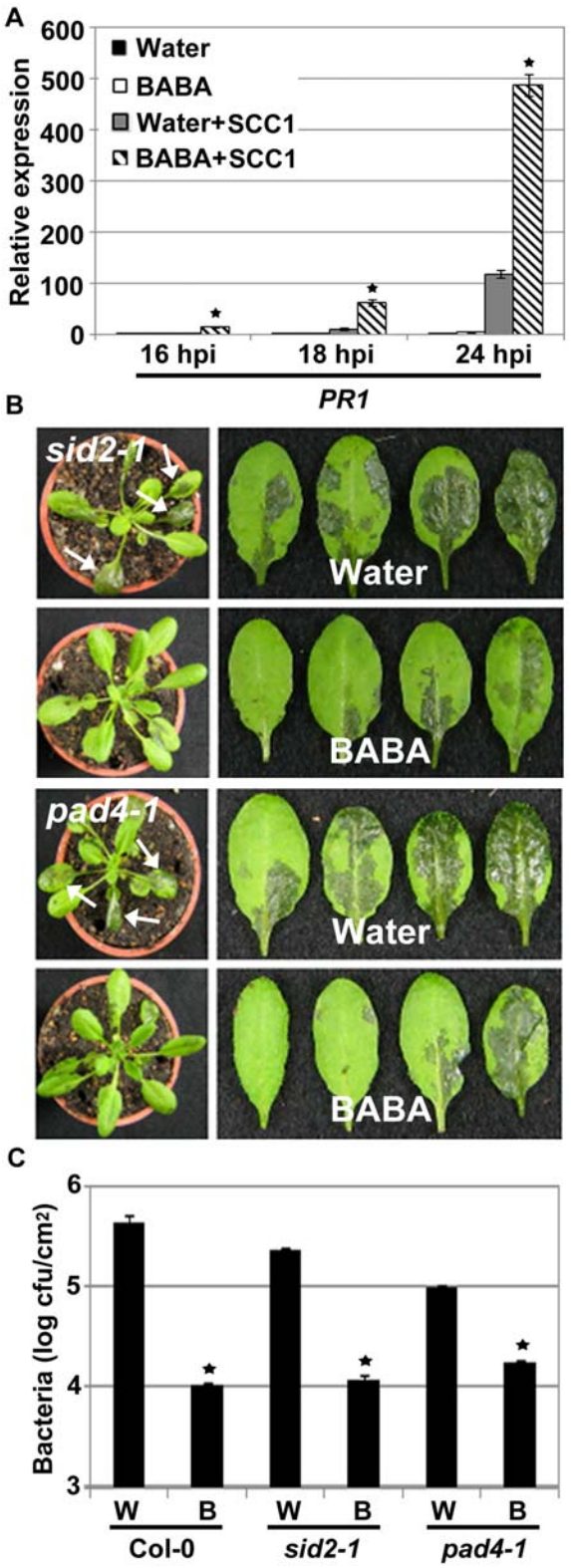

Fig. 3 Involvement of salicylic acid (SA) signalling in β-aminobutyric acid (BABA)-induced resistance to *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1. (A) Expression analyses of the SA-responsive *PATHOGENESIS RELATED 1* (*PR1*). Arabidopsis plants were treated with water or BABA and challenged 48 h later with *Pcc* SCC1 at a concentration of 5×10^6 colony-forming units (cfu)/mL. RNAs were harvested 16, 18 and 24 h post-inoculation (hpi). Transcript levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). Asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.01$). (B) Pathogen-induced lesion formation in SA-defective mutants. Five-week-old *SA induction deficient 2-1* (*sid2-1*) and *phytoalexin deficient 4-1* (*pad4-1*) mutant plants were dip inoculated in 5×10^6 cfu/mL *Pcc* SCC1 and photographs were taken 1 day later. White arrows indicate *Pcc* SCC1-mediated water-soaked lesions. (C) *Pcc* SCC1 proliferation in Col-0 and SA-defective mutants. Water (W)- and BABA (B)-treated plants were dip-inoculated as in (B) and bacterial titres were evaluated at 1 day post-inoculation (dpi). Average values ± SD from three independent experiments are presented ($n = 9$). Asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.05$).

wild-type levels of BABA-induced resistance against *Pcc* SCC1 infection. Collectively, these data suggest that JA/ET signalling is not critical for BABA-mediated Arabidopsis resistance to *Pcc*.

Priming of the SA-dependent defence signalling cascade by BABA upon *Pcc* SCC1 infection

BABA primes the expression of SA-responsive genes upon infection with hemibiotrophic *Pst* DC3000 bacteria (Ton *et al*., 2005; Tsai *et al*., 2011; Zimmerli *et al*., 2000) and the necrotrophic fungus *B. cinerea* (Zimmerli *et al*., 2001). To determine whether BABA also potentiates the Arabidopsis SA defence signalling cascade on *Pcc* infection, we monitored the transcript accumulation of *PR1* by qRT-PCR at 16, 18 and 24 hpi with the wild-type *Pcc* SCC1. Expression levels of the SA-responsive *PR1* were higher in *Pcc*-inoculated BABA-treated plants than in water controls, suggesting that BABA potentiates *PR1* expression upon *Pcc* SCC1 infection (Fig. 3A). To further evaluate the role of SA signalling in BABA-induced Arabidopsis resistance to *Pcc*, we tested the responses of SA biosynthesis (*sid2-1*) (Nawrath and Métraux, 1999) and SA signalling (*phytoalexin deficient 4-1*, *pad4-1*) (Glazebrook *et al*., 1997) mutants to *Pcc* SCC1 infection. As expected, *sid2-1* and *pad4-1* did not show up-regulation or BABA-mediated priming of *PR1* at 18 hpi with *Pcc* SCC1 (Fig. S2, see Supporting Information). However, both BABA-treated SA-defective mutants demonstrated a reduction in the formation of *Pcc* SCC1-mediated water-soaked lesions when compared with water-treated controls (Fig. 3B). In addition, BABA treatment induced a reduction in bacterial titres in BABA-treated *sid2-1* and *pad4-1*, suggesting a functional BABA-induced resistance against *Pcc* SCC1 infection in these SA-defective mutants (Fig. 3C). Collectively, these data

suggest that, although BABA primes SA-dependent defence responses upon *Pcc* SCC1 infection, SA signalling is not critical for BABA-induced resistance to *Pcc* SCC1.

BABA-induced potentiation of PTI-responsive gene expression and callose deposition

PTI is the first active defence layer in plant immunity (Jones and Dangl, 2006), and our data suggest that JA/ET and SA defence signalling cascades are not critical for BABA-induced Arabidopsis resistance to *Pcc* (Figs 2,3). To test whether the chemical BABA primes the PTI response in Arabidopsis, expression levels of the PTI-responsive genes *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*) and *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) (Boudsocq *et al*., 2010) were analysed after inoculation with *Pcc* strain WPP17. This *Pcc* strain is deficient in several *hypersensitive response and pathogenicity* (*hrp*) and *hrp conserved* (*hrc*) genes, and thus mainly activates the host PTI response (Yap *et al*., 2004). Accordingly, strain *Pcc* WPP17 is much less infective than the wild-type *Pcc* SCC1 (Fig. S3, see Supporting Information). On *Pcc* WPP17 infection, BABA-treated Arabidopsis accumulated more *FRK1*, *NHL10* and *CYP81F2* mRNAs than the water controls, suggesting that BABA primes bacterial-mediated PTI marker gene expression (Fig. 4A). Priming of the expression of PTI-responsive genes was also observed after treatment with the peptide flg22, the biologically active epitope of the bacterial MAMP flagellin (Fig. 4B), and with the purified bacterial MAMP EF-Tu (elf26) (Fig. 4C).

As the accumulation of callose is a typical marker for the PTI response (Gómez-Gómez *et al*., 1999), we compared callose deposition in water- and BABA-treated Arabidopsis after inoculation with *Pcc* WPP17. Aniline blue staining and image analysis revealed a higher level of callose deposition in BABA-treated Arabidopsis than in water controls at 6 hpi with *Pcc* WPP17 (Fig. 4D,E). Callose deposition was also enhanced in BABA-treated Arabidopsis leaves after treatment with MAMPs flg22 or elf26 (Fig. 4F–I). In addition, BABA-mediated priming of PTI was evaluated upon infection with the wild-type *Pcc* SCC1. As expected, PTI-responsive *FRK1*, *NHL10* and *CYP81F2* expression and callose deposition were primed in BABA-treated Arabidopsis (Fig. S4A,B, see Supporting Information). Collectively, these data suggest that BABA primes the Arabidopsis PTI response.

Reopening of stomata upon *Pcc* infection is blocked by BABA

Plants close stomata during bacterial invasion. Stomatal closure occurs through MAMP recognition and is therefore considered as an early PTI response (Melotto *et al*., 2006; Zeng and He, 2010). Virulent bacteria, such as *Pst* DC3000, reopen stomata in a COR-dependent manner (Melotto *et al*., 2006; Zeng and He, 2010). BABA blocks the COR-dependent reopening of stomata upon *Pst* DC3000 infection (Tsai *et al*., 2011). To test whether BABA also inhibits stomatal reopening upon *Pcc* infection, stomatal movements were observed in epidermal peels from water- and BABA-treated Arabidopsis kept in a solution containing *Pcc* bacteria. Similar to *Pst* DC3000 (Melotto *et al*., 2006; Zeng and He, 2010), *Pcc* bacteria induced stomatal closure at 1.5 hpi and stomatal reopening was observed at 5 hpi with *Pcc* in epidermal peels from water-treated Arabidopsis (Fig. 5). By contrast, at 5 hpi, stomatal guard cells from BABA-treated Arabidopsis were still closed (Fig. 5). Thus, as on *Pst* DC3000 inoculation (Tsai *et al*., 2011), BABA inhibited the reopening of stomata in contact with *Pcc* SCC1. These results further support the idea that BABA potentiates the PTI response in Arabidopsis.

Priming of the PTI response in SA-defective mutants upon *Pcc* infection

Expression of the SA-responsive gene *PR1* is primed by BABA (Fig. 3A), but SA-defective mutants show wild-type levels of BABA-mediated resistance to *Pcc* (Fig. 3B,C). Our data suggest that BABA primes the PTI response upon *Pcc* infection. Wild-type levels of BABA-mediated resistance in *sid2-1* and *pad4-1* could therefore be attributed to a potentiated PTI response. To test whether a primed PTI response is observed in SA-defective mutants upon *Pcc* infection, expression levels of the PTI-responsive gene *FRK1* were evaluated 2 h after infiltration–inoculation with 1×10^8 colony-forming units (cfu)/mL *hrp*- and *hrc*-deficient bacterial mutant *Pcc* WPP17 or at 6 hpi with 5×10^6 cfu/mL of wild-type *Pcc* SCC1. BABA-treated SA-defective mutants demonstrated a primed *FRK1* expression upon bacterial infection (Figs 6A and S5A, see Supporting Information). In addition, BABA-treated SA-defective mutants accumulated more callose deposits than did water-treated controls after *Pcc* inoculation (Figs 6B and S5B). Upon inoculation with *Pcc* WPP17, levels of callose deposition in SA-defective mutants were lower than in wild-type Col-0 controls (Fig. 6B). Reduced callose accumulation in SA-deficient mutants is, however, not observed upon infection with the nonhost bacterium *Pseudomonas syringae* pv. *phaseolicola* (Ham *et al*., 2007). To further evaluate the role of BABA on the PTI response of SA-defective mutants, PTI-mediated stomatal movements upon *Pcc* SCC1 infection were evaluated in *sid2-1* and *pad4-1*. Similar to *Pst* DC3000 (Melotto *et al*., 2006), *Pcc* SCC1 did not induce stomatal closure in SA-defective mutants (Fig. 6C). This observation demonstrates the importance of the SA signalling cascade in *Pcc*-mediated stomatal closure. Although BABA did not induce constitutive stomatal closure in mock-treated wild-type controls (Figs 5,6C; Tsai *et al*., 2011), the stomata of BABA-treated SA-defective mutants were constitutively closed (Fig. 6C). Similar to what is observed in wild-type controls (Figs 5,6C), BABA

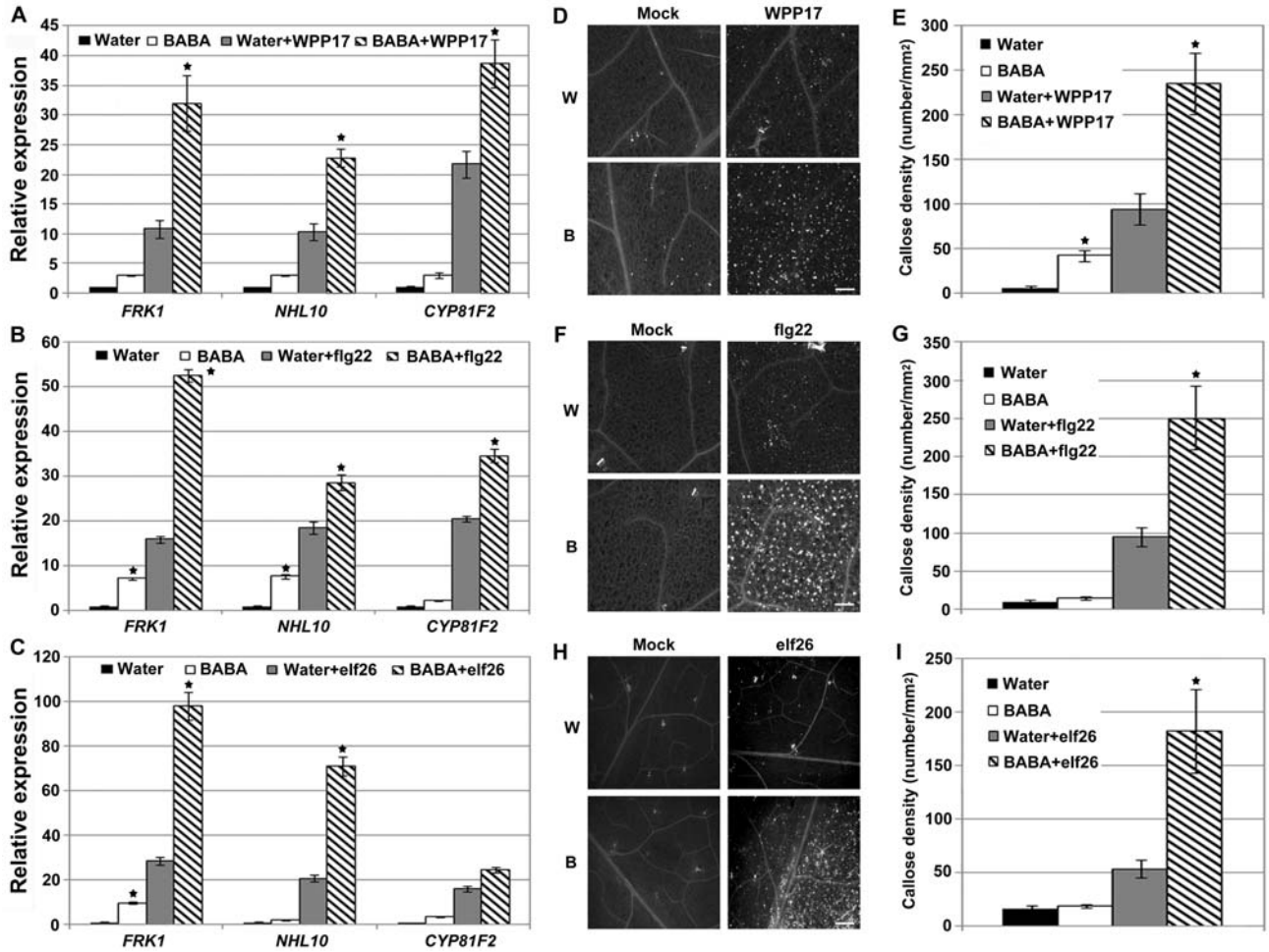

Fig. 4 Effect of β-aminobutyric acid (BABA) treatments on the Arabidopsis pattern-triggered immunity (PTI) response. (A) BABA potentiates the expression of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*) and *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) on *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) WPP17 infection. Transcript levels were analysed at 2 h post-inoculation (hpi) with 1×10^8 colony-forming units (cfu)/mL *Pcc* WPP17. (B,C) Potentiated expression of PTI-responsive genes 30 min after infiltration with 1 μM flg22 (B) or elf26 (C). For (A–C), transcript levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). (D–I) BABA primes callose deposition upon *Pcc* WPP17 infection (D,E), flg22 (F,G) and elf26 (H,I). Leaves were syringe infiltrated with *Pcc* WPP17 (1×10^8 cfu/mL), 1 μM flg22 or elf26, and samples were collected 6 h later for aniline blue staining. Graph represents the average number of deposits observed per square millimetre. Biological triplicates were averaged ± SD ($n = 27$). For A, B, C, E, G and I, asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.01$). White bars, 200 μm. B, BABA treated; W, water treated.

blocked the *Pcc*-mediated reopening of stomata in *sid2-1* and *pad4-1* (Fig. 6C). Taken together, these results indicate that BABA primes or reinforces the PTI response in SA-deficient mutants.

BABA induces chromatin modifications associated with transcriptional activation

Chromatin modifications at the N-terminal tail of histone H3 are associated with Arabidopsis pathogenesis (Jaskiewicz *et al*., 2011; Luna *et al*., 2012; Zhou *et al*., 2005). To address whether histone modifications occur in Arabidopsis treated with the priming agent BABA, we performed chromatin immunoprecipitation (ChIP) analyses of the promoter of PTI-responsive *FRK1*, *NHL10*, *CYP81F2* and *PR1*, and *ACTIN 2/7* as a negative control. Within the standard histone code, acetylation of histone H3 at lysine 9 and lysine 14 (H3K9K14Ac), and dimethylation at lysine 4 (H3K4Me2), are correlated with transcriptional activation (Lusser, 2002). BABA treatment was associated with enrichment (1.5–4.0-fold) of H3K9K14Ac and H3K4Me2 in promoter regions of PTI marker genes, whereas no enrichment was observed in the *ACTIN 2/7* control (Fig. 7). BABA treatment thus induces chromatin modifications associated with increased transcriptional capacity.

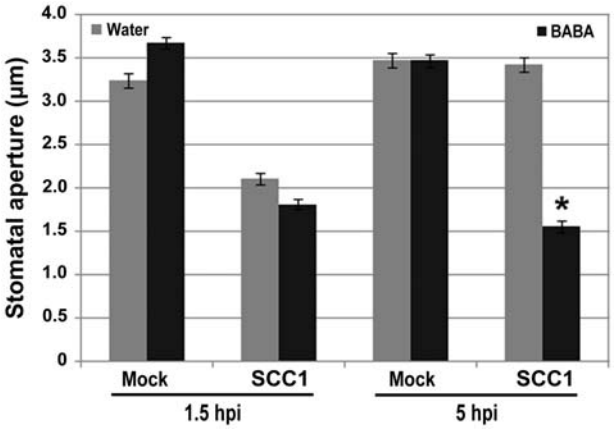

Fig. 5 β-Aminobutyric acid (BABA) effect on stomatal movements upon *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1 infection. Stomatal apertures in epidermal peels exposed to buffer $MgSO_4$ (Mock) or *Pcc* SCC1 [1 × 10^6 colony-forming units (cfu)/mL] were analysed at 1.5 and 5 h post-inoculation (hpi). Results are shown as the mean ($n > 60$ stomata) ± standard error (SE). Asterisks indicate a significant difference from the water control based on Student's *t*-test analysis ($P < 0.001$). Experiments were repeated three times with similar results.

DISCUSSION

The nonprotein amino acid BABA enhances plant resistance to numerous pathogens, notably microbial pathogens (Cohen, 2002; Jakab *et al*., 2001), nematodes (Oka *et al*., 1999) and insects such as aphids (Hodge *et al*., 2005). The spectrum of action of BABA against microbial pathogens is broad, with observed protection in numerous crops against very divergent groups of microbial pathogens, such as oomycetes (Cohen, 1994; Cohen *et al*., 2011; Hamiduzzaman *et al*., 2005; Papavizas, 1964; Slaughter *et al*., 2008), biotrophic (Shailasree *et al*., 2001; Worrall *et al*., 2012) and necrotrophic (Jakab *et al*., 2001) fungi and viruses (Siegrist *et al*., 2000). In the model plant Arabidopsis, BABA enhances resistance to necrotrophic fungi, such as *B. cinerea*, *A. brassicicola* and *Plectosphaerella cucumerina* (Ton and Mauch-Mani, 2004; Zimmerli *et al*., 2001), oomycetes such as *H. arabidopsidis* and hemibiotrophic *Pst* bacteria (Zimmerli *et al*., 2000). In this study, we have demonstrated that BABA enhances Arabidopsis resistance to *Pcc* at both symptom and titre levels, widening the spectrum of action of BABA in Arabidopsis to necrotrophic bacteria. BABA-induced resistance against *Pcc* is observed in calla lily (Luzzatto *et al*., 2007). Arabidopsis resistance to *Pcc* is usually related to concomitant activation of the JA/ET defence signalling pathways (Kariola *et al*., 2003; Norman-Setterblad *et al*., 2000). BABA did not prime the expression of the JA/ET-responsive *PDF1.2*, suggesting that the JA/ET-dependent defence responses are not potentiated by BABA upon *Pcc* infection. By contrast, BABA primed the expression of the SA marker *PR1* upon *Pcc* infection, indicating that SA signalling might be responsible for BABA-induced Arabidopsis resistance to *Pcc*. Similarly, BABA primes the expression of *PR1*, but not *PDF1.2*, in Arabidopsis infected with the necrotrophic fungal pathogen *B. cinerea* (Zimmerli *et al*., 2001). Together, these observations suggest that BABA does not potentiate the JA/ET signalling that is usually associated with Arabidopsis resistance to necrotrophic microbial pathogens, such as *Pcc* (Kariola *et al*., 2003; Norman-Setterblad *et al*., 2000), but rather primes the SA defence signalling that is responsive to hemibiotrophic microbial pathogens (Ton *et al*., 2005; Tsai *et al*., 2011; Zimmerli *et al*., 2000) and has limited effects on necrotrophs (Ferrari *et al*., 2003; Zimmerli *et al*., 2001). Mutants defective in SA- or JA/ET-dependent defence signalling pathways were fully protected against *Pcc* by BABA. These observations further suggest that JA/ET signalling cascades are not necessary for BABA-induced protection against *Pcc* and, although the SA response is boosted by BABA treatment, other layers of Arabidopsis defence may compensate and play a crucial role for full resistance in SA-defective mutants. Such independence towards the SA and JA/ET defence signalling cascades is also observed in BABA-treated Arabidopsis upon infection with *H. arabidopsidis* (Zimmerli *et al*., 2000) or *P. cucumerina* (Ton and Mauch-Mani, 2004). In both cases, BABA pretreatments induce fast and strong callose accumulations upon pathogen attack, suggesting that BABA-mediated cell wall reinforcement at infection sites efficiently blocks these pathogens (Ton and Mauch-Mani, 2004; Zimmerli *et al*., 2000).

The PTI response is the first defence layer in plant immunity (Jones and Dangl, 2006). Similar to BABA-induced resistance to *Pcc*, PTI-induced Arabidopsis resistance mediated by flg22 is independent of SA or JA/ET defence signalling cascades (Zipfel *et al*., 2004). We therefore questioned whether BABA could prime the PTI response in Arabidopsis challenged with *Pcc*. Our data indicate that bacterial-, flg22- and elf26-induced PTIs are primed by BABA pretreatment, as illustrated by potentiated *PR1*, *FRK1*, *CYP81F2* and *NHL10* expression and callose deposition. BABA also primes the Arabidopsis PTI response upon infection with the hemibiotrophic bacterium *Pst* DC3000 (Singh *et al*., 2012), suggesting that BABA priming of Arabidopsis PTI is a common mechanism for the enhancement of Arabidopsis resistance to bacteria. BABA pretreatments induced an 'open chromatin' configuration at promoter regions of PTI-responsive genes. Such enrichment of histone H3 activating marks may favour potentiation of PTI marker gene expressions. Chromatin structure reprogramming and priming are also correlated upon treatment with the systemic acquired resistance (SAR) chemical inducer acibenzolar *S*-methyl (Jaskiewicz *et al*., 2011) and during transgenerational SAR (Luna *et al*., 2012). As suggested recently (van den Burg and Takken, 2009), modification of chromatin structures may regulate the plant defence response. Occasionally, we observed a direct BABA-mediated up-regulation of PTI-responsive gene expression or callose deposition after infiltration of buffer (mock controls) (Fig. 4). As the infiltration of buffer in leaves induces wounding stress (Jaskiewicz

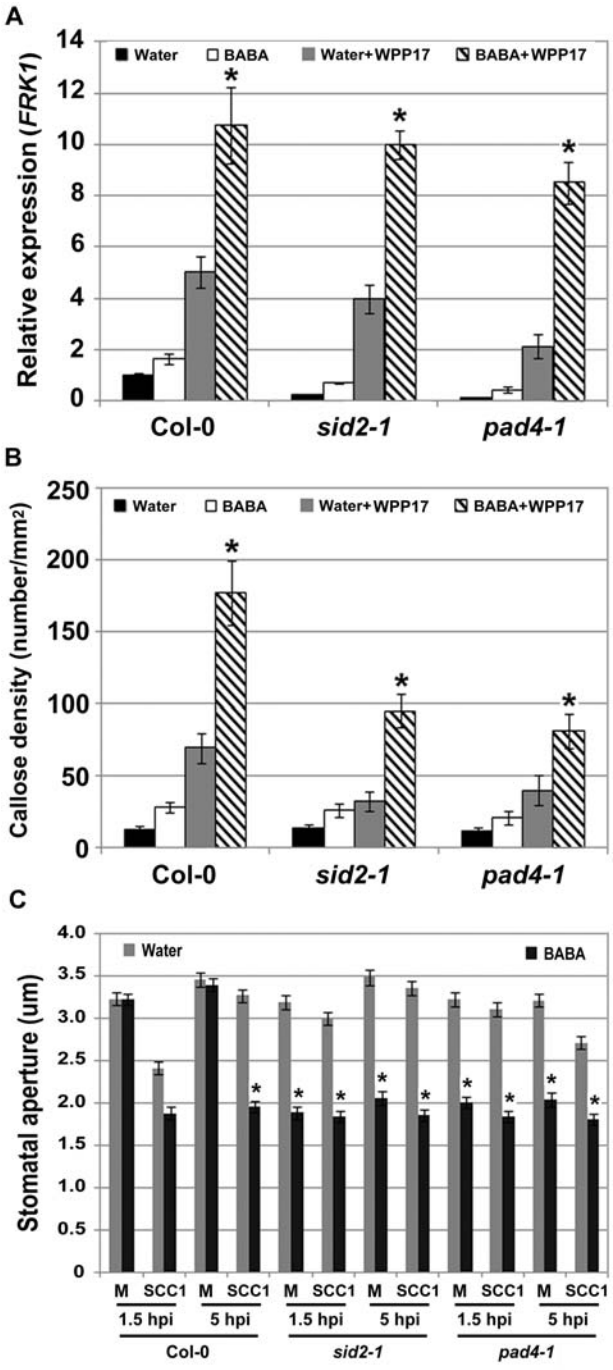

Fig. 6 β-Aminobutyric acid (BABA) primes the pattern-triggered immunity (PTI) response in salicylic acid (SA)-defective mutants. (A) BABA-mediated priming of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*) expression in Col-0, *SA induction deficient 2-1* (*sid2-1*) and *phytoalexin deficient 4-1* (*pad4-1*) after inoculation with *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) WPP17. Transcript levels of *FRK1* were analysed 2 h post-inoculation (hpi) with *Pcc* WPP17 [1×10^8 colony-forming units (cfu)/mL]. *FRK1* expression levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with Col-0 water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). (B) Callose deposition is primed in SA-defective mutants. Leaves were syringe infiltrated with 1×10^8 cfu/mL *Pcc* WPP17 and samples were collected 6 h later for aniline blue staining. Graph represents the average number of deposits observed per square millimetre. Biological triplicates were averaged ± SD ($n = 27$). For (A) and (B), asterisks indicate a significant difference from the water controls based on Student's *t*-test ($P < 0.01$). (C) Role of BABA on stomatal movements in *Pcc* SCC1-inoculated SA-defective mutants. Stomatal apertures in epidermal peels exposed to buffer or *Pcc* SCC1 (1×10^6 cfu/mL) were analysed at 1.5 and 5 hpi in Col-0, *sid2-1* and *pad4-1*. Results are shown as mean ($n > 60$ stomata) ± standard error (SE). Asterisks indicate a significant difference from the water control based on Student's *t*-test analysis ($P < 0.001$). Experiments were repeated three times with similar results.

et al., 2011), direct up-regulation of *FRK1*, *CYP81F2*, *NHL10* or callose deposition by BABA after buffer infiltration may actually reflect priming of the wounding response. Accordingly, no increase in callose deposition was observed in BABA-treated plants that were not subjected to buffer infiltration (Fig. S6, see Supporting Information). The SA-dependent defence response is usually associated with the Arabidopsis PTI response (Tsuda *et al*., 2008). Priming by BABA of the SA marker *PR1* thus further suggests that BABA boosts the PTI response in Arabidopsis. The observed protective effect of BABA against *Pcc* in SA-defective mutants may therefore be associated with a primed PTI response, as wild-type levels of PTI priming were observed in *sid2-1* and *pad4-1* mutants (Fig. 6A,B). The PTI response is multilayered (Schwessinger and Zipfel, 2008) and complex, and the removal of one branch of the PTI response (i.e. SA signalling) may not be sufficient to alter BABA-induced resistance in these SA-defective mutants. In this context, priming of PTI-mediated callose deposition and PTI marker gene expression, and inhibition of stomatal reopening, could be sufficient for BABA-induced resistance to be fully functional in SA-defective mutants.

Stomata are microscopic pores that control CO_2 uptake for photosynthesis and water loss during transpiration (Acharya and Assmann, 2009). Stomatal closure upon activation of Arabidopsis PTI in response to hemibiotrophic *Pst* DC3000 or *Pseudomonas syringae* pv. *syringae* (*Pss*) infections, known as 'stomatal immunity', is critical for plant protection (Melotto *et al*., 2006, 2008; Schellenberg *et al*., 2010). Our data indicate that the necrotrophic bacterium *Pcc*, similar to hemibiotrophic bacteria, induces a rapid SA-dependent closure of stomata. *Pst* DC3000 and *Pss* reopen Arabidopsis stomata in a COR- or Syringolin A-dependent manner, respectively (Melotto *et al*., 2006; Schellenberg *et al*., 2010; Zeng *et al*., 2010). Although reopening mechanisms upon *Pcc* infection are still unknown, our data demonstrate that Arabidopsis stomata reopen at 5 hpi with *Pcc* (Fig. 5). BABA treatment inhibited the reopening of stomata, further suggesting that BABA potentiates or reinforces the Arabidopsis PTI response.

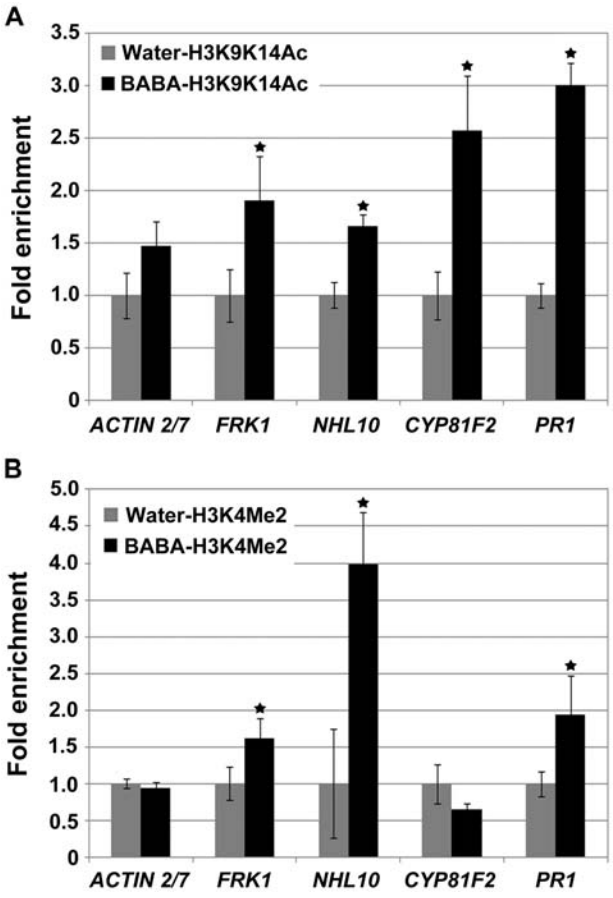

Fig. 7 Modifications of histone H3 after β-aminobutyric acid (BABA) treatment. Levels of H3K9K14Ac (A) and H3K4Me2 (B) at the promoter region of pattern-triggered immunity (PTI)-responsive *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*), *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) and *PATHOGENESIS RELATED 1* (*PR1*) mutants and in the nonresponsive control *ACTIN 2/7*. Relative enrichment levels of H3K9K14Ac and H3K4Me2 were determined in plants treated with 200 μM BABA for 2 days or in water-treated controls. The water-treated Col-0 levels were set to unity after normalization by internal control *TUB2*. Biological duplicates were averaged and the statistical significance was determined by Student's *t*-test (**P* < 0.01) (comparison between BABA- and water-treated plants). Error bars are the standard deviation (SD) ($n = 6$).

BABA demonstrates a similar effect upon *Pst* DC3000 COR-dependent reopening of stomata (Tsai *et al*., 2011), suggesting a common mechanism of action of BABA on both necrotrophic and hemibiotrophic bacteria, possibly through the inhibition of effector actions, as already suggested for *Pst* DC3000 (Tsai *et al*., 2011). Both the BABA-treated SA biosynthesis mutant *sid2-1* and SA signalling mutant *pad4-1* demonstrated closed stomata even in the absence of *Pcc* bacteria (Fig. 6C). This observation is surprising and indicates that functional SA signalling is necessary for wild-type levels of stomatal opening in BABA-treated mock plants. As SA antagonizes abscisic acid (ABA) signalling (de Torres Zabala *et al*., 2009), BABA may activate directly ABA-mediated stomatal closure when SA signalling is defective. Our data suggest that BABA-mediated efficient and long-lasting stomatal closure upon bacterial attack represents one important aspect of BABA-induced resistance against bacterial pathogens.

In conclusion, the Arabidopsis PTI response appears to have a central role in BABA-induced resistance to *Pcc*. As shown, the JA/ET defence signalling cascade, which is usually associated with Arabidopsis resistance to *Pcc* (Norman-Setterblad *et al*., 2000), was not primed by BABA treatment. JA- and ET-defective mutants, such as *coi1-16* or *ein2-1*, were also fully protected by BABA, further suggesting that BABA-induced resistance to *Pcc* is independent of JA/ET. BABA primed SA-dependent defence signalling, but SA-defective *sid2-1* and *pad4-1* mutants still demonstrated a functional BABA-induced resistance. These SA-defective mutants showed primed PTI-mediated callose deposition, primed PTI-responsive gene expression and BABA blocked stomatal reopening in SA-defective mutants. We thus propose a model in which BABA restricts *Pcc* infection in Arabidopsis through priming of the PTI response (Fig. 8). Microarray analyses revealed that BABA up-regulates directly numerous genes coding for putative kinases, notably leucine-rich repeat protein kinases (LRR-PKs) (Zimmerli *et al*., 2008). Pattern recognition receptors, such as FLS2 and EFR1, which perceive MAMPs, are LRR-PKs (Ausubel, 2005; Boller and Felix, 2009). Direct up-regulation by BABA of putative positive regulators of the PTI response, such as LRR-PKs, could thus prepare Arabidopsis plants for a faster and stronger activation of the PTI response upon pathogen challenge. Pre-stress deposition of defence signalling components is indeed known to be critical for priming (Beckers *et al*., 2009; Singh *et al*., 2012). Alternatively, BABA-mediated opening of chromatin at defence-related genes may account for the primed response of PTI marker genes.

EXPERIMENTAL PROCEDURES

Biological materials

Arabidopsis thaliana (L. Heyhn.) ecotype Columbia (Col-0) and derived mutant lines were grown as described previously (Tsai *et al*., 2011). The Col-0 background mutant *sid2-1* was provided by C. Nawrath (University of Lausanne, Lausanne, Switzerland); *pad4-1* and *ein2-1* were obtained from the Arabidopsis Biological Resource Centre, and the Col-6 background mutant *coi1-16* with a functional PEN2 (Westphal *et al*., 2008) was a gift from Dr M. R. Grant (University of Exeter, Exeter, UK). We obtained wild-type *Pcc* SCC1 bacteria (Kariola *et al*., 2005) expressing the green fluorescent protein (GFP) (Kwon *et al*., 2009) from O. K. Park (Korea University, Seoul, Korea), and *Pcc hrp*- and *hrc*-deficient strain WPP17 (Yap *et al*., 2004) was a gift from A. O. Charkowski (University of Wisconsin, Madison, WI, USA).

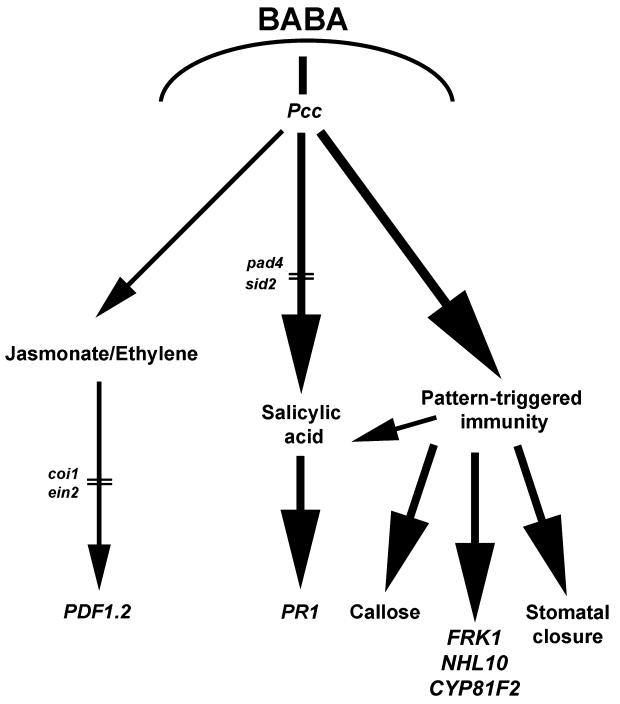

Fig. 8 Hypothetical model illustrating β-aminobutyric acid (BABA)-induced resistance to *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*). This model is based on the information provided in this study and references cited in the discussion. Thick bold black arrows represent priming, and thin arrows indicate no priming. Upon *Pcc* infection, BABA primes salicylic acid (SA)-dependent signalling and the pattern-triggered immunity (PTI) response. Priming leads to a potentiated expression of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*), *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) and *PATHOGENESIS RELATED 1* (*PR1*), increased callose deposition and blockage of reopening of stomata. Even when the SA defence signalling cascade is disrupted in SA-defective *SA induction deficient 2-1* (*sid2-1*) and *phytoalexin deficient 4-1* (*pad4-1*), priming of other PTI-dependent defence responses may be sufficient for a functional BABA-induced resistance. *coi1*, *coronatine-insensitive 1*; *ein2*, *ethylene-insensitive 2*.

Bacterial inoculations

Pcc bacteria were cultivated at 28 °C, 340 rpm in Luria–Bertani (LB) medium (Bioman Scientific Co, Taipei, Taiwan) for 16–18 h without selection (*Pcc* WPP17) or with ampicillin (*Pcc* SCC1). *Pcc* were then collected by centrifugation, suspended in 10 mM $MgSO_4$ at $A_{600} = 0.25$, corresponding to a concentration of 10^8 cfu/mL. Five-week-old Arabidopsis rosettes were dipped in a bacterial solution of 5×10^6 cfu/mL (for *Pcc* SCC1) or 1×10^8 cfu/mL (for *Pcc* WPP17) in 10 mM $MgSO_4$ containing 0.01% Silwet L-77 for 15 min. After inoculation, plants were kept at 100% relative humidity and symptoms were evaluated 1 day later. Bacterial titres were determined by serial dilutions on LB plates, as described previously for *Pst* DC3000 (Zimmerli *et al*., 2000).

qRT-PCR

qRT-PCR experiments were performed as described previously (Wu *et al*., 2010). Briefly, leaf samples from three to five plants per treatment were harvested at the indicated time points, flash frozen in liquid N_2 and kept at −80 °C. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Qiagen, Carlsbad, CA, USA) for 15 min. cDNA was synthesized from 2 μg of total RNA using oligo(dT) primers and the reverse transcriptase from the M-MLV kit (Invitrogen; http://www.invitrogen.com/). The iCycler sequence detection system (Bio-Rad; http://www.bio-rad.com/) and SYBR Green PCR Master Mix (Bio-Rad, Hercules, CA, USA) were used for real-time PCR analysis. The thermal cycling programme was 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s. Melting curve analysis was used to ensure the specificity of the product. Normalization of gene expression across different samples was performed with *EF-1a* and *UBQ10* as internal controls. Primer sequences were as follows: *UBQ10* (At4g05320) F, 5′-GGCCTTGTATAATCCCTGATGA-3′/R, 5′-AAAGAGATAACAGGAACGGAAA-3′; *EF-1a* (At5g60390) F, 5′-TGAGCACGCTCTTCTTGCTTTC-3′/R, 5′-GGTGGTGGCATCCATCTTGTTA-3′; *FRK1* (AT2G19190) F, 5′-GCCAACGGAGACATTAGAG-3′/R, 5′-CCATAACGACCTGACTCATC-3′; *NHL10* (AT2G35980) F, 5′-TTCCTGTCCGTAACCCAAAC-3′/R, 5′-CCCTCGTAGTAGGCATGAGC-3′; *CYP81F2* (AT5G57220) F, 5′-AAATGGAGAGAGCAACACAATG-3′/R, 5′-ATCGCCCATTCCAATGTTAC-3′; *PR1* (AT2G14610) F, 5′-AAAACTTAGCCTGGGGTAGCGG-3′/R, 5′-CCACCATTGTTACACCTCACTTTG-3′; *PDF1.2a* (AT5G44420) F, 5′-AGAAGTTGTGCGAGAAGCCAAG-3′/R, 5′-GTGTGCTGGGAAGACATAGTTGC-3′.

BABA treatment

Five-week-old Arabidopsis plants were soil drenched with BABA (Fluka, Steinheim, Germany) dissolved in water at a final concentration of 200 μM, or with water as control, 2 days before bacterial inoculation or MAMP treatments. Samples were collected at the indicated time points after bacterial inoculation.

MAMP treatments

The peptides representing the MAMPs flagellin (flg22) and EF-Tu (elf26) were synthesized by Biomer Technology (Pleasanton, CA, USA); 1 μM flg22 or elf26 was dissolved in 10 mM $MgSO_4$ and syringe infiltrated in leaves of 5-week-old Arabidopsis. Buffer $MgSO_4$ only was used as a control. Leaf samples from three to five plants per treatment were harvested at the indicated time points for further gene expression or callose deposition analyses.

Callose staining

Leaves from 5-week-old Arabidopsis plants were syringe infiltrated with 5×10^6 cfu/mL *Pcc* SCC1, 1×10^8 cfu/mL *Pcc* WPP17, 1 μM flg22 or 1 μM elf26 in 10 mM $MgSO_4$. Control plants were syringe infiltrated with 10 mM $MgSO_4$ only. At the indicated time points, nine leaf discs from three different plants were collected for analyses. Harvested leaf discs were cleared overnight by soaking in 95% ethanol at room temperature and

then washed three times (2 h for each washing) with sterilized water. Cleared leaves were stained with 0.05% aniline blue in 0.07 M phosphate buffer (pH 8) overnight. Callose deposits were visualized under ultraviolet illumination using an Olympus BX51 microscope digital camera and application software DP2-BSW (Olympus, Hamburg, Germany). Callose deposits were counted using the 'analyze particles' function of ImageJ (http://rsb.info.nih.gov/ij/).

Stomatal assay

Plants were maintained under light (approximately 100 μmol/m^2/s) for at least 3 h to allow the opening of stomata before the performance of the experiments. The epidermis of three fully expanded leaves from three plants was peeled off and placed in a plastic well containing the stomatal buffer only (10 mM $MgSO_4$) or bacterial suspension in buffer (1×10^6 cfu/mL *Pcc* SCC1 in 10 mM $MgSO_4$). At various time points, photographs were taken randomly using an Olympus BX51 microscope digital camera and application software DP2-BSW (http://www.olympusglobal.com/). The width of the stomatal aperture was measured using the 'measure' function of ImageJ (http://rsb.info.nih.gov/ij/).

ChIP assay

The ChIP assay was carried out as described by Gendrel *et al*. (2005). Chromatin extracts were prepared from 5-week-old plants treated with formaldehyde. The chromatin was sheared to an average length of 500 bp by sonication and immune precipitated with specific antibodies, including anti-acetylated histone H3K9K14 (catalogue no. 06-599; Millipore, Temecula, CA, USA) and anti-dimethyl histone H3K4 (catalogue no. 07-030; Millipore). Relative enrichments in the promoter region of *ACTIN 2/7*, *FRK1*, *NHL10*, *CYP81F2* and *PR1* in Col-0 pretreated with water or BABA were determined after normalization to *TUB2*. The DNA sequence from *ACTIN 2/7*, which is constitutively expressed in Arabidopsis, was used as a negative control (Johnson *et al*., 2002). Real-time PCR was used to determine the amounts of genomic DNA immune precipitated in the ChIP experiments. The primer pairs used were as follows: *TUB2* (AT5G62690) F, 5′-ACAAACACAGAGAGGAGTGAGCA-3′/R, 5′-ACGCATCTTCGGTTGGATGAGTGA-3′; *ACTIN 2/7* (AT5G09810) F, 5′-CGTTTCGCTTTCCTTAGTGTTAGCT-3′/R, 5′-AGCGAACGGATCTAGAGACTCACCTTG-3′; *FRK1* (AT2G19190) F, 5′-AACCTTAGAAGATGGTTGGTTGA-3′/R, 5′-CAGAAGAGCAAAGCTTGTGAA-3′; *NHL10* (AT2G35980) F, 5′-AAACTCCCGTTGGAGTTGGT-3′/R, 5′-GTGATGTTGCCCCAAACTTC-3′; *CYP81F2* (AT5G57220) F, 5′-AATTTTGTATCGTGTGTAAAATTTAGCTT-3′/R, 5′-AAGTTAGGTTTCGTAAGCATGCC-3′; *PR1* (AT2G14610) F, 5′-TCGGTCACCTAGAGTTTTTCAA-3′/R, 5′-CCGCCACATCTATGACGTAAG-3′.

Accession numbers

FRK1 (AT2G19190); *NHL10* (AT2G35980); *CYP81F2* (AT5G57220); *PR1* (AT2G14610); *PDF1.2a* (AT5G44420); *UBQ10* (At4g05320); *EF-1a* (At5g60390); *TUB2* (AT5G62690); *ACTIN 2/7* (AT5G09810).

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SUPPORTING INFORMATION

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

Fig. S1 β-Aminobutyric acid (BABA)-mediated enhanced resistance at 48 h post-inoculation (hpi) with *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1. Arabidopsis plants were treated with water (A) or BABA (B) and challenged 48 h later with *Pcc* SCC1 at a concentration of 5×10^6 colony-forming units (cfu)/mL. Photographs were taken 2 days later. White arrows indicate *Pcc* SCC1-mediated water-soaked lesions.

Fig. S2 Defective *PATHOGENESIS RELATED 1* (*PR1*) up-regulation in salicylic acid (SA)-defective mutants. Arabidopsis plants were treated with water or BABA and challenged 48 h later with *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1 at a concentration of 5×10^6 colony-forming units (cfu)/mL. RNAs were harvested at 18 h post-inoculation (hpi). Transcript levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). Asterisks indicate a significant difference from the water controls based on Student's *t*-test ($P < 0.01$).

Fig. S3 The *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) *hypersensitive response and pathogenicity* (*hrp*)- and *hrp conserved* (*hrc*)-deficient mutant strain WPP17 is less virulent than the wild-type *Pcc* SCC1 strain. Five-week-old Col-0 plants were dip inoculated with 5×10^6 colony-forming units (cfu)/mL *Pcc* SCC1 (A) or *Pcc* WPP17 (B) and photographs were taken 1 day later. White arrows indicate *Pcc*-mediated water-soaked lesions.

Fig. S4 β-Aminobutyric acid (BABA) primes the pattern-triggered immunity (PTI) response upon infection with wild-type *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1 bacteria. (A) BABA potentiates the expression of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*) and *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) upon *Pcc* SCC1 infection. Transcript levels were analysed at 4 h post-inoculation (hpi) with 5×10^6 colony-forming units (cfu)/mL *Pcc* SCC1. Transcript levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). (B) BABA primes callose deposition upon *Pcc* SCC1 infection. Leaves were syringe infiltrated with *Pcc* SCC1 (5×10^6 cfu/mL) and samples were collected 12 h later for aniline blue staining. The graph represents the average number of deposits observed per square millimetre. Biological triplicates were the average ± SD ($n = 27$). Asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.01$).

Fig. S5 β-Aminobutyric acid (BABA)-mediated priming of pattern-triggered immunity (PTI) upon *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) SCC1 infection in salicylic acid (SA)-defective mutants. (A) *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*) expression is primed in BABA-treated Col-0, *SA induction deficient 2-1* (*sid2-1*) and *phytoalexin deficient 4-1* (*pad4-1*) after inoculation with *Pcc* SCC1. Transcript levels of *FRK1* were analysed at 6 h post-inoculation (hpi) with 5×10^6 colony-forming units (cfu)/mL *Pcc* SCC1. *FRK1* expression levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and normalized to both *UBQ10* and *EF-1*. Relative gene expression levels were compared with Col-0 water controls (defined value of one). Data represent the means and standard deviation (SD) of three independent biological replicates ($n = 9$). (B) Callose deposition is primed in SA-defective mutants upon *Pcc* SCC1 infection. Leaves were syringe infiltrated with *Pcc* SCC1 (5×10^6 cfu/mL), and samples were collected 12 h later for aniline blue staining. Graph represents the average number of deposits observed per square millimetre. Biological triplicates were averaged ± SD ($n = 27$). Asterisks indicate a significant difference from water controls based on Student's *t*-test ($P < 0.01$).

Fig. S6 Callose deposition in nonbuffer-infiltrated Arabidopsis leaves. Arabidopsis plants were soil drenched with water or β-aminobutyric acid (BABA), and leaves were analysed for callose deposition 2 days later. The graph represents the average number of deposits observed per square millimetre. Biological triplicates were averaged ± standard deviation (SD) ($n = 27$). Values of BABA-treated plants were not significantly different from those of water-treated controls based on Student's *t*-test ($P < 0.01$).