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The tomato UV-damaged DNA-binding protein-1 (DDB1) is implicated in pathogenesis-related (*PR*) gene expression and resistance to *Agrobacterium tumefaciens*

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SUMMARY

Plants defend themselves against potential pathogens via the recognition of pathogen-associated molecular patterns (PAMPs). However, the molecular mechanisms underlying this PAMP-triggered immunity (PTI) are largely unknown. In this study, we show that tomato *HP1/DDB1*, coding for a key component of the CUL4-based ubiquitin E3 ligase complex, is required for resistance to *Agrobacterium tumefaciens*. We found that the DDB1-deficient mutant (*high pigment-1*, *hp1*) is susceptible to nontumorigenic *A. tumefaciens*. The efficiency of callus generation from the *hp1* cotyledons was extremely low as a result of the necrosis caused by *Agrobacterium* infection. On infiltration of nontumorigenic *A. tumefaciens* into leaves, the *hp1* mutant moderately supported *Agrobacterium* growth and developed disease symptoms, but the expression of the pathogenesis-related gene *SlPR1a1* and several PTI marker genes was compromised at different levels. Moreover, exogenous application of salicylic acid (SA) triggered *SlPR1a1* gene expression and enhanced resistance to *A. tumefaciens* in wild-type tomato plants, whereas these SA-regulated defence responses were abolished in *hp1* mutant plants. Thus, HP1/DDB1 may function through interaction with the SA-regulated PTI pathway in resistance against *Agrobacterium* infection.

INTRODUCTION

Plants are constantly exposed to potential pathogens, but are resistant to the majority of them. This is largely because plants use a complex of immune systems to defend themselves against pathogen invasion. One important component of the immune system is innate immunity, also called basal defence in many cases (Dangl and Jones, 2001). Innate immunity is elicited by the perception of pathogen-associated molecular patterns (PAMPs), conserved among pathogens, by pattern recognition receptors (PRRs) in host plants. This leads to the activation of a series of cellular events to eventually prevent pathogen colonization, including the production of reactive oxygen species (ROS) and ethylene (ET), callose deposition at the cell wall, activation of the mitogen-activated protein kinase (MAPK) cascade and the induction of defence-related genes (Asai *et al*., 2002; Ausubel, 2005; Boller and Felix, 2009). In a given plant species, multiple PAMP-triggered immunity (PTI) pathways may exist and are integrated into the complex of immune systems that is sufficient to prevent the colonization of plants by most pathogens.

Although a number of PAMPs and corresponding PRRs have been established (Zipfel *et al*., 2004, 2006), the downstream signalling leading to cellular events against pathogen colonization remains to be elucidated. Recently, an emerging body of evidence has suggested that the general stress signalling molecules, including salicylic acid (SA), jasmonic acid (JA) and ET, play a role in the PTI pathway after recognition of PAMPs by PRRs (Chen *et al*., 2009; Halim *et al*., 2009; Liu *et al*., 2010). Despite the existing cross-talk between signalling pathways mediated by these molecules, it is generally thought that SA-regulated defence signalling is associated with defence responses against biotrophic pathogens, whereas JA- and ET-mediated defence signalling is mainly responsible for resistance against necrotrophic pathogens, both of which are usually characterized by the induction of specific pathogenesis-related (*PR*) gene expression on pathogen challenge (Durrant and Dong, 2004). For instance, induction of the *PR-1* gene has been established as a cellular marker for the activation of the SA-regulated signalling pathway (Durrant and Dong, 2004).

Agrobacterium tumefaciens is a soil-borne bacterium that causes crown-gall disease in many plant species. The molecular basis of pathogenesis involves the transfer of the T-DNA part of the tumour-inducing (Ti) plasmid into the plant nucleus by *Agrobacterium*, followed by integration into the plant chromosome. The T-DNA contains plant hormone genes that stimulate the

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infected plant tissue to overgrow, with the formation of tumour tissue (Gelvin, 2003). The molecular interactions between plants and *Agrobacterium* are very complicated. On the one hand, *Agrobacterium* can trigger PTI: plants perceive PAMPs from *Agrobacterium* and mount a defence response, including the activation of the MAPK cascade; MPK3 phosphorylates the VirE2-interacting protein 1 (VIP1) transcription factor and promotes VIP1 shuttling into the nucleus, where it activates the transcription of *PR-1* and other stress genes (Djamei *et al*., 2007; Pitzschke *et al*., 2009). On the other, *Agrobacterium* can hijack this plant defence system by associating with VIP1 to move the T-complex into the nucleus and target chromatin (Djamei *et al*., 2007; Pitzschke *et al*., 2009). In the mean time, *Agrobacterium* takes advantage of the host VBF ubiquitin E3 ligase, which is also activated by *Agrobacterium* during the early time course of defence responses, to degrade VIP1 and other *Agrobacterium* T-DNA-associated proteins to expose the T-DNA for integration into the host chromatin (Zaltsman *et al*., 2010). In addition, as pathogen-induced *PR-1* expression is mainly regulated by SA, it appears that SA also plays a significant role in the later stage of the defence response to *Agrobacterium* infection (Anand *et al*., 2008; Pruss *et al*., 2008). Exogenous application of SA in *Nicotiana benthamiana* enhanced the resistance to *Agrobacterium* infection, whereas decreasing the endogenous SA level by expressing the bacterial *NahG* (encoding salicylate hydroxylase) gene or silencing the genes involved in SA biosynthesis resulted in plants hypersusceptible to *Agrobacterium* infection (Anand *et al*., 2008).

Tomato (*Solanum lycopersicum*) is an economically and experimentally important crop. Several photomorphogenic mutants with exaggerated photoresponsiveness and elevated pigmentation, such as the monogenic recessive high-pigment mutants *hp1* and *hp2*, have been described in tomato (Kendrick *et al*., 1997). These mutants are characterized by higher anthocyanin levels, shorter hypocotyls and greater fruit pigmentation than their semi-isogenic wild-type counterparts (Mustilli *et al*., 1999; Yen *et al*., 1997). Previously, we have characterized the *HP1* gene, revealing that it encodes a protein homologous to (mammalian) UV-DAMAGED DNA-BINDING PROTEIN-1 (DDB1) (Lieberman *et al*., 2004; Liu *et al*., 2004; Wang *et al*., 2008). Conserved from fission yeast to higher eukaryotes, the CUL4–DDB1 complex has been identified as a cullin-RING finger ubiquitin ligase that is involved in the regulation of genome stability, DNA repair, the cell cycle and histone modification, and can be subverted by pathogenic viruses to benefit viral infection (Angers *et al*., 2006; Braun *et al*., 2011; Centore *et al*., 2010; Jin *et al*., 2006; Li *et al*., 2006; Petroski and Deshaies, 2005).

In this study, we demonstrate that, in addition to the well-established functions involved in photomorphogenesis and pigment development, tomato HP1/DDB1 is required for the resistance to *Agrobacterium*. We found that the *hp1* mutant is susceptible to nontumorigenic *Agrobacterium* infection, as manifested by necrosis on excised cotyledons, enhanced *Agrobacterium* growth and the development of disease symptoms on leaves, and increased efficiency of *Agrobacterium*-mediated transient expression. The expression of *SlPR1a1* and several PTI marker genes was compromised in the *hp1* mutant. Moreover, *SlPR1a1* induction and enhanced resistance to *Agrobacterium* by exogenous SA were abolished in the *hp1* mutant, suggesting that HP1/DDB1 may act through interaction with the SA-mediated PTI pathway.

RESULTS

The excised cotyledons of the *hp1* mutant are hypersusceptible to the nontumorigenic *Agrobacterium* strain

In an attempt to generate transgenic *hp1* mutant plants by *A. tumefaciens*-mediated transformation, we found that the excised cotyledons of the *hp1* mutant were too susceptible to *A. tumefaciens* infection to produce callus. During the standard *Agrobacterium*-mediated transformation (Fillatti *et al*., 1987), the excised cotyledon explants of the wild-type (WT) AC^+ and *hp1* mutant were infected with nontumorigenic *A. tumefaciens* GV2260 containing the binary vector pBI121 (Jefferson, 1987). The pBI121 vector carried a kanamycin resistance gene for selection of positive transformation. To our surprise, under our experimental conditions (1000 lx light for 16 h, dark for 8 h), necrosis started to develop on cotyledon explants of the *hp1* mutant at 10 days post-inoculation (dpi), whereas only a few of the cotyledon explants of WT AC^+ showed this cell death phenotype (Fig. 1A). At 30 dpi, most excised cotyledons of the *hp1* mutant had died without generating any callus. However, the majority of cotyledon explants of WT AC^+ were still alive and produced callus (Fig. 1A). The number of cotyledon explants that generated callus at this time point was also scored. As shown in Fig. 1B, only 5.8% of the *hp1* cotyledon explants were able to generate callus, whereas more than 50% of the WT AC^+ cotyledon explants developed callus. These results indicate that the *hp1* mutant is hypersusceptible to nontumorigenic *Agrobacterium*, which dramatically reduces the rate of callus generation for transformation.

The *hp1* mutant supports bacterial growth and develops disease symptoms on *Agrobacterium* infection

In general, nontumorigenic *Agrobacterium* is not pathogenic to tomato plants. It does not cause disease symptoms or multiply to a large extent in leaves. The susceptibility of the *hp1* cotyledon to *Agrobacterium* infection prompted us to examine the responses of *hp1* mutant plant leaves to nontumorigenic *Agrobacterium*. We sought to determine whether the *hp1* mutant could develop disease symptoms or support bacterial growth after *Agrobacte-*

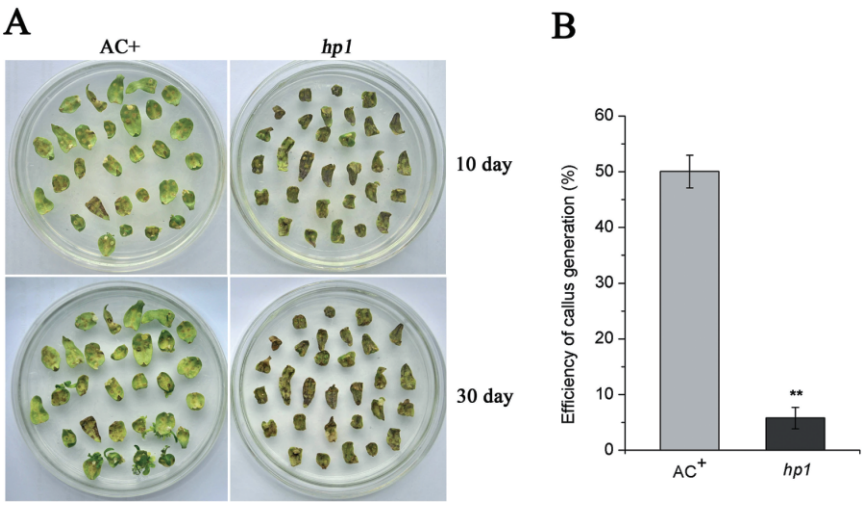

Fig. 1 The detached cotyledons of the *hp1* mutant are hypersusceptible to nontumorigenic *Agrobacterium tumefaciens* infection. (A) Necrosis on cotyledons caused by *A. tumefaciens* GV2260. Cotyledons detached from wild-type (WT) AC$^+$ and *hp1* mutant seedlings, germinated on half-strength Murashige and Skoog (MS) medium, were inoculated with *A. tumefaciens* GV2260 carrying the binary vector pBI121 (containing the kanamycin resistance gene) and incubated on regeneration medium under 1000 lx. Photographs were taken 10 and 30 days after infection. (B) Quantification of kanamycin-resistant callus produced on the cotyledons. Each data point consists of at least three samples. Error bars indicate standard deviation. The statistical significance of the difference was confirmed by Student's *t*-test (**$P < 0.01$). Similar results were obtained in at least two independent experiments.

rium infection. We first assessed possible disease symptom development by inoculating WT AC$^+$ and *hp1* mutant plant leaves with *A. tumefaciens* GV2260 at an inoculum of 10^8 colony-forming units (cfu)/mL. At 4 dpi, more than 17% of the leaves in *hp1* mutant plants had wilted, whereas no WT AC$^+$ leaves showed wilting-like disease symptoms (Fig. 2A). In order to differentiate the subtle effect of *hp1* on *Agrobacterium* growth in leaves, we inoculated WT AC$^+$ and *hp1* mutant plant leaves with *A. tumefaciens* GV2260 using a low inoculum of 10^4 cfu/mL. *Agrobacterium* populations in plant leaves were scored at 2 and 4 dpi. As expected, *A. tumefaciens* GV2260 showed only basal level multiplication in WT AC$^+$ leaves. However, *A. tumefaciens* GV2260 multiplied five times more strongly in *hp1* mutant leaves than in WT AC$^+$ leaves (Fig. 2B). A consistently similar result was obtained when WT AC$^+$ and *hp1* mutant plant leaves were inoculated with another nontumorigenic *A. tumefaciens* strain EHA105 (Fig. 2C). Taken together, our results suggest that *HP1/DDB1* is required for resistance to *A. tumefaciens*.

Agrobacterium-mediated transient transformation in *hp1* leaves is more efficient than in WT AC$^+$ leaves

The efficiency of *Agrobacterium*-mediated transient transformation has been widely used to evaluate the susceptibility of plants to *Agrobacterium* infection (Anand *et al*., 2007, 2008; Wroblewski *et al*., 2005; Zipfel *et al*., 2006). We next sought to determine whether *hp1* affects the efficiency of transient transformation mediated by nontumorigenic *A. tumefaciens* GV2260 strain. *Agrobacterium* harbouring the binary vector pBISN1 expressing a *β-glucuronidase* (*GUS*) gene (Nam *et al*., 1999) was vacuum infiltrated into WT AC$^+$ and *hp1* leaves, and the transformation efficiency was determined by the GUS activity derived from the transformed leaf tissue. The GUS activity assay was conducted 2 days after *Agrobacterium* infiltration. As shown in Fig. 3A, GUS activity, indicated by the intensity of 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) staining of leaves, was significantly greater in *hp1* mutant leaves than in WT AC$^+$ leaves. GUS activity was also quantified. GUS activity in *hp1* leaves infiltrated with *Agrobacterium* was about 70% greater than that in WT AC$^+$ leaves with the same treatment (Fig. 3B). Together, our data suggest that *hp1* plants are more susceptible to *Agrobacterium* infection, and this susceptibility facilitates the efficiency of transient transformation mediated by the nontumorigenic *A. tumefaciens* strain.

HP1/DDB1 is required for the induction of the *SlPR1a1* gene by *A. tumefaciens*

PR genes play a significant role in plant defence responses and have been widely used as molecular markers for the defence reaction in plant–pathogen interactions (Durrant and Dong, 2004). The infiltration of nontumorigenic *Agrobacterium* into *Nicotiana benthamiana* or Arabidopsis leaves triggers strong defence responses, which are characterized by the induction of the *PR-1* gene at late time courses after infiltration (Pitzschke *et al*., 2009; Pruss *et al*., 2008). Significantly, the PR-1-characterized host responses are sufficient to confer resistance to subsequent

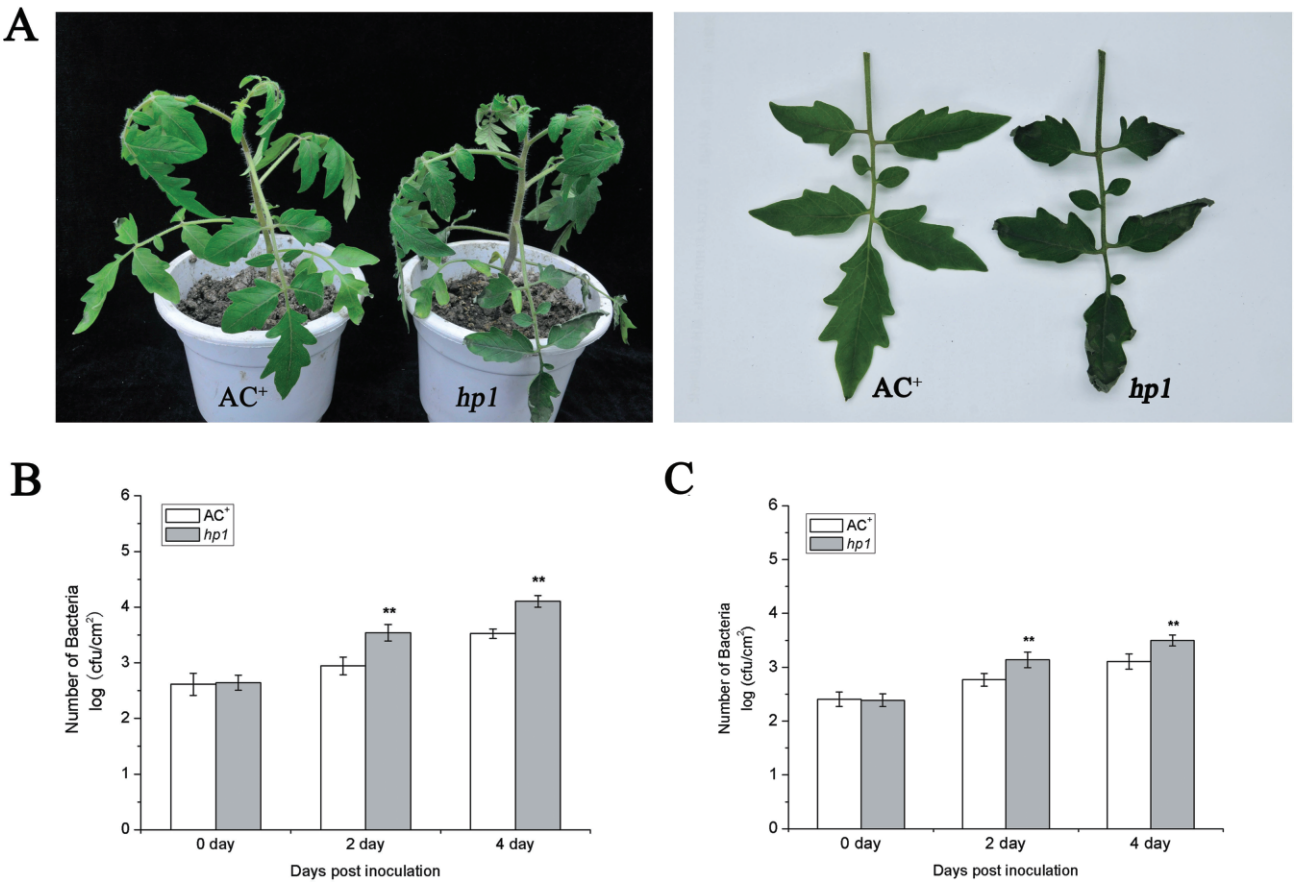

Fig. 2 Disease symptoms and bacterial growth in leaves of wild-type (WT) AC+ and *hp1* mutant plants infected with *Agrobacterium tumefaciens* GV2260 or EHA105. Six-week-old WT AC+ and *hp1* mutant plants were vacuum infiltrated with a suspension of *A. tumefaciens* GV2260 or EHA105, and maintained under light conditions of 200 lx 16 h/dark 8 h. (A) WT AC+ and *hp1* mutant plants were vacuum infiltrated with a suspension of *A. tumefaciens* GV2260 [optical density at 600 nm (OD_{600}) = 0.1]. Photographs were taken 4 days after infection. (B) WT AC+ and *hp1* mutant plants were vacuum infiltrated with a suspension of *A. tumefaciens* GV2260 (OD_{600} = 0.00001). (C) WT AC+ and *hp1* mutant plants were vacuum infiltrated with a suspension of *A. tumefaciens* EHA105 (OD_{600} = 0.00001). Bacterial growth was determined at 0, 2 and 4 days post-inoculation (dpi). Each data point consists of at least six samples. Error bars indicate standard deviation. The statistical significance of the difference was confirmed by Student's *t*-test (**$P < 0.01$). Similar results were obtained in at least two independent experiments.

infection with other pathogens, such as tobacco mosaic virus (Pruss *et al*., 2008). Thus, it is possible that the susceptibility of the *hp1* mutant to *Agrobacterium* infection is a result of the abolishment of defence reactions, such as *PR-1* gene induction. To test this hypothesis, we sought to determine the tomato *PR-1* (*SlPR1a1*) mRNA expression pattern in *A. tumefaciens*-inoculated AC+ and *hp1* mutant plants by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) at different time points. As shown in Fig. 4A, *A. tumefaciens* treatment induced *SlPR1a1* expression in WT AC+ plants at 2 dpi, and *SlPR1a1* induction was maintained over the next 2 days. The induction pattern was similar to that reported for the induction of *PR-1* by *Agrobacterium* in tobacco (Pruss *et al*., 2008). In contrast, the induction of *SlPR1a1* expression in *hp1* mutant plants was completely abolished throughout all time points examined. It is also notable that, even prior to *Agrobacterium* infection, the basal level expression of *SlPR1a1* was lower in WT AC+ than in the *hp1* mutant (Fig. 4A). These results indicate that *HP1/DDB1* is essential for the induction of the *SlPR1a1* gene by *Agrobacterium*. They also suggest a correlation between HP1/DDB1-dependent *SlPR1a1* induction and resistance to *Agrobacterium*.

HP1/DDB1 is required for several PTI marker genes induced by *A. tumefaciens*

Recent publications have demonstrated that plants express *PR-1* and other defence-related genes in response to *Agrobacterium* infection through the MAPK-mediated PTI pathway (Djamei *et al*., 2007; Pitzschke *et al*., 2009). We next verified whether the PTI signalling pathways were compromised in the *hp1* mutant by

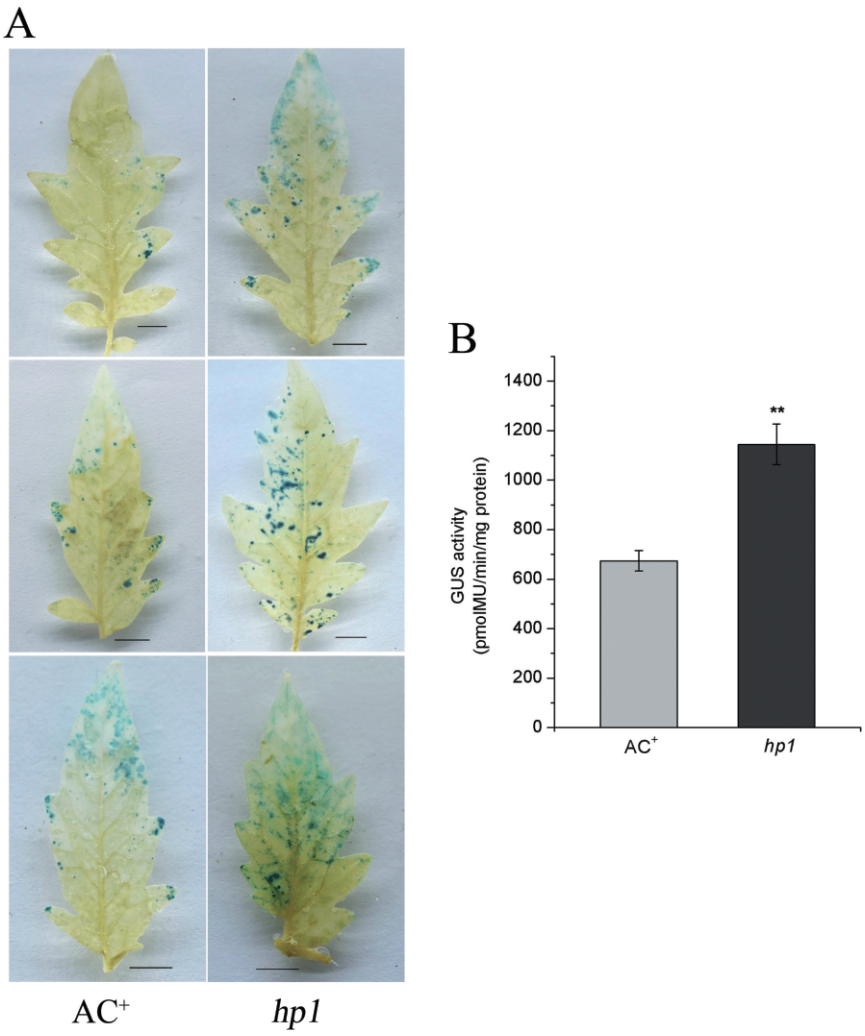

Fig. 3 *Agrobacterium*-mediated transient transformation in the leaves of wild-type (WT) AC$^+$ and *hp1* mutant plants. (A) Randomly selected representative leaves of WT AC$^+$ and *hp1* mutant plants stained with 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) at 2 days after infiltration with *A. tumefaciens* GV2260 expressing the *β-glucuronidase* (*GUS*) gene at an inoculum of $OD_{600} = 0.1$ (optical density at 600 nm). Bars, 1 cm. (B) Quantification of GUS activity in WT AC$^+$ and *hp1* mutant leaves. The GUS activity of infected leaves was measured by recording the fluorescence of 4-methylumbelliferone (MU) at 2 days post-inoculation (dpi). The data presented are the means with standard deviations of three independent experiments. The statistical significance of the difference was confirmed by Student's *t*-test (**$P < 0.01$).

examining the induction pattern of several PTI marker genes by *Agrobacterium*. To this end, we took advantage of several PTI marker genes characterized in tomato, including *SlPti5*, *SlGras2*, *SlLrr22* and *SlWrky28* (Nguyen *et al*., 2010). To monitor gene induction by *Agrobacterium*, WT AC$^+$ and *hp1* mutant plants were vacuum infiltrated with a bacterial suspension of *A. tumefaciens* GV2260 at a concentration of 10^8 cfu/mL. The mRNA abundance of the PTI marker genes was determined by quantitative RT-PCR at 0, 4, 12 and 24 hpi. As shown in Fig. 4B, E, all four PTI marker genes were rapidly induced by *Agrobacterium* infection in WT AC$^+$ plant leaves. However, the expression of these markers in *hp1* mutant leaves showed different patterns in response to *Agrobacterium* challenge: *SlPti5* and *SlLrr22* were not induced; *SlGras2* and *SlWrky28* were induced, but the induction level was significantly lower than that in WT AC$^+$ plant leaves (Fig. 4B, E). These results suggest that *HP1/DDB1* plays a significant role in certain PTI signalling pathways.

SlPR1a1 expression and enhanced resistance to *A. tumefaciens* by exogenous SA is abolished in the *hp1* mutant

It is thought that *PR-1* gene induction by bacteria is mainly regulated through the SA signalling pathway (Durrant and Dong, 2004). Recent studies have also suggested that SA-mediated defence signalling plays a major role in the resistance to *Agrobacterium* in *N. benthamiana* (Anand *et al*., 2008). In particular, exogenous application of SA can induce *PR* expression and activate defence responses to bacteria, including *Agrobacterium* (Anand *et al*., 2008). Therefore, it was logical to determine whether HP1/DDB1 functions through interaction with SA signalling in the resistance to *Agrobacterium* by examining whether SA-regulated defence signalling and resistance to *Agrobacterium* were compromised in the *hp1* mutant. First, we tested the induction of the *SlPR1a1* gene by exogenous SA in *hp1* mutant plants. As shown in

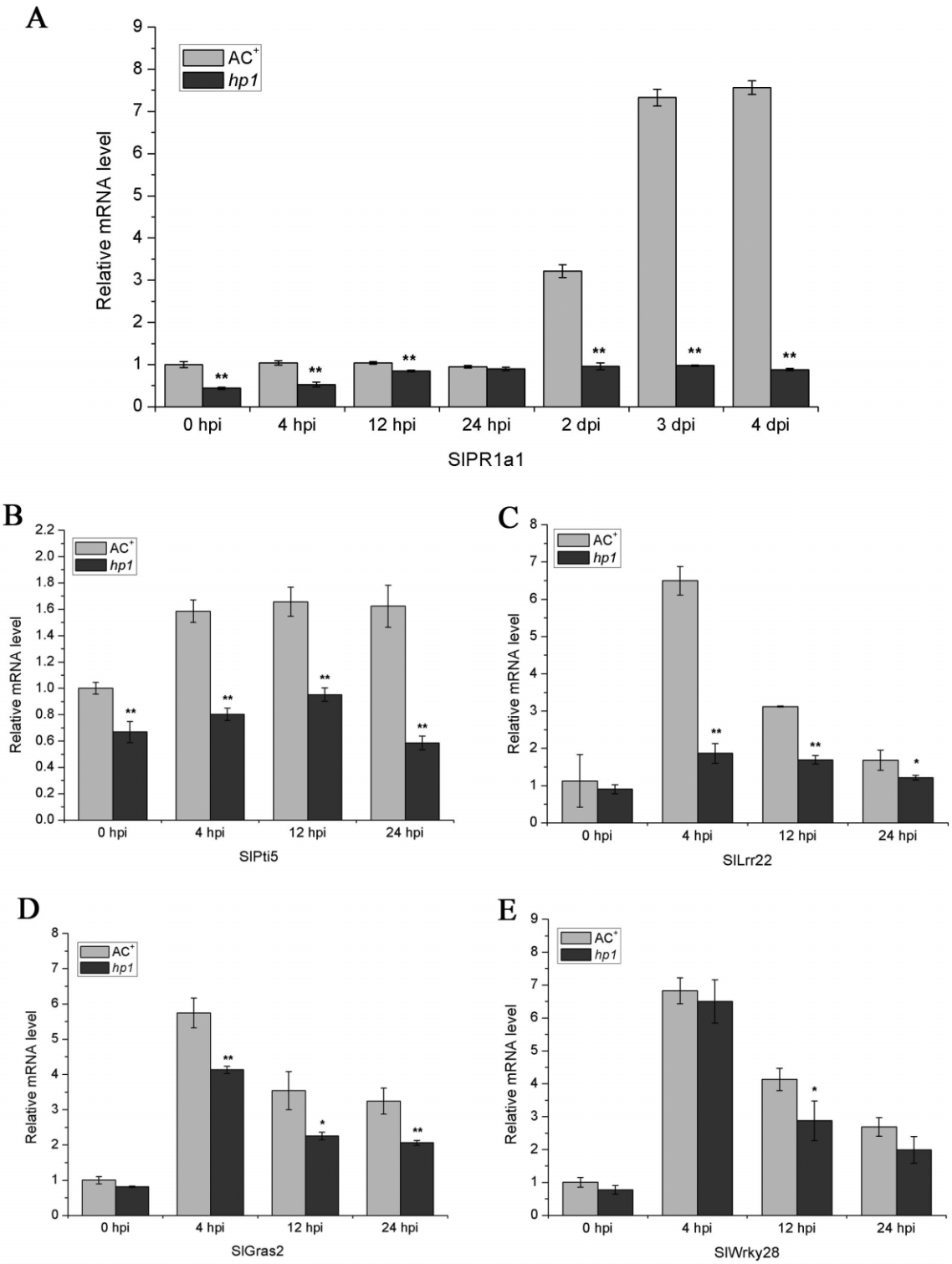

Fig. 4 Effect of HP1/DDB1 on the induction of *SlPR1a1* (A), *SlPti5* (B), *SlLrr22* (C), *SlGras2* (D) and *SlWrky28* (E) by *Agrobacterium tumefaciens*. Six-week-old wild-type (WT) AC^+ and *hp1* mutant plants were vacuum infiltrated with *A. tumefaciens* GV2260 [optical density at 600 nm (OD_{600}) = 0.1]. Total RNA was extracted at the indicated time points for quantitative reverse transriptase-polymerase chain reaction (RT-PCR). *SlUBI3* was used as an internal control. Data represent the mean ± standard deviation of three independent experiments. The statistical significance of the difference was confirmed by Student's *t*-test (*$P < 0.05$; **$P < 0.01$). hpi, hours post-inoculation.

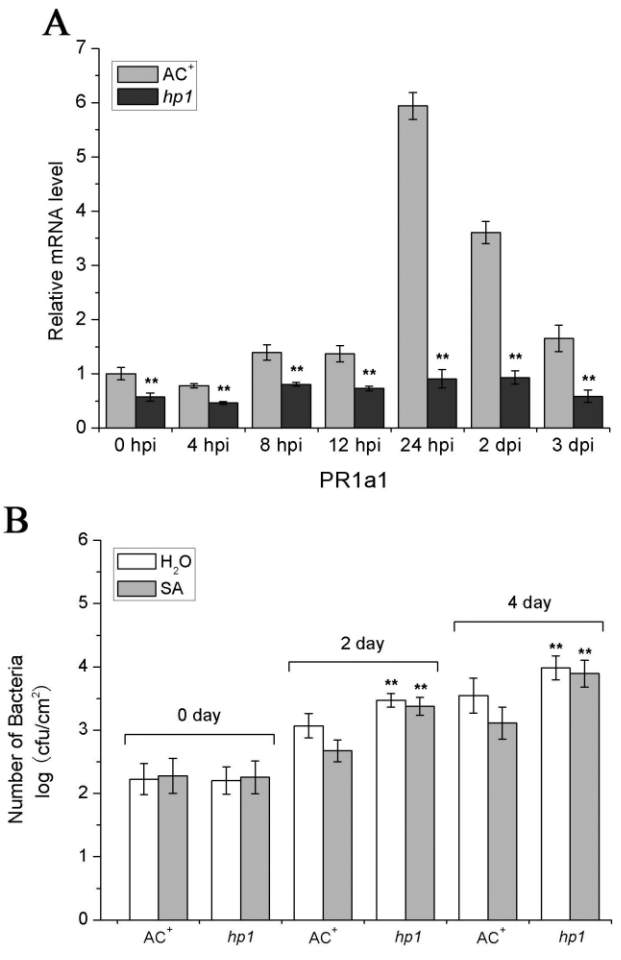

Fig. 5 Influence of salicylic acid (SA) treatment on *SlPR1a1* expression and the susceptibility of the *hp1* mutant to *Agrobacterium tumefaciens*. (A) *SlPR1a1* expression in wild-type (WT) AC+ and *hp1* mutant after SA treatment. Leaf samples sprayed with 0.5 mM SA solution were collected for quantitative reverse transriptase-polymerase chain reaction (RT-PCR) analysis at the indicated time points. *SlUBI3* was used as an internal control. Data represent the mean ± standard deviation from three independent experiments. The statistical significance of the difference was confirmed by Student's *t*-test (***P* < 0.01). (B) Bacterial growth in infected leaves of WT AC+ and *hp1* mutant plants treated with SA. Leaves were presprayed with 1 mM SA or mock solution, 48 h before inoculation with *A. tumefaciens* GV2260 at an inoculum of 1×10^4 colony-forming units (cfu)/mL [optical density at 600 nm (OD_{600}) = 0.00001]. Bacterial growth was determined at 0, 2 and 4 days post-inoculation (dpi). Each data point consists of at least six samples. Error bars indicate standard deviation. The statistical analysis of the difference between the *hp1* mutant and WT AC+ for both buffer and SA treatment was confirmed by Student's *t*-test (***P* < 0.01). Similar results were obtained in at least two independent experiments.

Fig. 5A, exogenous application of SA on WT AC+ leaves strongly induced *SlPR1a1* expression, whereas this induction by SA was completely abolished in *hp1* mutant leaves. Next, we assessed the SA-induced resistance to *Agrobacterium* in the *hp1* mutant. We pretreated leaves of WT AC+ and *hp1* mutant plants with SA solution and tested their response to *A. tumefaciens*. Again, to differentiate the subtle effect on *Agrobacterium* growth in tomato leaves, we inoculated WT AC+ and *hp1* mutant plant leaves with *A. tumefaciens* GV2260 using a low inoculum of 10^4 cfu/mL. The results showed that, in WT AC+ leaves, pretreatment with SA enhanced significantly the resistance to *Agrobacterium* growth, as manifested by about five-fold fewer bacterial populations in SA-pretreated leaves relative to mock-treated leaves, suggesting that SA can activate resistance to *Agrobacterium*. However, in the case of the *hp1* mutant, there was no significant difference in *Agrobacterium* populations in SA-pretreated and mock-treated leaves, suggesting that the SA-triggered resistance was abolished (Fig. 5B). Taken together, our results suggest that HP1/DDB1 is required for SA-regulated *SlPR1a1* induction and resistance to *Agrobacterium* infection in tomato.

Functional deficiency of HP1/DDB1 (*hp1*) affects tumour formation by tumorigenic *Agrobacterium*

Tumorigenesis is the characteristic pathogenesis of virulent *A. tumefaciens* strains. However, tumour development during plant–*Agrobacterium* interactions is determined by both *Agrobacterium* and the host plant, as it involves the virulence of *Agrobacterium* and the regulation of tumour formation in the host by plant genes, in which, in particular, the regulation of plant cell division plays a critical role (Anand *et al*., 2007; Gelvin, 2003; Lee *et al*., 2009). To further investigate the role of *HP1/DDB1* in the tumorigenesis by *Agrobacterium*, we inoculated the stems of WT AC+ and *hp1* mutant plants with virulent *A. tumefaciens* A348 strain and assessed tumour development. Four weeks after inoculation, tumours had developed on the stems of both WT AC+ and *hp1* mutant plants (Fig. 6A). However, the sizes of the tumours on the stems of *hp1* mutant plants were significantly smaller than those on WT AC+ plants. We also quantified the biomass of tumours formed on the stems by measuring the fresh and dry weights of the tumours. As shown in Fig. 6B,C, the average fresh and dry weights of the tumours formed on *hp1* mutant stems were 57 mg and 5.4 mg, respectively, which accounted for only 70% fresh weight and 50% dry weight of tumours developed in WT AC+ stems. We speculate that the weaker tumorigenesis in the *hp1* mutant is a result of the defect of cell division caused by *hp1* mutation (Caspi *et al*., 2008). Further experiments on the tumour cell division rate are needed to verify this hypothesis. Thus, our results show the complexity of tumour formation during plant–*Agrobacterium* interactions, in which cell division plays a critical role.

DISCUSSION

The interplay between *Agrobacterium* infection and plant immunity is very complicated. In general, it is thought that plants can

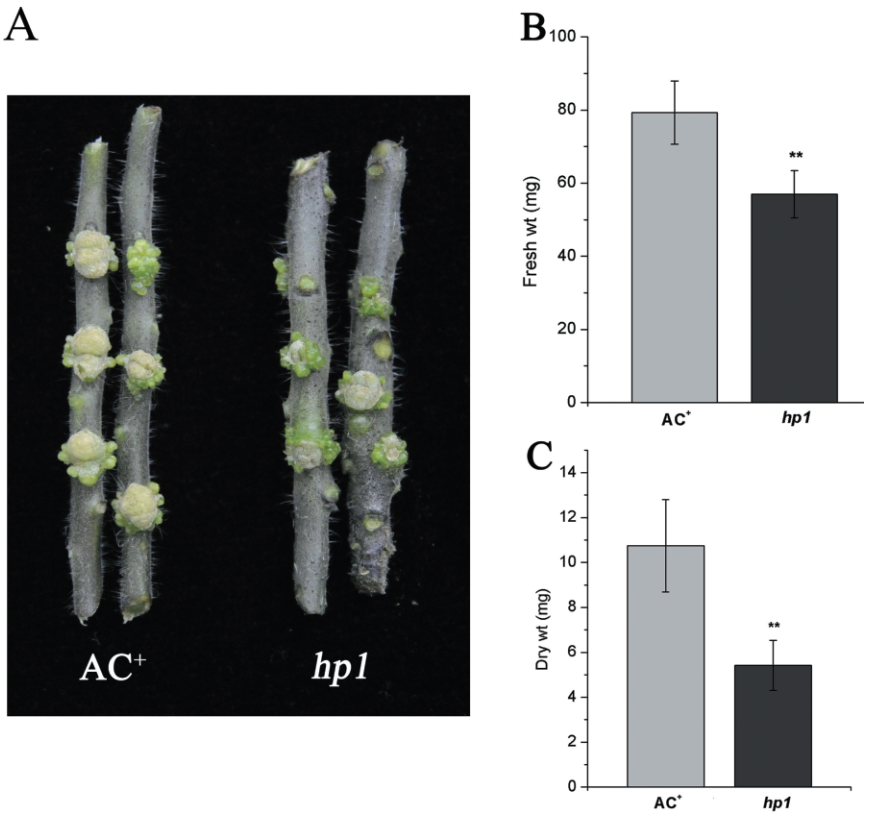

Fig. 6 *In planta* tumorigenesis assays in wild-type (WT) AC$^+$ and *hp1* mutant plants. (A) Tumours formed on stems of WT AC$^+$ and *hp1* mutant plants inoculated with the tumorigenic strain *Agrobacterium tumefaciens* A348. The photographs of the stems with tumours were taken 4 weeks after inoculation. (B, C) Quantification of the biomass of the tumours. The fresh and dry weights of the tumours developed on the stems of WT AC$^+$ and *hp1* mutant plants were measured 4 weeks after inoculation. The experiments were repeated twice with at least six samples. Error bars indicate standard deviation. The statistical significance of the difference was confirmed by Student's *t*-test (**$P < 0.01$).

recognize PAMPs from *Agrobacterium* and mount basal level defence—PTI (Zipfel *et al*., 2006). However, virulent *Agrobacterium* has evolved to overcome basal defence by hijacking the PTI signalling pathway to transfer T-DNA into host chromatin and, consequently, to cause tumour formation (Djamei *et al*., 2007; Pitzschke *et al*., 2009; Zaltsman *et al*., 2010). Disarmed *Agrobacterium* strains without tumour-triggering gene expression no longer cause tumorigenesis, but retain the ability to transfer T-DNA into the plant chromosome. Thus, these nontumorigenic strains have been widely used as vectors to transfer genes of interest into the plant chromosome and, subsequently, to express the encoding proteins, either via stable transformation of the transgenic plants or transient transformation in the plant leaves (Gelvin, 2003). However, nontumorigenic *Agrobacterium* normally does not cause typical disease symptoms or proliferate to a great extent in leaves (Zipfel *et al*., 2006). In this study, we found that a tomato DDB1-deficient mutant (*high pigment-1*, *hp1*), which was originally identified as a spontaneous mutation with enhanced pigmentation, was hypersusceptible to nontumorigenic *Agrobacterium* infection. The majority of excised *hp1* cotyledons infected with *A. tumefaciens* GV2260 died without developing callus (Fig. 1), suggesting that *hp1* compromises the resistance to *Agrobacterium*. When inoculated with *A. tumefaciens* GV2260 by infiltration into leaves, the *hp1* mutant moderately supported *Agrobacterium* growth and developed disease symptoms (Fig. 2). A consistently similar result was obtained when WT AC$^+$ and *hp1* mutant plant leaves were inoculated with another nontumorigenic *A. tumefaciens* strain EHA105 (Fig. 2C). Unfortunately, we were unable to test whether complementation of the *hp1* mutant could restore the resistance phenotype. It was difficult to obtain restored transgenic plants overexpressing *DDB1* driven by the 35S promoter in the *hp1* mutant background (Y. Liu and J. Giovannoni, unpublished data), probably because the function of *HP1/DDB1* is strictly controlled by the rate-limiting expression level. We are currently conducting complementation experiments using tomato *DDB1* as native promoter. Once the transgenic lines become available, it would be interesting to determine whether overexpression of this gene contributes to the increased level of resistance to *Agrobacterium*.

It is notable that there is a discrepancy between our results and data from a previous publication, in which it was shown that *A. tumefaciens* did not multiply after infiltration (Pruss *et al*., 2008). These contradictory observations could be caused by the different inoculation titres used in the experiments. A high inoculation titre, which was used by Pruss and colleagues, may saturate *Agrobacterium* growth and affect bacterial propagation because of a lack of nutrients in the harsh conditions of plant tissue. Interestingly, the *Arabidopsis efr2* mutant is susceptible to *Agrobacterium*, as manifested by the increased transformation efficiency and chlorosis symptoms after infiltration with *A. tumefaciens* GV3101 strain,

but does not support *Agrobacterium* growth in leaves (Zipfel *et al*., 2006). EFR recognizes the PAMP EF-Tu from *Agrobacterium* and mounts a basal defence against *Agrobacterium* infection (Zipfel *et al*., 2006). Thus, from our observations, it seems that the *hp1* mutation has a greater severity of effect than *efr2* on PTI-mediated resistance to *Agrobacterium*. As EFR is the PRR receptor to one particular PAMP EF-fu from *Agrobacterium*, functional abrogation of one particular PTI pathway may only partially affect the resistance because of the redundancy of PTI pathways. Given the fact that plants belonging to the Solanum family, including tomato, do not contain a functional *EFR* gene (Zipfel *et al*., 2006), our results imply that HP1/DDB1 might function as an important signalling component essential for many PTI pathways other than the EF-Tu/EFR pathway. Supporting the effect of *hp1* on the PTI signalling triggered by *Agrobacterium*, several PTI marker genes exhibited less response to *A. tumefaciens*. Among the four PTI marker genes examined, *SlPti5* and *SlLrr22* were not induced, and *SlGras2* and *SlWaky28* showed reduced induction in *hp1* mutant leaves after infiltration with *A. tumefaciens* GV2260 strain (Fig. 4B, E). Based on the fact that *SlPti5*, at least, is induced by multiple PAMPs, including flg22 and chitin (Nguyen *et al*., 2010), it is likely that *hp1* mutation affects several PTI pathways, probably by compromising a common factor essential for PTI signalling.

The *PR-1* gene is a well-known marker gene for defence reactions. In this study, we showed a correlation between the abolishment of *SlPR1a1* expression and loss of resistance to *Agrobacterium* in the *hp1* mutant (Fig. 5). Significantly, it has been demonstrated that the *Escherichia coli* DH5α strain can also induce *PR-1* expression in tobacco, even though it is nonpathogenic on plants, suggesting that *PR-1* induction by *Agrobacterium* is triggered by the perception of PAMPs (Pruss *et al*., 2008). A working model for PAMP-triggered PR-1 activation can be proposed on the basis of recent publications (Chinchilla *et al*., 2007; Djamei *et al*., 2007; Lu *et al*., 2010; Pitzschke *et al*., 2009; Zhang *et al*., 2010; Zipfel *et al*., 2006): PAMPs from *Agrobacterium* are first perceived by PRRs; PRRs coordinate with other factors, such as BAK1 and BIK1, to activate the MAPK cascade (Chinchilla *et al*., 2007; Lu *et al*., 2010; Zhang *et al*., 2010), triggering MAPK-dependent activation of the VIP1 transcription factor, which, in turn, shuttles into the nucleus, where it indirectly activates *PR-1* expression (Djamei *et al*., 2007; Pitzschke *et al*., 2009). Thus, any step involved in *PR-1* induction could be a potential target of *HP1/DDB1* for interference with defence signalling.

It is thought that the *PR-1* gene is mainly regulated through SA, an important component of the complex plant defence signalling network against pathogens (Durrant and Dong, 2004). Increasing evidence has indicated that SA is also involved in PAMP-triggered basal defence signalling (Chen *et al*., 2009; Halim *et al*., 2009; Liu *et al*., 2010). However, at least in tomato, it remains unknown how the PAMP-activated MAPK–VIP pathway and SA pathway coordinate to activate defence responses, such as the induction of the *PR-1* gene. It is unclear whether SA functions in a MAPK-dependent manner or SA functions parallel to the MAPK–VIP pathway after recognition of PAMPs by PRRs. In this study, we investigated the functional relationship between SA and the *hp1* mutation in terms of *SlPR1a1* expression and resistance to *Agrobacterium* infection. We found that the exogenous application of SA on tomato leaves can induce *SlPR1a1* expression and confer enhanced resistance to nontumorigenic *A. tumefaciens* GV2260. However, the *hp1* mutant was insensitive to SA treatment, as revealed by the abolishment of *SlPR1a1* induction and attenuation of the resistance to *A. tumefaciens* GV2260 (Fig. 5). Thus, our results indicate that HP1/DDB1 functions downstream of SA in defence signalling leading to *SlPR1a1* induction. They also suggest that the deficiency of SA-regulated defence signalling in the *hp1* mutant is caused by interference in SA signal transduction, rather than by a defect in SA biosynthesis. However, we cannot rule out the possibility that the SA-independent signalling pathway also contributes to HP1/DDB1-dependent resistance, as we were unable to test the critical requirement of SA for *SlPR1a1* induction or resistance to *Agrobacterium* because of a lack of the *NahG* transgenic line of WT AC$^+$ which inhibits SA accumulation.

Consistent with the results of the *Agrobacterium* growth and disease symptom assay, the efficiency of *Agrobacterium*-mediated transient expression in *hp1* leaves was higher than that in WT AC$^+$ leaves (Fig. 3), again indicating that the *hp1* mutant is susceptible to *Agrobacterium* infection. However, the *hp1* mutant did not show enhanced tumorigenesis by tumorigenic *A. tumefaciens* A348 strain with regard to the size and biomass of the tumours formed in stems (Fig. 6). This is probably because of the role of HP1/DDB1 in the regulation of cell growth. In the *hp1* mutant plant, cell growth is reduced significantly compared with that in the WT AC$^+$ plant, probably as a result of the decreased cell division rate in the *hp1* mutant (Caspi *et al*., 2008). Thus, although *hp1* facilitates *Agrobacterium*-mediated transformation, the cell growth rate of the tumour may be restricted and, consequently, result in smaller sized tumours.

HP1/DDB1 is an important component of the CUL4-mediated ubiquitin E3 ligase complex, which has multiple functions in plastid division (Liu *et al*., 2004; Wang *et al*., 2008), photomorphogenesis (Chen *et al*., 2010) and stress responses (Lee *et al*., 2010). Moreover, recent studies have demonstrated that HP1/DDB1 is also involved in the regulation of gene expression by epigenetic modifications (Higa *et al*., 2006; Pazhouhandeh *et al*., 2011; Zhao *et al*., 2010). As described above, PR-1-associated resistance is activated through the MAP3–VIP1 pathway and is also regulated through SA signalling. HP1/DDB1 probably contributes to the defence response via interaction with MAP3–VIP1 signalling, SA signalling, or both. We speculate that HP1/DDB1 might regulate the factor(s) downstream of SA in defence signalling, leading to *SlPR1a1* expression, either through ubiquitination of the protein for degradation or via histone and/or DNA methylation of the

corresponding gene for expression repression. Further experiments, such as the identification of the CUL4–HP1/DDB1 E3 substrate(s), are needed to verify this hypothesis.

EXPERIMENTAL PROCEDURES

Plant material and tomato cotyledon transformation

Tomato plants [wild-type AC$^+$ (Ailsa Craig) and *hp1* mutant] were grown in the glasshouse under standard conditions (26 °C day, 18 °C night; 16 h light, 8 h dark). The light intensity was monitored by a luxmeter (TES-1334A). *Agrobacterium tumefaciens* GV2260, carrying the binary vector pBI121, was grown at 28 °C in Luria–Bertani (LB) medium containing rifampicin (50 μg/mL) and kanamycin (50 μg/mL). Tomato cotyledon transformation was carried out according to the method described by Fillatti *et al*. (1987). The efficiency of callus induction was expressed as the quotient of the number of cotyledons having callus divided by the total number of cotyledons used for transformation in each plate.

Leaf infection with *Agrobacterium tumefaciens*

Agrobacterium tumefaciens GV2260 or EHA105 strain was propagated at 28 °C in LB medium with 50 μg/mL rifampicin. For leaf inoculation, overnight cultures were subcultured into fresh medium (LB, acetosyringone at 20 μM and appropriate antibiotics), and grown at 28 °C to an optical density at 600 nm (OD_{600}) of 1–1.8. Cells were harvested by centrifugation, resuspended in buffer (10 mM $MgCl_2$, 10 mM 2-(*N*-morpholino)ethanesulphonic acid (MES), pH 5.6, and 0.006% Silwet L-77) to $OD_{600} = 0.00001$ (approximately 1×10^4 cfu/mL). Three six-week-old tomato plants were vacuum infiltrated, and well-expanded leaves were collected for analysis at 0, 2 and 4 dpi. To assess bacterial populations, eight 0.2826-cm^2 leaf discs were punched and ground in 1 mL of 10 mM $MgCl_2$, diluted and plated on LB medium containing appropriate antibiotics. The number of bacteria (cfu/cm^2) was counted 2 days after the plates had been kept at 28 °C. In the case of expression assessments of *HP1*, *SlPR1a1*, *SlPti5*, *SlGras2*, *SlWrky28* and *SlLrr22*, the inoculum of *A. tumefaciens* GV2260 at $OD_{600} = 0.1$ (approximately 1×10^8 cfu/mL) was used.

Agrobacterium tumefaciens-mediated transient transformation of tomato leaves

The media and culture conditions for induction and infection are detailed in Anand *et al*. (2007). Briefly, overnight cultures of *A. tumefaciens* GV2260 carrying the binary vector pBISN1 were washed with distilled water, and induced on agro-induction medium supplemented with acetosyringone (150 μg/mL) at room temperature (24 °C) for 14–16 h. The induced cultures were washed with sterile distilled water and resuspended in buffer (10 mM $MgCl_2$, 10 mM MES, pH 5.6, and 0.006% Silwet L-77) to $OD_{600} = 0.1$ (approximately 1×10^8 cfu/mL). Six-week-old plants were vacuum infiltrated, and well-expanded leaves were washed with water and immediately stained with X-Gluc staining solution [0.1 M sodium phosphate buffer, pH 7.0, 0.1% Triton X-100, 1 mM potassium ferricyanide, 1 mM potassium ferrocyanide, 10 mM ethylenediaminetetraacetic acid (EDTA), 200 mL/L methanol and 2 mM X-Gluc] for 1 day at 37 °C in the dark. Stained leaves were placed in 95% ethanol and incubated at 37 °C until the leaves were cleared of chlorophyll, with the ethanol being replaced as necessary until the clearing was complete.

GUS activity was analysed using fluorometric assays (Jefferson *et al*., 1987). Protein extracts were prepared by grinding five to eight leaf discs from the *Agrobacterium*-infected WT AC$^+$ or *hp1* mutant plants in a microcentrifuge tube containing GUS extraction buffer [50 mM sodium phosphate buffer, 1% sodium dodecylsulphate (SDS), 10 mL/L, 10 mM EDTA, 200 mL/L methanol, 0.1% Triton X-100, 0.1% β-mercaptoethanol], and two aliquots were assayed for each of the extracts to determine the protein concentration and GUS activity, following the method described previously (Jefferson *et al*., 1987). The protein concentration of plant extracts was determined spectrophotometrically using the Gene Quant pro (Amersham Biosciences, Piscataway, NJ, USA) based on the Bradford method (Bradford, 1976). The fluorescence of 4-methylumbelliferone was measured with a Fluoroskan ascent FL2-6 (Thermo Electron Corporation, Waltham, MA, USA).

Quantitative RT-PCR assay for PTI marker gene expression

Six-week-old tomato plants were vacuum infiltrated with *A. tumefaciens* GV2260 in buffer (10 mM $MgCl_2$, 10 mM MES, pH 5.6, and 0.006% Silwet L-77) at an inoculum of $OD_{600} = 0.1$, or with buffer only as the mock inoculation. Leaf tissue was harvested at different time points after inoculation for RNA isolation. The 0-hpi sample was harvested immediately prior to vacuum infiltration. Total RNAs were extracted using Trizol reagent according to the protocol provided by the manufacturer (Invitrogen, Carlsbad, CA, USA; http://www.Invitrogen.com/), and treated with DNaseI (TaKaRa, Dalian, Liaoning, China; http://www.takara-bio.com).

Primers for real-time RT-PCR were designed for *SlPR1a1* (SGN-U577839; SlPR1a1-F, 5′-TGCTGGTGCTGTGAAGATGTG-3′; SlPR1a1-R, 5′-CAGACTTTACCTGGAGCACACG-3′), *SlPti5* (SGN-U571539; SlPti5-F, 5′-ATTCGCGATTCGGCTAGACATGGT-3′; SlPti5-R, 5′-AGTAGTGCCTTAGCACCTCGCATT-3′), *SlLrr22* (SGN-U585837; SlLrr22-F, 5′-AAGATTGGAGGTTGCCATTGGAGC-3′; SlLrr22-R, 5′-ATCGCGATGAATGATCGGTGGAGT-3′), *SlGras2* (SGN-U567396; SlGras2-F, 5′-TAATCCAAGGGATGAGCTTCT-3′; SlGras2-R, 5′-CCACCAACGTGACCACCTT-3′), *SlWrky28* (SGN-U586086; SlWrky28-F, 5′-ACAGATGCAGCTACCTCATCCTCA-3′; SlWrky28-R, 5′-GTGCTCAAAGCCTCATGGTTCTTG-3′), *SlUBI3* (GenBank accession no. X58253; SlUBI3-F, 5′-AGGTTGATGACACTGGAAAGGTT-3′; SlUBI3-R, 5′-AATCGCCTCCAGCCTTGTTGTA-3′). Real-time PCR was performed using an SsoFast EvaGreen Supermix (Bio-Rad catalogue no. 172-5203, Hercules, CA, USA). Each sample was amplified in triplicate and all PCRs were performed on an Applied Biosystems StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA; http://www.appliedbiosystems.com.cn/). Dissociation curve analysis was performed at the end of each run to ensure that unique products were amplified. The tomato *SlUBI3* gene was used as a reference. The RT-PCR conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. The expression level was normalized to the *SlUBI3* control, and relative expression values were determined against the buffer-treated sample or WT AC$^+$ sample using the $2^{-\Delta\Delta Ct}$ method. To confirm the specificity of the PCR, PCR products were verified on a 1% agarose gel for the accurate amplification product size. A pairwise Student's *t*-test was performed to obtain the *P* values indicated in the figures.

SA treatment

SA treatment assay was performed according to Wu and Yang (2010). Briefly, plants were sprayed with a solution of SA containing 0.006% Silwet L-77. SA solution at a concentration of 0.5 mM was used to determine *SlPR1a1* expression, and a 1-mM solution was used to induce resistance to *A. tumefaciens* GV2260. Control plants were sprayed with water containing the same concentration of Silwet L-77. Plants were covered with clear transparent plastic foil for 4 h to retain moisture.

In planta tumour assay

Tumorigenic *A. tumefaciens* A348 strain containing the octopine type Ti plasmid (pTiA6) was cultured as described above in transient transformation methods. Stems of WT Ailsa Craig or *hp1* mutant plants were inoculated by slight injury to the stem, using a needle dipped in *A. tumefaciens* A348 suspension culture ($OD_{600} = 0.1$). Tumours on shoots were scored after 4 weeks.

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REFERENCES

Anand, A., Vaghchhipawala, Z., Ryu, C.M., Kang, L., Wang, K., del-Pozo, O., Martin, G.B. and Mysore, K.S. (2007) Identification and characterization of plant genes involved in *Agrobacterium*-mediated plant transformation by virus-induced gene silencing. *Mol. Plant–Microbe Interact.* **20**, 41–52.

Anand, A., Uppalapati, S.R., Ryu, C.M., Allen, S.N., Kang, L., Tang, Y. and Mysore, K.S. (2008) Salicylic acid and systemic acquired resistance play a role in attenuating crown gall disease caused by *Agrobacterium tumefaciens*. *Plant Physiol.* **146**, 703–715.

Angers, S., Li, T., Yi, X., MacCoss, M.J., Moon, R.T. and Zheng, N. (2006) Molecular architecture and assembly of the DDB1–CUL4A ubiquitin ligase machinery. *Nature*, **443**, 590–593.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M. and Sheen, J. (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature*, **415**, 977–983.

Ausubel, F.M. (2005) Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* **6**, 973–979.

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.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72**, 248–254.

Braun, S., Garcia, J.F., Rowley, M., Rougemaille, M., Shankar, S. and Madhani, H.D. (2011) The Cul4-Ddb1^{Cdt2} ubiquitin ligase inhibits invasion of a boundary-associated antisilencing factor into heterochromatin. *Cell*, **144**, 41–54.

Caspi, N., Levin, I., Chamovitz, D.A. and Reuveni, M. (2008) A mutation in the tomato *DDB1* gene affects cell and chloroplast compartment size and CDT1 transcript. *Plant Signal. Behav.* **3**, 641–649.

Centore, R.C., Havens, C.G., Manning, A.L., Li, J.M., Flynn, R.L., Tse, A., Jin, J., Dyson, N.J., Walter, J.C. and Zou, L. (2010) CRL4(Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. *Mol. Cell*, **40**, 22–33.

Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., Cai, R., Zuo, J., Tang, X., Li, X., Guo, H. and Zhou, J.M. (2009) ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 repress *SALICYLIC ACID INDUCTION DEFICIENT2* expression to negatively regulate plant innate immunity in *Arabidopsis*. *Plant Cell*, **21**, 2527–2540.

Chen, H., Huang, X., Gusmaroli, G., Terzaghi, W., Lau, O.S., Yanagawa, Y., Zhang, Y., Li, J., Lee, J.H., Zhu, D. and Deng, X.W. (2010) *Arabidopsis* CULLIN4-damaged DNA binding protein 1 interacts with CONSTITUTIVELY PHOTOMORPHOGENIC1-SUPPRESSOR OF PHYA complexes to regulate photomorphogenesis and flowering time. *Plant Cell*, **22**, 108–123.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G. and Boller, T. (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, **448**, 497–500.

Dangl, J.L. and Jones, J.D.G. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, **411**, 826–833.

Djamei, A., Pitzschke, A., Nakagami, H., Rajh, I. and Hirt, H. (2007) Trojan horse strategy in *Agrobacterium* transformation: abusing MAPK defense signaling. *Science*, **318**, 453–456.

Durrant, W.E. and Dong, X. (2004) Systemic acquired resistance. *Annu. Rev. Phytopathol.* **42**, 185–209.

Fillatti, J.J., Kiser, J., Rose, R. and Comai, L. (1987) Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. *Nat. Biotechnol.* **5**, 726–730.

Gelvin, S.B. (2003) *Agrobacterium*-mediated plant transformation: the biology behind the 'gene-jockeying' tool. *Microbiol. Mol. Biol. Rev.* **67**, 16–37.

Halim, V.A., Altmann, S., Ellinger, D., Eschen-Lippold, L., Miersch, O., Scheel, D. and Rosahl, S. (2009) PAMP-induced defense responses in potato require both salicylic acid and jasmonic acid. *Plant J.* **57**, 230–242.

Higa, L.A., Wu, M., Ye, T., Kobayashi, R., Sun, H. and Zhang, H. (2006) CUL4–DDB1 ubiquitin ligase interacts with multiple WD40-repeat proteins and regulates histone methylation. *Nat. Cell Biol.* **8**, 1277–1283.

Jefferson, R.A. (1987) Assaying chimeric genes in plants: the GUS gene fusion system. *Plant Mol. Biol. Rep.* **5**, 387–405.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.

Jin, J., Arias, E.E., Chen, J., Harper, J.W. and Walter, J.C. (2006) A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. *Mol. Cell*, **22**, 709–721.

Kendrick, R.E., Kerckhoffs, L.H.J., Van Tuinen, A. and Koornneef, M. (1997) Photomorphogenic mutants of tomato. *Plant Cell Environ.* **20**, 746–751.

Lee, C.W., Efetova, M., Engelmann, J.C., Kramell, R., Wasternack, C., Ludwig-Muller, J., Hedrich, R. and Deeken, R. (2009) *Agrobacterium tumefaciens* promotes tumor induction by modulating pathogen defense in *Arabidopsis thaliana*. *Plant Cell*, **21**, 2948–2962.

Lee, J.H., Yoon, H.J., Terzaghi, W., Martinez, C., Dai, M., Li, J., Byun, M.O. and Deng, X.W. (2010) DWA1 and DWA2, two *Arabidopsis* DWD protein components of CUL4-based E3 ligases, act together as negative regulators in ABA signal transduction. *Plant Cell*, **22**, 1716–1732.

Li, T., Chen, X., Garbutt, K.C., Zhou, P. and Zheng, N. (2006) Structure of DDB1 in complex with a paramyxovirus V protein: viral hijack of a propeller cluster in ubiquitin ligase. *Cell*, **124**, 105–117.

Lieberman, M., Segev, O., Gilboa, N., Lalazar, A. and Levin, I. (2004) The tomato homolog of the gene encoding UV DAMAGED DNA BINDING protein 1 (DDB1) underlined as the gene that causes the *high pigment-1* mutant phenotype. *Theor. Appl. Genet.* **108**, 1574–1581.

Liu, P.P., Yang, Y., Pichersky, E. and Klessig, D.F. (2010) Altering expression of *benzoic acid/salicylic acid carboxyl methyltransferase 1* compromises systemic acquired resistance and PAMP-triggered immunity in *Arabidopsis*. *Mol. Plant–Microbe Interact.* **23**, 82–90.

Liu, Y., Roof, S., Ye, Z., Barry, C., van Tuinen, A., Vrebalov, J., Bowler, C. and Giovannoni, J. (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proc. Natl. Acad. Sci. USA*, **101**, 9897–9902.

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L. and He, P. (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc. Natl. Acad. Sci. USA*, **107**, 496–501.

Mustilli, A.C., Fenzi, F., Ciliento, R., Alfano, F. and Bowler, C. (1999) Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. *Plant Cell*, **11**, 145–157.

Nam, J., Mysore, K.S., Zheng, C., Knue, M.K., Matthysse, A.G. and Gelvin, S.B. (1999) Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. *Mol. Gen. Genet.* **261**, 429–438.

Nguyen, H.P., Chakravarthy, S., Velasquez, A.C., McLane, H.L., Zeng, L., Nakayashiki, H., Park, D.H., Collmer, A. and Martin, G. (2010) Methods to study PAMP-triggered immunity using tomato and *Nicotiana benthamiana*. *Mol. Plant–Microbe Interact.* **23**, 991–999.

Pazhouhandeh, M., Molinier, J., Berr, A. and Genschik, P. (2011) MSI4/FVE interacts with CUL4–DDB1 and a PRC2-like complex to control epigenetic regulation of flowering time in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **108**, 3430–3435.

Petroski, M.D. and Deshaies, R.J. (2005) Function and regulation of cullin-RING ubiquitin ligases. *Nat. Rev. Mol. Cell Biol.* **6**, 9–20.

Pitzschke, A., Djamei, A., Teige, M. and Hirt, H. (2009) VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. *Proc. Natl. Acad. Sci. USA*, **106**, 18 414–18 419.

Pruss, G.J., Nester, E.W. and Vance, V. (2008) Infiltration with *Agrobacterium tumefaciens* induces host defense and development-dependent responses in the infiltrated zone. *Mol. Plant–Microbe Interact.* **21**, 1528–1538.

Wang, S., Liu, J., Feng, Y., Niu, X., Giovannoni, J. and Liu, Y. (2008) Altered plastid levels and potential for improved fruit nutrient content by downregulation of the tomato DDB1-interacting protein CUL4. *Plant J.* **55**, 89–103.

Wroblewski, T., Tomczak, A. and Michelmore, R. (2005) Optimization of *Agrobacterium*-mediated transient assays of gene expression in lettuce, tomato and *Arabidopsis*. *Plant Biotechnol. J.* **3**, 259–273.

Wu, L. and Yang, H.Q. (2010) CRYPTOCHROME 1 is implicated in promoting R protein-mediated plant resistance to *Pseudomonas syringae* in *Arabidopsis*. *Mol. Plant*, **3**, 539–548.

Yen, H.C., Shelton, B.A., Howard, L.R., Lee, S., Vrebalov, J. and Giovannoni, J.J. (1997) The tomato high-pigment (hp) locus maps to chromosome 2 and influences plastome copy number and fruit quality. *Theor. Appl. Genet.* **95**, 1069–1079.

Zaltsman, A., Krichevsky, A., Loyter, A. and Citovsky, V. (2010) *Agrobacterium* induces expression of a host F-box protein required for tumorigenicity. *Cell Host Microbe*, **7**, 197–209.

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., Mengiste, T., Zhang, Y. and Zhou, J.M. (2010) Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe*, **7**, 290–301.

Zhao, Y., Shen, Y., Yang, S., Wang, J., Hu, Q., Wang, Y. and He, Q. (2010) Ubiquitin ligase components Cullin4 and DDB1 are essential for DNA methylation in *Neurospora crassa*. *J. Biol. Chem.* **285**, 4355–4365.

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.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D., Boller, T. and Felix, G. (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, **125**, 749–760.