The bile acid deoxycholate elicits defences in Arabidopsis and reduces bacterial infection

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SUMMARY

Disease has an effect on crop yields, causing significant losses. As the worldwide demand for agricultural products increases, there is a need to pursue the development of new methods to protect crops from disease. One mechanism of plant protection is through the activation of the plant immune system. By exogenous application, 'plant activator molecules' with elicitor properties can be used to activate the plant immune system. These defence-inducing molecules represent a powerful and often environmentally friendly tool to fight pathogens. We show that the secondary bile acid deoxycholic acid (DCA) induces defence in Arabidopsis and reduces the proliferation of two bacterial phytopathogens: *Erwinia amylovora* and *Pseudomonas syringae* pv. *tomato*. We describe the global defence response triggered by this new plant activator in Arabidopsis at the transcriptional level. Several induced genes were selected for further analysis by quantitative reverse transcription-polymerase chain reaction. We describe the kinetics of their induction and show that abiotic stress, such as moderate drought or nitrogen limitation, does not impede DCA induction of defence. Finally, we investigate the role in the activation of defence by this bile acid of the salicylic acid biosynthesis gene *SID2*, of the receptor-like kinase family genes *WAK1–3* and of the NADPH oxidase-encoding *RbohD* gene. Altogether, we show that DCA constitutes a promising molecule for plant protection which can induce complementary lines of defence, such as callose deposition, reactive oxygen species accumulation and the jasmonic acid and salicylic acid signalling pathways.

Keywords: bile acid, elicitor, *Erwinia amylovora*, plant defence, *Pseudomonas syringae*, SID2, WAK.

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INTRODUCTION

Plants have developed sophisticated protection mechanisms against the biotic and abiotic stresses to which they are exposed. The plant immune system consists of two closely interconnected defensive levels (Tsuda and Katagiri, 2010). The first line of defence is induced by the recognition of conserved molecular structures of pathogens, such as lipopolysaccharide (LPS), flagellin and chitin, which are collectively called microbe (or pathogen)-associated molecular patterns (MAMPs or PAMPs). This first line of defence is defined as PAMP-triggered immunity (PTI) and confers a wide-spectrum protection against phytopathogens. PTI is triggered on perception of MAMPs by plasma membrane pattern recognition receptors (PRRs) (Zipfel, 2008).

The second defensive level, defined as effector-triggered immunity (ETI), is specifically induced by pathogens that have evolved strategies to overcome PTI using protein effectors that function inside the plant cell. The recognition of such molecules, mediated by specific R proteins, is initiated by a more rapid and stronger type of response than PTI (Jones and Dangl, 2006). PTI and ETI are associated with common downstream responses (Tsuda *et al.*, 2009). In particular, transcriptional reprogramming, an early oxidative burst at the site of infection and changes in the concentration of hormones, such as salicylic acid (SA) and jasmonic acid (JA), have been observed. Other phytohormones, such as abscisic acid (ABA), cytokinins and ethylene (ET), are involved in the control of these signalling pathways (Pieterse *et al.*, 2012). Furthermore, another important defensive strategy related to ETI is the hypersensitive response (HR) (Jones and Dangl, 2006). The HR consists of the persistent production of several signal compounds, such as reactive oxygen species (ROS), nitric oxide (NO) and SA, coupled to Ca^{2+} cytoplasmic influxes, leading to a programmed cell death (PCD) at the site of infection (Mur *et al.*, 2008). To date, ETI has been found to be effective almost exclusively against biotrophic and hemibiotrophic pathogens, but not against necrotrophic pathogens (Mengiste, 2012).

Pests and pathogens affect the potential yield of crops worldwide, in terms of both quantity and quality. It has been estimated

that, in spite of current crop protection methods, pests and pathogens reduce crop production by 20%–40% (Oerke and Dehne, 2004; Savary *et al.*, 2012). Furthermore, the effect of disease on crop yield is heterogeneous in time and space as a result of environmental factors, such as edaphic and climatic factors. Because crop yields are significantly affected by disease and because the worldwide demand for agricultural products is increasing, there is a need to pursue the development of new methods to protect crops from disease, as well as to understand the underlying mechanisms. In parallel with classical and molecular breeding strategies to reduce crop losses caused by pathogen attacks, novel strategies are currently being developed to increase host recognition of the pathogen, interfering with the virulence arsenal and improving plant immunity (Gust *et al.*, 2010). One method of plant protection against disease is through the activation of its immune system. Indeed, the plant immune system can be activated by the exogenous application of molecules with elicitor properties (Noutoshi *et al.*, 2012c; Schreiber and Desveaux, 2008; Zarattini *et al.*, 2015). These defence-inducing molecules include pathogen-derived molecules, such as glucan, *N*-deacetylated chitin (chitosan) and pathogen effectors (Gust *et al.*, 2010), as well as other natural substances not derived from the pathogen, such as plant hormones. These molecules are collectively defined as 'plant activators' and represent a powerful and often environmentally friendly tool to fight pathogens (Noutoshi *et al.*, 2012a,b, c). Moreover, in contrast with antimicrobial compounds, such as fungicides or antibiotics, which limit microbe propagation, plant activators act on plant resistance, thus avoiding the occurrence of pathogen resistance (Noutoshi *et al.*, 2012c; Zarattini *et al.*, 2015). In this context, bile acids have recently attracted increasing attention in plant science for their capacity to elicit plant defences (Koga *et al.*, 2006). Bile acids are part of the steroid class of lipids that play key roles in animals. For example, it is well established that bile acids can dissolve cholesterol and other lipids (Rotunda *et al.*, 2004), enhance lipid absorption into the small intestine (Hofmann, 1999) and play a role in signalling to control certain aspects of metabolism (Li and Chiang, 2011).

It has been described by Koga *et al.* (2006) that bile acids, if applied directly on rice plants, can protect rice against the fungus *Magnaporthe grisea*. These authors showed that increased protection against the pathogen in rice plants was associated with the accumulation of an antimicrobial phytoalexin molecule, phytocassane, increased glucanase activity and electrolyte leakage. In soybean, bile acids trigger superoxide ($O_2^{\bullet-}$) and hydroxyl radical ($\cdot OH$) accumulation and increase the content of the antioxidant glutathione (GSH) and superoxide dismutase (SOD) enzymes (Kevresan *et al.*, 2009; Malencic *et al.*, 2012), suggesting that bile acids trigger an HR in plant cells. These reports show that bile acids can elicit plant defences; however, the knowledge of their elicitor properties in plants is still limited.

Bile acids are divided into two categories based on their precursor. Primary bile acids are formed directly from cholesterol, whereas secondary bile acids are derived from primary bile acids (Okoli *et al.*, 2007). Deoxycholic acid (DCA) is a secondary bile acid that is preventatively removed during the industrial production of ursodeoxycholic acid (UDCA). Thus, DCA is a resulting industrial waste product (roughly 20% of bovine bile). The possibility to use an industrial waste product as a new tool to protect plants against pathogens, thanks to its elicitor properties, is appealing. Therefore, we decided to use Arabidopsis as a model plant to characterize the elicitor properties of DCA.

In this article, we show that DCA triggers defence activation in Arabidopsis and reduces the proliferation of two bacterial phytopathogens. We describe the global defence response triggered by DCA in Arabidopsis at the transcriptional level. Several induced genes were selected for further analysis by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and we describe the kinetics of their induction. Finally, we demonstrate that, in response to bile acid treatment, defence activation is affected in the SA-deficient *sid2-1* mutant and NahG transgenic line but not in the *wak1-1*, *wak2-1* and *wak3-1* mutants. We also show that ROS production in response to DCA is reduced in NADPH-defective *rbohD* mutant seedlings compared to wild-type seedlings.

RESULTS

DCA elicits defences in Arabidopsis

To evaluate the potential of DCA to prevent disease in plants, we first analysed the defence reactions in plants sprayed with an aqueous solution of DCA. We chose two concentrations of DCA: 20 μM, which is the concentration of cholic acid (CA) that is able to protect rice against *Magnaporthe grisea*, and 200 μM, which is the lowest concentration of CA that induces the maximum defence response (Koga *et al.*, 2006).

The first mechanical barrier encountered by pathogens during plant invasion is the cell wall. To evaluate whether DCA treatment is able to reinforce the cell wall, we investigated the formation of callose deposits through aniline blue staining of DCA-treated leaves. Five-week-old Arabidopsis Col-0 plants were sprayed with 20 μM DCA, 200 μM DCA or mock solution, and then stained with aniline blue at 24 h post-treatment (hpt). In response to treatment with 200 μM DCA, a visible amount of callose deposits could be observed in treated leaves compared with mock-treated leaves (Fig. 1A). The counting of callose spots using ImageJ software indicated that treatment with 200 μM DCA induced significantly ($P < 0.05$) more callose deposits than in mock-treated leaves (Fig. 1A). However, in response to treatment with 20 μM DCA, no visible callose deposits were observed in leaves (Fig. 1A), except in the hydathodes (Fig. S1, see Supporting Information).
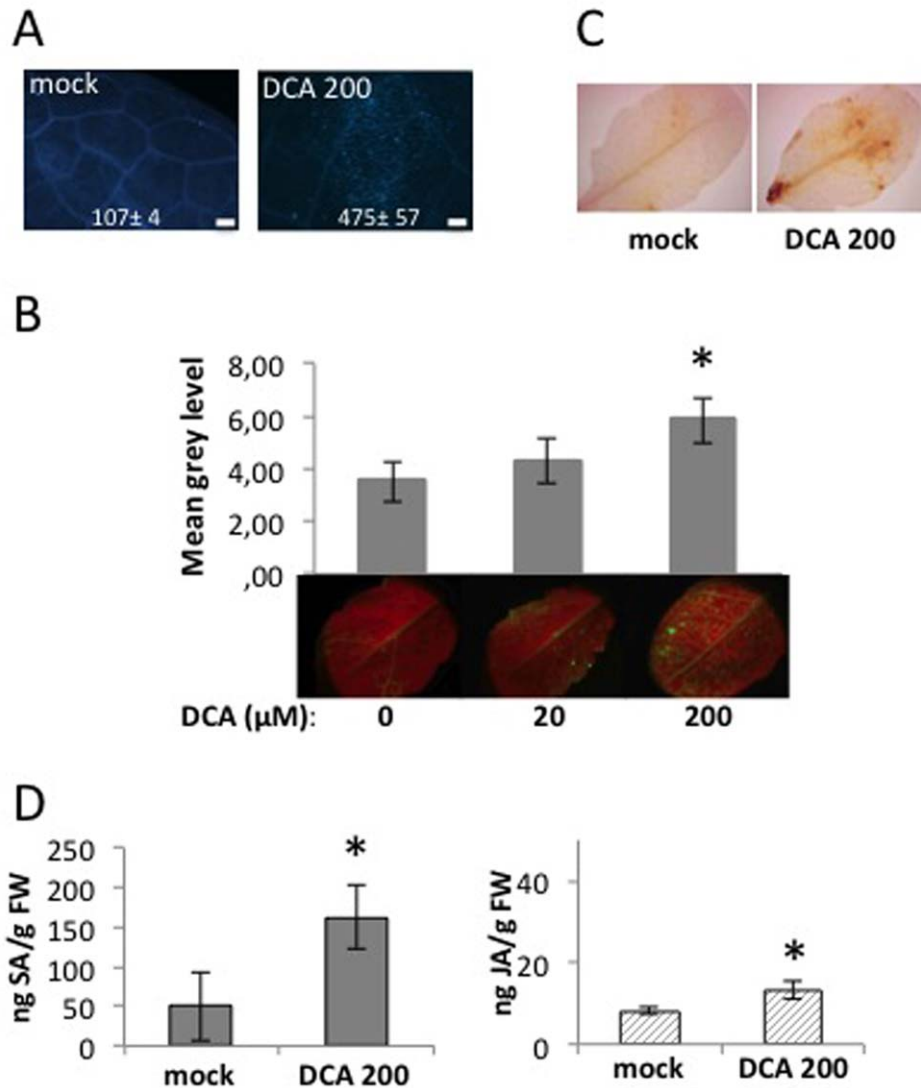
Fig. 1 Deoxycholic acid (DCA) induces defence accumulation in Arabidopsis leaves. Arabidopsis leaves of 5-week-old plants were sprayed with DCA at the indicated concentration (20 or 200 μM) or mock-treated. (A) Leaves were stained with aniline blue at 24 h post-treatment (hpt) to detect callose deposition. The bar represents 200 μm. The numbers in each photograph indicate the mean number of spots per photograph $\pm$ standard error of the mean (SEM). (B) Leaves were sampled at 16 hpt and inoculated with 2',7'-dichlorofluorescein diacetate (DCFH-DA). The fluorescence level of DCFH-DA, which reveals the presence of H_2O_2, was measured using ImageJ ($n = 9$). The bars represent the mean $\pm$ SEM. (C) Leaves were collected at 6 hpt and infiltrated with 3,3'-diaminobenzidine (DAB). The brown colour reveals the presence of H_2O_2. (A–C) Nine leaves ($n = 9$) from three plants were used for each condition. Three independent experiments were perfomed that gave similar results. Representative photographs are shown. (D) Quantification of salicylic acid (SA) and jasmonic acid (JA) contents using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). For each independent experiment, 20 leaves from four plants were collected ($n = 4$) at 24 hpt and ground for JA and SA extraction. The bars represent the standard deviation (SD). For SA quantification, two independent experiments were perfomed that gave similar and statistically significant results. For JA quantification, the results of three independent experiments were pooled. FW, fresh weight. (B, D) The asterisk indicates a significant difference from mock-treated leaves according to the Mann–Whitney test ($P < 0.05$).

One of the earliest cellular responses implemented by plants after pathogen recognition is the production of ROS, which is referred to as the oxidative burst. To estimate the effect of DCA treatment on ROS levels in Arabidopsis leaves, we first performed a specific histochemical assay using 2',7'-dichlorofluorescein diacetate (DCFH-DA) which fluoresces in the presence of intracellular hydrogen peroxide (H_2O_2). DCFH-DA staining was carried out at 16 hpt. The leaves with the lowest concentration of DCA (20 μM) showed only a weak increase in DCFH-DA fluorescence compared with mock-treated leaves, indicating that 20 μM DCA did not trigger a strong intracellular production of ROS. However, leaves treated with the highest DCA concentration tested (200 μM)

showed stronger DCFH-DA staining than mock or 20 μM DCA-treated leaves, indicating that 200 μM DCA induced noticeable ROS production (Fig. 1B). Quantification of DCFH-DA fluorescence using ImageJ software confirmed that only 200 μM DCA-treated leaves showed a significantly ($P < 0.05$) stronger fluorescence than mock-treated leaves, indicating that 200 μM DCA induced significant intracellular ROS production (Fig. 1B). These results indicate that, in Arabidopsis plants, DCA triggers significant ROS accumulation. As DCFH-DA has been shown to detect reactive nitrogen species, as well as ROS (Crow, 1997; Gomes *et al.*, 2005), we completed our analysis with a second histochemical assay using 3,3′-diaminobenzidine (DAB) which stains both intra- and extracellular H_2O_2. H_2O_2 production was examined at 6 hpt by DAB staining. Strong brown precipitates were observed in leaves treated with 200 μM DCA, whereas, in mock- (Fig. 1C) and 20 μM DCA-treated leaves (data not shown), only a light brown staining could be observed. These results confirm that DCA induces the production of H_2O_2 in Arabidopsis leaves.

Many studies have shown the importance of the SA- and JA-mediated defence responses in plant–pathogen interactions (Okada *et al.*, 2015; Pieterse *et al.*, 2012). To determine whether leaf endogenous phytohormone content is altered by DCA treatment, JA and SA levels were quantified 24 h after 200 μM DCA or mock application. As shown in Fig. 1D, a three-fold increase in SA concentration was observed in Arabidopsis plants treated with DCA compared with mock-treated plants. Moreover, a slight, but significant ($P < 0.05$), increase in JA accumulation was observed following treatment with 200 μM DCA (Fig. 1D).

As CA has been found to trigger necrosis in rice (Koga *et al.*, 2006), we determined whether DCA induced a similar reaction in Arabidopsis. To do so, we analysed necrosis induced by DCA treatment. Arabidopsis plants were treated with increasing DCA concentrations (0, 20, 100 and 200 μM). Necrosis was then estimated visually 24 h and 5 days after DCA treatment, assessing the symptom using the severity scale reported in Degrave *et al.* (2008). After 24 h, no necrosis was observed in any of the treated leaves. After 5 days, no visible necrosis could be observed in water- or 20 μM DCA-sprayed leaves; however, we observed slight necrosis symptoms in leaves sprayed with 100 μM DCA and more extensive necrosis with 200 μM DCA (Fig. S2, see Supporting Information).

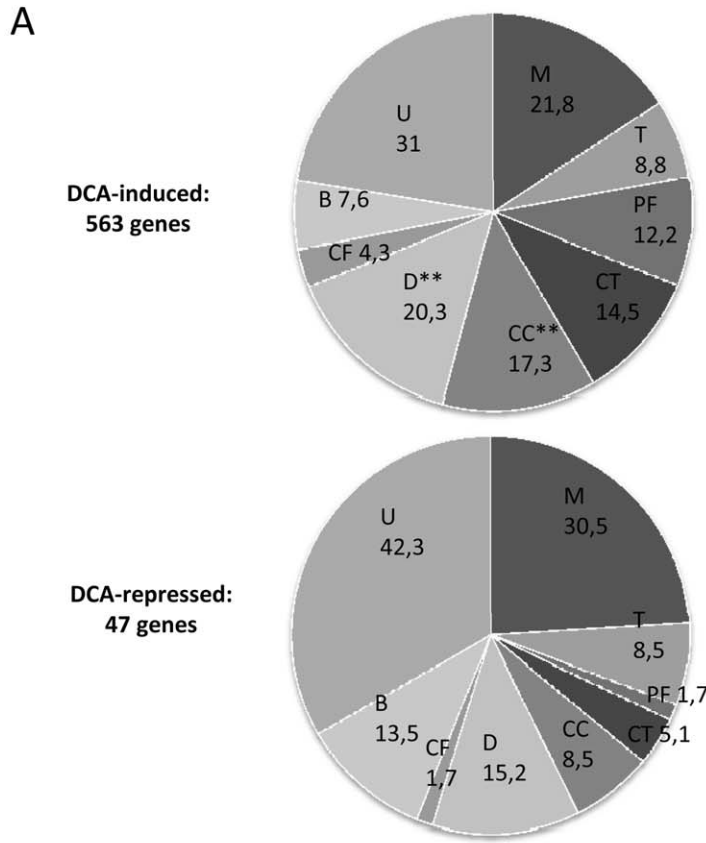Altogether, our results show that DCA is able to induce several lines of defence in Arabidopsis leaves.

DCA triggers changes in the transcriptome of Arabidopsis

In order to understand the molecular changes induced by DCA treatment, we analysed the transcriptome of Arabidopsis leaves following DCA treatment. Rosette leaves of 5-week-old Arabidopsis Col-0 plants were sprayed with 200 μM DCA or mock-treated,

and collected at 24 hpt. RNA was extracted and the gene expression level was analysed using Complete Arabidopsis Transcriptome Microarray (CATMA) chips, as described previously (Moreau *et al.*, 2012). Two biological replicates and a dye swap were performed; a third biological replicate was performed to analyse the profile of selected genes by qRT-PCR as presented later (see Figs 4–6). Statistical analysis of the transcriptome data revealed that 563 and 47 genes were significantly up- and down-regulated, respectively, 24 h after DCA treatment (Table S2, see Supporting Information).
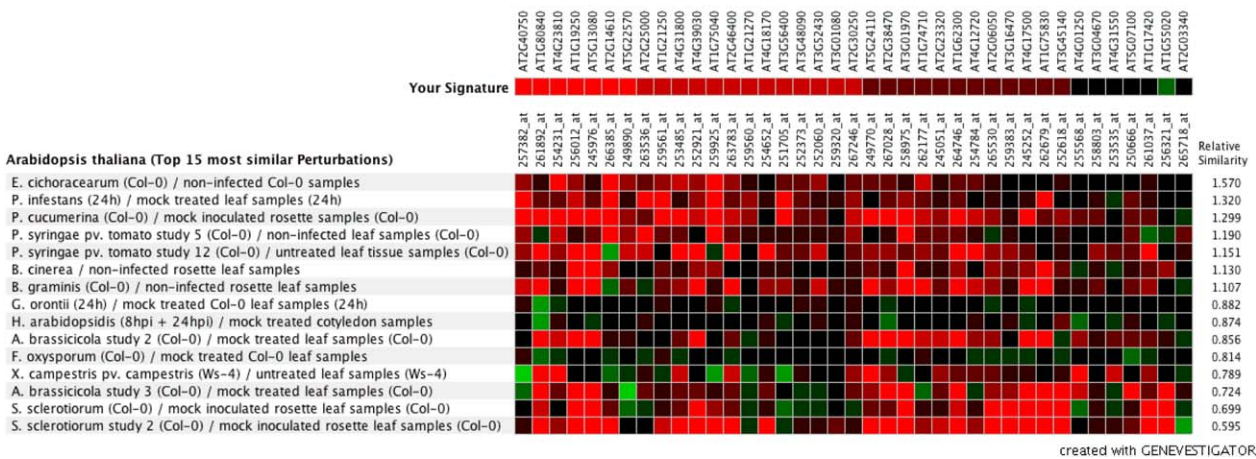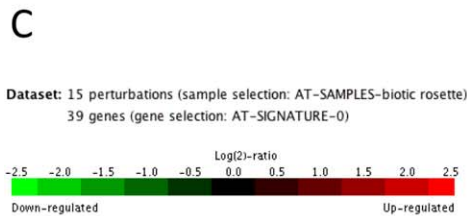
In order to gain an insight into the impact of DCA treatment on plants, we determined the putative functions of the genes modulated following DCA treatment using the MIPS Functional Catalogue Database (FunCatDB) (Ruepp *et al.*, 2004). This analysis revealed that a very large proportion of the 610 DCA-modulated genes (70%) were annotated as encoding proteins of known or predicted function (Fig. 2A), which is consistent with our previous analysis of pathogen response transcriptome datasets (Moreau *et al.*, 2012). Among the induced genes (Fig. 2A, top), the most represented functional categories were metabolism (21.8%), defence (20.3%), cellular communication (17.3%) and cellular transport (14.5%). The defence and cellular communication functional categories were both over-represented compared with the distribution of genes among the different functional categories in the Arabidopsis genome ($P < 0.005$). Among the repressed genes (Fig. 2A, bottom), the most represented categories were metabolism (30.5%), defence (15.2%) and biogenesis of cellular components (13.5%); however, none of these categories was over-represented compared with the whole genome ($P < 0.005$). Altogether, our results indicate that DCA triggers substantial changes in the gene expression of Arabidopsis and, in particular, induces changes in the expression of defence-associated genes.

As DCA induces the accumulation of defence-associated molecules, such as SA, JA, ROS and callose (Fig. 1), we analysed our transcriptome data to determine whether genes known to be associated with these defence responses were modulated by DCA treatment. *ICS1* encodes the SA biosynthesis enzyme isochorismate synthase and *EDS5* encodes the MATE-transporter required for the export of SA from the chloroplast (Serrano *et al.*, 2013). *LOX2* and *LOX3* encode lipoxygenases, and *OPR3* encodes a 12-oxo-phytodienoic acid (OPDA) reductase involved in JA biosynthesis (Dave and Graham, 2012; Wasternack and Hause, 2013). We also analysed genes that are known to be markers for the activation of these defence pathways, such as *PR1*, *PAD4*, *WAK1* and *WAK2* for the SA-dependent pathway and *PDF1*, *JR1* and *ATERF1* for the JA-dependent pathway. The results (Fig. 2B, DCA column) clearly indicate that the expression of both SA- and JA-associated genes is induced following DCA treatment, although the SA-associated genes appear to be more strongly induced. In particular, genes dependent on EDS1 are also induced by DCA. We also

A

DCA-induced: 563 genes

Pie chart slices: M 21,8; T 8,8; PF 12,2; CT 14,5; CC** 17,3; D** 20,3; CF 4,3; B 7,6; U 31

DCA-repressed: 47 genes

Pie chart slices: M 30,5; T 8,5; PF 1,7; CT 5,1; CC 8,5; D 15,2; CF 1,7; B 13,5; U 42,3

B

SA response/biosynthesis		DCA	*Ea*
EDS5	AT4G39030	2,61	1,31
PAD4	AT3G52430	2,11	3,25
ICS1	AT1G74710	1,71	2,66
PR1	AT2G14610	3,12	#N/A
WAK1	AT1G21250	2,76	#N/A
WAK2	AT1G21270	2,45	1,52
WAK3	AT1G21240	1,73	3,00
PR2	AT3G57260	4,98	
PR5	AT1G75040	2,59	
ET/JA response and biosynthesis			
ATERF-1	AT4G17500	1,22	1,32
LOX3	AT1G17420	0,83	1,32
OPR3	AT2G06050	1,44	
JR1	AT3G16470	1,39	
LOX2	AT3G45140	1,00	
LCR67/PDF1.1	AT1G75830	1,16	
LOX1	AT1G55020	-1,66	2,31
EDS1 pathway			
FMO1	AT1G19250	3,43	2,70
EDS1	AT3G48090	2,26	1,20
AtNUDT7	AT4G12720	1,51	2,10
WRKY TFs			
WRKY22	AT4G01250	0,99	2,96
WRKY75	AT5G13080	3,40	2,94
WRKY51	AT5G64810	3,45	2,89
WRKY46	AT2G46400	2,52	2,68
WRKY18	AT4G31800	2,66	2,45
WRKY38	AT5G22570	3,01	2,43
WRKY40	AT1G80840	3,72	2,26
WRKY25	AT2G30250	2,03	2,01
WRKY54	AT2G40750	3,81	1,99
WRKY70	AT3G56400	2,34	1,98
WRKY45	AT3G01970	1,75	1,92
WRKY6	AT1G62300	1,52	1,61
WRKY15	AT2G23320	1,61	1,47
WRKY53	AT4G23810	3,52	1,41
WRKY39	AT3G04670	0,96	1,26
WRKY11	AT4G31550	0,94	1,25
WRKY60	AT2G25000	2,80	1,05
WRKY28	AT4G18170	2,45	0,80
WRKY50	AT5G26170	3,86	
WRKY30	AT5G24110	1,98	
WRKY26	AT5G07100	0,55	
WRKY58	AT3G01080	2,04	
WRKY33	AT2G38470	1,78	#N/A
WRKY3	AT2G03340	-0,86	

C

Dataset: 15 perturbations (sample selection: AT-SAMPLES-biotic rosette)
39 genes (gene selection: AT-SIGNATURE-0)

Log(2)-ratio
-2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 2.5
Down-regulated   Up-regulated

Your Signature

Arabidopsis thaliana (Top 15 most similar Perturbations) — Relative Similarity

E. cichoracearum (Col-0) / non-infected Col-0 samples	1.570
P. infestans (24h) / mock treated leaf samples (24h)	1.320
P. cucumerina (Col-0) / mock inoculated rosette samples (Col-0)	1.299
P. syringae pv. tomato study 5 (Col-0) / non-infected leaf samples (Col-0)	1.190
P. syringae pv. tomato study 12 (Col-0) / untreated leaf tissue samples (Col-0)	1.151
B. cinerea / non-infected rosette leaf samples	1.130
B. graminis (Col-0) / non-infected rosette leaf samples	1.107
G. orontii (24h) / mock treated Col-0 leaf samples (24h)	0.882
H. arabidopsidis (8hpi + 24hpi) / mock treated cotyledon samples	0.874
A. brassicicola study 2 (Col-0) / mock treated leaf samples (Col-0)	0.856
F. oxysporum (Col-0) / mock treated Col-0 leaf samples	0.814
X. campestris pv. campestris (Ws-4) / untreated leaf samples (Ws-4)	0.789
A. brassicicola study 3 (Col-0) / mock treated leaf samples (Col-0)	0.724
S. sclerotiorum (Col-0) / mock inoculated rosette leaf samples (Col-0)	0.699
S. sclerotiorum study 2 (Col-0) / mock inoculated rosette leaf samples (Col-0)	0.595

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analysed in more detail the transcriptome data of the WRKY family of transcription factors (TFs), one of the largest families of plant transcriptional regulators. Although WRKY TFs play a role in many plant processes, the major role of this family is in the control of the response of plants to biotic stress (Rushton *et al.*, 2010). Our data indicate that DCA induced the expression of 23 WRKY-

encoding genes, several of which have been shown previously to be involved in the plant's immune response, such as WRKY18, WRKY33, WRKY40, WRKY46 and WRKY70 (Pandey and Somssich, 2009; Rushton *et al.*, 2010).

In order to determine whether the plant's transcriptional response to DCA resembled the plant's immune response, we compared the data obtained in response to DCA treatment with previously described data. We used data obtained in response to different pathogens available in the Genevestigator database (Fig. 2C), as well as our data obtained in response to *Erwinia amylovora* inoculation (Fig. 2B, *Ea* column; Moreau *et al.*, 2012). Values represent the $\log_2$(ratio) of the fold modulation of selected Arabidopsis genes in response to DCA treatment or pathogen inoculation (as described in the figure) compared with mock treatment (Fig. 2B,C). We compared the list of defence-associated genes identified as being induced by DCA (Fig. 2B, DCA column) with our previously obtained transcriptome data (Fig. 2B, *Ea* column) or with public datasets using the signature tool in the Genevestigator database (Fig. 2C). The results show an important similarity between the response of Arabidopsis to several pathogens, including the model pathogen *Pseudomonas syringae* pv. *tomato* (Fig. 2C, lines 4 and 5) and *E. amylovora* (Fig. 2B), and the response of Arabidopsis to DCA.

Altogether, our results indicate that treatment of Arabidopsis leaves with DCA triggers a transcriptional reprogramming that bears similarities to the plant's immune response.

DCA protects against pathogen infection

Previous reports have indicated that CA is able to induce defence activation in rice and to protect rice plants against infection by the fungal pathogen *Magnaporthe grisea* (Koga *et al.*, 2006). To determine whether DCA treatment is also able to protect plants against pathogen infection, we analysed the capacity of DCA to reduce bacterial proliferation in Arabidopsis leaves. We chose *P. syringae* pv. *tomato* DC3000 and *E. amylovova* CFBP1430 as models of a compatible interaction and a non-host interaction, respectively, as our analysis indicates some similarity between the transcriptional response to DCA and the transcriptional response to both of these bacterial phytopathogens (Fig. 2B,C). Arabidopsis

leaves were treated with 200 μM DCA or mock solution and, 24 h later, were inoculated with the virulent *P. syringae* DC3000 strain and the *E. amylovora* CFBP1430 strain. The bacterial count in the leaves was carried out at 0 and 24 h post-infection (hpi), as indicated in Fig. 3. Consistent with previous reports, we found that, in control plants, both *P. syringae* and *E. amylovora* were able to proliferate, although to a much lesser extent in the case of *E. amylovora* as it is not adapted to Arabidopsis (Moreau *et al.*, 2012). In plants pretreated with DCA, we observed a significantly ($P < 0.05$) lower bacterial count *in planta* at 24 hpi for both
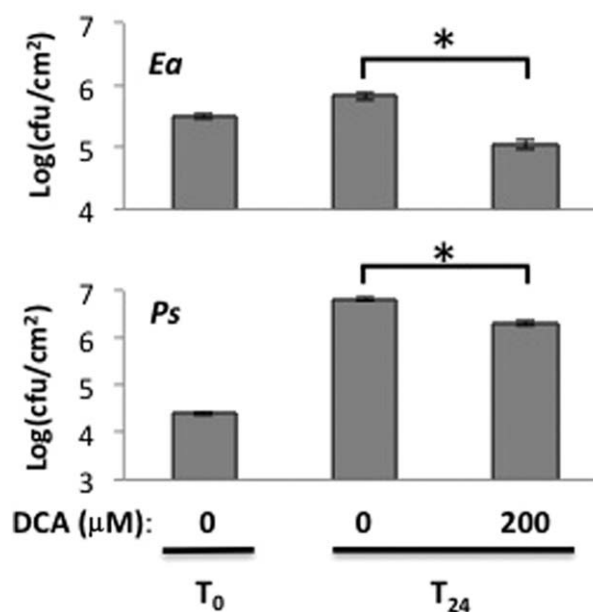
Fig. 3 Deoxycholic acid (DCA) reduces the *in planta* growth of phytopathogens. Arabidopsis leaves were sprayed with 200 μM DCA 24 h prior to infection with *Pseudomonas syringae* pv. *tomato* [*Ps*; optical density (OD) = 0.01, 10^6 colony-forming units (cfu)/mL] or *Erwinia amylovora* (*Ea*; OD = 0.1, 10^7 cfu/mL). For each experiment, 10 leaves ($n = 10$) were collected immediately after infection (T_0) or 24 h post-infection (T_{24}), ground and cfu/cm² was determined. Bars show the mean number of cfu/cm² of leaf ± standard error of the mean (SEM). Experiments were repeated three times with similar results. The asterisk indicates a significant difference from the mock-treated plants at the same time point according to the Mann–Whitney test ($P < 0.05$).

bacteria tested. For both bacterial species, the bacterial count in plants pretreated with DCA was approximately 10 times lower than in plants that had not been pretreated with DCA. In the case of *E. amylovora*, which is not adapted to Arabidopsis and thus shows only weak bacterial multiplication in control plants, DCA pretreatment led to an absence of multiplication (Fig. 3). In the case of *P. syringae*, which is virulent on Arabidopsis, DCA pretreatment did not prevent bacterial multiplication, but reduced it significantly (Fig. 3).

Our results show that the treatment of plants with DCA before inoculation reduces the proliferation of two phytopathogenic bacteria.

Dose response of gene modulation by DCA treatment and effect of abiotic stress

In order to better understand the response of Arabidopsis to DCA, we monitored, by qRT-PCR, the expression of selected defence marker genes in response to increasing concentrations of DCA. We chose *WRKY46* and *WRKY70*, two WRKY TFs, *PDF1.2a* and *CHI-B* as markers of the JA/ET-dependent pathway, and *PR1*, *WAK2* and *EDS1* as markers of the SA-dependent pathway. Five-week-old Arabidopsis leaves were sprayed with different concentrations of DCA or mock-treated. Leaves were collected at 24 hpt and gene expression was assayed using qRT-PCR. All genes analysed showed an induction of their mRNA accumulation in response to the highest concentration of DCA (200 μM), confirming our transcriptome data (Fig. 4). In response to 100 μM only, the mRNA levels of *PR1* and *CHI-B* were induced significantly. However, exposure of Arabidopsis plants to 20 μM DCA did not induce the expression of any of the tested genes (Fig. 4).

As plant growth conditions are not always optimal, especially water availability, we evaluated the impact of drought and nitrogen limitation on the induction of molecular defences by DCA. Five-week-old Arabidopsis plants subjected to water deficit were treated with 200 μM DCA and gene expression at 24 hpt was evaluated by qRT-PCR. The expression of the drought marker genes *RAB18* and *RD29B* increased during water stress (Fig. S3, see Supporting Information), as expected (Harb *et al.*, 2010). In plants subjected to water deficit, most of the defence genes tested retained the ability to be induced by DCA, although the level of induction was affected slightly (Fig. 5). In contrast, the TFs *WRKY46* and *WRKY70* did not respond to DCA in plants subjected to drought. We also determined whether DCA was able to induce defence in Arabidopsis plants that were grown under nitrogen limitation. Five-week-old Arabidopsis plants subjected to nitrogen limitation (2 mM NO_3^-) or not (10 mM NO_3^-), as described in Fagard *et al.* (2014), were treated with 200 μM DCA or mock-treated. Leaves were sampled at 24 hpt and defence gene expression was evaluated by qRT-PCR (Fig. 5B). We found that both the SA path-

way and JA pathway marker genes, *PR1* and *PDF1.2*, respectively, were induced by DCA in plants grown under nitrogen limitation.

In summary, we observed that DCA treatment induces defence marker genes in a dose-dependent manner, even in the presence of drought or nitrogen limitation.

## Kinetics of gene modulation by DCA treatment

In order to assess the capacity of DCA to protect plants over time, we analysed gene expression over several time points from 6 hpi to 7 days post-infection. Five-week-old Arabidopsis leaves were sprayed with 200 μM DCA or mock-treated. We observed a peak of mRNA accumulation between 24 and 48 hpt, depending on the gene, and thereafter the mRNA level decreased gradually within 72 h and then remained constant until 7 days after DCA treatment (Fig. 6). For the SA-dependent marker genes *EDS1* and *PR1*, the peak of mRNA accumulation was at 24 hpt, whereas it was at 48 hpt for the JA-dependent genes *CHI-B* and *PDF12a*. mRNA accumulation in response to DCA started at 6 hpt for *WRKY46*, *WRKY70*, *EDS1* and *WAK2*. The expression of *WAK2* showed the most rapid increase in mRNA accumulation in response to DCA.

Altogether, we show that gene induction following DCA treatment exhibits a peak between 6 and 48 h depending on the gene, whereas almost no gene induction remains 7 days after treatment.

Defence induction by DCA requires *SID2* and *RbohD*

In order to further characterize the action of DCA, we analysed the induction of the SA marker gene *PR1* following DCA treatment in the SA biosynthesis-deficient *sid2-1* mutant (Nawrath and Métraux, 1999). As a control, we also used the NahG-B15 transgenic line that expresses a salicylate hydroxylase-encoding bacterial gene and displays very low levels of SA (Lawton *et al.*, 1995). Approximately 20 seedlings of each genotype were grown *in vitro* on one-fifth strength Murashige and Skoog medium and sprayed with 200 μM DCA or mock-treated, and sampled at 24 hpt. The expression of the *PR1* gene was assessed by RT-PCR as shown in Fig. 7A. The expression of the constitutive gene *Elongation factor-1α* (*EF1α*) was used as a loading control. No expression of the *PR1* gene was detected in the mock-treated plants. Our results show that DCA treatment can induce the expression of the *PR1* gene in seedlings grown *in vitro* as it does in plants grown in soil. Furthermore, our results clearly indicate that the induction of the *PR1* gene by DCA is completely abolished in the *sid2-1* mutant, as well as in the NahG transgenic line, indicating that *PR1* induction by DCA requires *SID2*-dependent SA production.

WAK (wall-associated kinase) genes encode receptor-like kinase (RLK) genes that are thought to be involved in the response of plants to their extracellular environment. Among the five Arabidopsis WAK genes, DCA treatment induced the expression of *WAK1*, *WAK2* and *WAK3* (Fig. 2). Moreover, *WAK1* and
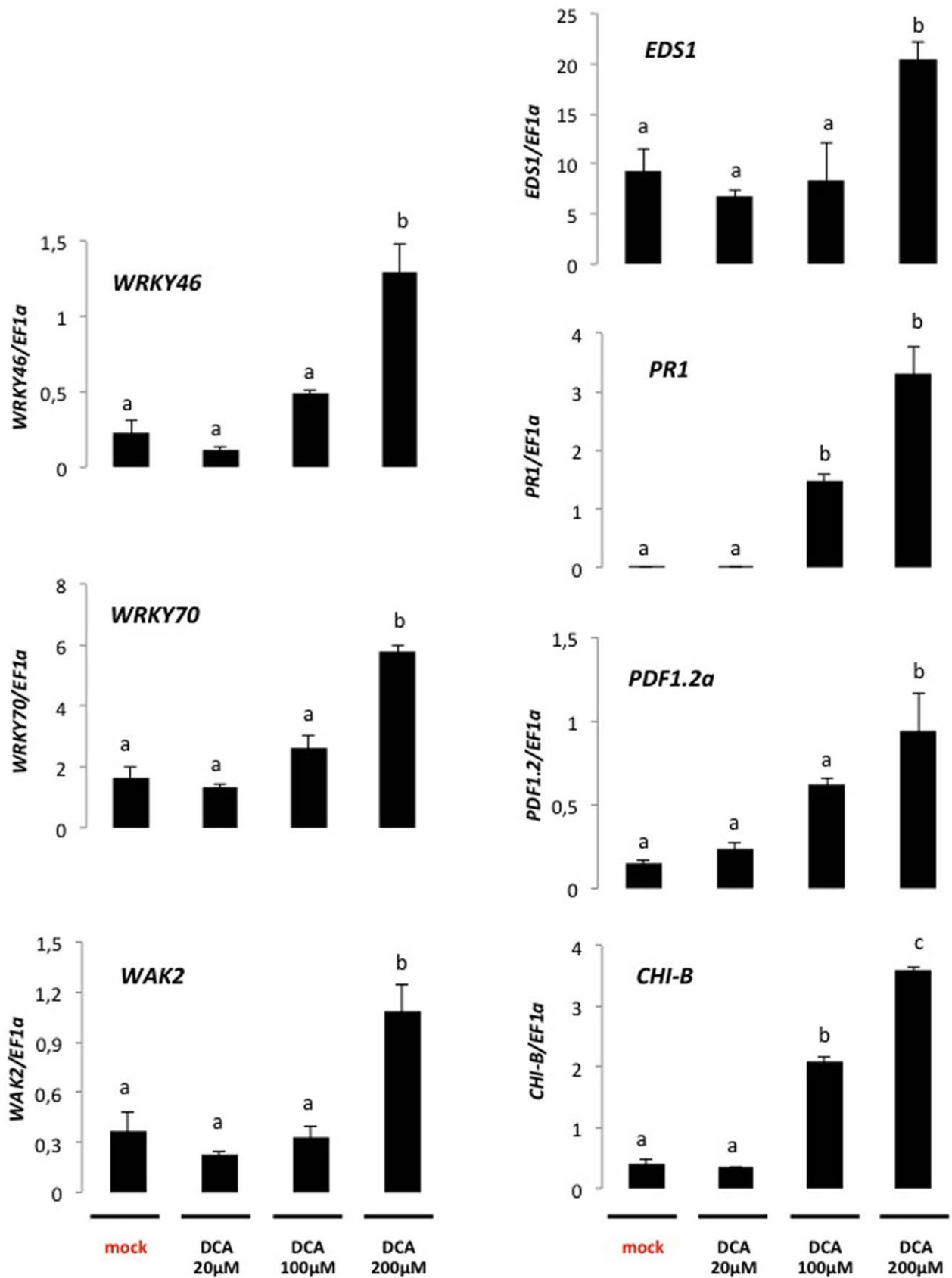
Fig. 4 Deoxycholic acid (DCA) induces defence-related gene expression in a dose-dependent manner. The expression of selected defence genes was evaluated 24 h following mock treatment or treatment with increasing concentrations of DCA (as indicated). Nine leaves of three plants were collected for RNA extraction ($n = 3$). Transcript accumulation was determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Bars represent the mean expression $\pm$ standard deviation (SD). Bars with the letter 'a' correspond to expression levels that are not significantly different from expression in mock-treated leaves according to the Mann–Whitney test ($P < 0.05$). Bars with the letters 'b' and 'c' correspond to expression levels that are significantly different from expression in mock-treated leaves and from each other according to the Mann–Whitney test ($P < 0.05$). The experiment was repeated twice with similar and statistically significant results.

WAK2 are involved in defence activation (He *et al.*, 1998; Kohorn *et al.*, 2009). In order to determine whether these receptors play a role in the induction of defence following DCA treatment, T-DNA insertion knock-out mutants were retrieved for *WAK1* and *WAK3*. The insertion of a T-DNA in the corresponding gene and the

absence of cDNA were confirmed for each line by PCR and RT-PCR, respectively (data not shown). For *WAK2*, we used the previously described *wak2-1* null allele (Kohorn *et al.*, 2006). Plants were treated with 200 μM DCA or mock-treated, and sampled at 24 hpt. Gene expression was assessed using qRT-PCR. Our results

Fig. 5 Effect of drought on the deoxycholic acid (DCA)-mediated induction of defence. (A) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of selected genes 24 h after mock (–) or 200 μM DCA (+) treatment in Arabidopsis Col-0 plants under well-watered (WW) or water deficit (WD) conditions, as described in Bouchabke *et al.* (2008). (B) qRT-PCR analysis of selected genes 24 h after mock (–) or 200 μM DCA (+) treatment in Arabidopsis Col-0 plants grown in limiting (2 mM NO$_3^-$) or non-limiting (10 mM NO$_3^-$) nitrate. (A, B) Nine leaves from three plants ($n = 3$) were collected 24 h after DCA or mock treatment. Bars represent the mean expression $\pm$ standard deviation (SD). The experiments were repeated twice with similar and statistically significant results. The asterisk indicates significant difference according to the Mann–Whitney test ($P < 0.05$).
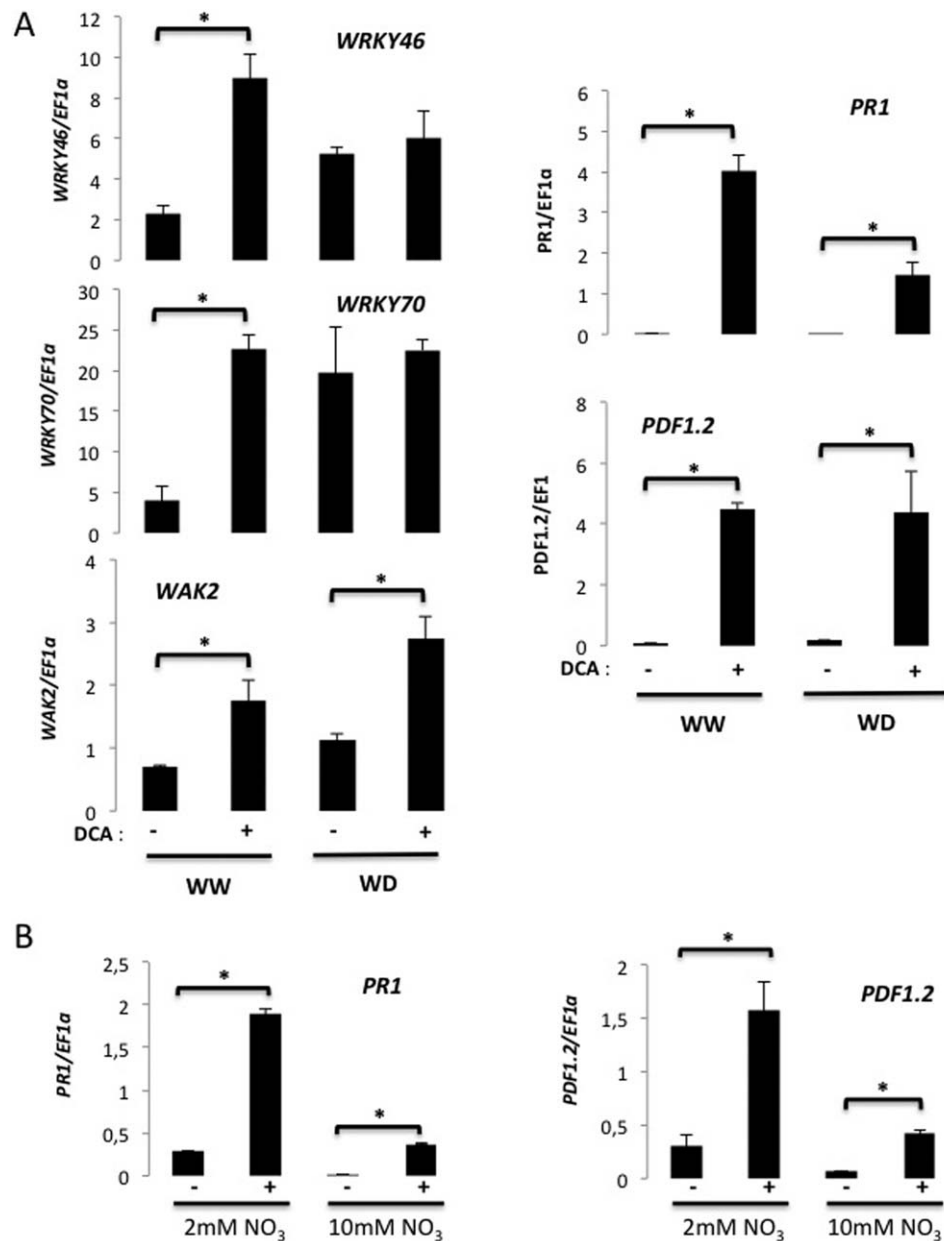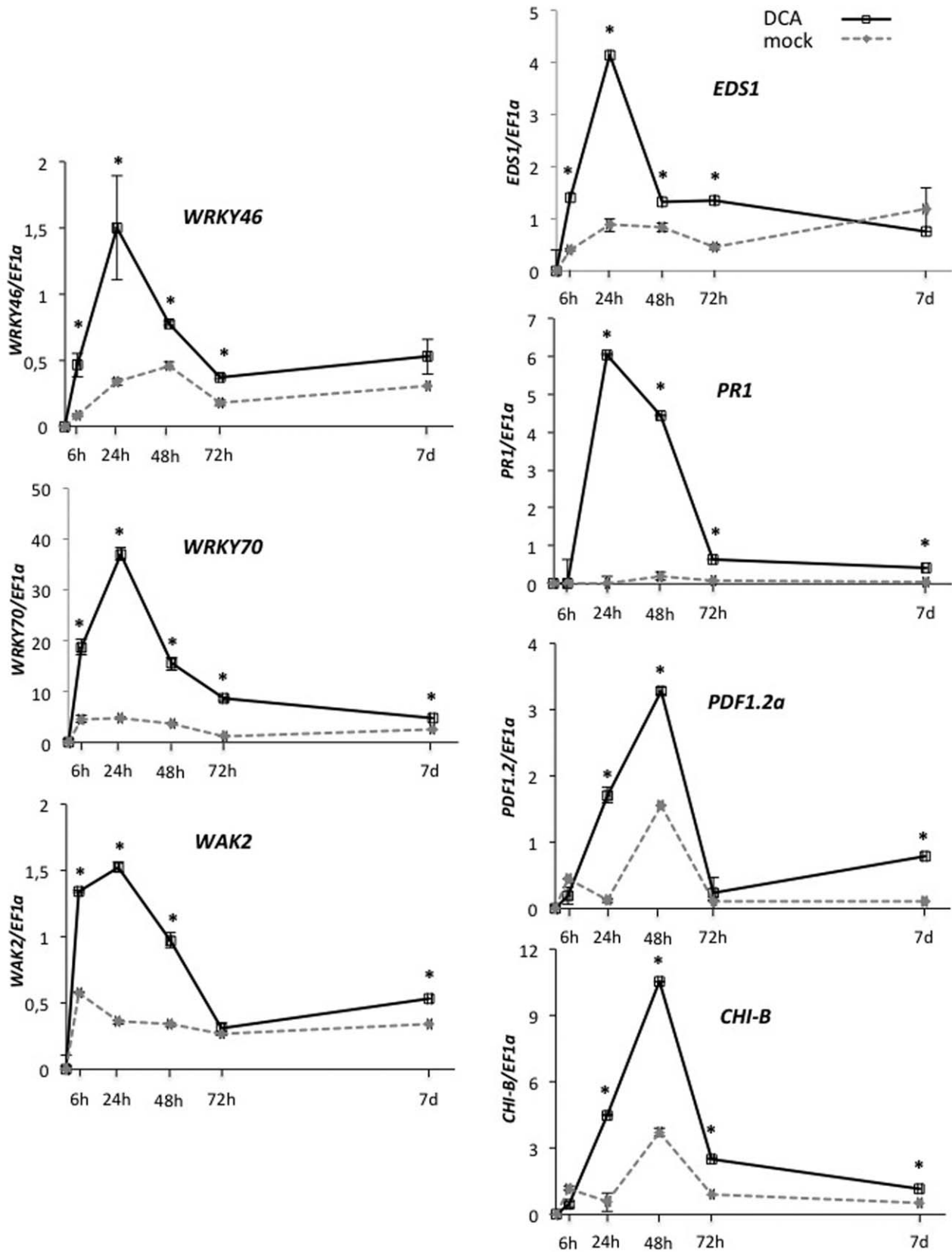
Fig. 6 Kinetics of defence induction triggered by deoxycholic acid (DCA) treatment. The expression of selected defence genes was monitored at different time points between 6 h and 7 days following mock (broken line with diamonds) or 200 μM DCA (full line with squares) treatment. Nine leaves from three plants (*n* = 3) were collected at the indicated time point following mock treatment or treament with 200 μM DCA. Transcript accumulation was determinated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Values represent the mean expression ± standard deviation (SD). The experiments were repeated twice with similar and statistically significant results. The asterisk indicates a significant difference from mock-treated plants according to the Mann–Whitney test (*P* < 0.05).

showed that the *wak1-1*, *wak2-1* and *wak3-1* mutant lines responded to DCA treatment in terms of defence gene induction, as did the Col-0 control plants (Fig. 7). These results indicate that none of the three *WAK* genes analysed is solely responsible for *PR1* induction following DCA treatment.

In *Arabidopsis*, the NADPH oxidase encoded by *RbohD* plays a predominant role in ROS production following pathogen infection (Torres *et al.*, 2002). We analysed H_2O_2 production in seedlings grown *in vitro* following DCA or mock treatment using DAB staining. No DAB staining could be detected in mock-treated plants. In contrast, DCA induced ROS accumulation in wild-type plants grown *in vitro* (Fig. 7B), as it does in soil-grown plants (Fig. 1C). In the *rbohD* mutant, ROS induction following DCA treatment was much weaker than in wild-type plants, indicating that ROS induction by DCA is at least partly dependent on *RbohD*.
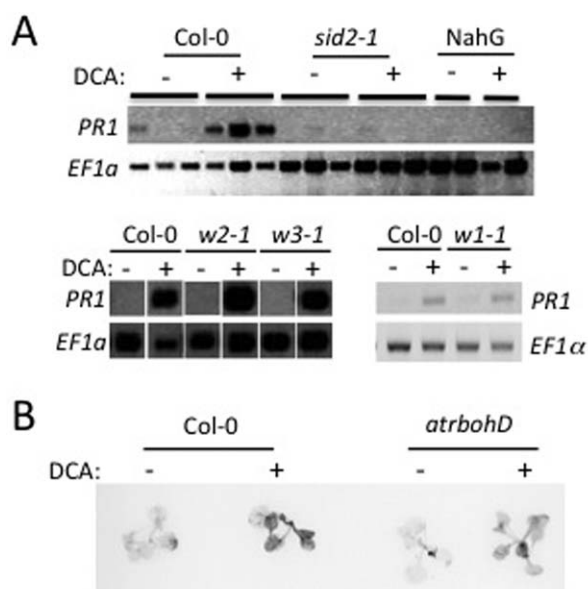
Fig. 7 *SID2* and *RbohD* are required for defence activation by deoxycholic acid (DCA). (A) Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the expression of *PR1* in 2-week-old *in vitro*-grown seedlings treated (+) or not (−) with 200 μM DCA and sampled 24 h after treatment. The *EF1α* gene was used as an internal standard for the quantity of cDNA. Each lane corresponds to a pool of 10 seedlings and the experiment was conducted three times. *w1-1*, *wak1-1*; *w2-1*, *wak2-1*; *w3-1*, *wak3-1*. The figure was rearranged for clarity; the original image is available in Fig. S4 (see Supporting Information). (B) 3,3′-Diaminobenzidine (DAB) staining of 2-week-old seedlings treated with 200 μM DCA (+) or mock-treated (−). The experiment was performed on 20 seedlings and the figure shows photographs of representative a seedlings.

DISCUSSION

Several studies have reported plant defence elicitor properties for bile acids when applied to rice or soybean plants (Kevresan *et al.*, 2009; Koga *et al.*, 2006; Malencic *et al.*, 2012). It has been shown that the primary bile acid CA elicits cell death, PR protein synthesis, phytoalexin accumulation, ROS accumulation and protects rice against the fungus *Magnaporthe grisea* (Kevresan *et al.*, 2009; Koga *et al.*, 2006; Malencic *et al.*, 2012). Although these studies focused on CA, it has been suggested that the secondary bile acid DCA, which is one of the three major constituents of bile, also has elicitor properties, as it induces phytoalexin accumulation in rice (Koga *et al.*, 2006). The present work focuses on the elicitor properties of DCA in plants. We investigated the effect of DCA treatment on defence elicitation in *Arabidopsis*. We first investigated the effect of DCA treatment on ROS accumulation and callose deposition, two representative cellular defence responses. Indeed, the first mechanical barrier encountered by pathogens during plant invasion is the cell wall and plants are able to reinforce their own cell wall by depositing callose, a β-(1-3)-glucan polymer, at the site of attack (Luna *et al.*, 2011). We found that DCA induces callose deposition and both intracellular and extracellular ROS accumulation in leaves (Fig. 1). Both callose and ROS accumulation were observed only in response to the highest concentration of DCA tested, consistent with previous reports that callose accumulation can be correlated with the oxidative burst (Luna *et al.*, 2011). DCA treatment was also able to induce the accumulation of SA and JA phytohormones (Figs 1 and S2). Consistent with the capacity of DCA to trigger defences in *Arabidopsis* leaves, we found that DCA reduces the capacity of two pathogenic bacteria to multiply in DCA-pretreated leaves (Fig. 3).

Previous studies have shown that CA can induce the gene expression required for phytoalexin biosynthesis in rice (Shimizu *et al.*, 2008), suggesting that defence activation by bile acids in plants could require transcriptional activation of the corresponding defence genes. However, no genome-wide analysis of the impact of bile acids on plants has been performed to date. In order to gain an insight into the elicitor properties of bile acids, we analysed the transcriptome of plants treated with DCA. Our results indicated that DCA strongly affected the plant's transcriptome, including many functional categories of genes (Fig. 2A). Interestingly, the vast majority of genes affected by DCA were induced (92%) and not repressed (8%), although it is not obvious whether this has any functional significance. Furthermore, an important number of genes from the WRKY family of TFs were induced following DCA treatment (Fig. 2B), which is consistent with a

transcriptional activation of defence by DCA following its perception by the plant by an as yet to be identified mechanism. Our data also showed that DCA induces the expression of a large number of defence-associated genes, including known defence genes that are induced in response to a wide set of pathogens (Fig. 2C). Thus, DCA has an important impact on the plant's transcriptome in a similar manner to two well-characterized PAMPs: the flagellin-derived flg22 peptide and oligogalacturonides (OGs). Indeed 359 of the 610 DCA-modulated genes were also modulated by flg22 or OGs (Denoux *et al.*, 2008). Furthermore, we showed that DCA affects gene expression as early as 6 hpi, as revealed by our qRT-PCR data (Fig. 6).

In accordance with our observation that SA and JA phytohormones accumulate following DCA treatment, transcriptome analysis of DCA-treated plants revealed an activation of genes dependent on the SA pathway and the JA pathway (Figs 3 and 4). In Arabidopsis, *PR2* encodes an SA-dependent pathogen-inducible β-1,3-glucanase (Gallego-Giraldo *et al.*, 2011). Koga *et al.* (2006) reported that CA treatment in rice increases β-1,3-glucanase activity. Consistent with the observations of Koga *et al.* (2006) on rice, *PR2* is induced in Arabidopsis by DCA (Fig. 2). Furthermore, although it is generally thought that SA strongly antagonizes the JA-mediated defensive pathway (Pieterse *et al.*, 2012), the interactions between SA and JA hormones are strongly dependent on the concentration of these molecules: applied exogenously at low concentrations, SA and JA act synergistically to activate defence gene expression, whereas antagonism is observed at higher concentrations (Mur *et al.*, 2006). Taken together, it appears that DCA treatment induces a balanced SA/JA accumulation that could be useful for plant protection against both necrotrophic and biotrophic pathogens (Okada *et al.*, 2015). Another interesting feature of DCA is that it can activate defence in plants grown under abiotic stress. Indeed, we showed that DCA induces defence in plants subjected to drought (Fig. 5A), as well as in plants grown under limiting nitrogen (Fig. 5B). Two genes, *WRKY46* and *WRKY70*, did not respond to DCA under moderate drought; however, this could be a result of the fact that both genes were more highly expressed in mock-treated plants subjected to drought stress than in untreated control plants.

WAK genes encode a family of RLKs that have attracted attention in recent years. *WAK2* has been shown to be necessary for normal cell enlargement, as a reduction in WAK2 protein levels in transgenic plants results in dwarf plants (Wagner and Kohorn, 2001). The extracellular domain of WAK1 and WAK2 binds pectin *in vitro* (Kohorn *et al.*, 2009). Furthermore, the *wak2-1* mutant is affected in MAPK activation by pectin in protoplasts and the *wak2-1* mutant is defective in the activation of numerous genes in protoplasts treated with pectin (Kohorn *et al.*, 2009). Among the five Arabidopsis *WAK* genes, *WAK1–3* are induced by SA or its analogue 2,6-dichloroisonicotinic acid (INA) (He *et al.*, 1998,

1999). The expression of *WAK1* is also induced by *P. syringae* infection and its role in resistance to lethal SA levels has been demonstrated (He *et al.*, 1998). Our results showed that the single mutants *wak1-1*, *wak2-1* and *wak3-1* displayed an induction of defence in response to DCA similar to the wild-type plants. These results indicate that, although they are rapidly activated at the transcriptional level, *WAK1*, *WAK2* and *WAK3* are individually dispensable for defence activation by DCA. Interestingly, we found that the *wak1-1* knock-out mutant was not affected in root length, whereas the *wak3-1* knock-out mutant displayed a slight reduction in root length (data not shown), suggesting that *WAK3* could play a role in cell growth with *WAK2* (Kohorn *et al.*, 2006).

Altogether, we have shown that DCA constitutes a potent plant defence elicitor in Arabidopsis, and have demonstrated the role of *SID2* and *RbohD* in the activation of defence following treatment with this elicitor. The present work confirms the potential of bile acids as good candidate elicitors for a balanced defence response in both dicots and monocots, and in response to different classes of pathogen. Furthermore, our data indicate that defence activation by DCA occurs in plants grown in different conditions, including under abiotic stress, suggesting that bile acids could be robust elicitors that are not strongly affected by the physiology of the treated plant, and could be used in crop production. The development of sustainable agriculture will greatly benefit from the development of new plant defence elicitors, such as DCA, together with a better understanding of their mode of action for an optimal use of these molecules.

EXPERIMENTAL PROCEDURES

Plant growth and DCA treatment

Seeds of *A. thaliana* Col-0 were obtained from the INRA Versailles collection. The SA-deficient *sid2-1* null mutant (Nawrath and Métraux, 1999) and NahG-B15 transgenic line (Lawton *et al.*, 1995), the *wak2-1* null mutant (Kohorn *et al.*, 2006) and the *rbohD* mutant (Torres *et al.*, 2002) are all in the Col-0 background. The *wak1-1* and *wak3-1* alleles correspond to lines SALK_107175 (Col-0) and SALK_071999 (Col-0), respectively. These lines possess a T-DNA insertion in exon3 of AT1G21250 and exon2 of AT1G21240, respectively. We checked by qRT-PCR that both *wak1-1* and *wak3-1* are null alleles (data not shown).

Plants were grown for 5 weeks in soil and were subjected to an 8-h light and 16-h dark cycle at 21°C (day)/18°C (night) with 65% relative humidity (Figs 1–6), or were grown for 7 days on one-fifth strength Murashige and Skoog medium plus 1% sucrose in a 16-h light/8-h dark cycle at 21°C with 50% relative humidity (Fig. 7). Plants were sprayed with different concentrations of DCA (Industria Chimica Emiliana, Reggio Emilia, Italy) dissolved in 0.01% Tween-20 (as described in Koga *et al.*, 2006) or with a mock solution (0.01% Tween-20).

Drought treatment was performed as described in Bouchabke *et al.* (2008). Briefly, soil water content was fixed at 60% of maximal water content as a control in well-watered (WW) conditions. The water deficit (WD) treatment was fixed at 30% of the maximal water content. Pots

were weighed daily and adjusted to a fixed weight corresponding to the water contents described above. The two watering regimes were applied 2 weeks after sowing. Nitrogen limitation was performed as described in Fagard *et al.* (2014). Briefly, plants were grown for 5 weeks in sand and watered with a nutrient solution containing ample (10 mM NO_3^-) or limiting (2 mM NO_3^-) nitrogen. In all cases, 5-week-old stressed or control plants were mock- or DCA-treated as described in the text.

Pathogen infections

Five-week-old Arabidopsis leaves were sprayed with 200 μM DCA or mock-treated and, 24 h later, inoculated with *E. amylovora* CFBP1430 or *P. syringae* pv. *tomato* DC3000. Bacterial suspensions were prepared in sterile water for *E. amylovora* (10^7 cfu/mL) or in 10 mM $MgCl_2$ for *P. syringae* (10^6 cfu/mL). At 24 hpi, we performed bacterial counting by grinding infected leaves using glass beads in a TissueLyser (Qiagen/Retsch, Hilden, Germany). The bacterial suspensions were used to prepare serial dilutions, which were plated on Lysogeny Broth (LB) medium and, after 1 or 2 days, the colonies formed were counted to evaluate the initial number of bacteria.

Callose staining

Leaves of 5-week-old Arabidopsis plants were sprayed with the indicated concentration of DCA or mock-treated. Leaves were collected at 24 hpt and placed overnight in a lactophenol clearance solution. Callose deposits were stained using 0.01% aniline blue in 150 mM K_2HPO_4 (pH 9.5) buffer for 30 min, as described previously (Degrave *et al.*, 2008). Leaves were examined by stereofluorescence microscopy with an Azio Zoom V.16 (Carl Zeiss Inc., Oberkochen, Germany). Experiments were repeated three times with similar results and representative photographs are shown. The number of spots of callose deposit per photograph was determined using ImageJ software in 25–30 photographs corresponding to more than five independent leaves for each treatment.

H$_2$O$_2$ detection

Intracellular H_2O_2 was detected using DCFH-DA. At 16 h after treatment with 20 or 200 μM DCA (0.01% Tween-20) or mock, leaves of 5-week-old Arabidopsis plants were cut off, immersed in 300 μM DCFH-DA solution and vacuum infiltrated. Whole-leaf images were taken using an Olympus SZX12 binocular (Olympus Corporation, Shinjuku, Japan). Green fluorescence was detected with an HQ510 1p emission filter. In parallel, we used DAB staining to detect both intra- and extracellular H_2O_2. Leaves of 5-week-old Arabidopsis plants were sprayed with 20 or 200 μM DCA or mock-treated. Leaves were then collected at 6 hpt and immediately vacuum infiltrated with DAB (1 mg/mL). We analysed 15–20 photographs per treatment and representative photographs were taken with a binocular (Leica MZFLIII, Wetzlar, Germany).

SA and JA phytohormone quantification

Following treatment with DCA or mock treatment, rosette leaves of 5-week-old plants were immediately frozen after harvest and ground in liquid nitrogen. For each treatment, 20 leaves from four plants were collected and 100 mg of material was freeze–dried for hormone extraction.

Hormone detection and quantification were performed by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS), as described previously (Le Roux *et al.*, 2014).

RNA extraction and qRT-PCR analysis

For RNA extraction, nine leaves of three plants (DCA- or mock-treated) were collected at the indicated time point after treatment, pooled and immediately frozen in liquid nitrogen. Total RNA was extracted from 100 mg of frozen ground leaves using Trizol® reagent (Invitrogen Life Technologies, Saint-Aubin, France). RNA quality was evaluated by an electrophoretic run on 1% agarose gel. For the qRT-PCR analysis, first-strand cDNA was synthesized using Superscript reverse transcriptase SSII (Invitrogen) from 1 μg of DNase-treated (Invitrogen) total RNA in a 20-μL reaction volume. qPCRs were performed using SYBR® Selected MasterMix 2x (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA), following the manufacturer's protocol. The cycling conditions consisted of an initial 5 min at 95°C, followed by 40 three-step cycles at 94°C for 15 s, 60°C for 30 s and 72°C for 30 s. Melting curve analysis was performed after cycle completion to validate amplicon identity. Relative expression levels were calculated following the standard curve-based method (Larionov *et al.*, 2005). Expression of the *EF1α* reference gene (Czechowski *et al.*, 2005; Degrave *et al.*, 2013; Moreau *et al.*, 2012) was used for normalization of each target gene studied. For each treatment, two biological replicates were analysed and each qRT-PCR was carried out in triplicate. The gene-specific primers used in this analysis are indicated in Table S1 (see Supporting Information).

Transcriptome studies

Microarray analysis (Fig. 3A,B; Table S2) was performed with CATMA chips containing 24 756 gene-specific tags corresponding to 22 089 genes from Arabidopsis (Crowe *et al.*, 2003; Hilson *et al.*, 2004). Two independent biological replicates were produced. Leaves of 5-week-old plants were sprayed with 200 μM DCA or mock-treated, collected 24 h after treatment and immediately frozen in liquid nitrogen. Total RNA was extracted using the Qiagen RNeasy kit according to the manufacturer's instructions. RNA integrity, cDNA synthesis hybridization and array scanning were performed as described previously (Lurin *et al.*, 2004). Statistical analysis was based on two dye swaps (Gagnot *et al.*, 2008). To determine differentially expressed genes, a paired *t*-test was performed on the log ratios. Spots displaying extreme variance (too small or too large) were excluded. The raw *P* values were adjusted by the Bonferroni method. Genes with a Bonferroni $P \leq 0.05$ were considered to be differentially expressed (pink, red and green boxes in Fig. 3B). Enrichment of FunCatDB categories (Fig. 3A) was assessed by employing hypergeometric distribution with a *P*-value cut-off of 5×10^{-3} (http://mips.helmholtz-muenchen.de/funcatDB/). For selected defence-associated genes, the transcriptomic data obtained following DCA treatment (this study) were compared with the transcriptomic data obtained following *E. amylovora* inoculation (Fig. 3B; Moreau *et al.*, 2012) and with data available in the Genevestigator database (Hruz *et al.*, 2008) obtained following inoculation with other pathogens, including *P. syringae* pv. *tomato* (Fig. 3C). The 15 datasets most similar to the DCA dataset for the selected defence-associated genes were identified using the Genevestigator 'Signature' tool (https://genevestigator.com/).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Deoxycholic acid (DCA) induces callose accumulation in the hydathodes.

Fig. S2 Symptoms induced by spraying increasing concentrations of deoxycholic acid (DCA) on Arabidopsis leaves.

Fig. S3 Expression of drought marker genes in water-deprived plants.

Fig. S4 Original images corresponding to Fig. 7.

Table S1 Detail of primers used in this study.

Table S2 Genes significantly up- and down-regulated 24 h after deoxycholic acid (DCA) treatment.