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Deep Sequencing Reveals Early Reprogramming of *Arabidopsis* Root Transcriptomes Upon *Ralstonia solanacearum* Infection

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Bacterial wilt caused by the bacterial pathogen *Ralstonia solanacearum* is one of the most devastating crop diseases worldwide. The molecular mechanisms controlling the early stage of *R. solanacearum* colonization in the root remain unknown. Aiming to better understand the mechanism of the establishment of *R. solanacearum* infection in root, we established four stages in the early interaction of the pathogen with *Arabidopsis* roots and determined the transcriptional profiles of these stages of infection. A total 2,698 genes were identified as differentially expressed genes during the initial 96 h after infection, with the majority of changes in gene expression occurring after pathogen-triggered root-hair development observed. Further analysis of differentially expressed genes indicated sequential activation of multiple hormone signaling cascades, including abscisic acid (ABA), auxin, jasmonic acid, and ethylene. Simultaneous impairment of ABA receptor genes promoted plant wilting symptoms after *R. solanacearum* infection but did not affect primary root growth inhibition or root-hair and lateral root formation caused by *R. solanacearum*. This indicated that ABA signaling positively regulates root defense to *R. solanacearum*. Moreover, transcriptional changes of genes involved in primary root, lateral root, and root-hair formation exhibited high temporal dynamics upon infection. Taken together, our results suggest that successful infection of *R. solanacearum* on roots is a highly programmed process involving in hormone crosstalk.

Keywords: bacterial wilt, lateral root formation, plant hormones, *Ralstonia solanacearum,* RNA sequencing, root defense, root growth inhibition, root-hair formation, transcriptome profiling

Ralstonia solanacearum, a soil-borne phytopathogen, causes devastating bacterial wilt disease on crops and leads to huge economic losses (Mansfield et al. 2012). The bacterium enters the root epidermis through natural openings or wounds, crosses the cortex and endodermis, and, finally, reaches the root xylem. In the xylem, *R. solanacearum* starts extensive colonization, spreads to the aerial part of the infected plant along the vascular system and, finally, kills the host by blocking water transport from root to shoot, which causes the wilting symptom (Genin and Denny 2012). Due to its wide host range, long persistence in the soil, and broad geographical distribution, *R. solanacearum* was ranked as the second most important bacterial plant pathogen (Mansfield et al. 2012).

The interaction between *R. solanacearum* and *Arabidopsis* has been successfully used as a model to study plant defense for more than 20 years (Deslandes et al. 1998). However, knowledge about the molecular mechanisms of *Arabidopsis* defending against *R. solanacearum* is still limited. *RRS1-R* is the only *R. solana*cearum resistance gene cloned from *Arabidopsis* and encodes a Toll interleukin 1 receptor (TIR) nucleotide binding site-leucine rich repeat (NBS-LRR) resistance protein with a C-terminal WRKY DNA-binding motif (Deslandes et al. 2002). In the absence of PopP2, an effector from *R. solanacearum* GMI1000, RRS1-R forms a heterodimer complex with RPS4, another NBS-LRR protein, localizes in the nucleus, and binds DNA through the WRKY domain. When PopP2 is delivered into the host cell through the type III secretion system (T3SS), it directly interacts with the RPS4/RRS1-R resistance complex and acetylates the WRKY domain of RRS1-R through its acetyltransferase activity, blocking RPS4/RRS1-R DNA-binding activity and activating RPS4-mediated plant resistance (Le Roux et al. 2015; Sarris et al. 2015).

It is widely recognized that phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play determinant

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roles in plant defense to diverse pathogenic insects, bacteria, and fungi. However, it is still not clear what is the precise role of these hormones in response to *R. solanacearum*. *Arabidopsis* mutants deficient in biosynthesis or signaling of SA, JA, and ET have been used to investigate their sensitivities to *R. solanacearum.* Sometimes their roles in plant defense to *R. solanacearum* are contradictory. For instance, while either an increase or decrease of endogenous SA levels did not alter plant sensitivity to *R. solanacearum* (Hirsch et al. 2002), depletion of SA in the *walls are thin 1* (*wat1*) mutant through overexpression of the bacterial SA hydroxylase gene *NahG* restored plant susceptibility to *R. solanacearum* (Denance et al. 2013). Mutation of EIN2, an important component in ET signal transduction, dramatically delayed bacterial wilt on *Arabidopsis*, which did not happen on the other ET-insensitive mutants *etr1-3*, *ein4-1*, and *eni3-1* (Hirsch et al. 2002). In addition, the *jar1-1* mutant deficient in the bioactive JA-isoleucine (JA-Ile) shows the same sensitivity to *R. solanacearum* as wild-type plants (Hirsch et al. 2002), but loss-of-function of the JA receptor COI1 enhances plant defense against *R. solanacearum* (Hernández-Blanco et al. 2007).

WRKY transcription factors, critical players in modulating plant resistance to phytopathogens, were also reported to function in plant defense to *R. solanacearum*. WRKY27 mutation delays disease symptom development by modulating signaling between the phloem and the xylem (Mukhtar et al. 2008). Inactivation of WRKY53 also reduces the wilting symptom caused by *R. solanacearum* (Hu et al. 2008).

The cell wall is the first physical layer of plant defense against pathogens. It is demonstrated that alteration of the cell wall affects *Arabidopsis* defense to *R. solanacearum*. Cellulose synthases are required for secondary cell-wall formation. Mutations of cellulose synthase genes (*CESA4*, *CESA7*, and *CESA8*) conferred enhanced resistance to *R. solanacearum* in an abscisic acid (ABA)-dependent way (Hernández-Blanco et al. 2007). Similarly, the *WAT1* gene is essential for secondary cell-wall deposition. A mutation in *WAT1* led to reduction in cell elongation and secondary wall thickness but increased SA content and plant defense to vascular *R. solanacearum* (Denance et al. 2013). Furthermore, pectin homogalacturonan in the root cell wall has been reported to be degraded after *R. solanacearum* infection (Digonnet et al. 2012).

Transcriptional profiles by RNA-seq have been employed to look for important events in plant defense against *R. solanacearum* in *Arabidopsis*. The *R. solanacearum* GMI1000 *ΔhrpB* mutant has a dysfunctional T3SS and loses the ability to invade host plants (Vasse et al. 2000). Plants infected with this mutant exhibited enhanced plant defenses to subsequent virulent strain infection. Microarray analysis of transcriptional changes in aerial parts of plants treated with GMI1000 *ΔhrpB* indicated that 26% of upregulated genes were involved in the metabolism and signaling of ABA (Feng et al. 2012). In addition, comparison of transcriptional profiles from the aerial part of *Arabidopsis* Col-0 inoculated with GMI1000 at several timepoints identified many differentially expressed genes (DEGs) associated with the ABA signaling pathway (Hu et al. 2008).

However, the majority of previous microarray studies focused on transcriptional changes in the aerial parts of *Arabidopsis* root-inoculated with *R. solanacearum*. Since *R. solanacearum* is soil-borne and infects plant roots, direct investigation of transcriptional changes in infected plant roots at a series of timepoints will help to disclose the molecular mechanism of *R. solanacearum* infection. In this study, by means of high-resolution temporal analysis of host global transcriptional changes following pathogen infection, we identified several important events, such as the activation of the biosynthesis and signaling of different hormones, and further connected root structure changes to transcriptional reprogramming following *R. solanacearum* infection. Our data provides a cornerstone to understand complicated regulation networks during the infection process of *R. solanacearum* in the root.

RESULTS

Characterization of root morphology changes following GMI1000 infection.

As previously reported, *Arabidopsis* seedling roots exhibited primary root growth retardation, de novo root-hair formation, and cell death appearance around the root tip at 9 days after GMI1000 treatment (Lu et al. 2018). To define appearance time of the three root phenotypes, we investigated the root structure changes of *Arabidopsis* seedling after infection with GMI1000 over time. Primary roots kept growing during the first 24 h postinoculation (hpi). At 48 hpi, primary root growth was found to be inhibited by GMI1000 (Fig. 1A). Root hair close to the root tip appeared around 24 hpi, at a time they did not come out on water-treated seedling roots (Fig. 1B). Roots were immersed in a DNA/RNA dye, propidium iodide, and cell integrity was observed under a confocal microscope. Cells in the root meristem area were alive at 24 hpi but were already dead at 48 hpi (Fig. 1C). In addition, lateral roots emerged from primary roots treated with GMI1000 at 72 hpi and became clear at 96 hpi. The number of these secondary roots on *Arabidopsis* root treated with GMI1000 was four- to fivefold higher than on water-treated plants (Fig. 1A; Supplementary Fig. S1). According to these root structure changes over time, we divided the initial root infection by *R. solanacearum* into four phenotypic stages: NS, no symptoms at 0 to 12 hpi; RH, root-hair emergence at 12 to 24 hpi; PC, primary root-growth arrest and cell death at 24 to 48 hpi; and LR, lateral root emergence at 48 to 72 hpi.

Time series of global transcriptional reprogramming in roots challenged with GMI1000.

To understand the events taking place at different infection stages of *R. solanacearum*, we infected, in vitro, 7-day-old seedling roots and collected root samples at 0, 6, 12, 24, 48, and 96 hpi, extracted total RNA, and sequenced the global transcripts of GMI1000-infected roots. Around 600 seedling roots were pooled into one sample. Three biological replicates per timepoint were directly subjected to Illumina RNA sequencing. An average of 33.9 million clean reads (ranging from 26.9 to 41.5 million) with Q30 >90% were obtained per sample. More than 94% of clean reads were mapped to the *Arabidopsis* genome (Supplementary Table S1). Aiming to disclose the molecular mechanism of the early infection process of *R. solanacearum*, we compared *R. solanacearum*–infected root transcriptomes at different infection timepoints with those obtained in water-treated roots after 96 h and in GMI1000-treated roots at 0 h. The time series expression profiles identified a total of 2,698 *Arabidopsis* genes as DEGs based on their significance in fold-change expression (*padj* < 0.05) and at least a twofold change in expression level ($-1 > \log_2 > 1$) (Fig. 2; Supplementary Dataset 1).

To analyze the overall patterns in gene expression during *R. solanacearum* infection, the 2,698 DEGs were clustered into 11 hierarchical clusters, based on their expression patterns over time (Fig. 2). The list of genes in each cluster was presented in Supplementary Dataset 2. These clusters of genes showed sequentially induced upon pathogen challenge over time. The cluster VI genes started increasing at 12 hpi and peaked at the root hair emergence (RH) stage (24 hpi), then, slowly dropped back to basal level, which was the most rapid response to

R. solanacearum infection. The maximum level of cluster IV and V genes was at the RH and PC stages (48 hpi), 24 h later than that of cluster VI. Then, cluster V quickly decreased. Compared with the relatively long-lasting expression pattern of cluster V and IV, the highest expression level of cluster III genes was more limited in the PC stage. Cluster I and II genes increased to maximum level at the LR stage (72 hpi) and at 96 hpi, which were the last induction clusters. The down-regulated genes also showed a temporally modulated expression pattern. The earliest repressed-gene clusters were cluster VIII and XI, which happened at the RH stage. Interestingly, unlike cluster VIII, which maintained at a lower expression, a few genes in cluster XI suffered a second induction at the LR stage. The lowest expression level of cluster IX and cluster X occurred at the LR stage. The expressions of cluster VII genes were down-regulated at the LR stage and at 96 hpi (Fig. 2).

To check whether the coexpressing genes in the same cluster participated in similar biological processes, we investigated over-representation of gene ontology (GO) terms in these groups. The selected over-represented GO terms are shown at the right of each gene expression cluster in Figure 2. The cluster I genes were enriched in GO terms 'glucosinalte biosynthetic process' and 'response to JA'. Cluster IV contained major GO terms 'tryptophan metabolic process', 'auxin metabolic process', and 'glucosinolate biosynthetic process', which shared major components in their biosynthesis and peaked at the RH and PC stages. GO term 'response to auxin' was over-represented in clusters II and III and was strongly induced during PC and LR stages (48 to72 hpi), 24 h later than GO term 'auxin metabolic process' in cluster VI (Fig. 2). Additionally, GO terms such as 'response to abiotic stress', 'response to heat', and 'response to hydrogen peroxide' were also over-represented in clusters II and III. Cell-wall organization genes were enriched in cluster VI and were up-regulated before the appearance of root hair (12 hpi), reaching their highest level at the RH stage, in response to the pathogen. Interestingly, GO terms 'cell to cell junction' and 'cell wall organization' also were enriched in cluster VIII and were significantly down-regulated at PC and LR stages. Moreover, another significantly enriched cell wall–related GO term was 'lignin metabolic

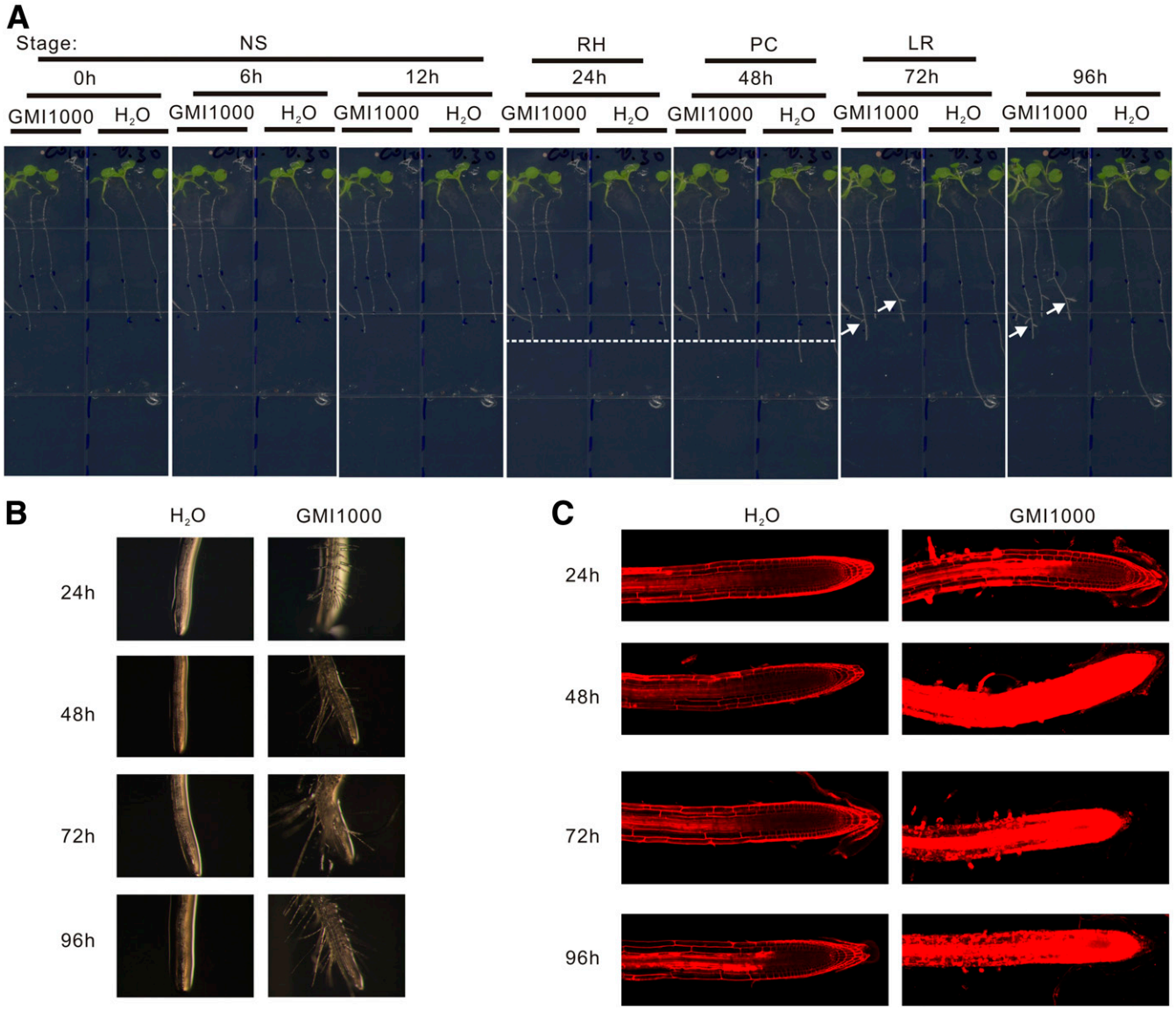

Fig. 1. Time series of root structure after GMI1000 infection. **A,** Root growth was measured and digital images were taken at indicated timepoints. Arrows indicate lateral roots. Dashed line indicates root growth arrest. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence. **B,** Root hair images were taken with an OLYMPUS SZX16 microscope at the indicated timepoints. **C,** Cell death on the root tip was stained with a propidium iodide solution and images were directly taken with an Olympus confocal microscope.

process' in cluster V. These suggest cell-wall remodeling probably has a specific role in the plant response to GMI1000 infection. GO terms related to plant defense, such as 'response to chitin', 'response to bacterium', 'response to SA', and 'defense response' were enriched in cluster VII and were significantly down-regulated during the LR stage (72 hpi). Cluster X genes were significantly related to GO term 'root hair cell differentiation' and were suppressed at the LR stage.

Biological processes that take place during *R. solanacearum* infection are likely to affect the outcome of the plant-pathogen interaction. Therefore, we further investigated enriched GO terms in the DEGs at single timepoints irrespective of the previous clustering (Supplementary Fig. S2). This analysis revealed that cell wall organization–associated genes were enriched in the upregulated DEGs at the NS and RH stages, suggesting that these genes probably contribute to modulation of the cell wall and cell-to-cell junctions, which may play a role in establishment of *R. solanacearum* infection. The term 'tryptophan metabolic process' was over-represented in the upregulated DEGs at the RH stage, which may promote auxin biosynthesis in the tryptophan-dependent pathway. 'Response to biotic stimulus' was a GO term over-represented in upregulated DEGs at the RH and PC stages. 'Response to hormones' was over-represented in genes specifically upregulated

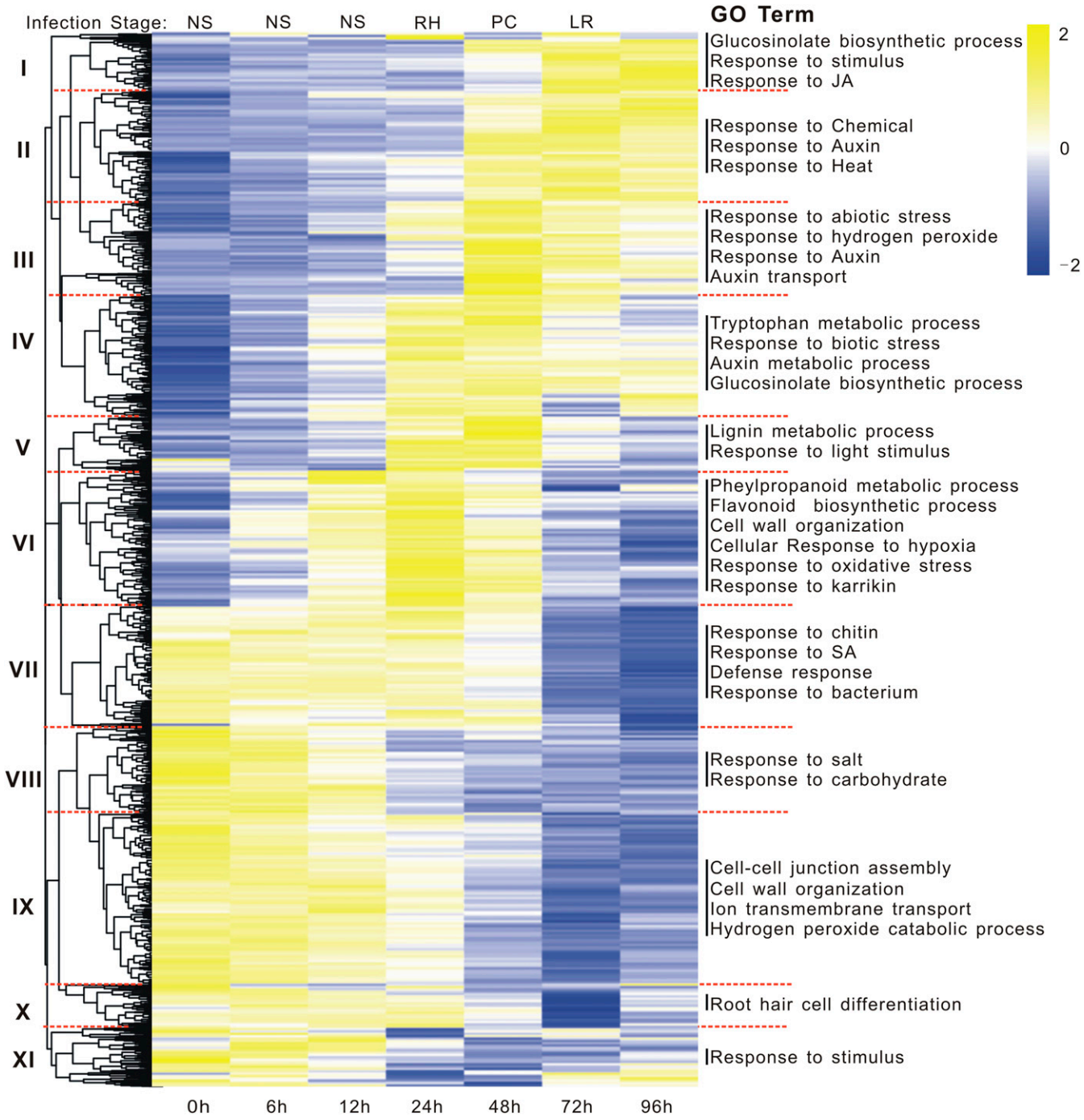

Fig. 2. Clustering analysis of RNA-seq data. The heat map represents the expression patterns of 2,698 differentially expressed genes identified in our RNA-seq data. The vertical axis organizes genes according to coexpression patterns. The horizontal axis displays timepoints. The selected over-presented gene ontology terms in each cluster were shown on the left. The heat map depicts FPKM (fragments per kilobase per million reads) value after $\log_{10}$ transformation. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence.

at PC and LR stages (48 and 72 hpi, respectively), which may reflect the root structure changes that take place at the LR stage. The GO term 'response to abiotic stimulus' was also highlighted in the upregulated DEGs at the PC stage. The upregulated 'glucosinolate biosynthetic process' term spanned from the LR stage to 96 hpi. JA is involved in root development and regulation of plant defense. The upregulated genes at 96 hpi were related to 'response to JA' term. In downregulated DEGs, the terms 'transport', 'cell wall organization', and 'root development' terms were over-represented at the PC and LR stages. These sequentially over-represented GO terms during early *R. solanacearum* infection indicate that infection is a programmed dynamic event from the very beginning of the plant-pathogen interaction.

ET-, JA-, auxin-, and ABA-dependent signaling are altered following *R. solanacearum* Infection.

The first and rate-limiting step in ET biosynthesis is the conversion of *S*-adenosyl methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). Five of the nine *ACS* genes in the *Arabidopsis* genome (*ACS2*, *ACS6*, *ACS7*, *ACS8*, and *ACS9*) were induced at the PC stage. Interestingly, the expression of *ACS5* was inhibited at the same time (Supplementary Fig. S3A). No ACC oxidase gene was identified in our RNA-seq data, but the expression of its regulator SHYG was up-regulated at the RH and PC stages (Rauf et al. 2013). Moreover, ET responsive factor (*ERF*) transcriptional factors including *ORA59* and *ERF71* in response to ET were up-regulated or down-regulated following GMI1000 infection. These findings suggest that ET biosynthesis and signaling are regulated upon *R. solanacearum* infection.

The expression of several genes involved in JA biosynthesis and degradation was also altered in our RNA-seq data. For example, 13-lipoxygenase (LOX), encoded by *LOX1* and *LOX2*, were induced at the PC stage. LOXs are responsible for converting α-linolenic acid to 13-hydroxyperoxy-octadecatrienoic acid in plastids, which is the first step in the production of the JA precursor (Wasternack and Hause 2013). Acyl-coenzyme A oxidase and 3-ketoacyl-CoA thiolase (KAT5) catalyze JA biosynthesis from this precursor (Li et al. 2005). Their expression was repressed after *R. solanacearum* inoculation. Three of four *Arabidopsis* jasmonate-induced oxygenase genes, whose products inactivate JA through hydroxylation (Caarls et al. 2017), were highly expressed in our data. Similarly, the expression of *ST2A* encoding a hydroxyjasmonate sulfotransferase that inactivates JA functions (Gidda et al. 2003) was highly induced at the LR stage. Jasmonate ZIM domain genes (*JAZ1*, *JAI3*, *JAI5*, and *JAZ10*), key negative regulators of the JA signaling pathway, were strongly up-regulated at the LR stage and at 96 hpi. In summary, a decrease in JA biosynthesis and an increase in JA degradation and negative regulators suggests this pathway may be turned off at the late infection stage of *R. solanacearum*.

Components in auxin metabolism, auxin signaling and auxin transport, were up-regulated from the NS stage to the LR stage (Fig. 3). *TRP4*, *TRP5*, *TRP1*, *TRP3*, and *TSB2* encode key components in the transformation of chorismate to the auxin precursor tryptophan (Zhao 2010). All of them were up-regulated at the RH stage (Fig. 3A and B). Another five genes involved in tryptophan-dependent auxin synthesis pathways described in *Arabidopsis* (Ruiz Rosquete et al. 2012; Zhao 2010) were up-regulated at the RH stage (genes *CYP79B2*, *CYP79B3*, *NIT1*, *NIT3*, and *YUC9*) (Fig. 3A and B). In addition, the expression of *DAO1* and *DAO2*, encoding enzymes that oxidate indole 3-acetic acid (IAA) to oxIAA, and GH3 family genes, encoding enzymes that conjugate amino acids to IAA (Ruiz Rosquete et al. 2012), were all induced at the RH and PC stages (Fig. 3A and B). Accumulation of auxin-responsive transcripts such as *SAURs* and *Aux/IAAs* was observed at the PC stage (Woodward and Bartel 2005), which was 24 h later than the expression peak of auxin synthesis genes (Fig. 3B; Supplementary Fig. S4). The expression of auxin response factors such as *ARF4* increased during infection (Fig. 3C). The expression of auxin efflux transporters (*PINs* and *ABCB4*) (Ruiz Rosquete et al. 2012) increased at the PC and LR stages (Fig. 3D). Moreover, a few regulators controlling stability of auxin transporters (*PATL2*, *RAM2*, *PBP1*, *PILS7*, *SMXL8*, and *PID*) were also differentially expressed in our data.

Our RNA-seq data also identified a group of genes that were associated with ABA metabolism and signaling (Fig. 4A). The expression of *CYP707A*, which oxidizes and inactivates ABA (Saito et al. 2004), was induced at all timepoints after 24 hpi. Expression of the ABA receptor *PYL5* was inhibited after infection (Fig. 4A). And *ABI2*, *HAB1*, and *PP2C5* genes, encoding protein phosphatases that suppress ABA signaling through dephosphorylation of SNRK2 proteins (Umezawa et al. 2009), were up-regulated at the PC stage. Expression of *OST1*, essential for ABA signaling (Fujii et al. 2009), was up-regulated at RH, PC, and LR stages and, then, quickly decreased at LR stage. On the contrary, other *SNRK* family genes were down-regulated at PC and LR stages (Fig. 4A). Finally, expression of the ABA-dependent transcription factor *ABF2* (Fujita et al. 2005) was up-regulated at the RH stage. (Fig. 4A). In addition, the expression trends of *OST1*, *ABI2*, *ABF1*, and *ABF2* were validated by qRT-PCR (Supplementary Fig. S5).

ABA signaling is involved in plant defense to *R. solanacearum*.

Next, we investigated whether disruption of ABA perception had an impact on plant responses to this pathogen. To this end, we took advantage of available *Arabidopsis* mutants, i.e., the quintuple *pyl1/pyl2/pyl4/pyl5/pyl8* (*12458*) and sextuple *pyr1/pyl1/pyl2/pyl4/pyl5/pyl8* (*112458*) mutants, which are devoid of multiple ABA receptors and show reduced vegetative growth and seed production (Gonzalez-Guzman et al. 2012). We grew the Col-0 accession and ABA receptor mutants and tested their responses to *R. solanacearum* infection. Both mutant lines showed increased wilting symptoms at 15 days postinoculation compared with their wild-type counterparts (Fig. 4B). This was translated into significantly higher plant mortality rates in the mutants than in wild-type plants (Fig. 4C). These results indicate interruption of ABA signal perception promotes leaf wilting symptom development triggered by *R. solanacearum*, which is consistent with the increased susceptibility of ABA-insensitive mutants *abi1*-1 and *abi2*-1 to the pathogen (Feng et al. 2012; Hernández-Blanco et al. 2007). We further tested if ABA signaling could affect the previously described root morphology changes induced by the bacterium. The sextuple mutant exhibited root morphogenetic responses similar to wild-type plants (Fig. 5), suggesting that ABA signaling is not required for *R. solanacearum*–induced root structural changes.

Regulation of plant defense response genes in *R. solanacerum*-infected roots.

Among the 2,698 genes differentially expressed after *R. solanacearum* infection, 109 genes have been reported to be involved in plant defense (Supplementary Fig. S6; Supplementary Dataset 3). RLK3, RD19, and WRKY27 have been reported to regulate plant defense to *R. solanacearum*. The expression of *RLK3* encoding a cysteine-rich repeat receptor-like kinase was induced in the *Arabidopsis* ecotype Niederzenz (Nd-1) infected with *R. solanacearum* GMI1000 (Czernic et al. 1999). In our transcriptome, it was induced at 12 hpi and

reached a peak at the PC stage in infected plants. Surprisingly, the expression of RD19, a cysteine protease required for RRS1-R-mediated resistance to *R. solanacearum* (Bernoux et al. 2008), was strongly inhibited upon infection. Similarly, expression of *WRKY27*, which was shown to promote disease symptom development (Bernoux et al. 2008), was repressed upon infection. The negative regulators of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), *PUB22*

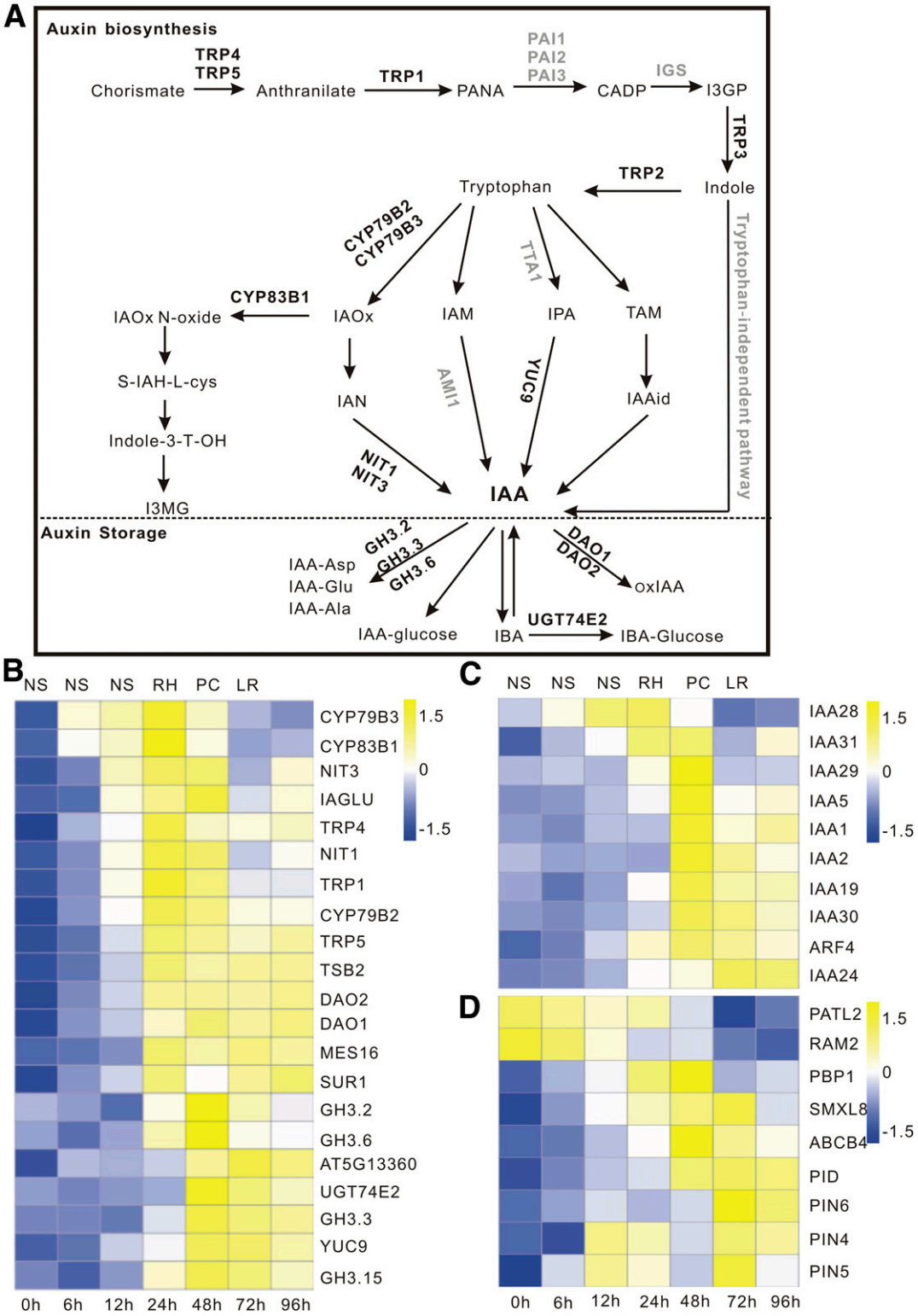

Fig. 3. Expression patterns of genes related to auxin biosynthesis, signaling, and transport. **A,** Auxin biosynthesis processes and metabolic processes. Differentially expressed genes encoding enzymes related to auxin biosynthesis or signaling found in our RNA-seq data are shown in black bold, otherwise enzymes are shown in gray bold. **B,** Expression patterns of differentially expressed auxin biosynthetic genes in response to GMI1000 infection. **C,** Expression patterns of differentially expressed auxin signaling genes in response to GMI1000. **D,** Expression patterns of differentially expressed auxin transport genes in response to GMI1000. The heat map depicts FPKM (fragments per kilobase per million reads) values after $\log_{10}$ transformation. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence.

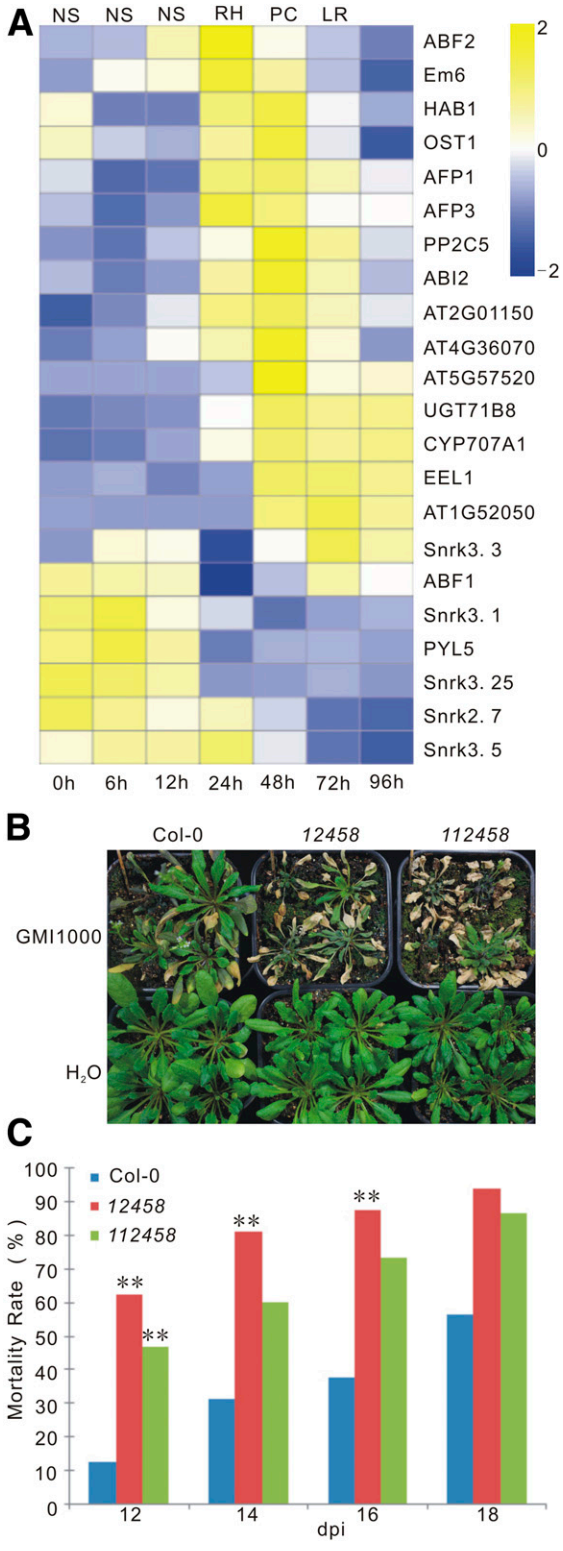

Fig. 4. Abscisic acid (ABA) receptor mutants *12458* and *112458* showed more sensitivity to GMI1000. **A,** Temporal dynamics of ABA signal components after GMI1000 treatment. Heat map depicts the expression patterns of differentially expressed ABA-responsive genes. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence. **B,** Wilt symptoms were digitally imaged at 15 days postinoculation. **C,** Mortality rate of the infected plants was recorded at indicated times. A total of 20 plants were used in three independent experiments. Asterisks (**) indicate $P < 0.001$ (Student's t test) with respect to Col-0.

and *PUB23* (Trujillo et al. 2008), were differentially expressed after infection. The transcript level of *LYK4* participating in sensing chitin was induced at 12 h after infection and the expression of other PTI regulators (*PEP1* and *MPK11*) were strongly induced at the RH and LR stages. Interestingly, these genes were inhibited at the LR stage. Finally, the genes encoding key modulators of plant immunity, such as WRKY, ERF, and ANAC transcription factors, were also identified as DEGs in our experiments.

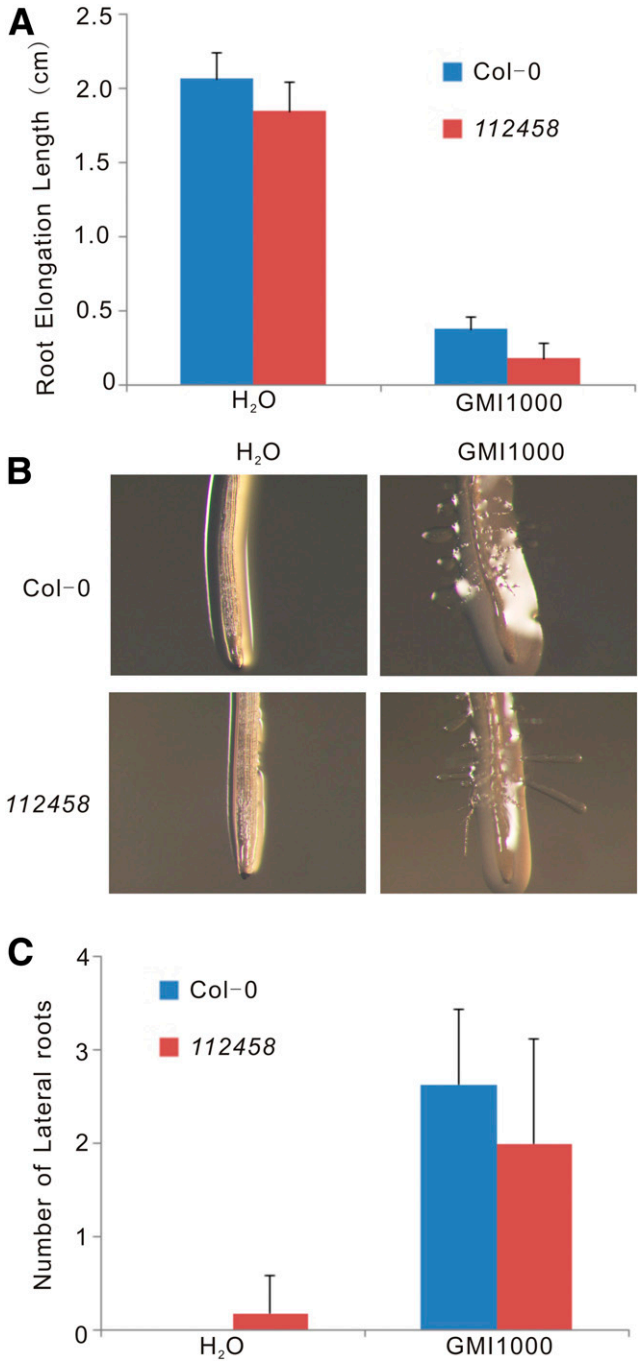

Fig. 5. Mutations in abscisic acid (ABA) receptors did not abolish root architecture changes caused by GMI1000. **A,** Inhibition of *112458* root growth. Primary root elongation length after infection was measured at 4 days postinoculation (dpi). **B,** Root hair formation on *112458* root tips. The images were taken with an Olympus microscope. **C,** Lateral roots on *112458*. Lateral roots per seedling were counted at 4 dpi. At least eight plants were used to measure root length and root hairs and to count lateral roots in three independent experiments.

Transcriptional regulation of programmed plant cell death (PCD) genes in *R. solanacearum*-infected roots.

PCD in root tip cells was initiated around 24 hpi and was completed around 48 hpi (Fig. 1C). In line with the appearance of cell death in the root meristem zone, many regulators of PCD were differentially expressed (Fig. 6). For instance, expression of two negative regulators of cell death, *MC2* and *SYP122* (Zhang et al. 2008) (Coll et al. 2010), were strongly down-regulated by *R. solanacearum* from the RH stage onward (Fig. 6A). Consistent with downregulation of *SYP122*, the expression of mono-oxygenase1 (*FMO1*), required for SYP22-dependent lesion formation, reached a peak at 24 h after infection (Fig. 6A). Expression of autoinhibited Ca^{2+}-ATPase 4 (*ACA4*), involved in regulation of PCD (Boursiac et al. 2010), was repressed at 24 to 48 hpi (Fig. 6A). In addition, we noticed plant senescence genes associated with PCD were differentially expressed (Fig. 6B). The transcripts of *Oresara1* (*ORE1*) and *WRKY57*, two hormone-mediated cell-death regulators (Jiang et al. 2014), were both strongly induced at the RH stage, the latter one began to increase at 12 hpi and decreased at the PC stage (Fig. 6B).

Root architecture responses to *R. solanacearum* infection.

Root-hair formation was induced at the RH stage at the root tip (Fig. 1B). We thus scrutinized our transcriptomes for DEGs described in the literature to play a role in this process. We found expression of *zinc finger protein 5* (*ZFP5*) and the *Oxidative signal-inducible 1* (*OXI1*) kinase, which are required for normal root-hair development (An et al. 2012; Rentel et al. 2004), was induced at 6 hpi, peaking at the RH stage and returning to basal levels at the LR stage (Fig. 7A). The *ERU*, *EXP7*, and *LRX1* genes, involved in root-hair elongation (Baumberger et al. 2001; Lin et al. 2011; Schoenaers et al. 2018), were also quickly turned on at the NS stage (6 to 12 hpi) and were inactivated at the PC stage (Fig. 7A). According to these data, root hair should appear on root tips just after 12 hpi. Thus, we analyzed the appearance of root hair in further detail by observing infected root tips at 6, 12, 18, and 24 hpi. Appearance of root hair around the root tip was observed at 18 h after infection (Fig. 7B), which correlates to the changes in root-hair gene expression patterns.

Another dramatic response to *R. solanacearum* infection is root growth inhibition. In our transcriptome data, many regulators involved in primary root growth were identified (Fig. 8). The expression of several negative regulators of root growth increased after infection, reaching the highest levels at the PC stage. These included *CLV3/ESR-related peptide 20* (*CLE20*) (Meng and Feldman 2010), methyltransferase *PXMT1* (Chung et al. 2016), the triterpene synthesis genes *THAH1*, *THAD1*, and *THAS1* (Field and Osbourn 2008), the *LRP1* gene involved in root growth retardation induced by phosphate deficiency (Svistoonoff et al. 2007), and *EFR* controlling rice primary-root elongation (Xiao et al. 2016). On the contrary, positive root growth regulators GA3ox and CLE6 were repressed at the PC stage. GA3ox

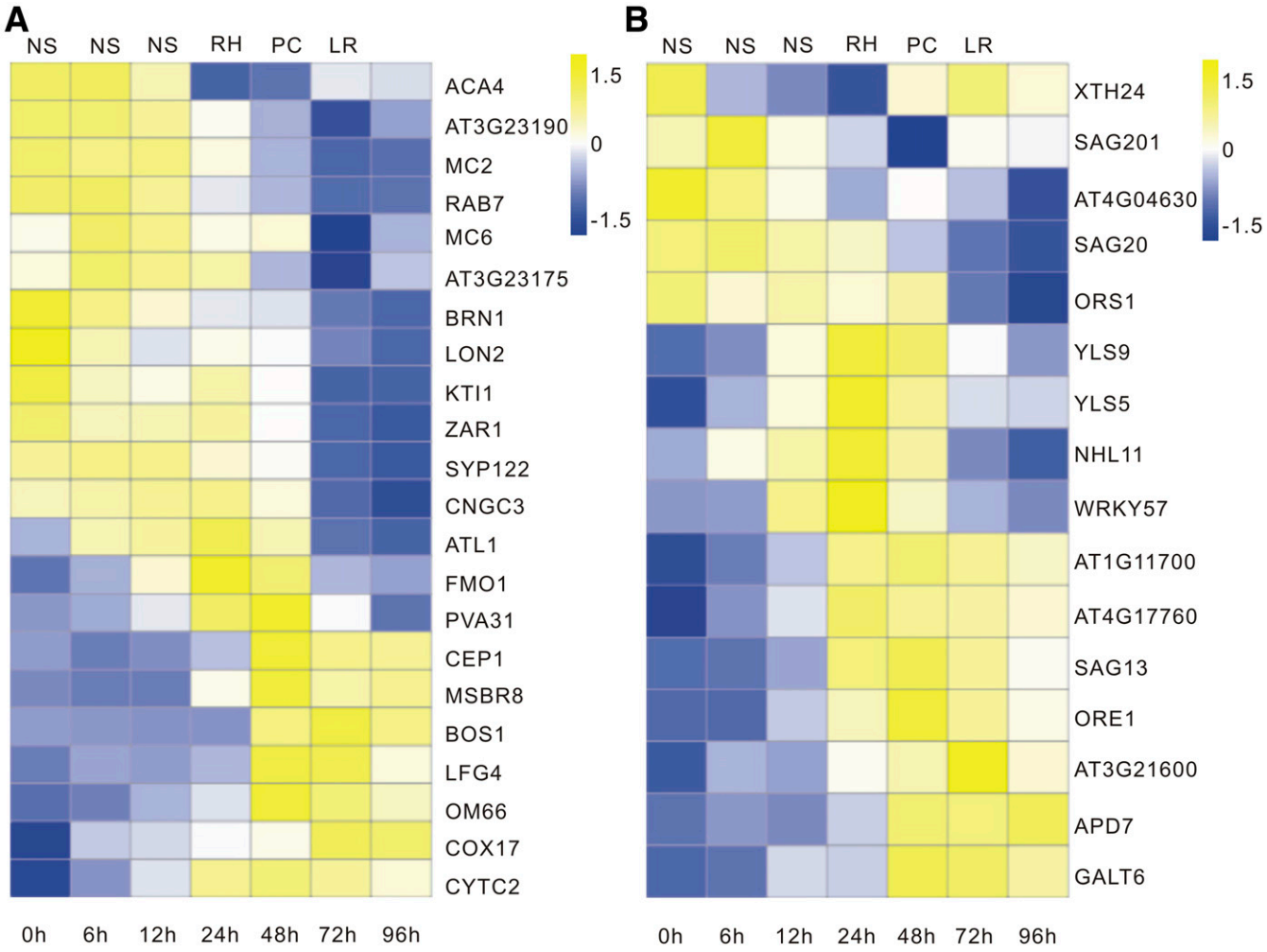

Fig. 6. Expression dynamics of components of programmed cell death over the infection time. **A,** Heat map depicting differentially expressed genes in effector-triggered hypersensitive responses. **B,** Heat map representation of differentially expressed components of senescence. Heat map values represent $\log_{10}$-transformed FPKM (fragments per kilobase per million reads) values. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence.

catalyzed the final step in gibberellic acid biosynthesis (Mitchum et al. 2006). *CLE6* overexpression in a *ga3ox* mutant partially restored primary root growth (Bidadi et al. 2014). Therefore, coordinated expression of positive and negative regulators may control root growth inhibition induced by *R. solanacearum*.

The last morphogenetic change observed in infected roots was enhanced appearance of secondary roots at 72 hpi. The transcript levels of the lateral root formation repressors CLE1, CLE3, and GLIP2 (Lee et al. 2009; Araya et al. 2014) were significantly decreased during the LR stage. In addition, the transcript of secondary root positive regulator *GATA23* was induced at 24 hpi and was repressed from 48 to 96 hpi (Fig. 8). Interestingly, the effect of *GATA23* on secondary root formation is auxin-mediated (De Rybel et al. 2010; Lally et al. 2001; Lee and Kim 2013; Xie et al. 2000), which suggests that auxin might control this root response to *R. solanacearum*.

DISCUSSION

R. solanacearum causes genome-wide transcriptional reprogramming in *Arabidopsis*.

Transcriptional reprogramming in aboveground tissue following soil-drenching with *R. solanacearum* has been previously reported in *Arabidopsis* (Feng et al. 2012; Hu et al. 2008). Leaf transcriptome analysis from susceptible plants showed that 40% of the upregulated genes were involved in ABA biosynthesis and signaling (Hu et al. 2008), which is in line with our root transcriptome results. Similarly, Feng and colleagues (2012) found that 26% of the upregulated genes in the leaf transcriptome pretreated with a nonpathogenic *Ralstonia* strain were also involved in ABA biosynthesis and signaling. These indicate ABA signaling is triggered by pathogenic and nonpathogenic invasion and may function in root defense against *R. solanacearum*. Very few SA-associated genes were found in our root transcriptome, which also happened in the leaf transcriptome (Hu et al. 2008), corroborating the notion that SA does not have a key role in plant responses against many root-pathogenic bacteria. Moreover, several genes involved in auxin signaling were down-regulated in the leaf transcriptome (Hu et al. 2008). In contrast, the auxin biosynthesis, signaling, and transport pathways were significantly induced in the root transcriptome reported here. This discrepancy in results could be partly caused by the different inoculation methods employed (soil drench versus in-vitro infection) and different tissues used in the experiment (leaf versus root). The commonalities and differences in global transcriptional responses between root and shoot occurred during *Plasmodiophora brassicae*, *Magnaporthe oryzae*, and

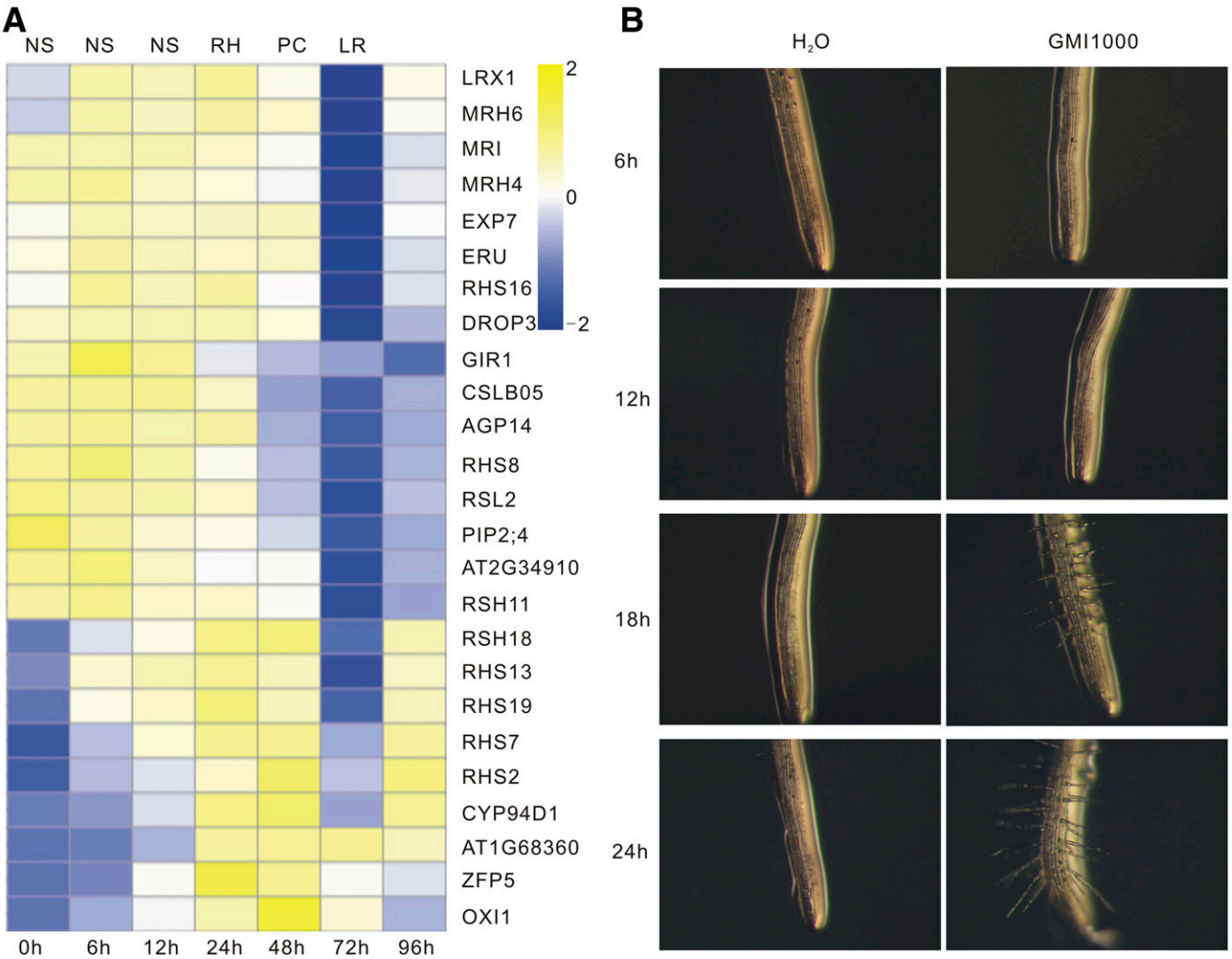

Fig. 7. Expression of genes related to root-hair formation. **A,** Heat map representation of differentially expressed genes in root-hair formation after GMI1000 infection. Heat map values represent $\log_{10}$-transformed FPKM (fragments per kilobase per million reads) values. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence. **B,** Root hair appeared at 18 h after GMI1000 infection. The pictures were taken with an Olympus microscope at the indicated time after infection.

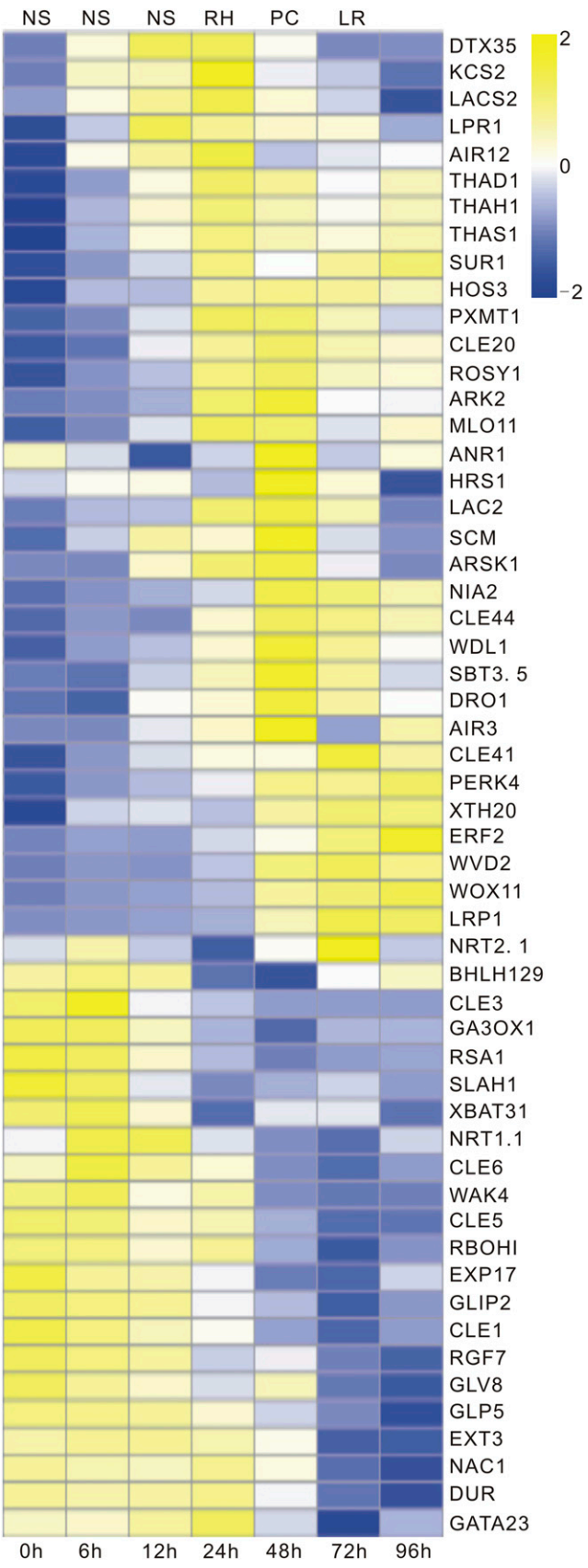

Fig. 8. Transcriptional dynamic changes of differentially expressed genes in root architecture. Heat map values represent $\log_{10}$-transformed FPKM (fragments per kilobase per million reads) values. Phenotypic stages: NS = no symptoms, RH = root-hair emergence, PC = primary root growth arrest and cell death, and LR = lateral root emergence.

Fusarium oxysporum infection (Irani et al. 2018; Lyons et al. 2015; Marcel et al. 2010), all of which indicates plant defense response is tissue-specific, suggesting more attention should be paid on plant root defense instead of leaf defense when the interaction between plant and soil-borne parasites is studied.

Root transcriptomes of *Arabidopsis* mutants *wat1* and *clv2* in response to *R. solanacearum* have been investigated (Denance et al. 2013; Hanemian et al. 2016). The GO term 'response to abiotic or biotic stress' over-represented in our transcriptome was also enriched in the root transcriptome data of *wat1* and *clv2* in response to *R. solanacearum*. Interestingly, the genes encoding enzymes and regulators of the indole glucosinolate biosynthetic pathway downregulated in noninoculated *wat1* roots were up-regulated during the RH infection stage but was not detected in the *clv2* root transcriptome. Similarly, the genes involved in JA biosynthesis and signaling that were up-regulated at the LR stage in our transcriptome were down-regulated in the *wat1* root transcriptome (Denance et al. 2013). The opposite expression pattern between our root transcriptome and *wat1* root transcriptome may result from the sensitivity difference of Col-0 and *wat1* to *R. solanacearum*. NF-YA transcriptional factors upregulated in *clv2* root were not identified in either our transcriptome or the *wat1* root transcriptome (Hanemian et al. 2016). It could be that NF-YAs are specific for CLV2-mediated plant defense against *R. solanacearum*. Recently, *Arabidopsis* root transcriptome response to soil-borne oomycete and fungi has been studied (Irani et al. 2018; Iven et al. 2012; Le Berre et al. 2017). Cell wall organization–related genes were over-represented in our transcriptome and were categorized into upregulated cluster IV and downregulated cluster IX, implying cell-wall modulation plays an important role for the interaction between *R. solanacearum* and *Arabidopsis*. In root response to obligate parasite *Plasmodiophora brassicae* infection, cell wall–modification genes linked to cell-wall loosening were up-regulated while the genes encoding cell-wall degradation were strongly down-regulated (Irani et al. 2018). The genes involved in cell wall loosening were also detected in root during the onset of *Phytophthora parasitica* infection (Le Berre et al. 2017). Cell wall was also highlighted in the category 'cellular compartment' in the root transcriptome infected with the fungal vascular pathogen *Verticillium longisporum* (Iven et al. 2012). Therefore, it seems that alteration of cell wall structure happens in root response to diverse soil-borne pathogens, such as bacterium, oomycetes, and fungi, implying the importance of the cell wall in plant root defense to soil-borne pathogens. The phenylpropanoid pathway up-regulated during the RH stage in our transcriptome was also found in the *Plasmodiophora brassicae*–infected root but not in *Phytophthora parasitica*– and *Verticillium longisporum*–infected root (Irani et al. 2018; Iven et al. 2012; Le Berre et al. 2017). Glucosinolate biosynthetic genes were transcriptionally activated in *Arabidopsis* root transcriptome early response to *R. solanacearum* and *Verticillium longisporum* but not to *Phytophthora parasitica* and *Plasmodiophora brassicae* (Irani et al. 2018; Iven et al. 2012; Le Berre et al. 2017), indicating the secondary metabolism in root after infection shows some species-specificity. The ET, JA, ABA, and auxin signaling pathway genes were differentially expressed in response to *R. solanacearum* in our transcriptome. JA, ABA, and auxin signaling but not the ET response pathways were activated in *Arabidopsis* root infected with *Plasmodiophora brassicae* (Irani et al. 2018). Only JA- and ET- mediated pathways in root were altered in the early infection of *Phytophthora parasitica* (Le Berre et al. 2017). Interestingly, very few SA-related genes were detected in the root transcriptome in response to *R. solanacearum*, *Plasmodiophora brassicae*, *Phytophthora parasitica*, and *Verticillium longisporum* (Irani et al. 2018; Iven

et al. 2012; Le Berre et al. 2017). All of these observations indicate hormones play different roles in successful infection of different soil-borne pathogen.

R. solanacearum manipulates different plant hormonal pathways.

Plant hormones are well-known to synergistically or antagonistically affect each other's output, leading to plant resistance or susceptibility to various pathogens (Berens et al. 2017). Therefore, phytopathogens have acquired abilities to hijack plant hormones to promote their proliferation in the host (Ma and Ma 2016). ET and JA signals have been shown to be the main target of many virulence factors produced by biotrophic and hemibiotrophic phytopathogens, due to their negative roles in plant immunity against biotrophic pathogens via SA antagonism (Berrocal-Lobo et al. 2002; Kloek et al. 2001). ET is produced by many plant pathogens, including the bacterial pathogens *Pseudomonas syringae* and *R. solanacearum* (Valls et al. 2006; Weingart and Volksch 1997). Disruption of ET production affects the virulence of *P. syringae* on soybean and bean (Weingart et al. 2001). In *R. solanacearum,* mutation of an ET-forming enzyme (*RsEFE*) did not affect its proliferation on its plant host (Valls et al. 2006). ET insensitivity in tobacco and *Arabidopsis* decreases plant defense against different soilborne fungi pathogens (Berrocal-Lobo and Molina 2004; Berrocal-Lobo et al. 2002; Geraats et al. 2002). However, plants defective in ET signaling (*ein2* mutants) show delayed wilting symptoms after *R. solanacearum* infection (Hirsch et al. 2002). Our transcriptome data showed that *R. solanacearum* highly induces expression of *ACS* genes in the roots, which suggests that, besides directly producing ET, *R. solanacearum* employs another, unknown strategy to activate endogenous ET.

P. syringae virulence factors such as coronatine, HopZ1a, HopX1, and AvrB activate JA signaling by promoting degradation of JAZ proteins in JA signaling (Gimenez-Ibanez et al. 2014; Jiang et al. 2013; Melotto et al. 2006; Zhou et al. 2015). Activation of JA signaling leads to entry of the phytopathogen into apoplast by reopening closed stomata and attenuates SA-dependent plant defense (Melotto et al. 2006; Zhou et al. 2015). Hernández-Blanco et al. (2007) reported that mutation in the JA-Ile receptor gene *COI1* conferred plant resistance to *R. solanacearum*. Our data showed JA biosynthesis and degradation genes (*LOX1, LOX4*, and *KAT5*) were differentially expressed at the RH stage and *JAZ* genes were mainly induced at the LR stage and at 96 hpi, suggesting that the JA signaling pathway was activated and then quickly inhibited during *R. solanacearum* infection. This sequential activation and inactivation of JA signaling was also found in plant root response to *Plasmodiophora brassicae* (Gravot et al. 2012; Irani et al. 2018; Lemarié et al. 2015). The *jai3-1*, *jar1-1*, and *dde2* mutants with disabled JA biosynthesis or signaling showed similar root architectures as wild-type plants in response to this pathogen (Lu et al. 2018), indicating that JA may be involved in plant response to the bacteria but not in the root morphogenesis changes caused by *R. solanacearum*.

Auxin signaling and transport has been reported to be manipulated by phytopathogens to suppress activation of SA-dependent defense. The *P. syringae* effector AvrRpt2 activated auxin biosynthesis and induced expression of auxin-response genes by promoting degradation of key negative regulators of auxin signaling, AUX/IAAs. The effector HopM1 from *P. syringae* and PSE1 from *Phytophthora parasitica* disrupted auxin transport by affecting expression or localization of different PIN auxin transporters, which promotes pathogen infection by antagonizing SA signaling (Chen et al. 2007; Cui et al. 2013; Evangelisti et al. 2013; Nomura et al. 2006; Tanaka et al. 2013). Many plant pathogens, including *R. solanacearum*, produce auxin-like molecules that may alter auxin homeostasis and affect auxin signaling in the host plants (Glickmann et al. 1998; Manulis et al. 1994; Robert-Seilaniantz et al. 2007; Valls et al. 2006). Interestingly, we observed that auxin biosynthesis genes were up-regulated at the RH stage. Auxin signaling and transport genes were also up-regulated at the PC and LR stages. In line with our data, the expression of *DR5*, a marker gene of the auxin signaling pathway, was strongly induced in root vascular after *R. solanacearum* GMI1000 infection (Lu et al. 2018). Moreover, the *dgl1*-1 tomato mutant with disordered auxin transport was highly resistant to *R. solanacearum* (French et al. 2018). Together, these data strongly support that auxin signaling plays a negative role in root defense against *R. solanacerum*. A deeper understanding of the role of auxin signaling in plant susceptibility to *R. solanacearum* awaits further investigation.

ABA also plays an important role in attenuating plant defense, possibly by inhibiting SA signaling (Cao et al. 2011). The increase of the ABA level in infected plants enhanced plant susceptibility to bacterial pathogen *P. syringae*, fungus *Magnaporthe grisea*, and nematode *Hirshcmaniella oryzae* (de Torres-Zabala et al. 2007; Jiang et al. 2010; Nahar et al. 2012). In turn, various pathogenic fungi have been shown to produce ABA or manipulate plant ABA signaling (Ma and Ma 2016). The effectors AvrPtoB and HopAM1, produced by *P. syringae*, enhanced plant susceptibility to the bacterial infection by promoting ABA biosynthesis or affecting ABA signaling (de Torres-Zabala et al. 2007; Goel et al. 2008). ABA also could positively regulate plant defense to *P. syringae*. For example, ABA induced stomata closure, locking the pathogen outside the host upon pathogen encounter and protecting the plant from pathogen infection (Melotto et al. 2006). A large number of ABA-responsive genes were up-regulated in plants infected with the nonvirulent *R. solanacerum* mutant *ΔhrpB* and in CESA4/CESA7/CESA8-mediated resistance to *R. solanacearum* (Feng et al. 2012; Hernández-Blanco et al. 2007). *abi1-1* and *abi2-1*, two ABA-insensitive mutants, exhibited more sensitivity to *R. solanacearum* and disabled *ΔhrpB*-triggered plant resistance, indicating ABA plays a very important role in plant defense to *R. solanacearum* (Feng et al. 2012; Hernández-Blanco et al. 2007). Here, we show that ABA signaling in the root is turned on at the PC stage, much earlier than activation of ABA signaling in leaf. Further genetic analysis demonstrated that simultaneous disruption of ABA receptors (*12458* and *112458*) dramatically accelerated plant wilting symptoms after *R. solanacerum* infection. Coincidently five ABA receptor genes (*PYR1*, *PYL1*, *PYL2*, *PYL4*, and *PYL8*) are highly expressed in the stele of roots (Antoni et al. 2013; Gonzalez-Guzman et al. 2012). Interestingly, ABA receptor mutants were still sensitive to root-hair formation, root growth inhibition, and lateral root formation caused by *R. solanacearum*. This indicates that ABA signaling is not essential for *R. solanacearum*–triggered root architecture changes. However, the precise mechanism by which ABA promotes defense to this bacteria still needs to be further elucidated.

All these data indicate that the interplay between *R. solanacearum* and *Arabidopsis* is mediated by a complex interplay of hormones. In particular, a synergistic effect among JA, ET, SA, ABA, and auxin seem to determine the outcome of the interaction between *R. solanoncearum* and plants. Our data provides new insight into the signaling network that occurs in the root host in response to root pathogen.

R. solanaceaum infection triggers specific defense responses in the root.

PTI and ETI (effector-triggered immunity) are the two layers of defense that plants pose to phytopathogens (Jones and Dangl

2006). In our RNA-seq data, we identified several components of both defense branches that are consistent with reports that PAMPs elicit transcriptional changes and callose deposition in *Arabidopsis* roots and the effector RBP1 from root nematode *Globodera pallida* triggers Gpa2-dependent resistance and cell death (Millet et al. 2010; Sacco et al. 2009). The transcript levels of PTI signaling components *LYK4*, *PUB22*, *PUB23*, *PEP1* and *MPK11*, are quickly induced upon infection. Interestingly, all these PTI-related genes were inhibited at the LR stage, suggesting that PTI in the root probably is activated by sensing PAMPs from *R. solanacearum*, then, quickly turned off after infection. In addition, we also found around 19 NBS-LRR resistance genes in DEGs, including ZAR1. ZAR1 detects acetylated hopz-ETI-deficient 1 (ZED1) by the *P. syringae* effector HopZ1a and triggers ETI (Lewis et al. 2010, 2013). This suggests that this NBS-LRR might be involved in *R. solanacearum* effector recognition.

Hypersensitive response (HR) a local cell death at the attempted entry site of pathogens, often accompanies ETI. Cell death was observed on root tips at the PC stage of *R. solanacearum* infection. Interestingly, the occurrence of *R. solanacearum*–mediated cell death at the root tip was dependent on the presence of a functional T3SS (Lu et al. 2018), indicating that this cell death occurs via effector recognition. Thus, cell death after infection probably results from ETI. HR in leaf is thought to directly kill invaders and/or to interfere biotrophic pathogen with acquisition of nutrients (Heath 2000). But cell death in root seems not to affect the virulence of GMI1000 on *Arabidopsis*, as GMI1000 is a compatible strain on *Arabidopsis*. Necrotrophic pathogen triggers cell death in order to obtain more nutrients that helps them accomplishing their life cycle (Glazebrook 2005). What are the benfits *R. solanacearum* gets from cell death needs to be answered.

Root morphogenesis changes triggered by *R. solanacearum* infection are accompanied by deep transcriptional reprogramming of genes involved in root architecture.

The root is embedded in the soil and its architecture determines the efficiency for nutrient uptake and aboveground growth. Root architecture is often shaped by biotic stress and abiotic stress such as interaction with mutualist microbes and elements deficiency (Le Fevre et al. 2015). *Arabidopsis* roots after GMI1000 infection also showed root architecture changes such as root-hair formation, primary root growth inhibition, and lateral root formation. In accordance with this, the transcription levels of genes participated in regulating root architecture were up- or down-regulated in our transcriptome data. Besides GMI1000, several other *R. solanacearum* strains also cause root morphological changes (Lu et al. 2018). Furthermore, the *R. solanacearum*–triggered root morphogenesis changes have been observed in tomato (Vasse et al. 1995), petunia (Zolobowska and Van Gijsegem 2006), *Medicago truncatula* (Turner et al. 2009), and *Arabidopsis* (Digonnet et al. 2012; Lu et al. 2018). Inhibition of primary root growth after infection is a common feature in all plant species investigated. Swelling of the root tip in tomato, petunia, and *M. truncatula* did not appear in *Arabidopsis* after infection. Promotion of lateral root growth after infection was only found in petunia and *Arabidopsis*. Root hair formation after infection only happened to *Arabidopsis*. These suggest, in addition to some common root features, phenomena associated with *R. solanacearum* are species-specific. In petunia, *R. solanacearum* infection resulted in root lateral structure formation. The structure resembled abnormal lateral roots and were efficient colonization sites (Zolobowska and Van Gijsegem 2006). Moreover, emerging lateral roots are found to be entry sites of *R. solanacearum* in tomato (Vasse et al. 1995). Therefore, it is possible that formation of lateral roots in *Arabidopsis* after infection offers more entry sites for *R. solanacearum*.

The root morphogenesis changes are reminiscent of root morphological changes triggered by plant growth–promoting bacteria and rhizobacteria or fungi (Verbon and Liberman 2016). These beneficial microbes affect cell division at the root meristem region and cell differentiation at sites of lateral root formation through manipulating endogenous hormone levels and hormone signaling, such as auxin biosynthesis and signaling, resulting in root structure changes (Verbon and Liberman 2016). Our transcriptomic analysis indicated that auxin synthesis, signaling, and transport in root are all activated by *R. solanacearum* colonization. The auxin-insensitive single mutant *tir1* and double mutant *tir1/afb2* were unable to form root hair in response to *R. solanacearum* infection (Lu et al. 2018). In consonance with this, IAA28, controlling the specification and identity of lateral root founder cells, was upregulated in our data (De Rybel et al. 2010). This suggests that activation of auxin signaling might be the reason for root morphogenesis changes in response to *R. solanacerum*. However, activation of auxin signaling enhanced plant sensitivity to *P. syringae*, *Xanthomonas oryzae*, and *Magnaporthe oryzae* (Kazan and Lyons 2014) In addition, destruction of polar auxin transport in tomato tremendously elevated plant resistance toward *R. solanacearum* infection. All of these suggest auxin signal plays critical roles in plant defense to diverse phytopathogens. This also poses the question of whether the observed *R. solanacearum*–triggered architecture changes are side effects of elevated auxin levels used by *R. solanacearum* to accomplish successful colonization or not. In addition, it is still not clear why *R. solanacearum* and plant growth–promoting rhizobacteria induce similar root architectures but exert two opposite influences on plant survival and what benefits (if any) *R. solanacearum* obtains by altering root structure.

MATERIALS AND METHODS

Plants materials.

In this study, *Arabidopsis thaliana* Col-0 and the ABA receptor mutants *12458* and *112458* were sown in soil and were grown in the chamber at 23°C, short day conditions (8 h of light, light intensity 12,000 lux), and 70% humidity. For *Arabidopsis* seedling growth, Col-0 seeds were sterilized with 30% bleach and 0.02% TritonX-100, were then sown on a Murashige Skoog (MS) without sucrose plates and were grown with the plates set vertically at 25°C and with long-day conditions (16 h of light, light intensity 9,000 lux for 6 to 7 days).

***R. solanacearum* infection.**

The strain *R. solanacearum* GMI1000 was used to infection in this study. For the soil-drench infection assay, 5-week-old plants were watered with a suspension of 1×10^8 CFU. One hour later, roots of the infected plants were wounded three times with a blade, were then grown into the chamber at 25°C, 16 h of light. Leaf-wilting symptoms were photographed at the indicated timepoint. Moreover, disease development for each infected plant was scored at the indicated time, using a disease index scale ranging from 0 to 4, according to the percentage of wilted leaves (0 = no symptoms, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100%) (Poueymiro et al. 2009). Mortality rate (the total number of completely wilted plants with disease index score of 4 divided by the total number of inoculated plants for each group) was used to exhibit plant sensitivity to *R. solanacearum*. Meantime disease index scores of each plant in each group were used for statistical tests.

For in vitro infection, we used the method previously described by Lu et al. (2018). Briefly, 6- to 7-day-old *Arabidopsis*

seedlings grown on MS plates were inoculated 1 cm away from the root tip with a droplet of a solution containing 1×10^7 CFU of *R. solanacearum* GMI1000, then, were kept in a growth chamber as detailed above. Root structures were photographed at indicated timepoints with an Olympus SZX16 microscopy camara and lateral roots were counted at the indicated times. For the cell-death assay, seedlings were immersed into a 0.1-mg/ml propidium iodide solution and were observed under an Olympus confocal microscope IX83-FV1200.

Sample preparations for RNA-seq.

Root samples were collected from around 600 infected seedlings at the indicated timepoint, were frozen in liquid nitrogen, and were then sent directly to Novogene Company, which performed RNA sequencing and data analysis (Supplementary Methods).

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