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Arabidopsis *wat1* (*walls are thin1*)-mediated resistance to the bacterial vascular pathogen, *Ralstonia solanacearum*, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism

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SUMMARY

Inactivation of Arabidopsis *WAT1* (*Walls Are Thin1*), a gene required for secondary cell-wall deposition, conferred broad-spectrum resistance to vascular pathogens, including the bacteria *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *campestris*, and the fungi *Verticillium dahliae* and *Verticillium albo-atrum*. Introduction of *NahG*, the bacterial salicylic acid (SA)-degrading salicylate hydroxylase gene, into the *wat1* mutant restored full susceptibility to both *R. solanacearum* and *X. campestris* pv. *campestris*. Moreover, SA content was constitutively higher in *wat1* roots, further supporting a role for SA in *wat1*-mediated resistance to vascular pathogens. By combining transcriptomic and metabolomic data, we demonstrated a general repression of indole metabolism in *wat1-1* roots as shown by constitutive down-regulation of several genes encoding proteins of the indole glucosinolate biosynthetic pathway and reduced amounts of tryptophan (Trp), indole-3-acetic acid and neoglucobrassicin, the major form of indole glucosinolate in roots. Furthermore, the susceptibility of the *wat1* mutant to *R. solanacearum* was partially restored when crossed with either the *trp5* mutant, an over-accumulator of Trp, or *Pro35S:AFB1-myc*, in which indole-3-acetic acid signaling is constitutively activated. Our original hypothesis placed cell-wall modifications at the heart of the *wat1* resistance phenotype. However, the results presented here suggest a mechanism involving root-localized metabolic channeling away from indole metabolites to SA as a central feature of *wat1* resistance to *R. solanacearum*.

Keywords: Arabidopsis, *Ralstonia solanacearum*, auxin, salicylic acid, vascular pathogen, indole glucosinolates.

INTRODUCTION

In nature, the constant exposure to pathogenic microorganisms has led plants to acquire sets of pre-formed and inducible defense mechanisms (for review, see Chisholm *et al.*, 2006; Jones and Dangl, 2006; Dodds and Rathjen, 2010). As the first cellular compartment encountered during plant–pathogen interactions, it is logical to assume that the cell wall plays a critical role in determining the success or failure of pathogen colonization. The plant cell wall is a complex composite made up of an intricate network of cellulose, non-cellulosic polysaccharides, proteins and aromatic substances that undergo dynamic changes in composition and inter-polymer assembly during normal growth and development or when exposed to environmental stress (for review, see Cosgrove, 2005; Scheller and Ulvskov, 2010; Carpita, 2011). In the last ten years, genetic approaches have demonstrated that altering the structure and/or composition of the plant cell wall results in changes in disease susceptibility. A noteworthy example is the screening of Arabidopsis mutants for resistance to virulent strains of powdery mildew fungi (Vogel and Somerville, 2000). Two of the *pmr* (powdery mildew-resistant) mutants were affected in cell-wall genes: *pmr4* is affected in a callose synthase, and *pmr6* is affected in a pectate lyase (Vogel *et al.*, 2002, 2004). Similarly, Arabidopsis *rat* mutants were isolated for their resistance to *Agrobacterium tumefaciens* root infection. One is affected in a cell-surface arabinogalactan protein (*rat1/agp17*), and another in a cellulose synthase-like (*rat4/atcslA9*), both of which are required for correct cell-wall assembly (Zhu *et al.*, 2003a,b; Gaspar *et al.*, 2004).

More recently, a link has been established between the secondary wall and the Arabidopsis response to *Ralstonia solanacearum* (Hernandez-Blanco *et al.*, 2007), a soil-borne pathogen that is responsible for bacterial wilt in over 200 plant species (Hayward, 2000). *Ralstonia solanacearum* invades host plants principally via the root apex, zones of secondary root emergence, and wounding sites (Vasse *et al.*, 1995; Vailleau *et al.*, 2007; Turner *et al.*, 2009; Digonnet *et al.*, 2012). A mutation in any one of the three secondary wall-specific cellulose synthase (CESA) genes [*IRX5* (*Irregular Xylem5*)/*CESA4*, *IRX1/CESA8* and *IRX3/CESA7*] led to full resistance towards the bacterium and the necrotrophic fungus *Plectosphaerella cucumerina*, via unique abscisic acid (ABA) signaling mechanisms (Hernandez-Blanco *et al.*, 2007).

This link established between the plant cell wall and the response to *R. solanacearum* prompted us to screen a panel of Arabidopsis cell-wall mutants that were originally selected based on gene expression studies performed in xylogenic *Zinnia elegans* cell cultures (Pesquet *et al.*, 2005). We demonstrate herein that one of them, *wat1* (*walls are thin1*), exhibits enhanced resistance to vascular plant pathogens including *R. solanacearum.* Even though the most conspicuous developmental phenotypes of *wat1* are related to cell-wall modifications (a decrease in cell elongation, changes in mechanical properties, and a severe reduction in stem fiber secondary wall thickness; Ranocha *et al.*, 2010), we provide evidence suggesting that cell wall-related mechanisms may not be the direct cause of *wat1*-mediated pathogen resistance, but instead that it is due to a mechanism involving redirection of tryptophan-derived metabolites to salicylic acid in *wat1.* Finally, this paper highlights the root as a central feature in resistance to *R. solanacearum.*

RESULTS

The Arabidopsis wat1 mutant exhibits increased resistance to *Ralstonia solanacearum*

A panel of putative secondary cell wall mutants was screened for altered sensitivity to *R. solanacearum*. Inoculations were performed by dipping roots into a bacterial culture, and disease symptoms were scored in leaves of each individual plant according to the following disease index rating: 0 = no symptoms, 1 = 25% wilted leaves, 2 = 50% wilted leaves, 3 = 75% wilted leaves, 4 = 100% wilted leaves (or dead plant). Two independent *wat1* allelic mutants, *wat1-1* and *wat1-3*, exhibited a delay in symptom development (leaf wilting) compared to Col-0 plants (Figure 1a). The altered response of *wat1* to *R. solanacearum* was clearly due to loss of *WAT1* function, as complementation of *wat1-1* with *ProWAT1:WAT1* led to fully restored susceptibility to the bacterium (Figure 1a). To determine whether the delay in visible symptoms in *wat1* was correlated with changes in bacterial multiplication *in planta*, *R. solanacearum* growth was measured in the aerial portions of inoculated plants following root inoculation. No difference in bacterial density was observed between *wat1* and Col-0 plants within the first 2 days of infection (Figure 1b), suggesting that bacterial movement from roots to leaves was not hindered in *wat1*. However, from 4 days post-inoculation (dpi) onward, *R. solanacearum* growth was strongly decreased in both *wat1-1* and *wat1-3* (Figure 1b). Together, these results allow us to conclude that *wat1* exhibits enhanced resistance to *R. solanacearum*.

In order to determine *WAT1* expression during disease development, quantitative RT-PCR experiments were performed in roots and leaves during a compatible interaction between Col-0 and *R. solanacearum* (Figure S1). *WAT1* transcript levels in leaves and roots were relatively stable during the early stages of infection and decreased only at the latter stages of disease development in both organs. The *WAT1* leaf expression data are in agreement with previously published microarray data from susceptible Col-0

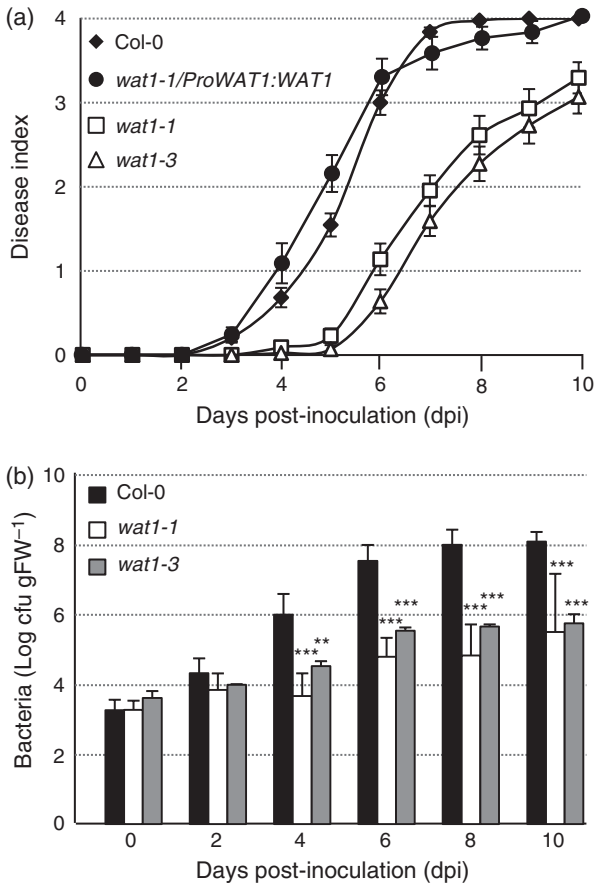


Figure 1. Inactivation of *WAT1* confers increased resistance to the vascular bacterium, *Ralstonia solanacearum*.
(a) Mean disease index (a measure of symptom severity) ± SE for Col-0 plants, *wat1-1*, *wat1-3* and the *wat1-1/ProWAT1:WAT1* complemented mutant after root inoculation with the GMI1000 strain of the bacterium.
(b) *Ralstonia solanacearum* GMI1000 growth [log cfu (colony-forming units)/g fresh weight] in Col-0, *wat1-1* and *wat1-3* plants 10 days post-inoculation. At least nine plants per genotype were tested at each time point. Values are mean bacterial growth ± SE. Four independent experiments were performed with similar results. Student's *t* test was used to determine significant differences between Col-0 and the *wat1-1* or *wat1-3* mutants (**$P < 0.01$; ***$P < 0.001$).

Arabidopsis leaves during *R. solanacearum* infection (Hu *et al.*, 2008), with a similar tendency occurring in roots.

wat1 mutants exhibit enhanced resistance to other vascular pathogens

To test whether the resistance of *wat1* is specific with regard to *R. solanacearum*, we challenged *wat1* with several bacterial and fungal pathogens with various lifestyles (Table 1). *wat1-1* and *wat1-3* were as susceptible as Col-0 plants to non-vascular pathogens [*Pseudomonas syringae* pv. *tomato* (*Pst*), *Botrytis cinerea* and *Colletotrichum higginsianum*] (Table 1). In contrast, *wat1-1* and *wat1-3* were more resistant to the three vascular pathogens tested: *Xanthomonas campestris* pv. *campestris* (*Xcc*), *Verticillium dahliae* and *Verticillium albo-atrum* (Table 1 and Figure S2). For all three, the decrease in visual symptoms in *wat1* was accompanied by a decrease in pathogen content (Figure S2). Similarly, the *wat1* mutants showed enhanced resistance to *Plectosphaerella cucumerina* (Figure S2), a necrotrophic fungus that colonizes Arabidopsis vascular tissues upon leaf infection (Llorente *et al.*, 2005). Together, these results indicate that *wat1* shows a specific, broad-spectrum resistance to vascular pathogens, but its susceptibility to non-vascular pathogens is similar to that of wild-type plants.

The fact that *wat1* was more resistant to all vascular pathogens tested suggests that resistance mechanisms of *wat1* plants may be associated with the vascular system. To further investigate this hypothesis, we compared two methods of vascular pathogen inoculation: piercing in the central leaf vein and mesophyll infiltration. Both of these methods of inoculation are routinely used for *Xcc* infection (Meyer *et al.*, 2005; Xu *et al.*, 2008), and leaf piercing inoculation has been used previously for *R. solanacearum*

Table 1 Response of *wat1* mutants to various pathogens

Pathogens (strain/lifestyle)	Vascular?	Arabidopsis response[a] Col-0	*wat1-1*	*wat1-3*
Bacteria				
Ralstonia solanacearum (GMI1000; hemi-biotrophic)	Yes	S	R	R
Xanthomonas campestris pv. *campestris* (8004*DXopAC*; hemi-biotrophic)	Yes	S	R	R
Pseudomonas syringae pv. *tomato* (DC3000; hemi-biotrophic)	No	S	S	S
Fungi				
Verticillium dahliae (JR2; hemi-biotrophic)	Yes	S	R	R
Verticillium albo-atrum (5431; hemi-biotrophic)	Yes	S	R	R
Plectosphaerella cucumerina (PcBMM; necrotrophic)	?[b]	S	R	R
Botrytis cinerea[c] (B0510; necrotrophic)	No	S	S	S
Colletotrichum higginsianum[c] (IMI349061; hemi-biotrophic)	No	S	S	nd

[a]The plant response to the pathogens was determined in at least two independent experiments for each case. S, fully susceptible: symptoms and fungal biomass/bacterial growth similar in *wat1* versus Col-0. R, enhanced resistance compared to Col-0: reduced symptoms and fungal biomass/bacterial growth in *wat1* versus Col-0. nd, not determined.
[b]*P. cucumerina*, a necrotrophic fungus, is currently not classified as a vascular pathogen, but is able to colonize vascular tissues of Arabidopsis (including the Col-0 ecotype) upon leaf infection (Llorente *et al.*, 2005).
[c]Fungal biomass was not determined.

(Hirsch *et al.*, 2002). When either *R. solanacearum* or *Xcc* was injected into the central leaf vein, i.e. directly into the vascular system, *wat1* resistance was maintained for both pathogens (Figure 2). In contrast, when the two pathogens were infiltrated directly into the mesophyll, *wat1* was as susceptible as Col-0 to both bacteria (Figure 2), reinforcing the hypothesis that mechanisms associated with *wat1* resistance to vascular pathogens are vascular system-localized.

wat1-associated resistance requires SA but not ET and JA

Jasmonic acid (JA), ethylene (ET) and/or salicylic acid (SA) signaling pathways are often involved in plant resistance mechanisms to biotrophic and necrotrophic pathogens (Glazebrook, 2005). ET has been shown to play a role in the response of Arabidopsis to *R. solanacearum* as ET-insensitive mutants (e.g. *ein2*) exhibit enhanced tolerance to the bacterium (Hirsch *et al.*, 2002). To determine whether any of the defense pathways are involved in *wat1*-mediated resistance, we generated *wat1-1 jar1-1* (JA) and *ein2-1 wat1-1* (ET) double mutants, and *NahG wat1-1* (SA) transgenic lines, and tested their response to *R. solanacearum* infection (Figure 3). The *wat1-1 jar1-1* double mutants exhibited similar resistance to *R. solanacearum* as *wat1*, indicating that JA signaling pathways are not essential for *wat1* resistance (Figure 3a,b). In the case of *ein2-1 wat1-1* plants, the double mutant exhibited an enhanced resistance to *R. solanacearum* compared to the single mutants individually, suggesting that *wat1*- and *ein2*-mediated resistance are independent of each other (Figure 3a,b). In contrast, the inactivation of the SA pathway in *NahG wat1-1* lines resulted in fully restored susceptibility to *R. solanacearum*, comparable to the wild-type both in terms of disease symptoms (Figure 3a) and bacterial growth (Figure 3b). The *NahG wat1-1* line was also inoculated with *Xcc* (Figure 3c). These plants also exhibited disease symptoms and bacterial growth similar to Col-0, suggesting a more generalized role for SA in *wat1*-mediated resistance to vascular pathogens.

The importance of SA in the response of the *wat1* mutant to *R. solanacearum* and *Xcc* prompted us to investigate SA content in *wat1* versus wild-type plants. Although no difference in SA content was observed in leaves between the two genotypes, the SA content was higher in *wat1* roots compared to Col-0 (Figure 4a). Finally, when the *cpr5* (*constitutive expression of PR genes 5*) mutant, a hyper-accumulator of SA (Bowling *et al.*, 1997), was challenged with *R. solanacearum*, it showed less severe wilting symptoms and reduced bacterial growth than in Col-0, similar to *wat1* (Figure 4b,c). These data reinforce the hypothesis that SA may be a key element of *wat1*-mediated resistance to *R. solanacearum*, and suggest a more general for SA role in conferring plant resistance to this pathogen.

Transcriptomic data suggest repression of indole metabolism in *wat1* roots

To determine whether other mechanisms in addition to SA are involved in *wat1* resistance to *R. solanacearum*, we performed comparative transcriptomic analyses on roots and leaves of *wat1-1* and Col-0 plants prior to inoculation and at 4 dpi (when the first wilting symptoms were observed in Col-0 but *wat1-1* remained asymptomatic). The complete datasets are shown in Tables S1–S4. Quantitative assessment of the data allowed us to draw several general conclusions (Figure S3). First, at both time points, the total number of de-regulated genes in leaves and roots of *wat1-1* compared to those of wild-type plants was relatively limited: 188 genes (of 22 089 on the microarray) in non-inoculated plants and 358 in bacterial-challenged (4 dpi) plants. Second, transcriptional de-regulation was more pronounced in *wat1-1* roots compared to leaves at both time points. Third, there was a clear tendency towards down-regulation in both *wat1-1* organs.

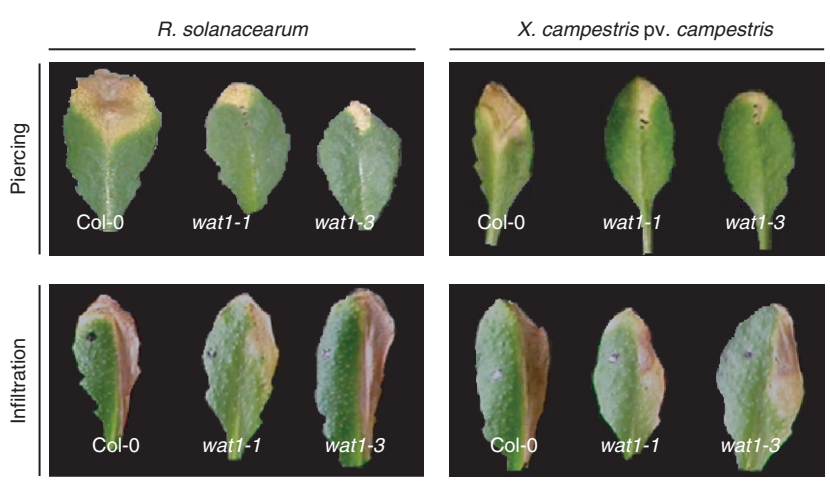
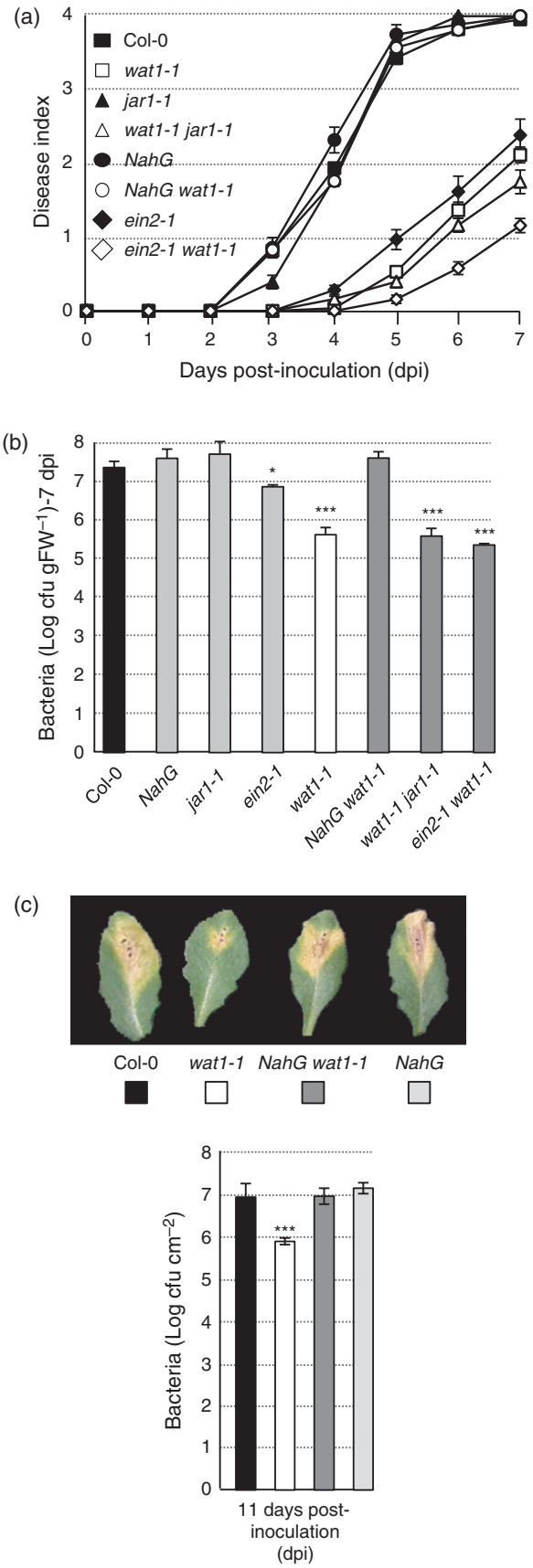


Figure 2. The *wat1* response to vascular pathogens is dependent on the inoculation mode. The response of Col-0, *wat1-1* and *wat1-3* plants towards two vascular bacteria, *R. solanacearum* (left panel) and *Xcc* (right panel), was tested using two infection techniques: piercing of the central vein in leaves (top) and direct infiltration into the leaf mesophyll (bottom). Photos were taken at 5 or 7 dpi for the piercing and mesophyll infiltration experiments, respectively. Similar results were obtained in three independent experiments.



A more in-depth examination of the data in non-inoculated roots and leaves indicated that the most abundant functional categories of differentially regulated genes in both organs are those involved in defense and abiotic and oxidative stress (Tables S1 and S2). Of the 40 de-regulated genes in leaves, 11 were also de-regulated in roots: eight genes were down-regulated and three genes were up-regulated in both *wat1* organs compared to wild-type plants (Tables S1 and S2). Among them, JA-related genes (*AOC1* and *JAR1*) were down-regulated in both organs, but, based on genetic data (Figure 3a,b), this is not likely to be crucial in explaining *wat1* resistance.

Based on genetic data, one may have expected to identify differentially expressed SA marker genes (e.g. *PR-1* and *PR-5*) in *wat1-1* versus Col-0, but this was not the case in either organ, at least for the two time points examined. Finally, as *wat1* was first identified as a cell-wall mutant, it was expected that genes involved in cell-wall synthesis, structure and modification would be de-regulated. However, this was not the case (Tables S1–S4), suggesting that, although cell-wall modifications cannot be completely ruled out as an essential feature in *wat1* resistance, major transcriptional reprogramming of cell-wall metabolism does not appear to be a pre-requisite.

In our view, perhaps the most interesting feature of the microarray datasets is the coordinated down-regulation of eight genes encoding enzymes and regulatory proteins of the indole glucosinolate (IGS) biosynthetic pathway in non-inoculated *wat1* roots (Figure 5a and Table S1). These include upstream enzymes of Trp biosynthesis such as anthranilate synthase (ASA1), the first committed step in Trp synthesis from chorismate, and ATR1 (altered tryptophan regulation 1), a MYB factor (MYB34) that regulates several genes encoding enzymes along the pathway, including ASA1 (Celenza *et al.*, 2005). A sulfotransferase (SOT16/AtST5a) involved in the final step of IGS core

Figure 3. *wat1*-associated resistance requires SA, but is independent of ET and JA.

(a) Mean disease index ±SE following *R. solanacearum* inoculation of Col-0 plants, the *wat1-1* mutant, and double mutants with defects in the SA (*NahG wat1-1*), ET (*ein2-1 wat1-1*) or JA (*wat1-1 jar1-1*) signaling pathways after root inoculation with the GMI1000 strain. *NahG*, *ein2-1* and *jar1-1* mutants were also tested.

(b) *Ralstonia solanacearum* GMI1000 density [log cfu (colony-forming units)/g fresh weight] at 7 dpi in Col-0 (black), the *wat1-1* mutant (white), double mutants (dark gray) and mutants for the ET, JA or SA signaling pathways (light gray). At least nine plants per genotype were tested at each time point, and data represent mean growth ± SE. Student's *t* test was used to determine significant differences between Col-0 and other genotypes (*$P < 0.05$; ***$P < 0.001$).

(c) Disease symptoms for Col-0 (black), *wat1-1* (white), *NahG wat1-1* (dark gray) and *NahG* (light gray) plants inoculated with *Xcc* at 11 dpi (top). *Xcc* density (log cfu cm^{-2}) at 11 dpi in the various genotypes (bottom). At least nine plants per genotype were tested at each time point, and data represent mean growth ± SE. Student's *t* test was used to determine significant differences between Col-0 and other genotypes (***$P < 0.001$).

structure synthesis was also down-regulated in *wat1* roots (Piotrowski *et al.*, 2004).

As transcriptional de-regulation was most apparent in the roots of *wat1*, we sought to identify the root cell type (s) most likely affected by the *WAT1* mutation. To this end, we mined the AREX database (http://www.arexdb.org; Birnbaum *et al.*, 2003; Brady *et al.*, 2007) for cell type-specific expression of each of the eight coordinately down-regulated genes of the IGS pathway in *wat1* roots. Hierarchal clustering according to gene expression suggested that the pericycle, lateral root primordium (LRP) and the quiescent center may be the preferential sites of Trp/IGS biosynthesis in roots (Figure 5b). Interestingly, *WAT1* exhibits an overlapping, but less restrictive, expression pattern. *WAT1* is highly expressed in the pericycle and lateral root primordium (but not the quiescent center), but also in protoxylem and metaxylem vessels (Figure 5b). Taken together, the *wat1* mutation appears to preferentially affect specific cell types (i.e. the pericycle and lateral root primordium) more than others (protoxylem and metaxylem), as suggested by the marked transcriptional repression of Trp/IGS biosynthesis in these cell types.

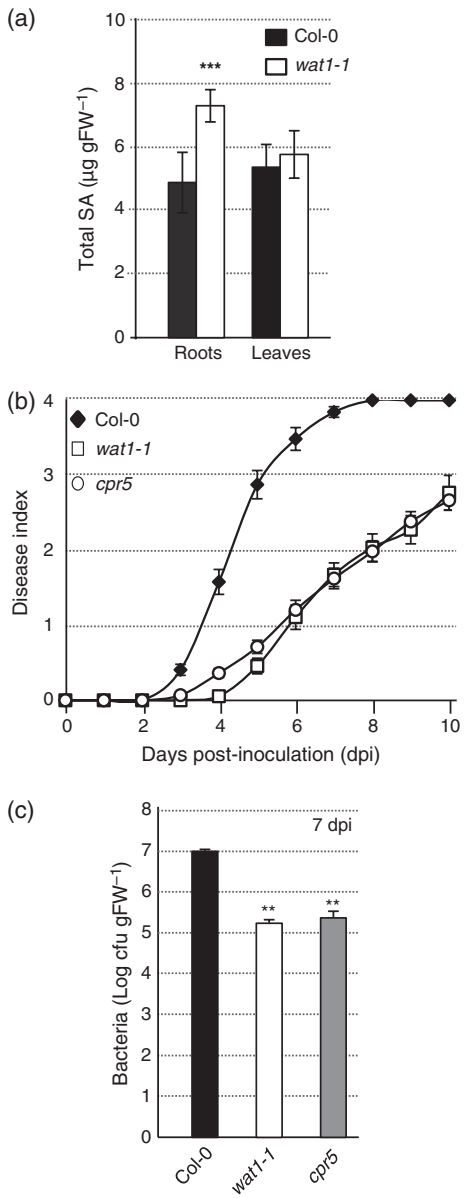


Figure 4. Salicylic acid content is positively correlated with increased resistance to *R. solanacearum*.
(a) SA content in roots and leaves of Col-0 and *wat1-1* plants prior to infection with *R. solanacearum*. Data (µg g^{-1} fresh weight) are means ± SE of three biological replicates. Student's *t* test was used to determine significant differences between the two genotypes (***$P < 0.001$).
(b) Mean disease index ± SE following *R. solanacearum* inoculation of Col-0 plants, the *wat1-1* mutant and the *cpr5* mutant, which over-produces SA.
(c) *Ralstonia solanacearum* GMI1000 density [log cfu (colony-forming units)/g fresh weight] at 7 dpi in Col-0, the *wat1-1* mutant and the *cpr5* mutant. At least nine plants per genotype were tested at each time point, and data represent mean growth ± SE. Student's *t* test was used to determine significant differences between Col-0 and *wat1-1* or *cpr5* mutants (**$P < 0.01$).

Metabolomic and genetic data suggest a key role for indole metabolism in *wat1*-associated resistance

Based on previously reported results for *wat1* (Ranocha *et al.*, 2010) and transcriptomic data presented herein, we investigated the role of indole metabolites in *wat1*-associated resistance to *R. solanacearum*. Levels of Trp, indole-3-acetic acid (IAA) and IGS were measured in roots and leaves of non-inoculated *wat1-1* and Col-0 plants, and at 4 dpi. In roots, Trp was detected at comparable levels in non-infected *wat1* and Col-0 plants (Figure 6a). At 4 dpi, Trp levels remained unchanged in *wat1-1*, but were slightly increased in Col-0 (Figure 6a). In leaves, although no significant differences were observed between the two genotypes at either time point, Trp levels were generally higher in infected versus non-infected leaves in both genotypes (Figure 6a). *wat1-1* was crossed with *trp5*, a mutant in which a single point mutation in *ASA1* inhibits the negative feedback control of Trp on its own biosynthesis, resulting in Trp accumulation (Li and Last, 1996). Although the *trp5* mutant itself did not respond differently to *R. solanacearum* compared to Col-0 plants, *wat1-1 trp5* exhibited partially restored susceptibility to the bacterium, in terms of both disease symptoms and bacterial density (Figure 6b,c).

With regard to for auxin, the level in non-inoculated *wat1* roots was significantly lower than in Col-0 roots (Figure 7a). Lower auxin content has also been reported in *wat1* stems (Ranocha *et al.*, 2010). At 4 dpi, auxin content remained low in *wat1*, but increased in Col-0 (Figure 7a), further accentuating the difference between *wat1* and Col-0. In leaves, no differences in auxin content were observed between genotypes at either time point in the presence or absence of *R. solanacearum* (Figure 7a). To further analyze the role of auxin in *wat1*-mediated resistance, *wat1-1* was crossed with *Pro35S:AFB1-myc*, a line in the *tir1-1* (*transport inhibitor response 1-1*) genetic background in which

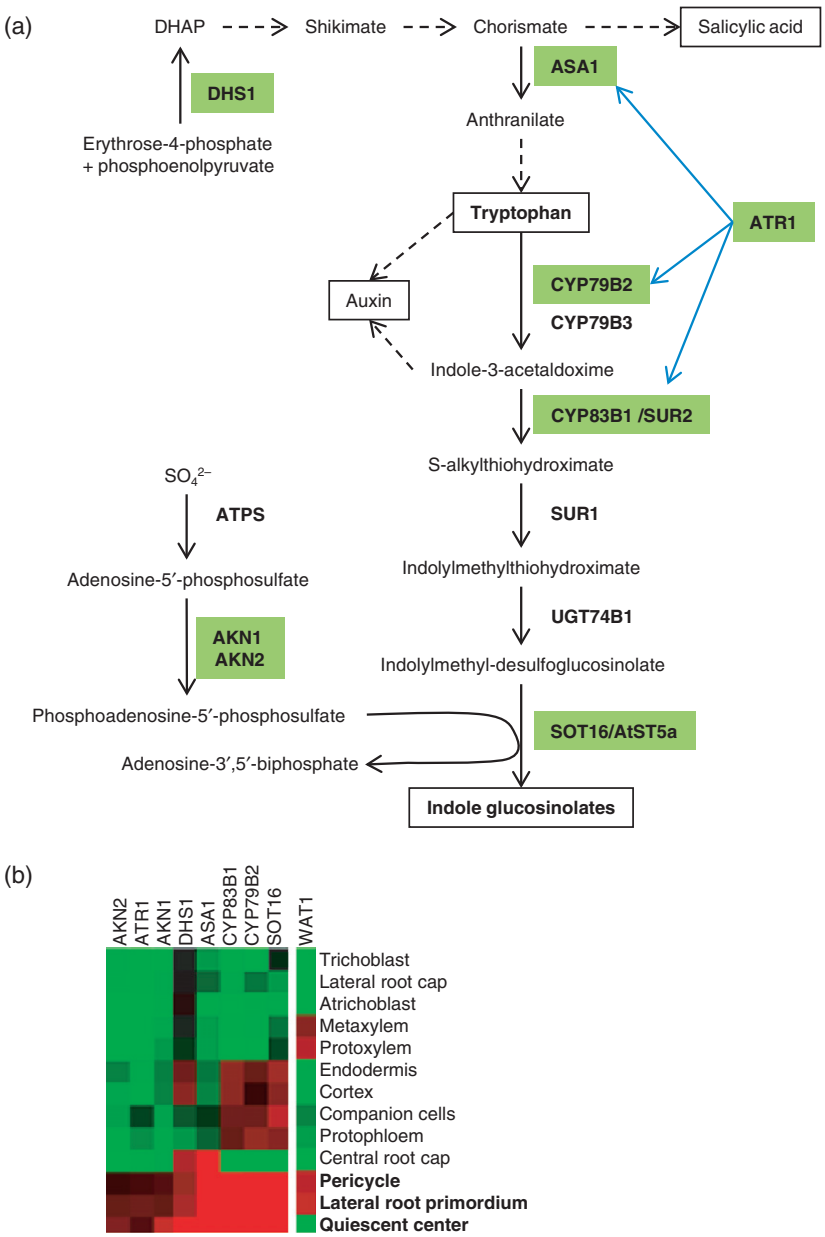


Figure 5. Tryptophan and indole glucosinolate biosynthetic and regulatory genes are repressed in *wat1* roots.
(a) Schematic view of the biosynthetic pathway of tryptophan (Trp) and indole glucosinolates (IGS) in Arabidopsis. Repressed genes in *wat1* roots are shown in green. Dotted arrows indicate related pathways. Blue arrows indicate the positive regulation of *ATR1* (*Altered Tryptophan Regulation1*) on Trp/IGS synthesis. DHS1, 2-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase; ASA1, anthranilate synthase α1; SUR1, Superroot1; SUR2, Superroot2 UGT74B1, UDP-glucosyl transferase 74B1; SOT16/AtST5a, sulfotransferase 16/Arabidopsis sulfotransferase 5a; ATPS, ATP-sulfurylase; AKN1/2, adenosine-5′-phosphosulfate kinase.
(b) Hierarchical clustering of *WAT1* and Trp/IGS biosynthetic genes (*DHS1*, *ASA1*, *ATR1*, *CYP79B2*, *CYP83B1*, *SOT16*, *AKN1* and *AKN2*) that are repressed in *wat1* roots. The color gradient from light green to red is proportional to expression levels (from low to high, respectively).

auxin signaling is constitutively activated (Dharmasiri *et al.*, 2005). Upon challenge with *R. solanacearum*, whereas the *tir1-1* mutant was as susceptible as Col-0, *Pro35S:AFB1-myc* plants were slightly more susceptible (Figure 7b). When the *Pro35S:AFB1-myc* construct was introduced into *wat1-1*, the resulting lines exhibited partially restored susceptibility to *R. solanacearum* (Figure 7b,c). These results show that *wat1* resistance may be abolished, at least in part, by constitutive activation of auxin signaling. *wat1-1 trp5* and *wat1-1 Pro35S:AFB1-myc* lines were also tested with *V. dahliae* (Figure S4). In contrast to the response observed with *R. solanacearum*, these lines did not exhibit partial restoration of susceptibility to this vascular fungus, suggesting that additional mechanisms are involved in the *wat1* response to *V. dahliae*.

With regard to transcriptomic data (Figure 5), we measured three IGSs [glucobrassicin (I3G), neoglucobrassicin (1MI3G) and 4-MeO-glucobrassicin (4MI3G)] (Figure 8). In roots, 1MI3G was the most abundant IGS in Col-0 (Figure 8b), in agreement with previously reported data (Petersen *et al.*, 2002; Brown *et al.*, 2003). Among the three IGS, only 1MI3G content in non-inoculated *wat1-1* roots was significantly lower compared to Col-0 roots (Figure 8a–c). At 4 dpi, all three IGS were less abundant in the *wat1* mutant (Figure 8a–c). In leaves, all three metabolites were present in similar quantities in *wat1* and Col-0 prior to

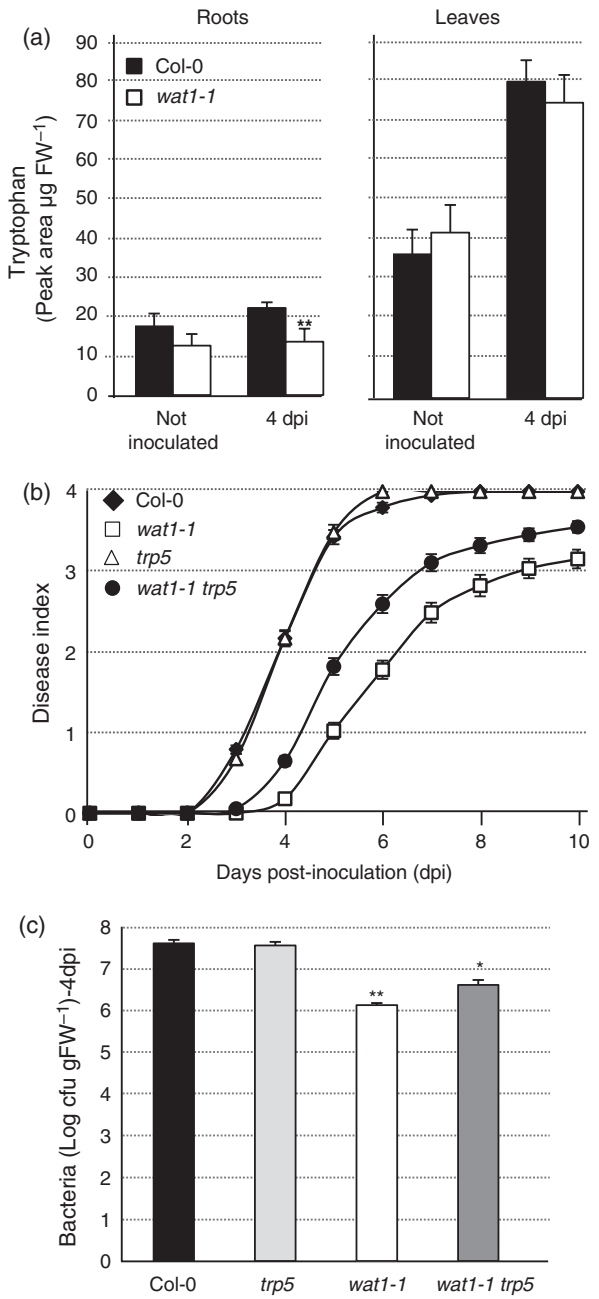


Figure 6. Role of tryptophan in *wat1*-mediated resistance.
(a) Semi-quantitative determination of Trp levels in roots and leaves of Col-0 and *wat1-1* plants prior to infection and at 4 dpi with *R. solanacearum*. Data represent means ± SE of three biological replicates. Student's *t* test was used to determine significant differences between the two genotypes (**$P < 0.01$).
(b) Mean disease index ± SE following *R. solanacearum* inoculation of Col-0 plants, *wat1-1*, *trp5* and the *wat1-1 trp5* double mutant.
(c) *Ralstonia solanacearum* GMI1000 density [log cfu (colony-forming units)/g fresh weight] at 4 dpi in Col-0, *trp5*, *wat1-1* and the *wat1-1 trp5* double mutant. At least nine plants per genotype were tested at each time point, and data represent mean growth ± SE. Two independent experiments were performed with similar results. Student's *t* test was used to determine significant differences between Col-0 and other genotypes (*$P < 0.05$; **$P < 0.01$). Note that data for *wat1-1* and *wat1-1 trp5* lines are significantly different ($P < 0.05$).

infection, whereas, at 4 dpi, I3G was less abundant in *wat1* (Figure 8a–c).

Finally, to determine whether the partial restoration of susceptibility to *R. solanacearum* in *wat1-1 trp5* and *wat1-1 Pro35S:AFB1-myc* lines was correlated with intermediary indole metabolite levels, Trp, IAA and 1MI3G content was measured in the roots of these lines (Figure S5). As expected, the Trp content in *trp5* roots was four times higher than that in Col-0. In *wat1-1 trp5* roots, intermediate levels (between Col-0 and *wat1-1*) for both auxin and 1MI3G were observed, but the Trp content was higher than Col-0. In *Pro35S:AFB1-myc* roots, the amounts of Trp, auxin and 1MI3G were similar to those of Col-0. Crossing *Pro35S:AFB1-myc* with *wat1-1* resulted in intermediate amounts (between Col-0 and *wat1-1*) for both auxin and 1MI3G, whereas the Trp content was comparable to *wat1-1*. These results indicate a negative correlation between indole metabolite content in roots, particularly auxin and 1MI3G, and *wat1*-mediated resistance to *R. solanacearum*.

DISCUSSION

Cell walls are not an obstacle to *R. solanacearum* root-to-shoot movement in wat1

The role of the plant cell wall in plant–pathogen interactions has become increasingly apparent within the last decade (Ellis *et al.*, 2002; Vorwerk *et al.*, 2004; Hernandez-Blanco *et al.*, 2007). Plant cell walls act not only as physical barriers against pathogen invasion but are also capable of sensing and converting pathogen-induced cell-wall modifications into intracellular signals. Here we show that, at early points of infection, pathogen content in the aerial portions of *wat1* was similar to that in Col-0 (Figure 1b), suggesting that *R. solanacearum* root-to-shoot movement is not impaired in *wat1*. The fact that *wat1* resistance is not merely a consequence of structural cell-wall modifications is perhaps not surprising given that the original cell-wall phenotype was uniquely detected in fiber cells and not in xylem vessels (Ranocha *et al.*, 2010), and that fiber cells are not yet present at the developmental stage at which pathogen inoculation was performed.

Transcriptomic data in both leaves and roots of *wat1-1* versus Col-0 did not indicate a major reprogramming of genes involved in cell-wall metabolism. That said, two expansins and four arabinogalactan proteins were down-regulated in non-inoculated and 4 dpi *wat1-1* roots, respectively. It is noteworthy that the auxin-responsive *GH3.8*-mediated repression of expansin gene expression in rice resulted in resistance to *Xanthomonas oryzae* pv. *oryzae* (Ding *et al.*, 2008). Moreover, a role for arabinogalactan proteins in plant–pathogen interactions was also demonstrated in the Arabidopsis mutant *rat1/agp17*, which is resistant to *Agrobacterium tumefaciens* due to lack of binding to plant cells that is essential for the initiation of

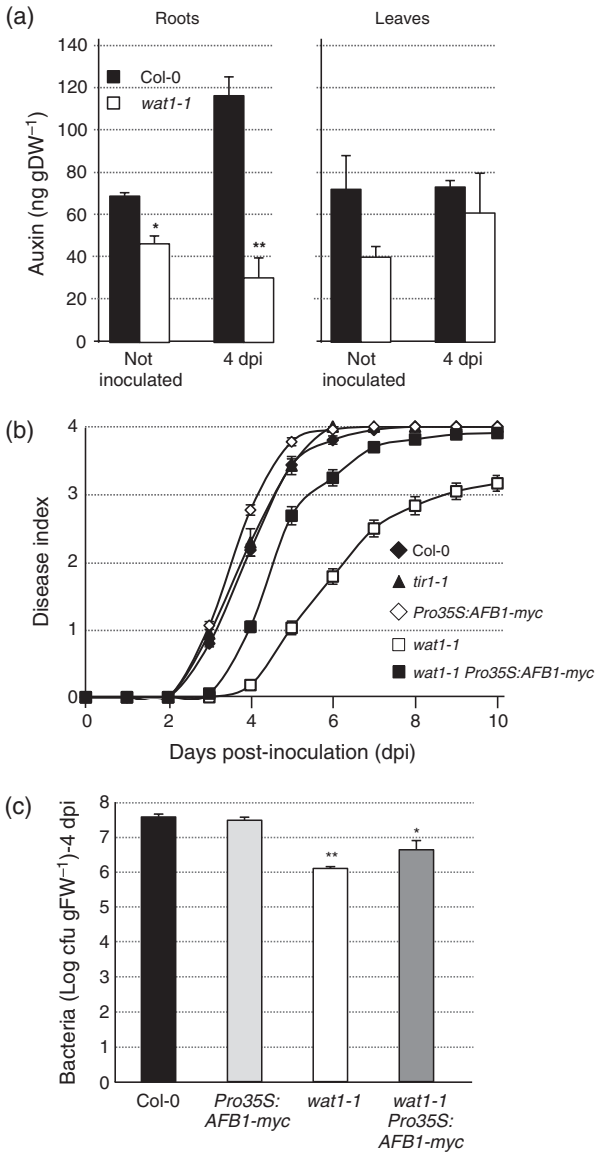


Figure 7. Role of auxin in *wat1*-mediated resistance.
(a) IAA content in roots and leaves of Col-0 and *wat1-1* plants prior to infection and at 4 dpi with *R. solanacearum*. Data (ng g^{-1} dry weight) are means ± SE of three biological replicates. Student's *t* test was used to determine significant differences between the two genotypes (**P* < 0.05; ***P* < 0.01).
(b) Mean disease index ± SE following *R. solanacearum* inoculation of Col-0 plants, *wat1-1*, *tir1-1*, *Pro35S:AFB1-myc* and *wat1-1 Pro35S:AFB1-myc*.
(c) *Ralstonia solanacearum* GMI1000 density [log cfu (colony-forming units)/g fresh weight] at 4 dpi in Col-0, *Pro35S:AFB1-myc*, *wat1-1* and the *wat1-1 Pro35S:AFB1-myc* transgenic line. At least nine plants per genotype were tested at each time point, and data represent mean growth ± SE. Two independent experiments were performed with similar results. Student's *t* test was used to determine significant differences between Col-0 and other genotypes (**P* < 0.05; ***P* < 0.01). Note that data for *wat1-1* and *wat1-1 Pro35S:AFB1-myc* lines are significantly different (*P* < 0.05).

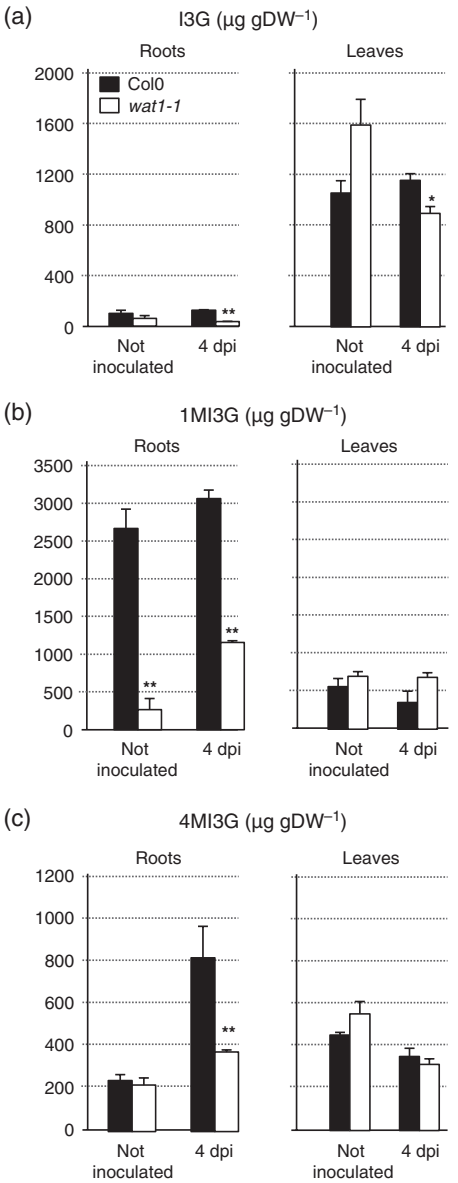


Figure 8. Indole glucosinolate content is constitutively lower in *wat1* roots. Quantitative determination of indole glucosinolate (IGS) content in roots and leaves of Col-0 and *wat1-1* plants prior to infection and 4 dpi with *R. solanacearum*. (a) neoglucobrassicin (1MI3G), (b) glucobrassicin (I3G), (c) 4-MeO-glucobrassicin (4MI3G). Data (μg g^{-1} dry weight) are means ± SE of three biological replicates. Student's *t* test was used to determine significant differences between the two genotypes (**P* < 0.05; ***P* < 0.01).

infection (Zhu *et al.*, 2003b; Gaspar *et al.*, 2004). Even though the *wat1* cell wall does not act as a physical barrier to *R. solanacearum* movement, we cannot categorically rule out the possibility that subtle modifications of cell-wall structure and/or composition, even perhaps only in certain cell types, may contribute to *wat1* resistance.

wat1 benefits from 'vascular immunity'

Although mutants exhibiting an altered response to more than one pathogen are common, systematic resistance to vascular pathogens and susceptibility to non-vascular pathogens is rare. Here we show that the *wat1* mutant was more resistant than the wild-type to all vascular pathogens

tested (Table 1). Moreover, in leaf inoculation assays, *wat1* was resistant to *R. solanacearum* and *Xcc* only when the bacteria were injected directly into the vascular system and not when they were infiltrated into the mesophyll tissue (Figure 2). Together, these data suggest that *wat1*-mediated resistance mechanisms may be located in the vascular system, representing what has been described as 'vascular immunity', as first reported for *XopAC*, a type 3 effector of *Xcc*, which confers avirulence to Arabidopsis Col-0 only when bacteria are targeted to the vascular system (Xu *et al.*, 2008).

Xylem sap is delivered from the roots to the aboveground organs, and is rich in organic substances, including proteins, amino acids, sugars and plant hormones (Satoh, 2006). Its biochemical composition has been characterized in the context of several plant–vascular pathogen interactions. For example, a recent proteomic study performed on xylem sap in *Fusarium*-treated tomato plants led to the identification of XSP10 (Xylem Sap Protein 10 kDa), a protein that exhibits structural similarities to lipid transfer proteins and is required for full susceptibility to the vascular fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (Krasikov *et al.*, 2011). In the present context, it is interesting to note that SA levels have recently been measured in the xylem sap of *Brassica napus*, and that these levels increase upon infection with the soil-borne fungal vascular pathogen *Verticillium longisporum* (Ratzinger *et al.*, 2009). The hypothesis that *wat1*-mediated vascular immunity may be a consequence of changes in xylem sap composition is attractive. One may imagine a lack of essential nutritional elements necessary for bacterial growth and/or an increased amount of antimicrobial compounds that are unfavorable for bacterial development. Even though the molecular mechanisms underlying *wat1* resistance remain to be elucidated, the results presented here strongly suggest some form of vascular immunity.

wat1-mediated resistance is SA-dependent

To date, the role of SA in wilt disease development remains poorly documented. In the case of the soil-borne vascular fungal pathogen *Fusarium oxysporum*, SA provides increased resistance in Arabidopsis (Berrocal-Lobo and Molina, 2004; Diener and Ausubel, 2005; Thatcher *et al.*, 2009). Two lines of evidence suggest that SA plays an important role in *wat1*-mediated resistance to *R. solanacearum* and *Xcc*. First, *wat1* roots contain constitutively higher amounts of SA compared to Col-0 roots. Second, *wat1*-associated resistance was completely abolished by introduction of *NahG*, a bacterial gene whose product converts SA to catechol, into *wat1-1*. Interestingly, the *cpr5* mutant, which over-produces SA (Bowling *et al.*, 1997), is more resistant to *R. solanacearum* than the wild-type, further suggesting that the response to the bacterium may correlate with SA levels in plants (Figure 4). In the case of *cpr5*, several *PR* genes, including *PR-1*, are constitutively expressed, indicating that defense mechanisms are in a primed state prior to infection. The fact that the *wat1* mutation is not accompanied by changes in expression of the SA marker genes *PR-1* and *PR-5* suggests that a transcriptional reprogramming of SA signaling pathways is not necessary for *wat1* resistance, and that the mechanisms involved in SA-mediated resistance in *wat1* and *cpr5* may not be conserved. Alteration of the SA levels in *wat1* plants may affect phytohormone balance, which is becoming increasingly recognized as crucial to the outcome of plant–pathogen interactions (Kazan and Manners, 2009).

wat1 is characterized by constitutive repression of indole metabolism in roots

Ralstonia solanacearum is a soil-borne pathogen that colonizes xylem vessels by penetration at the root. Although several pioneering studies based on cytological approaches suggested a potential key role for this organ in response to the bacteria, very few studies have focused on putative regulatory mechanisms occurring within the root itself. Transcriptomics and metabolomics analyses performed on both leaves and roots clearly show that the major differences between *wat1* and Col-0 exist in the root, not the leaves.

We previously reported that a mutation in *WAT1* resulted in a decrease in the Trp and IAA content in stems (Ranocha *et al.*, 2010). Here we show that the IAA content is constitutively lower in *wat1* roots, but not leaves. This decrease in auxin content did not appear to be transcriptionally regulated as genes involved in auxin biosynthesis were not down-regulated in *wat1*. Interestingly, *wat1* resistance to *R. solanacearum* was partially abolished when it was crossed with *trp5*, an over-producer of Trp, and *Pro35S:AFB1-myc*, a line characterized by activation of constitutive auxin signaling. Many genes along the IGS biosynthetic pathway were down-regulated in *wat1* roots, resulting in a significant reduction in the level of 1MI3G/neoglucobrassicin, the major IGS in roots (Figures 5 and 8). Together, these metabolomic and genetic data suggest that the repression of indole metabolism in *wat1* appears to be an advantage for the plant when challenged with *R. solanacearum*.

Indole metabolites play varying roles, both positive and negative, in plant–pathogen interactions, depending on the plant species and the pathogen considered. For example, the *cyp79b2 cyp79b3* double mutant, which is impaired in IGS biosynthesis, is susceptible to three *P. cucumerina* isolates that are normally unable to colonize Arabidopsis (Sanchez-Vallet *et al.*, 2010). Several auxin mutants have been reported to be compromised in their ability to establish systemic acquired resistance when challenged with *P. syringae* (Truman *et al.*, 2010). However, there are now many examples showing that, when auxin signaling and/or transport is repressed, pathogen resistance is enhanced (Kazan and Manners, 2009). For example, *axr1*, *axr2*, *axr3*, *axr4* and *aux1* are resistant to *Fusarium oxysporum*,

presumably via SA-independent mechanisms (Kidd *et al.*, 2011). *axr2* is also more resistant to the bacterium *P. syringae* pv. *maculicola* (Wang *et al.*, 2007). Repression of auxin signaling through miR393 over-expression also led to increased resistance to *P. syringae* (Navarro *et al.*, 2006). Thus, it is conceivable that the reduced auxin content of *wat1* at least partly explains its increased resistance to *R. solanacearum* and to vascular pathogens generally.

Altered SA and auxin levels in roots are key features of *wat1*-mediated resistance

Based on metabolomic data presented here, *wat1* roots contain lower levels of indole metabolites and more SA. An inverse correlation between SA and auxin content has also been reported in several Arabidopsis mutants and transgenic lines. On the one hand, the Arabidopsis SA-deficient mutant *sid2* (isochorismate synthase) and *NahG* transgenic lines showed increased auxin levels in seeds and leaves, respectively, during the reproductive stage (Abreu and Munné-Bosch, 2009). On the other hand, the SA-accumulating mutants *cpr6* and *snc1* had relatively low IAA levels and showed reduced sensitivity to exogenous auxin (Wang *et al.*, 2007). Given the proximity of their respective metabolic pathways, it is possible that these mutants, and perhaps *wat1*, have undergone a re-orientation of metabolic flux. We show here that genetically re-equilibrating this flux in *wat1* resulted in the expected disease response phenotype. A model combining metabolomic and genetic data to explain *wat1*-mediated resistance to *R. solanacearum* is proposed in Figure S6.

Beyond just a balance in relative abundance, recent reports indicate that SA and auxin signaling are mutually antagonistic in what appears to be a development versus defense trade-off (Kazan and Manners, 2009). There are several lines of evidence showing that SA inhibits pathogen growth partly through suppression of auxin signaling. Benzothiadiazole, an SA analog, was shown to repress expression of numerous genes involved in auxin perception, transport or signaling (Wang *et al.*, 2007). From a mechanistic perspective, this inhibition is accomplished through SA-mediated stabilization of auxin response repressors (Wang *et al.*, 2007). We believe that this shift in SA and auxin levels is a cornerstone of the reduced susceptibility of *wat1* to *R. solanacearum*, and, more widely, to other vascular pathogens. Finally, although plant compatibility remains poorly understood, this study illustrates the wide range of unexpected strategies that plants may use to limit pathogen infection.

EXPERIMENTAL PROCEDURES

Plant material and growth conditions

Arabidopsis (*A. thaliana*) wild-type Col-0 (accession number N1093) used in this study was obtained from the Nottingham Arabidopsis Stock Centre. *wat1-1* (Ranocha *et al.*, 2010) and *wat1-3* (line 799H03) T-DNA insertion mutants were isolated from the SALK (Alonso *et al.*, 2003) and GABI-kat (Kleinboelting *et al.*, 2012) collections, respectively. Seeds were germinated on MS medium (Murashige and Skoog, 1962), and plants were grown in Jiffy pots (Jiffy, http://www.jiffygroup.com) in a growth chamber at 22°C, with a 9 h light period and a light intensity of 190 mmol m^{-2} sec^{-1} and 50% relative humidity. Unless indicated otherwise, experiments were performed on 4-week-old plants.

Pathogen strains

Ralstonia solanacearum GMI1000 strain was grown at 28°C on BG medium (Plener *et al.*, 2010). *Xcc* 8004*DXopAC* strain was grown at 30°C on Moka medium (Blanvillain *et al.*, 2007). *Pst* DC3000 strain was grown at 28°C on King's B medium (King *et al.*, 1954). Rifampicin (50 μg ml^{-1} final concentrations) was used for *Xcc* and *Pst* strains. *B. cinerea* B0510, *P. cucumerina* PcBMM, *V. dahliae* JR2 and *V. albo-atrum* 5431 isolates were cultivated at 28°C on potato (*Solanum tuberosum*) Difco dextrose agar (Becton Dickinson, http://www.bd.com), and spores were harvested in sterile water and stored at −80°C in 20% glycerol (Berrocal-Lobo *et al.*, 2002; Valette-Collet *et al.*, 2003; Llorente *et al.*, 2005; Yadeta *et al.*, 2011). The *C. higginsianum* IMI 349061 isolate was grown at 17°C on Mathur medium (O'Connell *et al.*, 2004).

Pathogenicity assays

Ralstonia solanacearum root infections were performed as previously described (Deslandes *et al.*, 1998) by dipping roots of 4-week-old plants into a bacterial suspension (5×10^7 cfu ml^{-1}), with one exception: roots were not cut prior to infection. Disease index was scored in leaves as follows: 0 = no symptoms, 1 = 25% wilted leaves, 2 = 50% wilted leaves, 3 = 75% wilted leaves, 4 = 100% wilted leaves (or dead plant). Following inoculation, plants were transferred to a growth chamber and kept under the following conditions: 12 h photoperiod, 27°C, 80% relative humidity. For each experiment, at least 40 plants per genotype were analyzed, with a minimum of two independent replicates. Bacterial growth *in planta* was measured as previously described (Deslandes *et al.*, 1998).

Leaf inoculation assays with *Xcc* were performed by piercing the central vein using a needle dipped into a bacterial suspension (1×10^8 cfu ml^{-1}) as previously described (Meyer *et al.*, 2005). For each experiment, four leaves per plant (ten plants per genotype) were inoculated in two independent experiments. This technique was also used for *R. solanacearum*. Plants were then placed under the following conditions: 9 h photoperiod, 22°C, 70% relative humidity. Bacterial growth *in planta* was measured as previously described (Xu *et al.*, 2008).

Leaf infection with *Pst* was performed as reported previously by infiltrating the leaf mesophyll with a bacterial suspension (5×10^5 cfu ml^{-1}) using a syringe without a needle (Katagiri *et al.*, 2002). For each experiment, four leaves per plant (ten plants in total per genotype) were inoculated in two independent experiments. This technique was also used for *R. solanacearum* and *Xcc*. Plants were then placed under the following conditions: 9 h light period at 22°C and 70% relative humidity. Bacterial *in planta* multiplication was measured as described by Bouchez *et al.* (2007).

P. cucumerina infections were performed by spraying 3-week-old plants with a fungal spore suspension (4×10^6 spores ml^{-1}). At least 15 plants per genotype were inoculated in each experiment. Following inoculation, plants were covered to maintain high humidity and placed in a growth chamber at a temperature of

24°C during the day and 22°C at night. Fungal biomass was determined by quantitative RT-PCR analysis of fungal β-tubulin gene expression in inoculated plants. Real-time amplification and detection were performed as previously described using 100 ng genomic DNA (Hernandez-Blanco *et al.*, 2007). Arabidopsis *UBIQUITIN21* (At5g25760) expression was used to normalize the transcript level in each sample. The primers used were 5′-AAAGGACCTTCGGAGACTCCTTACG-3′ and 5′-GGTCAAGAATCGAACTTGAGGAGGTT-3′ for plant *UBIQUITIN21*, and 5′-CAAGTATGTTCCCCGAGCCGT-3′ and 5′-GAAGAGCTGACCGAAGGGACC-3′ for *P. cucumerina* β-tubulin.

For pathogen assays with *Verticillium* species, plants were grown as described previously (Yadeta *et al.*, 2011). For quantification of *Verticillium* biomass, the above-ground parts of at least three plants were harvested at 4 weeks post-inoculation, and DNA was extracted from the pooled samples. Real-time PCR was used for quantification of *Verticillium* colonization *in planta.* Primer pairs for the *Verticillium ITS1* gene (Ellendorff *et al.*, 2009) and the Arabidopsis large subunit of RuBisCo gene (Yadeta *et al.*, 2011) were used as a target and endogenous control, respectively. The real-time PCR conditions were as previously reported (Ellendorff *et al.*, 2009).

C. higginsianum was inoculated by pulverization of fungal spores (1×10^6 spores ml^{-1}) onto 2-week-old plantlets as previously reported (O'Connell *et al.*, 2004). Plantlets were then placed under 16 h light conditions at 25°C. Inoculation with *B. cinerea* was performed by depositing a fungal mycelium plug onto the adaxial side of detached leaves as previously reported (Valette-Collet *et al.*, 2003).

Gene expression analysis

Real-time RT-PCR reactions were performed as described previously (Hu *et al.*, 2008). The primer sets used in the experiments are listed in Table S5. Expression of each gene was calculated according to the efficiency-corrected calculation model (Ramakers *et al.*, 2003; Pfaffl, 2004) and the target gene expression was normalized to the geometric mean of three reference genes: At1g13320, At5g09810 and At1g18780 (Vandesompele *et al.*, 2002; Hu *et al.*, 2008).

Generation and selection of double/triple mutants

wat1-1 was crossed with the following mutant alleles or transgenic lines: *jar1-1* (Staswick *et al.*, 1992), *NahG* (Lawton *et al.*, 1995), *Pro35S:AFB1-myc* (Dharmasiri *et al.*, 2005), *ein2-1* (Roman *et al.*, 1995) and *trp5* (Li and Last, 1996). All of the lines are in the Col-0 genetic background, except *Pro35S:AFB1-myc* which is in the Col-0/*tir1-1* background. The mutations (*jar1-1*, *ein2-1* and *trp5*) and transgenes (*NahG* and *Pro35S:AFB1-myc*) were identified by PCR-based methods (Table S6), or by selection on MS plates containing 0.2 μM IAA to identify plantlets with root elongation greater than Col-0 due to the *tir1-1* mutation (Ruegger *et al.*, 1998), or on 0.5% sucrose, 300 μM 6-methylanthranilate and 25 μM L-Trp to identify the *trp5* mutation (Li and Last, 1996).

Transcriptome studies

Microarray analysis was performed using CATMA arrays containing 31 776 gene-specific tags corresponding to 22 089 genes from Arabidopsis (Crowe *et al.*, 2003; Hilson *et al.*, 2004). Two independent biological replicates were produced using RNA extracted from leaves and roots separately harvested from 4-week-old plants (Col-0 and *wat1-1*) inoculated or not with *R. solanacearum*. For each comparison, one technical replicate with fluorochrome reversal was performed. RNA integrity, cDNA synthesis, hybridization and array scanning were performed as described previously (Lurin *et al.*, 2004). Statistical analysis and identification of differentially expressed genes were performed as described previously (Gagnot *et al.*, 2008).

Metabolomic analyses

Tryptophan and indole acetic acid were quantified by GC-MS (Agilent 7890A GC coupled to 5975C MSD, http://www.home.agilent.com) and LC-MS, respectively, as described by Fiehn *et al.* (2006). Indole glucosinolate contents were determined by LC/ESI-MS (QuattroLC, Waters, http://www.waters.com) in negative-ionization, full-scan mode, with internal calibration by addition of 100 ng sinigrin standard (Sigma-Aldrich, http://www.sigma-aldrich.com). For determination of salicylic acid content, 300 mg tissue, either from leaves or roots of healthy 4-week-old plants, were ground in liquid nitrogen, and 50 ng of an internal standard (*o*-anisic acid) were added before extraction of total SA (Tronchet *et al.*, 2010). After extraction, analysis was performed by HPLC (Ultimate 3000, Dionex, http://www.dionex.com). Total SA and *o*-anisic acid contents were quantified using a Jasco FP-920 spectrofluorimeter (http://www.jascoinc.com), with excitation and emission wavelengths of 305 and 365 nm, respectively. The data were analyzed using Chromeleon 6.8 chromatography software (Dionex).

Construction of the *ProWAT1:WAT1* gene fusion and plant transformation

The *WAT1* promoter sequence (2980 bp) was amplified using primers 5′-TTGGTACCTCTACCATACACATCATCGC-3′ and 5′-CATGAATTCTGGATCGAGCTTAACAATC-3′ (*Kpn*I and *Eco*RI sites underlined). The *WAT1* coding sequence (1180 bp) was amplified using primers 5′-CCAGAATTCATGGCGGATAACACCGATA-3′ and 5′-GGGAGCTCTCAAACATTGTCCGTTGACT-3′ (*Eco*RI and *Sac*I sites underlined). After sub-cloning into pGEM-Teasy (Promega, http://www.promega.com), *ProWAT1* and *WAT1* were cloned in fusion (via the *Eco*RI site) at the *Kpn*I and *Sac*I sites of pGreenII 0179 (Hellens *et al.*, 2000). The resulting construct was introduced into *Agrobacterium tumefaciens* GV3101. *wat1-1* plants were transformed by floral dipping (Clough and Bent, 1998). Transformed seeds were selected on MS medium containing hygromycin (15 μg ml^{-1}).

ACCESSION NUMBER

Expression data from this paper were deposited according to MIAME standards at http://urgv.evry.inra.fr/CATdb/ under project name 'RS08-04_wat1-Ralstonia'.

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SUPPORTING INFORMATION

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

Figure S1. *WAT1* gene expression decreases following *R. solanacearum* infection in Col-0 plants.

Figure S2. *wat1* mutants exhibit an increased resistance to vascular pathogens.

Figure S3. Quantitative assessment of the effect of the *wat1* mutation on global gene expression in roots and leaves.

Figure S4. Role of tryptophan and auxin in *wat1*-mediated resistance to the vascular fungal pathogen, *Verticillium dahliae*.

Figure S5. Indole metabolite content in non-inoculated roots of *wat1-1 trp5* and *wat1-1 Pro35S:AFB1-myc*.

Figure S6. Working model for *wat1*-associated resistance to *R. solanacearum*.

Table S1. List of the 148 de-regulated genes in non-inoculated *wat1-1* roots compared to the wild-type.

Table S2. List of the 40 de-regulated genes in non-inoculated *wat1-1* leaves compared to the wild-type.

Table S3. List of the 210 de-regulated genes in *wat1-1* roots compared to the wild-type at 4 dpi.

Table S4. List of the 148 de-regulated genes in *wat1-1* leaves compared to the wild-type at 4 dpi.

Table S5. Primers used for analysis of *WAT1* gene expression by quantitative RT-PCR.

Table S6. Primers used to detect mutations for selection of double/triple mutants or transgene introduction in *wat1-1.*

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