

Genetic dissection of basal defence responsiveness in accessions of *Arabidopsis thaliana*

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ABSTRACT

Basal resistance involves a multitude of pathogen- and herbivore-inducible defence mechanisms, ranging from localized callose deposition to systemic defence gene induction by salicylic acid (SA) and jasmonic acid (JA). In this study, we have explored and dissected genetic variation in the responsiveness of basal defence mechanisms within a selection of *Arabidopsis* accessions. Responsiveness of JA-induced *PDF1.2* gene expression was associated with enhanced basal resistance against the necrotrophic fungus *Plectosphaerella cucumerina* and the herbivore *Spodoptera littoralis*. Conversely, accessions showing augmented *PR-1* induction upon SA treatment were more resistant to the hemi-biotrophic pathogen *Pseudomonas syringae*, and constitutively expressed defence-related transcription factor (TF) genes. Unexpectedly, accessions with primed responsiveness to SA deposited comparatively little callose after treatment with microbe-associated molecular patterns. A quantitative trait locus (QTL) analysis identified two loci regulating flagellin-induced callose and one locus regulating SA-induced *PR-1* expression. The latter QTL was found to contribute to basal resistance against *P. syringae*. None of the defence regulatory QTLs influenced plant growth, suggesting that the constitutive defence priming conferred by these loci is not associated with major costs on plant growth. Our study demonstrates that natural variation in basal resistance can be exploited to identify genetic loci that prime the plant's basal defence arsenal.

Key-words: basal resistance; genetic variation; herbivores; hormones; pathogens; priming of defence.

INTRODUCTION

The plant immune system governs a wide range of defence mechanisms that are activated after recognition of pathogen-associated molecular patterns (PAMPs). This PAMP-triggered immunity (PTI) protects the plant against

the majority of potentially harmful microorganisms (Jones & Dangl 2006). However, a small minority of virulent pathogens have evolved ways to suppress PTI by using effectors that interfere with PTI signalling components (Nomura, Melotto & He 2005), rendering the host plant susceptible. To counteract this effector-triggered susceptibility (ETS), plants have co-evolved the ability to recognize and respond to these pathogen effectors (Jones & Dangl 2006). This immune response is dependent on specific resistance (R) proteins that can recognize the presence or activity of effectors, resulting in effector-triggered immunity (ETI). Pathogens that are resisted by ETI can break this immune response by evolving alternative effectors that suppress ETI, or that are no longer recognized by the host's R proteins (Abramovitch *et al.* 2006; Fu *et al.* 2007; Cui, Xiang & Zhou 2009; Houterman *et al.* 2009). In this situation, ETI is reverted to basal resistance, which is too weak to protect against disease, thereby putting the susceptible host plant under selective pressure to evolve alternative R proteins. The resulting arms race between plants and their (a)virulent pathogens manifest as an ongoing oscillation in the effectiveness of plant defence and is referred to as the zigzag model (Jones & Dangl 2006).

PTI, ETI and basal resistance involve multiple defensive mechanisms that are activated at different stages of infection. Induced defence can already be active before the host tissue is colonized. Rapid closure of stomata can form a first pre-invasive defence barrier against bacterial pathogens (Melotto *et al.* 2006; Melotto, Underwood & He 2008). After successful entry of the host tissue, plant attackers often encounter early-acting post-invasive defence barriers, such as accumulation of reactive oxygen species, followed by depositions of callose-rich papillae (Eulgem *et al.* 1999; Torres, Jones & Dangl 2006; Ton, Flors & Mauch-Mani 2009; Luna *et al.* 2010). Upon further colonization, plants undergo a large-scale transcriptional reprogramming that coincides with the generation of long-distance defence signals and *de novo* biosynthesis of the regulatory plant hormones salicylic acid (SA) and jasmonic acid (JA) (Heil & Ton 2008). This relatively late-acting post-invasive defence involves expression of a wide range of local and systemic defence mechanisms. Hence, induced defence is a multilayered phenomenon

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that includes diverse resistance mechanisms, which are regulated by a complex cellular signalling network (Pieterse *et al.* 2009).

Arabidopsis thaliana displays substantial natural variation in basal resistance against a variety of pathogens, such as *Pseudomonas syringae* pv. *tomato* DC3000 (Kover & Schaal 2002; Perchevied *et al.* 2006; Van Poecke *et al.* 2007), *Erysiphe pathogens* (Adam *et al.* 1999), *Fusarium graminearum* (Chen *et al.* 2006), *Plectosphaerella cucumerina* (Llorente *et al.* 2005), *Botrytis cinerea* (Denby, Kumar & Kliebenstein 2004) and *Alternaria brassicicola* (Kagan & Hammerschmidt 2002). Quantitative trait locus (QTL) mapping analyses of this natural variation have identified novel defence regulatory loci. Llorente *et al.* (2005) revealed that genetic variation in basal resistance to *P. cucumerina* is largely determined by the *ERECTA* gene, which encodes for an LRR receptor like kinase protein. QTL analysis of natural variation in basal resistance against *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) has identified various QTLs that mapped to genomic regions containing putative R and/or PRR genes (Kover *et al.* 2005; Perchevied *et al.* 2006). This suggests that natural variation in basal resistance against *Pst* DC3000 is based on differences in the perception of the pathogen. However, downstream signal transduction components can contribute to natural variation in basal resistance as well. For instance, variation in basal resistance against necrotrophic fungi has been reported to originate from accumulation levels of the phytoalexin camalexin (Kagan & Hammerschmidt 2002; Denby *et al.* 2004), which are caused by variations in signalling, rather than synthesis *per se* (Denby *et al.* 2004). Furthermore, Koornneef *et al.* (2008) reported natural variation between *Arabidopsis* accessions in the level of cross-talk between SA and JA signalling, suggesting that differences in signalling downstream of plant hormones can contribute to natural variation in basal resistance.

The relative weakness of basal resistance imposes selective pressure on plants to evolve alternative defensive strategies (Ahmad *et al.* 2010). Apart from ETI, plants have evolved the ability to enhance their basal defence capacity after perception of selected environmental signals. This so-called priming of defence results in a faster and/or stronger expression of basal resistance upon subsequent attack by pathogenic microbes or herbivorous insects (Conrath *et al.* 2006). Priming is typically induced by signals that indicate upcoming stress, such as localized attack by pathogens (Van Wees *et al.* 1999; Jung *et al.* 2009), or wounding-induced volatiles that are released by neighbouring, insect-infested plants (Engelberth *et al.* 2004; Ton *et al.* 2007). However, there are also examples where interactions with plant beneficial microorganisms trigger defence priming, such as non-pathogenic rhizobacteria (Van Wees *et al.* 1999; Verhagen *et al.* 2004; Pozo *et al.* 2008) or mycorrhizal fungi (Pozo *et al.* 2009). Finally, most biologically induced priming phenomena can be mimicked by applications of chemicals, such as low doses of SA (Mur *et al.* 1996), methyl jasmonate (MeJA; Kaus *et al.* 1994) and β -aminobutyric acid (BABA; Jakab

et al. 2001). The primed defence state is associated with enhanced expression of defence regulatory protein kinases that remain inactive until a subsequent stress stimulus is perceived (Conrath *et al.* 2006; Beckers *et al.* 2009). Furthermore, we recently demonstrated that induction of rhizobacteria- and BABA-induced priming coincides with enhanced expression of defence-regulatory transcription factor (TF) genes (Van der Ent *et al.* 2009). Accumulation of these signalling proteins can contribute to an augmented induction of defence-related genes after pathogen attack.

Previously, we demonstrated that priming of defence is associated with minor fitness costs when compared to expression of induced defence (Van Hulten *et al.* 2006). In addition, we found that the costs of priming are outweighed by the benefits of protection under conditions of disease pressure (Van Hulten *et al.* 2006). Together, these findings suggest that defence priming entails a beneficial defence strategy in hostile environments. Accordingly, it can be predicted that selected plant accessions have adapted to hostile environments by acquiring a constitutively primed immune system (Ahmad *et al.* 2010). This hypothesis prompted us to investigate whether natural variation in basal resistance of *Arabidopsis* is associated with variation in responsiveness of basal defence mechanisms. To this end, we selected six *Arabidopsis* accessions that had previously been reported to differ in basal resistance against *Pst* DC3000 (Supporting Information Table S1) and tested them for basal resistance against different attackers and responsiveness to exogenously applied JA, SA and PAMPs. We show that natural variation in basal resistance against pathogens and herbivores is associated with variation in the sensitivity of basal defence responses. Further genetic dissection of this variation identified two QTLs controlling PAMP-induced callose and one QTL regulating SA-induced defence gene induction and basal resistance against *Pst* DC3000.

MATERIALS AND METHODS

Cultivation of plants, pathogens and herbivores

Arabidopsis accessions Col-0, Can-0, No-0, Bur-0, Sf-2 and Ws-2 (Supporting Information Table S1) from the Nottingham Arabidopsis Stock Centre (UK) were grown in sand for 2 weeks and subsequently transferred to 60 mL pots containing a compost soil/sand mixture, as described previously by Pieterse *et al.* (1998). Plants were cultivated in a growth chamber with an 8 h day (24 °C) and 16 h (20 °C) night cycle at 60–70% relative humidity (RH). *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000; Whalen *et al.* 1991) and luxCDABE-tagged *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000-lux; Fan, Crooks & Lamb 2008) were cultured as described by Van Wees *et al.* (1999), and *P. cucumerina* was cultured as described by Ton & Mauch-Mani (2004). *Spodoptera littoralis* eggs were provided by Dr. Ken Wilson (Lancaster University, UK) and reared on artificial diet as described (Shorey & Hale 1965).

***Pseudomonas syringae* pv. *tomato* DC3000 bioassays**

Five-week-old plants were inoculated by dipping the leaves in a bacterial suspension containing 10^8 cfu mL⁻¹ in 10 mM MgSO₄ and 0.01% (v/v) Silwet L-77 (Van Meeuwen Chemicals BV, Weesp, the Netherlands), or by pressure infiltration of a bacterial suspension containing 5×10^5 cfu mL⁻¹ in 10 mM MgSO₄. After inoculation, the plants were maintained at 100% RH. At 4 d after dip inoculation, the percentage of diseased leaves per plant was determined ($n = 35$). Leaves were scored as diseased when showing water-soaked lesions surrounded by chlorosis. Bacterial proliferation over a 3 d time interval was determined as described by Ton *et al.* (2005). Colonization by bioluminescent *Pst* DC3000-lux was quantified at 3 d after dip inoculation, using a liquid nitrogen-cooled CCD detector (Princeton Instruments, Trenton, NJ, USA) at maximum sensitivity. Digital photographs of inoculated leaves were taken under bright light (exposure time 0.1 s) and in darkness (exposure time 300 s), using WinView/32 software (Princeton Instruments, Trenton, NJ, USA) at fixed black and white contrast settings. Bacterial titres in each plant were expressed as the number of bioluminescent pixels in their leaves, standardized to the total number of leaf pixels from bright light pictures, using Photoshop CS3 software as described for digital quantification of callose (Luna *et al.* 2010).

***Plectosphaerella cucumerina* bioassays**

Five-week-old plants were inoculated by applying 6 μ L droplets containing 5×10^5 spores mL⁻¹ onto six to eight fully expanded leaves and maintained at 100% RH. Seven days after inoculation, each leaf was examined for disease severity. Disease rating was expressed as intensity of disease symptoms: I, no symptoms; II, moderate necrosis at inoculation site; III, full necrosis size of inoculation droplet; and IV, spreading lesion. Leaves were stained with lactophenol trypan blue and examined microscopically as described previously (Ton & Mauch-Mani 2004).

***Spodoptera littoralis* bioassays**

Two independent experiments were performed using 3.5- and 5-week-old plants ($n = 45$), divided over three 250 mL pots per accession. Third-instar *S. littoralis* larvae of equal size were selected, starved for 3 h, weighted and divided between the six different accessions (four caterpillars per pot; 12 caterpillars per genotype). After 18 h of infestation, the caterpillars were re-collected and weighted, and plant material was collected for photographic assessment of leaf damage. Caterpillar regurgitant was collected by anaesthetizing caterpillars with CO₂ and gently centrifuging at 800–1000 rpm for 5 min in 50 mL tubes containing fitted sieves to separate the regurgitant from caterpillars.

Statistical analysis of bioassays

Student's *t*-tests, χ^2 tests, analysis of variance (ANOVA) and multiple regression analysis were performed using IBM SPSS statistics 19 software (IBM, SPSS, Middlesex, UK).

RNA blot analysis of hormone-induced gene expression

Plant hormone treatments were performed by dipping the rosettes of 5- to 6-week-old plants in a solution containing 0.01% (v/v) Silwet L-77, and SA (sodium salicylate), JA or MeJA at the indicated concentrations. The plants were placed at 100% RH, and leaves from three to five rosettes were collected at 6 h (for SA) and 4 h (for JA or MeJA) after treatment. RNA extraction, RNA blotting and labelling of specific probes for *PR-1* and *PDF1.2* were performed as previously described by Ton *et al.* (2002a). Equal loading was verified by ethidium bromide staining of the gels.

Gene expression assays by RT-quantitative PCR (RT-qPCR)

Basal TF gene expression profiles in accessions were based on three biologically replicate samples, each consisting of three to five rosettes from 5-week-old plants. The TF gene expression profiling of water- and BABA-treated Col-0 plants were based on three similar biologically replicate samples, collected at 2 d after soil-drench treatment of 4-week-old plants with water or 80 μ M BABA. Analysis of *PDF1.2* and *VSP2* gene induction was based on three biologically replicate samples, each consisting of six leaves of similar age from three different plants of 5 weeks old, which were collected at the indicated time-points after spraying 0.01% (v/v) Silwet L-77 solution with 0, 200 or 500 μ M JA, or after mechanical wounding by forceps (one wounding site per leaf), with or without 5 μ L caterpillar regurgitant pipetted onto the wounded leaf areas. The gene expression analysis of recombinant inbred lines (RILs) was performed by cultivating 15–18 plants of each RIL (maximally 20 RILs per screen) along with both parental accessions. Leaves of 4-week-old plants were sprayed with water, 200 μ M JA or 0.5 mM SA, each supplemented with 0.01% (v/v) Silwet L-77. At 4 and 7 h after treatment, three biologically replicate samples, each consisting of six leaves from three different plants, were collected for analysis of *PDF1.2* and *PR-1* gene expression, respectively. RNA extraction, cDNA synthesis and qPCR reactions were performed as described by Van der Ent *et al.* (2009). Primers were similar as described previously (Czechowski *et al.* 2004, 2005), or designed using Primer3 software (<http://frodo.wi.mit.edu/primer3/input.htm>) with a T_m between 60.5 and 62, and a product size <175 bp. Two technical replicates of each sample were subjected to the qPCR reaction. The PCR efficiency (*E*) of primer pairs was estimated from data obtained from multiple amplification plots using the equation $(1 + E) = 10^{\text{slope}}$ (Ramakers *et al.* 2003), and was confirmed to consistently provide $(1 + E)$ values close to 2 (ranging from

1.92 to 2.0). Transcript levels were calculated relative to the reference genes *AtIG13320* or *GAPDH* (Czechowski *et al.* 2005), using the $2^{\Delta\Delta Ct}$ method, as described (Livak & Schmittgen 2001; Schmittgen & Livak 2008), or the $2^{\Delta Ct}$ method, where $\Delta Ct = Ct$ (reference gene) – Ct (gene of interest).

Callose assays

Vapour phase-sterilized seeds were cultivated in sterile 12-well plates, containing filter-sterilized MS medium (without vitamins), supplemented with 0.5% sucrose and 0.5% MES hydrate (pH = 5.7–5.8). Seedlings were cultivated at a 16 h/8 h day/night cycle at 20 °C 150 $\mu\text{M m}^{-2} \text{s}^{-1}$ light intensity. At day 7, the medium was replaced by fresh MS medium, and 1 d later the seedlings were treated with 0.01 mM flg22 or 0.01% chitosan. Cotyledons (8 to 15 from different plants) were collected at 24 h after PAMP treatment, stained with alinine blue and quantified for callose intensity as described by Luna *et al.* (2010).

Cluster analysis of TF gene expression profiles

TF gene profiles were analysed using TIGR MultiExperiment Viewer (TMEV) software (Saeed *et al.* 2003). Analyses were based on the log-transformed values of the fold inductions of each gene, relative to the mean expression value of three independent, un-induced Col-0 samples. Differences in TF gene expression between accessions were tested for statistical significance using Student's *t*-tests, or a non-parametric Wilcoxon Mann–Whitney test when values did not follow normal distributions.

QTL mapping analysis

QTL mapping was performed with the Bur-0 \times Col-0 core population from INRA Versailles Genomic Resource Centre (Bouchabke *et al.* 2008). This mapping population was genotyped with 87 molecular markers at an average genetic marker distance of 4.4 cM (~1.4 Mb) and has a global allelic equilibrium of 51.3% of Col-0 and 48.7% Bur-0 (<http://dbsgap.verailles.inra.fr/vnat/Documentation/20/DOC.html>). GenStat software (12th edition) was used for the analysis of genetic linkage. Gene expression values ($2^{\Delta Ct}$), callose intensities and rosette diameters for each RIL were standardized to corresponding average values from the parental accessions in each screen and uploaded as phenotypic data. After calculation of the genetic predictors, an initial genome scan produced candidate QTL positions by simple interval mapping, which were used as cofactors in a subsequent genome scan by composite interval mapping. A logarithm of odds (LOD) score of 2.94 was used as threshold of significance, corresponding to a genome-wide significance of $P = 0.05$ for normally distributed data.

Comparison of Col-0 and Bur-0 genomic sequences

Genomic polymorphisms between Col-0 and Bur-0 were based on the fully sequenced genome of accession Bur-0

(Ossowski *et al.* 2008) and visualized using POLYMORPH (<http://polymorph.weigelworld.org/>).

RESULTS

Natural variation in JA-induced *PDF1.2* expression is associated with basal resistance against the necrotrophic fungus *P. cucumerina* and the generalist herbivore *S. littoralis*

Six *Arabidopsis* accessions were selected on the basis of previously reported natural variation in basal resistance (Supporting Information Table S1). To test the response of these accessions to the defence hormone JA, induction of the JA-responsive marker gene *PDF1.2* was determined at 4 h after treatment with increasing concentrations of MeJA. At this early time-point, the reference accession Col-0 did not yet show detectable levels of *PDF1.2* expression upon treatment with 100 μM MeJA, which typically becomes detectable on Northern blots around 6–8 h after treatment (Koornneef *et al.* 2008). However, accession Bur-0 already showed significant levels of *PDF1.2* induction upon treatment with 100 μM MeJA, whereas all other accessions failed to mount detectable induction of *PDF1.2* (Fig. 1a). Hence, accession Bur-0 is primed to activate the *PDF1.2* gene upon exogenous application of MeJA.

If the observed variation in JA responsiveness influences basal resistance, it can be expected that accession Bur-0 is more resistant to pathogens or herbivores that are resisted by JA-dependent defences. To test this hypothesis, we quantified basal resistance of the six accessions to the necrotrophic fungus *P. cucumerina*, which is resisted by JA-dependent defence mechanisms (Berrocal-Lobo, Molina & Solano 2002). At 6 d after inoculation of 5-week-old plants, the accessions showed variation in symptom severity. Among all accessions, Ws-2 was most severely affected by the pathogen, whereas accession Bur-0 and, to a lesser extent, accession Sf-2 were more resistant than Col-0 (Fig. 1b). Microscopy analysis of lactophenol trypan blue-stained leaves confirmed that the differences in disease symptoms are consistent with differences in tissue damage and colonization by the fungus (Fig. 1b). To examine levels of basal resistance against JA-resisted herbivores, we quantified larval weight gain and leaf damage on 5-week-old plants upon 18 h of infestation by *S. littoralis*, a generalist herbivore that is resisted by JA-controlled defences (Mewis *et al.* 2005; Bodenhausen 2007; Bodenhausen & Reymond 2007). As is shown in Fig. 1c, larval growth on accession Bur-0 was the lowest of all combinations and differed statistically from the larval growth values on Col-0 plants (Fig. 1c). Furthermore, levels of leaf damage appeared relatively severe on accessions Ws-2 and Col-0, whereas Can-0, No-0 and Sf-2 showed intermediate levels of damage. Consistent with the larval weight gain values, accession Bur-0 showed the lowest degree of damage by the caterpillars (Fig. 1c). The caterpillar experiment yielded identical results when repeated with 3.5-week-old plants (data not shown), indicating that the variation in herbivore resistance is

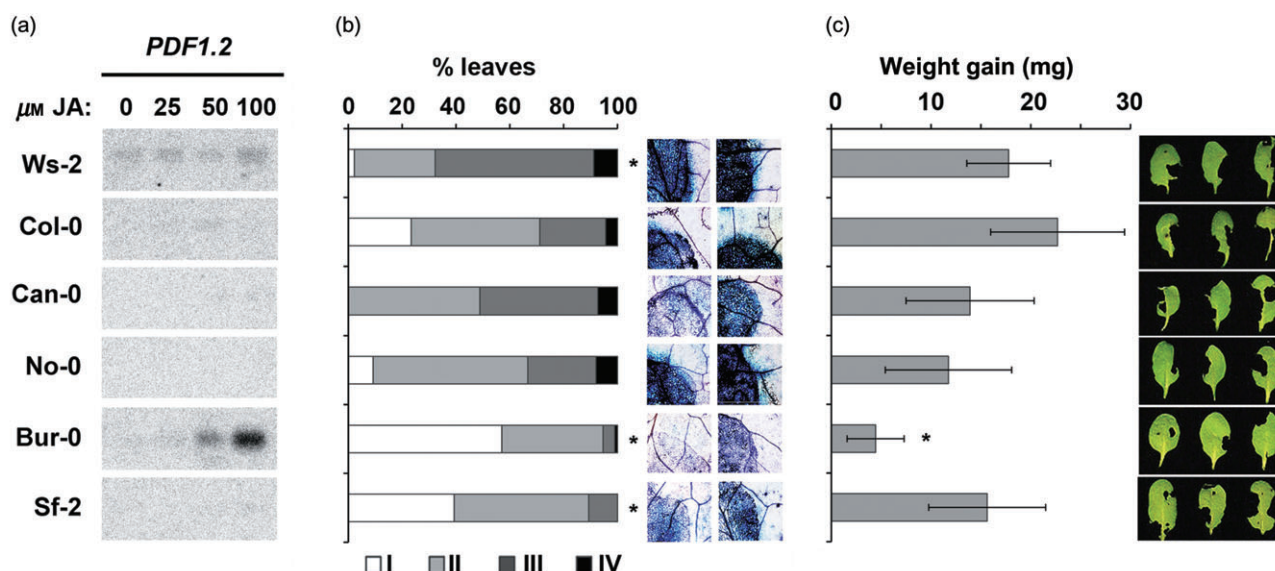


Figure 1. Natural variation in defence responsiveness to jasmonic acid (JA) between *Arabidopsis* accessions. (a) Northern blot analysis of *PDF1.2* gene expression in 5-week-old plants at 4 h after treatment of the leaves with increasing concentrations of JA. Equal loading was verified by ethidium bromide staining of the gels. (b) Natural variation in basal resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. Disease severity was scored at 7 d after drop inoculation with $6 \mu\text{L}$ droplets of 5×10^5 *P. cucumerina* spores mL^{-1} on leaves of 5-week-old plants. I, no symptoms; II, moderate necrosis at inoculation site; III, full necrosis size of inoculation droplet; and IV, spreading lesion. Asterisks indicate statistically significant different distributions of the disease classes compared to the reference accession Col-0 (χ^2 test; $\alpha = 0.05$; $n = 90$ leaves). Colonization by the pathogen and cell death were visualized by lactophenol trypan blue staining and light microscopy. Photographs show the mildest (left) and most severe (right) symptoms observed within each accession. (c) Natural variation in basal resistance to the generalist herbivore *Spodoptera littoralis*. Larval weight gain was based on an 18 h time interval of feeding. Asterisks indicate statistically significant differences in weight gain compared to the reference accession Col-0 (Fisher's LSD test; $\alpha = 0.05$; $n = 10$). Photographs show representative levels of feeding damage in leaves of similar age. The experiment was repeated with 3.5-week-old plants, yielding similar results.

unrelated to plant age. Hence, the enhanced responsiveness of accession Bur-0 to JA is associated with increased levels of basal resistance against attackers that are controlled by JA-dependent defence mechanisms.

Accession Bur-0 is primed to activate ERF1/ORA59-dependent *PDF1.2*, but is repressed in MYC2-dependent induction of *VSP2*

Induction of *PDF1.2* gene expression is regulated by the TFs ORA59 and ERF1, which integrate JA- and ET-dependent defence signals (Lorenzo *et al.* 2003; Pré *et al.* 2008). Conversely, induction of JA-dependent *VSP2* is regulated by MYC2, a TF that integrates JA- and abscisic acid (ABA)-dependent signals (Lorenzo *et al.* 2004). Both branches of the JA response pathway act antagonistically on each other (Lorenzo *et al.* 2004; Lorenzo & Solano 2005). To investigate whether the primed *PDF1.2* gene responsiveness of Bur-0 is caused by a shift in negative cross-talk between ERF1/ORA59- and MYC2-dependent signalling branches, we monitored the expression of *PDF1.2* and *VSP2* in Col-0 and Bur-0 at different time-points after the application of JA. Again, accession Bur-0 showed strongly augmented levels of JA-induced *PDF1.2* expression in comparison to Col-0 (Fig. 2a). In contrast, expression of *VSP2* remained

consistently lower in Bur-0 at all time-points after JA application. A similar transcription profile was observed after leaf wounding and combined treatment of wounding + *S. littoralis* regurgitant (Fig. 2b). The antagonistic induction profiles of *PDF1.2* and *VSP2* indicate that accession Bur-0 is primed to activate the ERF1/ORA59-dependent branch of the JA response, but is repressed in the MYC2-dependent JA response. Nevertheless, we did not detect consistent differences in expression levels of *ORA59*, *ERF1* or *MYC2* between Bur-0 and Col-0 (Supporting Information Fig. S1a), nor did we find differences in genomic coding sequences of these TF genes (Supporting Information Fig. S1b). Hence, the differentially regulated JA response in accession Bur-0 is caused by modulating factors acting up- or downstream of ORA59, ERF1 or MYC2.

Natural variation in SA-induced *PR-1* expression is associated with basal resistance against the hemi-biotrophic pathogen *Pst* DC3000

To assess natural variation in responsiveness to the plant defence hormone SA, the six accessions were examined for levels of *PR-1* gene induction at 6 h after treatment of the leaves with increasing concentrations of SA. In the first experiment, accessions Bur-0, Can-0 and Sf-2 showed

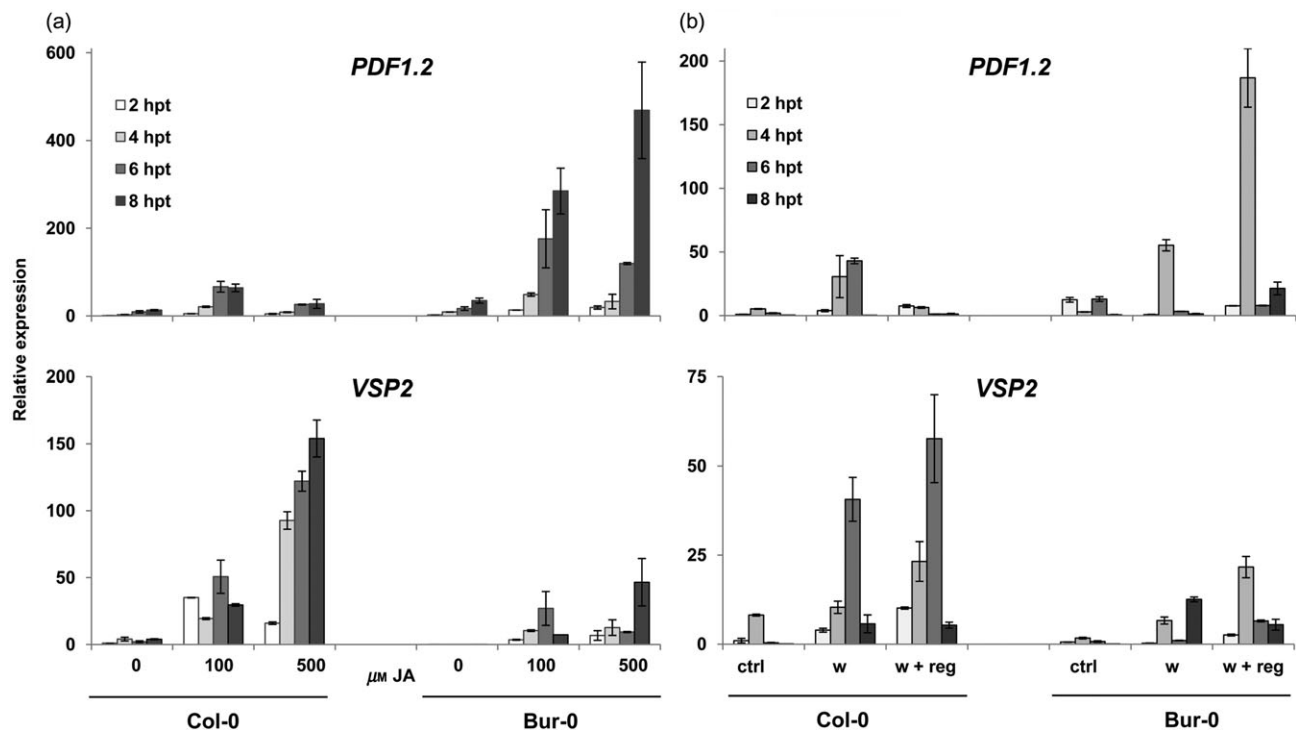


Figure 2. *Arabidopsis* accession Bur-0 is primed to activate *PDF1.2* gene expression, but is repressed in *VSP2* gene expression. (a) RT-qPCR analysis of *PDF1.2* and *VSP2* expression in 5-week-old Col-0 and Bur-0 plants at different time-points after application of increasing concentrations of jasmonic acid (JA) to the leaves. Data presented are average fold-change values ($n = 3$; \pm SEM) relative to the mean expression level in control-treated Col-0 at 2 hpt. (b) RT-qPCR analysis of *PDF1.2* and *VSP2* expression in 5-week-old Col-0 and Bur-0 plants at different time-points after leaf wounding, or combined treatment of wounding and *Spodoptera littoralis* regurgitant. Data presented are average fold-change values ($n = 3$; \pm SEM) relative to the mean expression level in control-treated Col-0 at 2 hpt.

enhanced levels of *PR-1* gene induction by SA, whereas accessions Col-0, No-0 and Ws-2 expressed lower levels of *PR-1* after treatment with SA (Fig. 3a). In an independent second experiment, similar results were obtained for all accessions except No-0, which showed constitutive *PR-1* gene expression in the control group. The latter finding is likely caused by the occasional development of spontaneous lesions in No-0 under our greenhouse conditions (data not shown). Thus, despite the variable behaviour of accession No-0, these results demonstrate consistent and substantial natural variation in *PR-1* gene responsiveness to exogenously applied SA.

To examine whether the observed natural variation in SA responsiveness has an effect on basal disease resistance, we evaluated under similar growth conditions basal resistance against the hemi-biotrophic pathogen *Pst* DC3000, which is resisted by SA-dependent defence mechanisms (Ton *et al.* 2002b; Glazebrook 2005). Plants were inoculated by dipping the rosettes into a bacterial suspension, and examined for disease symptoms and bacterial proliferation. Accessions Can-0, No-0, Bur-0 and Sf-2 developed significantly fewer disease symptoms (Fig. 3b), and allowed less bacterial growth in comparison to accession Col-0 (Fig. 3c). Conversely, accession Ws-2 allowed higher levels of bacterial growth than Col-0 (Fig. 3b,c). To examine whether these

differences are caused by pre-invasive early-acting defence barriers, we quantified bacterial proliferation upon pressure infiltration of the leaves. This experiment yielded similar differences in bacterial proliferation between the accessions (Supporting Information Fig. S2), suggesting that the genetic variation in basal resistance to *Pst* DC3000 is based on post-invasive defence mechanisms. Hence, primed SA responsiveness of accessions Can-0, Bur-0 and Sf-2 is associated with increased levels of basal resistance to *Pst* DC3000.

Accessions with primed *PR-1* gene responsiveness express enhanced levels of priming-related TF genes

Previously, we demonstrated that priming of SA-dependent defence upon treatment with the chemical priming agent BABA is marked by enhanced expression of 28 defence-regulatory TF genes (Van der Ent *et al.* 2009). Because Can-0, Bur-0 and Sf-2 are primed to respond to SA in comparison to Col-0 (Fig. 3a), we used RT-qPCR to determine whether this phenotype is similarly marked by enhanced expression of TF genes. Out of the 28 TF genes tested, 21, 23 and 24 genes showed enhanced expression in Bur-0, Can-0 and Sf-2, respectively

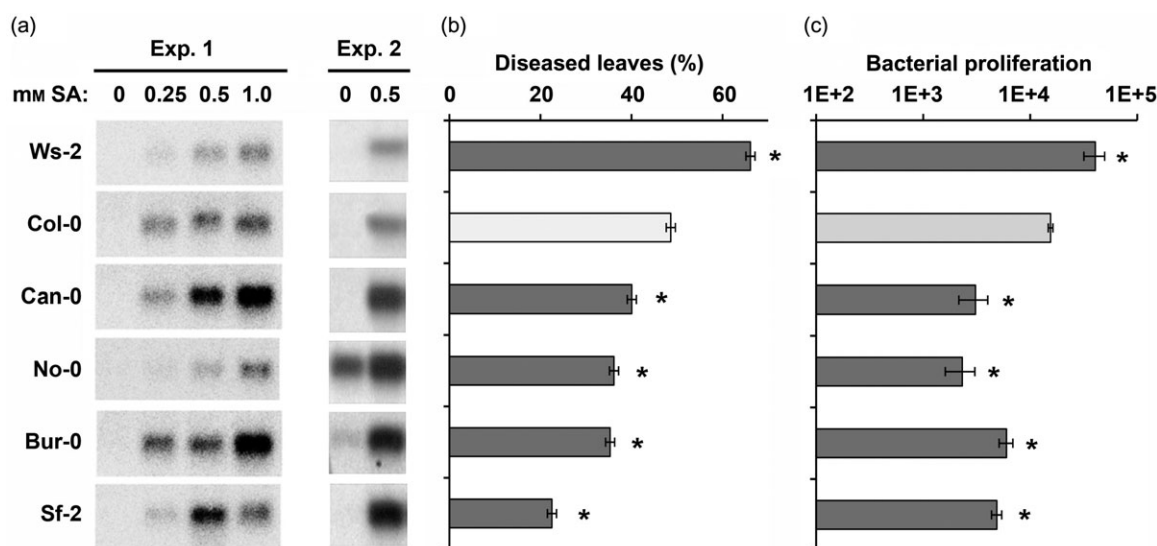


Figure 3. Natural variation in defence responsiveness to salicylic acid (SA) between *Arabidopsis* accessions. (a) Northern blot analysis of *PR-1* gene induction in 5-week-old *Arabidopsis* accessions at 6 h after treatment with different concentrations of SA. Equal loading was verified by ethidium bromide staining of the gels. (b) Disease symptoms caused by *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) at 4 d after dipping the leaves in a suspension containing 5×10^5 cfu mL⁻¹. Values represent the average percentage of leaves showing chlorotic symptoms per plant (\pm SEM; $n = 25$ –30). Asterisks indicate statistically significant differences compared to reference accession Col-0 (Student's *t*-test; $\alpha = 0.05$). (c) Bacterial proliferation of *Pst* DC3000 over a 3 d time interval after dip inoculation of the leaves. Shown are average values (\pm SE; $n = 5$ –10). Asterisks indicate statistically significant differences compared to reference accession Col-0 (Student's *t*-test; $\alpha = 0.05$).

($P < 0.05$; Fig. 4 and Supporting Information Fig. S3). In contrast, accession Ws-2, which exhibited similar or reduced SA responsiveness compared to Col-0 (Fig. 3a), displayed a mostly unaltered or decreased expression of these marker genes (Fig. 4). To examine whether the basal transcription patterns of the 28 TF genes in accessions Bur-0, Can-0 and Sf2 are similar to those of chemically primed Col-0 plants, we used multivariate cluster analysis. As expected, the transcription profiles of Bur-0, Can-0 and Sf-1 resembled the BABA-induced transcription profile of Col-0 (Supporting Information Fig. S3), demonstrating that the constitutively primed SA response in these accessions is marked by elevated expression of the same set of TF genes that are induced upon chemical defence priming by BABA.

Natural variation in responsiveness of callose deposition to PAMPs

To examine genetic variation in the sensitivity of locally expressed defence responses that are not directly under control by JA or SA, we quantified callose intensities upon treatment with the fungal PAMP elicitor chitosan and the bacterial PAMP elicitor flg22. Seedlings of the six accessions were cultivated in a hydroponic growth medium and examined for levels of callose deposition at 24 h after mock or PAMP treatment, using a standardized quantification method as described previously (Luna *et al.* 2010). Although all accessions showed a statistically

significant increase in callose deposition after chitosan treatment, there was substantial variation in the intensity of this defence response (Fig. 5). On average, Col-0 and Ws-2 deposited fivefold more callose than accessions Bur-0, No-0, Sf-2 and Can-0. Similar patterns were observed upon treatment with flg22 (Fig. 5), with the exception of accession Ws-2, which lacked flg22-induced callose because of a dysfunctional FLS2 receptor (Gómez-Gómez & Boller 2000). Interestingly, when comparing natural variation in PAMP-induced callose to natural variation in SA-induced *PR-1* expression (Fig. 3), an inverse relationship can be noted: accessions with a modest callose response are primed to respond to SA, while accessions with a primed callose response are relatively unresponsive to SA.

Identification of a locus regulating responsiveness of SA-induced *PR-1* expression

To dissect the genetic variation in defence gene responsiveness, we performed QTL mapping analysis, using a fully genotyped core population of 164 RILs from a cross between accessions Col-0 and Bur-0 (Simon *et al.* 2008). For each gene expression screen, 15 to 20 RILs were cultivated along with the parental accessions, and examined for *PDF1.2* or *PR-1* gene expression at 4 h after treatment with 200 μ M JA, or 7 h after treatment with 0.5 mM SA, respectively. At both time-points, leaves from mock-treated plants were collected to assess basal levels of

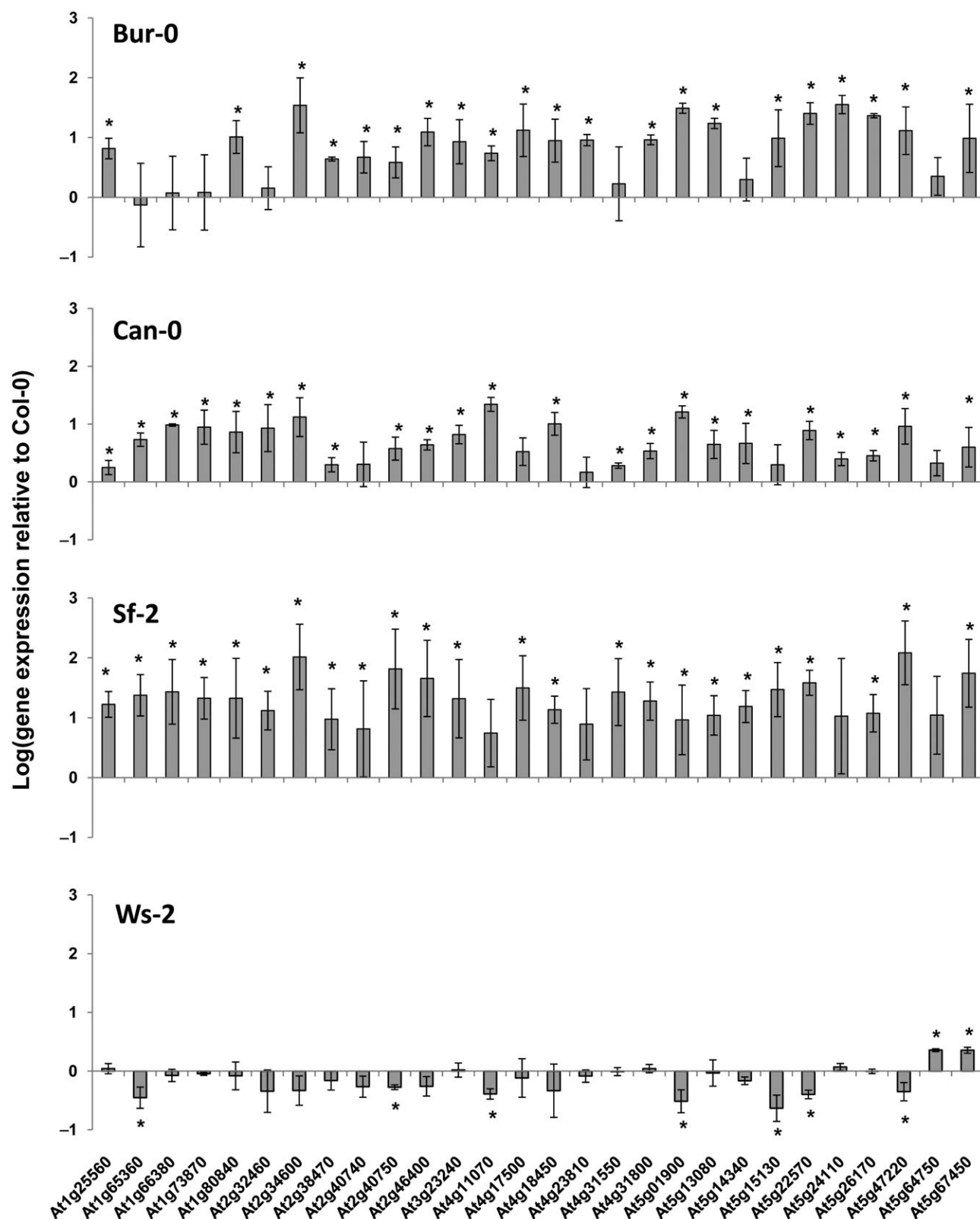


Figure 4. Accessions with primed responsiveness to salicylic acid (SA) show enhanced transcription of priming-related transcription factor (TF) genes. (a) Transcriptional levels of 28 priming-related TF genes, previously identified as markers for beta-aminobutyric acid (BABA)-induced priming of SA-dependent defence (Van der Ent *et al.* 2009). Data presented are log-transformed fold-change values (\pm SEM) relative to the mean expression level in accession Col-0. Asterisks indicate statistically enhanced levels of expression compared to Col-0 (Mann–Whitney–Wilcoxon test or Student's *t*-test; $\alpha = 0.05$).

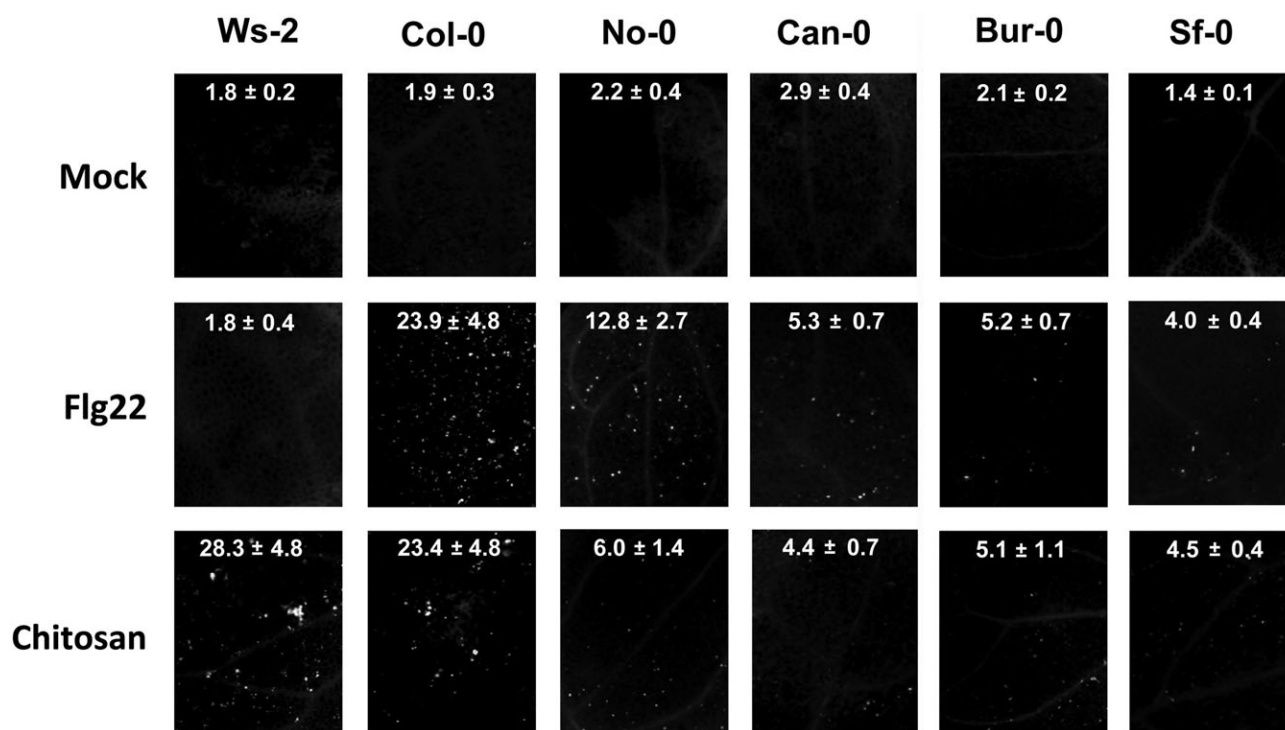


Figure 5. Natural variation in pathogen-associated molecular pattern (PAMP)-induced callose deposition. Eight-day-old seedlings were treated with either 0.1% chitosan or 1 μ M flg22. After 24 h, the cotyledons were stained for callose with aniline blue. Callose intensities were digitally quantified from photographs by UV epifluorescence microscopy. Values ($\times 10,000$) represent relative callose intensities, quantified as the number of fluorescent callose pixels divided by the number of pixels covering plant material ($n = 15$; \pm SEM).

PDF1.2 and *PR-1* expression. Although accession Bur-0 showed consistently higher levels of JA-induced *PDF1.2* expression than Col-0, this difference appeared to be too variable between screens to allow for reliable QTL analysis of the pooled data set (data not shown). On the other hand, inter-experiment variation in *PR-1* gene expression remained marginal. *PR-1* expression values in each RIL screen were standardized to the averaged value from the parental accessions (Col-0 and Bur-0). Composite interval mapping of these standardized values revealed statistically significant linkage between SA-induced *PR-1* expression and a locus at chromosome IV (LOD score = 5.64; Fig. 6 and Table 1). RILs carrying the Bur-0 alleles at this locus showed higher levels of SA-induced *PR-1* expression than RILs carrying the Col-0 alleles at this locus. This direction indicates either a suppressive effect from the Col-0 parent, or a stimulatory locus from the Bur-0 parent (Table 1). No genetic linkage was found with levels of basal *PR-1* gene expression (Fig. 6a). Hence, the locus at chromosome IV influences responsiveness of the *PR-1* gene to SA, but has no influence on basal levels of *PR-1* expression. Interestingly, the locus maps closely to a cluster of TIR-NB-LRR genes (At4g16860–At4g16990), of which 10 show non-synonymous polymorphisms between Col-0 and Bur-0 (Supporting Information Table S1). The locus also maps closely to the highly polymorphic *ACD6* gene (At4g14430;

Supporting Information Table S1), which was recently identified as a source of natural allelic variation causing vegetative growth reduction and SA-dependent disease resistance (Todesco *et al.* 2010).

Identification of QTLs regulating responsiveness of flg22-induced callose

To explore the genetic basis of natural variation in responsiveness of PAMP-induced callose, we screened the RIL population for levels of basal and flg22-induced callose. For each experiment, callose intensities were standardized to the averaged values from the parental accessions. Composite interval mapping of the pooled data did not reveal genetic linkage with basal callose levels in mock-treated seedlings. On the other hand, a strong linkage was found between flg22-induced callose and a locus at chromosome III (LOD score = 16.22; Fig. 6). An additional weaker influence was detected at a locus on the top of chromosome I (LOD score = 3.01; Fig. 6b). The direction of both QTLs suggests opposite effects (Table 1): the major-effect QTL at chromosome III mediates a repressive effect from the Bur-0 parent or a stimulatory effect from the Col-0 parent, whereas the weaker QTL at chromosome I exerts a stimulatory effect from Bur-0 or a repressive effect from Col-0.

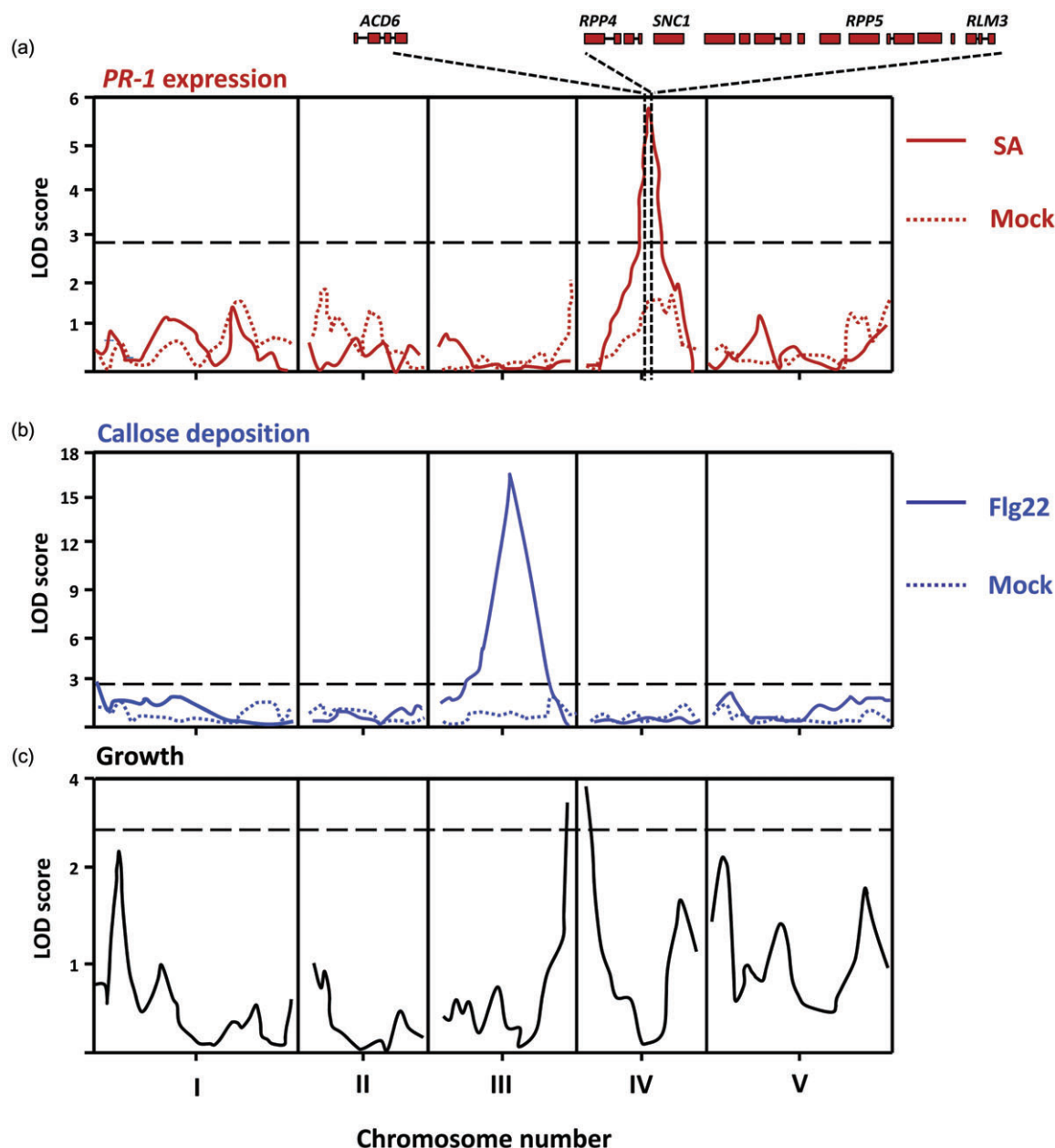


Figure 6. Logarithm of odds (LOD) scores and chromosome positions of quantitative trait loci (QTLs) influencing *PR-1* gene expression, callose deposition and plant growth. The QTL mapping analysis was based on 164 recombinant inbred lines (RILs) from a cross between accessions Col-0 and Bur-0. (a) QTLs controlling basal and salicylic acid (SA)-induced *PR-1* expression. (b) QTLs controlling basal and flg22-induced callose. (c) QTLs controlling plant growth estimated from rosette sizes of 4-week-old plants. The *ACD6* gene and the *RPP4/SNC1/RPP5* cluster of R genes are indicated at the top. Horizontal dashed lines represent threshold levels of statistically significant LOD scores (2.94).

QTLs regulating defence responsiveness are not associated with reduced growth

Induction of defence priming is associated with minor or no detectable costs on plant growth (Van Hulten *et al.* 2006; Walters *et al.* 2009). To examine whether the constitutive defence priming in accession Bur-0 is associated with costs on plant growth, we scored rosette sizes of the

RILs over a growth period of 4 weeks. Two relatively weak QTLs were identified at the bottom and top of chromosomes III and IV (LOD scores 3.19 and 3.76, respectively), which did not correspond to the QTLs controlling flg22-induced callose or SA-induced *PR-1* expression (Fig. 6; Table 1). This indicates that the priming-inducing loci from accession Bur-0 are not associated with major costs on plant fitness.

| Trait | Chromosome | cM | Position (bp) | LOD score | Direction |
|----------------------------------|------------|------|---------------|-----------|-----------|
| flg22-induced callose | I | 0.0 | 592 939 | 3.01 | Bur+Col- |
| flg22-induced callose | III | 34.2 | 10 995 664 | 16.22 | Bur-Col+ |
| Growth | III | 63.5 | 22 146 585 | 3.19 | Bur+Col- |
| Growth | IV | 0.0 | 641 363 | 3.76 | Bur+Col- |
| SA-induced <i>PR1</i> expression | IV | 30.9 | 8 929 959 | 5.64 | Bur+Col- |

Bur+Col-, a positive effect from the Bur-0 alleles or a negative effect from the Col-0 alleles;
Bur-Col+, a negative effect from the Bur-0 alleles or a positive effect from the Col-0 alleles.

Priming by the SA response locus on chromosome IV boosts basal resistance against *Pst* DC3000

The major callose QTL at chromosome III is based on either a repressive effect by the Bur-0 alleles, or a stimulatory effect from the Col-0 alleles (Table 1). Because accession Bur-0 is considerably more resistant against *Pst* DC3000 than accession Col-0 (Fig. 3), it is unlikely that this locus contributes to basal resistance against this pathogen. In contrast, the direction of the SA response locus at chromosome IV is consistent with a positive effect on basal resistance against *Pst* DC3000. To confirm this, we compared levels of basal resistance between five RILs carrying the Bur-0 alleles of the SA response locus, five RILs carrying the Col-0 alleles of this locus, and the two parental accessions. Basal resistance assays were performed using a bioluminescent luxCDABE-tagged *P. syringae* pv. *tomato* DC3000 strain (*Pst* DC3000-lux), which enables *in planta* quantification of bacterial colonization (Fan *et al.* 2008). At 3 d after dipping the leaves in the bacterial suspension, *Pst* DC3000-lux colonization in leaves of the Bur-0 lines was consistently lower than in leaves of the Col-0 lines (Fig. 7a). Furthermore, bacterial growth values in all genotypes were inversely related to levels of SA-induced *PR-1* transcription (Fig. 7b,c). Multiple regression analysis of the bacterial growth values confirmed a statistically significant effect of the SA response locus ($P < 0.001$), whereas variation between lines with similar genotypes for this locus had no significant influence on bacterial growth ($P = 0.258$). These results indicate that constitutive priming of the SA response by the locus on chromosome IV boosts basal resistance against *Pst* DC3000.

DISCUSSION

Plant resistance to biotic stress largely depends on inducible defence mechanisms. However, induced defence can be costly because of allocation of resources or toxicity to the plant's own metabolism. These contrasting benefits provide a classic trade-off between plant defence and development (Heil 2002; Heil & Baldwin 2002). Priming of induced defence is associated with relatively minor costs which are outweighed by the benefits of increased resistance under conditions of disease pressure (Van Hulst *et al.* 2006; Walters *et al.* 2009). Furthermore, priming provides

resistance against a broad spectrum of pathogens and herbivores (Conrath *et al.* 2006; Frost *et al.* 2008). It is therefore plausible that selected plant varieties have evolved constitutively primed immune systems to adapt to environments that impose constant pressure from a wide range of pathogens (Ahmad *et al.* 2010). In support of this, our present study revealed genetic variation in the sensitivity of basal defence reactions among naturally occurring *Arabidopsis* accessions. Genetic dissection of this variation identified QTLs that regulate the responsiveness of these basal defence (Fig. 6; Table 1). Interestingly, none of these QTLs had a detectable influence on plant growth (Fig. 6), which supports our notion that constitutively primed defence is not associated with major fitness costs.

Priming of defence can be based on enhanced expression of signalling proteins that remain inactive until an environmental stress signal is perceived by the plant (Conrath *et al.* 2006; Beckers *et al.* 2009). In agreement with this, we previously found that BABA-induced priming of SA-dependent defence is marked by enhanced expression of a set of defence-related TF genes (Van der Ent *et al.* 2009). In this study, we demonstrated that accessions Bur-0, Can-0 and Sf-2 express enhanced responsiveness to SA, are more resistant to *Pst* DC3000, and show elevated expression of BABA-inducible TF genes (Fig. 2). This suggests that the primed defence state of Bur-0, Can-0 and Sf-2 is based on similar signalling mechanisms as BABA-induced defence priming. In addition, these results illustrate that the set of BABA-inducible TF genes can serve as a marker to identify plants with primed immune systems.

Accessions with primed responsiveness to SA expressed relatively high basal resistance against *Pst* DC3000 (Fig. 3). Van Leeuwen *et al.* (2007) reported similar natural variation between *Arabidopsis* accessions in the transcriptional response to exogenously applied SA. In this study, we used QTL mapping analysis to dissect this natural variation, and identified a locus on chromosome IV that contributes to basal resistance against *Pst* DC3000 (Figs 6 & 7). Interestingly, this locus maps to a cluster of nine TIR-NB-LRR genes at chromosome IV (Fig. 6; Supporting Information Table S2), which includes the *Leptosphaeria maculans* R gene *RLM3* (Staal *et al.* 2008), the *Hyaloperonospora arabidopsidis* R genes *RPP4* and *RPP5* (Parker *et al.* 1997; Van der Biezen *et al.* 2002) and the *SUPPRESSOR OF npr1-1*, *CONSTITUTIVE 1* (*SNCI*) gene. Mutations in *SNCI* have been reported to constitutively activate a

Table 1. Chromosome locations, statistical significance and direction of quantitative trait loci (QTLs) influencing of salicylic acid (SA)-induced *PR1* expression, pathogen-associated molecular pattern (PAMP)-induced callose and plant growth

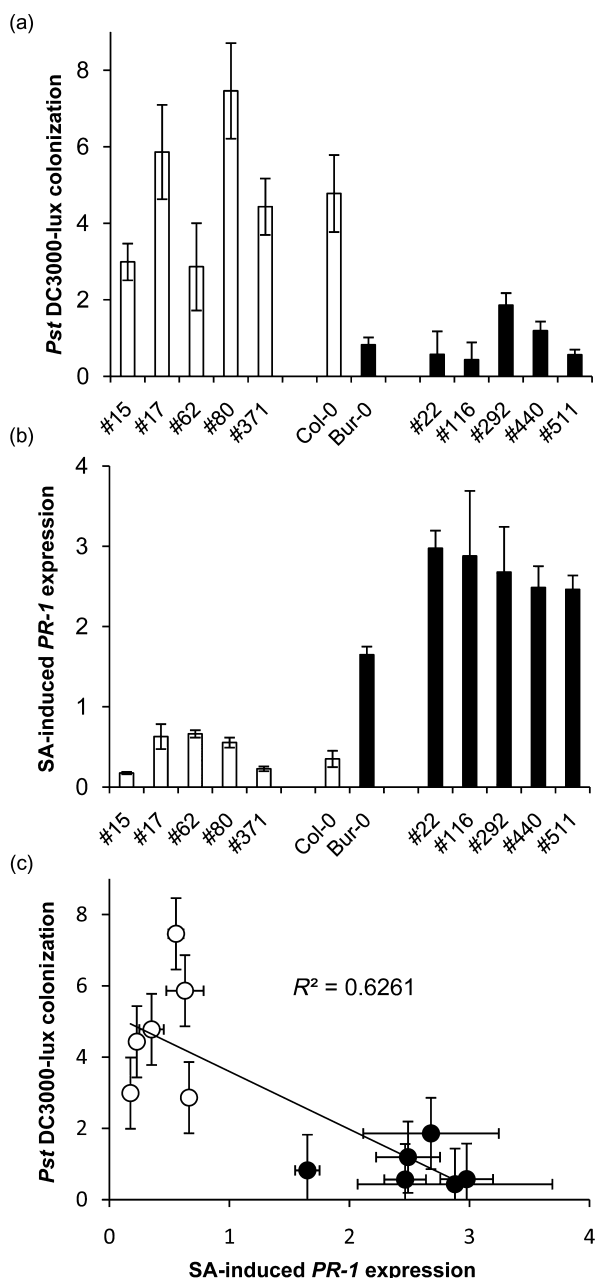


Figure 7. Basal resistance against *Pst* DC3000-lux and salicylic acid (SA)-induced *PR1* expression in five recombinant inbred lines (RILs) with the Col-0 alleles of the SA response quantitative trait locus (QTL) (lines #15, #17, #62, #80, #371), five RILs with the Bur-0 alleles of this locus (lines #22, #116, #292, #440, #511) and the parental accessions Col-0 and Bur-0. Black and white bars/symbols indicate genotypes carrying the Bur-0 and Col-0 alleles of the locus, respectively. (a) Colonization by *Pst* DC3000-lux at 3 d after dip inoculation of the leaves. Bacterial titres were based on *in planta* bioluminescence by the bacteria. Data shown represent average values ($n = 12$; \pm SEM) of bioluminescence-corresponding pixels in leaves relative to the number of pixels covering total leaf material in each plant. (b) Transcriptional levels of *PR-1* expression at 7 h after treatment of the leaves with 0.5 mM of SA. Data presented are average fold-change values ($n = 3$; \pm SEM) standardized to the mean expression value between Col-0 and Bur-0. (c) Correlation between bacterial colonization and SA-induced *PR-1* expression.

SA-dependent disease resistance (Li *et al.* 2001; Zhang *et al.* 2003). It is therefore possible that the non-synonymous polymorphisms between Col-0 and Bur-0 in this gene cluster (Supporting Information Table S1) are responsible for the differences in SA-induced *PR-1* gene expression and basal resistance against *Pst* DC3000 between these accessions. Furthermore, natural allelic variation in the *ACD6* gene (*At4g14420*) has recently been reported to cause constitutive expression of SA-dependent defence and reduced vegetative growth (Todesco *et al.* 2010). Not only does *ACD6* map closely to the SA response QTL, accessions Col-0 and Bur-0 also show 10 non-synonymous polymorphisms in the *ACD6*, of which four confer biochemically dissimilar amino acids. (Supporting Information Table S2). However, unlike accession Est-1, Bur-0 is not reduced in vegetative growth (Fig. 6) and does not express SA-dependent defence constitutively (Fig. 3). It remains, nonetheless, possible that the Bur-0 alleles of *ACD6* constitute a less extreme gene variant that merely primes for SA-dependent defences.

Jones & Dangl (2006) defined basal resistance as residual resistance that is activated by virulent pathogens after defence suppression by disease-promoting pathogen effectors (i.e. basal resistance = PTI – ETS + weakened ETI). A recent study by Zhang *et al.* (2010) suggested that basal resistance against *Pst* DC3000 is mostly determined by weakened ETI, while PTI has relatively little contribution. In agreement with this, we mapped a regulatory locus for SA responsiveness and basal resistance against *Pst* DC3000 to a cluster of ETI-associated TIR-NB-LRR genes. Furthermore, two previous studies on natural variation in basal resistance against *Pst* DC3000 identified QTLs at other genomic regions enriched in R and/or PRR genes (Kover *et al.* 2005; Perchepied *et al.* 2006). Together, these results suggest that natural variation in basal resistance against *Pst* DC3000 originates from the ETI component of basal resistance. Interestingly, accessions with relatively high basal resistance to *Pst* DC3000 deposited comparatively low levels of PAMP-induced callose (Figs 3 & 5). Moreover, our QTL analysis identified a major callose-promoting locus from the more susceptible Col-0 parent (Fig. 6; Table 1), suggesting that virulent *Pst* DC3000 is not significantly resisted by PAMP-induced callose. Indeed, other studies have demonstrated that *Pst* DC3000 is extremely efficient in suppressing callose deposition through type III effectors (Zhang *et al.* 2007; Guo *et al.* 2009; Xiang *et al.* 2010). In contrast, dissection of natural variation in non-host resistance against non-host *P. syringae* pv. *phaseolicola* identified a major influence from the flagellin receptor FLS2 (Forsyth *et al.* 2010), suggesting that PTI has a more prominent role in non-host resistance against *P. syringae* pathogens. It thus seems that natural variation in resistance against virulent *P. syringae* strains stems from ETI-related defence mechanisms, whereas natural variation in resistance against non-host *P. syringae* strains is based on PTI-related defence mechanisms.

Accession Bur-0 is primed to activate both SA- and JA-dependent defences (Figs 1–3), even though both

pathways are mutually antagonistic in *Arabidopsis* (Koornneef & Pieterse 2008). This phenotype demonstrates that enhanced responsiveness of SA- and JA-dependent defences is not affected by the negative cross-talk between both pathways. In support with this, Van Wees *et al.* (2000) demonstrated that simultaneous activation of rhizobacteria-mediated induced systemic resistance (ISR) and pathogen-induced systemic acquired resistance (SAR), which are based on priming of JA- and SA-dependent defences, respectively, yields additive levels of resistance. Interestingly, the primed *PDF1.2* response of accession Bur-0 to JA or wounding + caterpillar regurgitant coincided with a repressed induction of the *VSP2* gene (Fig. 2). Expression of *PDF1.2* and *VSP2* marks the activities of two antagonistically acting branches of the JA response, which are regulated by the TFs ERF1 and ORA59, and MYC2, respectively (Lorenzo *et al.* 2003, 2004; Pré *et al.* 2008). The ERF1/ORA59-dependent branch integrates JA and ET signals, whereas the MYC2-dependent branch integrates JA and ABA signals (Lorenzo *et al.* 2003; Anderson *et al.* 2004; Pré *et al.* 2008). Therefore, the primed *PDF1.2* response of Bur-0 indicates potentiation of the ERF1/ORA59-dependent JA response (Lorenzo *et al.* 2004). This branch of the JA response has been described to contribute to basal resistance against necrotrophic pathogens (Anderson *et al.* 2004; Lorenzo *et al.* 2004). Indeed, accession Bur-0 displayed enhanced resistance to the necrotrophic fungus *P. cucumerina* (Fig. 1b) and had previously been described as more resistant to the necrotrophic fungi *B. cinerea* and *Fusarium oxysporum* (Llorente *et al.* 2005). We also showed that Bur-0 is more resistant to feeding by the generalist herbivore *S. littoralis* (Fig. 1c). Although Lorenzo *et al.* (2004) proposed a dominant role for the MYC2-dependent JA branch in resistance against herbivory, mutations in MYC2 have no consistent effect on basal resistance against *S. littoralis* (Bodenhausen 2007). On the other hand, there is ample evidence that ET synergizes JA-dependent defences against herbivores (Von Dahl & Baldwin 2007). This supports findings by Van Oosten *et al.* (2008), who demonstrated that ISR-expressing Col-0 plants are primed to activate *PDF1.2* and display enhanced resistance against *S. exigua*. Involvement of ET in the JA-responsive phenotype of accession Bur-0 would also explain why we encountered high inter-experiment variation in JA-induced *PDF1.2* gene expression during our attempted QTL mapping of this trait.

Plants have evolved various strategies to defend themselves against pathogens and herbivores. Apart from the well-characterized zigzag evolution towards R protein-mediated ETI, there are alternative defence strategies that can be equally effective depending on the environmental conditions (Ahmad *et al.* 2010). Our study has provided genetic evidence that selected *Arabidopsis* accessions have evolved constitutive priming of basal defence mechanisms, which can boost resistance against virulent pathogens. Mining for similar genetic traits in ancestral crop species will allow for integration of this naturally evolved defence strategy in sustainable pest and disease management.

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REFERENCES

- Abramovitch R.B., Janjusevic R., Stebbins C.E. & Martin G.B. (2006) Type III effector AvrPtoB requires intrinsic E3 ubiquitin ligase activity to suppress plant cell death and immunity. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 2851–2856.
- Adam L., Ellwood S., Wilson I., Saenz G., Xiao S., Oliver R.P., Turner J.G. & Somerville S. (1999) Comparison of *Erysiphe cichoracearum* and *E. cruciferarum* and a survey of 360 *Arabidopsis thaliana* accessions for resistance to these two powdery mildew pathogens. *Molecular Plant–Microbe Interactions* **12**, 1031–1043.
- Ahmad S., Gordon-Weeks R., Pickett J. & Ton J. (2010) Natural variation in priming of basal resistance: from evolutionary origin to agricultural exploitation. *Molecular Plant Pathology* **11**, 817–827.
- Anderson J.P., Badruzaufari E., Schenk P.M., Manners J.M., Desmond O.J., Ehler C., Maclean D.J., Ebert P.R. & Kazan K. (2004) Antagonistic interaction between abscisic acid and jasmonate–ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *The Plant Cell* **16**, 3460–3479.
- Beckers G.J.M., Jaskiewicz M., Liu Y., Underwood W.R., He S.Y., Zhang S. & Conrath U. (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *The Plant Cell* **21**, 944–953.
- Berrocal-Lobo M., Molina A. & Solano R. (2002) Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. *The Plant Journal* **29**, 23–32.
- Bodenhausen N. (2007) *Arabidopsis thaliana* response to insect feeding: new components controlling defense gene expression and plant resistance. PhD thesis, University of Lausanne, Available at: http://www.unil.ch/webdav/site/dbmv/shared/Reymond/Natacha_Thesis/NatachaBodenhausen.pdf (accessed 2007, University of Lausanne).
- Bodenhausen N. & Reymond P. (2007) Signaling pathways controlling induced resistance to insect herbivores in *Arabidopsis*. *Molecular Plant–Microbe Interactions* **20**, 1406–1420.
- Bouchabke O., Chang F., Simon M., Voisin R., Pelletier G. & Durand-Tardif M. (2008) Natural variation in *Arabidopsis thaliana* as a tool for highlighting differential drought responses. *PLoS ONE* **3**, e1705.
- Chen X., Steed A., Harden C. & Nicholson P. (2006) Characterization of *Arabidopsis thaliana*–*Fusarium graminearum* interactions and identification of variation in resistance among ecotypes. *Molecular Plant Pathology* **7**, 391–403.
- Conrath U., Beckers G.J.M., Flors V., *et al.* (2006) Priming: getting ready for battle. *Molecular Plant–Microbe Interactions* **19**, 1062–1071.
- Cui H., Xiang T. & Zhou J.M. (2009) Plant immunity: a lesson from pathogenic bacterial effector proteins. *Cellular Microbiology* **11**, 1453–1461.
- Czechowski T., Bari R.P., Stitt M., Scheible W.R. & Udvardi M.K. (2004) Real-time RT-PCR profiling of over 1400 *Arabidopsis*

- transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. *The Plant Journal* **38**, 366–379.
- Czechowski T., Stitt M., Altmann T., Udvardi M.K. & Scheible W.-R. (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiology* **139**, 5–17.
- Denby K.J., Kumar P. & Kliebenstein D.J. (2004) Identification of *Botrytis cinerea* susceptibility loci in *Arabidopsis thaliana*. *The Plant Journal* **38**, 473–486.
- Engelberth J., Alborn H.T., Schmelz E.A. & Tumlinson J.H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 1781–1785.
- Eulgem T., Rushton P.J., Schmelzer E., Hahlbrock K. & Somssich I.E. (1999) Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. *EMBO Journal* **18**, 4689–4699.
- Fan J., Crooks C. & Lamb C. (2008) High-throughput quantitative luminescence assay of the growth in planta of *Pseudomonas syringae* chromosomally tagged with *Photobacterium luminescens* luxCDABE. *The Plant Journal* **53**, 393–399.
- Forsyth A., Mansfield J.W., Grabov N., de Torres M., Sinapidou E. & Grant M.R. (2010) Genetic dissection of basal resistance to *Pseudomonas syringae* pv. *phaseolicola* in accessions of *Arabidopsis*. *Molecular Plant–Microbe Interactions* **23**, 1545–1552.
- Frost C.J., Mescher M.C., Carlson J.E. & De Moraes C.M. (2008) Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology* **146**, 818–824.
- Fu Z.Q., Guo M., Jeong B.R., Tian F., Elthon T.E., Cerny R.L., Staiger D. & Alfano J.R. (2007) A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity. *Nature* **447**, 284–288.
- Glazebrook J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**, 205–227.
- Gómez-Gómez L. & Boller T. (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Molecular Cell* **5**, 1003–1011.
- Guo M., Tian F., Wamboldt Y. & Alfano J.R. (2009) The majority of the type III effector inventory of *Pseudomonas syringae* pv. *tomato* DC3000 can suppress plant immunity. *Molecular Plant–Microbe Interactions* **22**, 1069–1080.
- Heil M. (2002) Ecological costs of induced resistance. *Current Opinion in Plant Biology* **5**, 345–350.
- Heil M. & Baldwin I.T. (2002) Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends in Plant Science* **7**, 61–67.
- Heil M. & Ton J. (2008) Long-distance signalling in plant defence. *Trends in Plant Science* **13**, 264–272.
- Houterman P.M., Ma L., Van Ooijen G., de Vroomen M.J., Cornelissen B.J., Takken F.L. & Rep M. (2009) The effector protein Avr2 of the xylem-colonizing fungus *Fusarium oxysporum* activates the tomato resistance protein I-2 intracellularly. *The Plant Journal* **58**, 970–978.
- Jakab G., Cottier V., Toquin V., Rigoli G., Zimmerli L., Mettraux J.P. & Mauch-Mani B. (2001) Beta-aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology* **107**, 29–37.
- Jones J.D.G. & Dangl J.L. (2006) The plant immune system. *Nature* **444**, 323–329.
- Jung H.W., Tschaplinski T.J., Wang L., Glazebrook J. & Greenberg J.T. (2009) Priming in systemic plant immunity. *Science* **324**, 89–91.
- Kagan I.A. & Hammerschmidt R. (2002) *Arabidopsis* ecotype variability in camalexin production and reaction to infection by *Alternaria brassicicola*. *Journal of Chemical Ecology* **28**, 2121–2140.
- Kauss H., Jeblick W., Ziegler J. & Krabler W. (1994) Pretreatment of parsley (*Petroselinum crispum* L.) suspension cultures with methyl jasmonate enhances elicitation of activated oxygen species. *Plant Physiology* **105**, 89–104.
- Koornneef A. & Pieterse C.M.J. (2008) Cross talk in defense signaling. *Plant Physiology* **146**, 839–844.
- Koornneef A., Leon-Reyes A., Ritsema T., Verhage A., Den Otter F.C., Van Loon L.C. & Pieterse C.M.J. (2008) Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. *Plant Physiology* **147**, 1358–1368.
- Kover P.X. & Schaal B.A. (2002) Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 11270–11274.
- Kover P.X., Wolf J.B., Kunkel B.N. & Cheverud J.M. (2005) Genetic architecture of *Arabidopsis thaliana* response to infection by *Pseudomonas syringae*. *Heredity* **94**, 507–517.
- Li L., Li C.Y. & Howe G.A. (2001) Genetic analysis of wound signaling in tomato. Evidence for a dual role of jasmonic acid in defense and female fertility. *Plant Physiology* **127**, 1414–1417.
- Livak K.J. & Schmittgen T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(T) (−ΔΔC) method. *Methods* **25**, 402–408.
- Llorente F., Alonso-Blanco C., Sanchez-Rodriguez C., Jorda L. & Molina A. (2005) ERECTA receptor-like kinase and heterotrimeric G protein from *Arabidopsis* are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. *The Plant Journal* **43**, 165–180.
- Lorenzo O. & Solano R. (2005) Molecular players regulating the jasmonate signalling network. *Current Opinion in Plant Biology* **8**, 532–540.
- Lorenzo O., Piqueras R., Sánchez-Serrano J.J. & Solano R. (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *The Plant Cell* **15**, 165–178.
- Lorenzo O., Chico J.M., Sanchez-Serrano J.J. & Solano R. (2004) JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. *The Plant Cell* **16**, 1938–1950.
- Luna A., Pastor V., Robert J., Flors V., Mauch-Mani B. & Ton J. (2010) Callose deposition: a multifaceted plant defence response. *Molecular Plant–Microbe Interactions* **24**, 183–193.
- Melotto M., Underwood W., Koczan J., Nomura K. & He S.Y. (2006) Plant stomata in innate immunity against bacterial invasion. *Cell* **126**, 969–980.
- Melotto M., Underwood W. & He S.Y. (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. *Annual Review of Phytopathology* **46**, 101–122.
- Mewis I., Appel H.M., Hom A., Raina R. & Schultz J.C. (2005) Major signaling pathways modulate *Arabidopsis* glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiology* **138**, 1149–1162.
- Mur L.A.J., Naylor G., Warner S.A.J., Sugars J.M., White R.F. & Draper J. (1996) Salicylic acid potentiates defence gene expression in tissue exhibiting acquired resistance to pathogen attack. *The Plant Journal* **9**, 559–571.
- Nomura K., Melotto M. & He S.Y. (2005) Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Current Opinion in Plant Biology* **8**, 361–368.
- Ossowski S., Schneeberger K., Clark R.M., Lanz C., Warthmann N. & Weigel D. (2008) Sequencing of natural strains of *Arabidopsis thaliana* with short reads. *Genome Research* **18**, 2024–2033.

- Parker J.E., Coleman M.J., Szabo V., Frost L.N., Schmidt R., Van der Biezen E.A., Moores T., Dean C., Daniels M.J. & Jones J.D. (1997) The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the toll and interleukin-1 receptors with N and L6. *The Plant Cell* **9**, 879–894.
- Perchevied L., Kroj T., Tronchet M., Loudet O. & Roby D. (2006) Natural variation in partial resistance to *Pseudomonas syringae* is controlled by two major QTLs in *Arabidopsis thaliana*. *PLoS ONE* **1**, e123.
- Pieterse C.M.J., Van Wees S.C.M., Van Pelt J.A., Knoester M., Laan R., Gerrits H., Weisbeek P.J. & Van Loon L.C. (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *The Plant Cell* **10**, 1571–1580.
- Pieterse C.M.J., Leon-Reyes A., Van der Ent S. & Van Wees S.C.M. (2009) Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology* **5**, 308–316.
- Pozo M.J., Van Der Ent S., Van Loon L.C. & Pieterse C.M.J. (2008) Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *The New Phytologist* **180**, 511–523.
- Pozo M.J., Verhage A., García-Andrade J., García J.M. & Azcón-Aguilar C. (2009) Priming plant defence against pathogens by arbuscular mycorrhizal fungi. In *Mycorrhizas – Functional Processes and Ecological Impact* (eds C. Azcón-Aguilar, J.M. Barea, S. Gianinazzi & V. Gianinazzi-Pearson), pp. 1–13. Springer, Berlin/Heidelberg, Germany.
- Pré M., Atallah M., Champion A., De Vos M., Pieterse C.M.J. & Memelink J. (2008) The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiology* **147**, 1347–1357.
- Ramakers C., Ruijter J.M., Deprez R.H. & Moorman A.F. (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* **339**, 62–66.
- Saeed A., Sharov V., White J., et al. (2003) TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **34**, 374–378.
- Schmittgen T.D. & Livak K.J. (2008) Analyzing real-time PCR data by the comparative C-T method. *Nature Protocols* **3**, 1101–1108.
- Shorey H.H. & Hale R.L. (1965) Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *Journal of Economic Entomology* **58**, 522–524.
- Simon M., Loudet O., Durand S., Berard A., Brunel D., Sennesal F.-X., Durand-Tardif M., Pelletier G. & Camilleri C. (2008) Quantitative trait loci mapping in five new large recombinant inbred line populations of *Arabidopsis thaliana* genotyped with consensus single-nucleotide polymorphism markers. *Genetics* **178**, 2253–2264.
- Staal J., Kaliff M., Dewaele E., Persson M. & Dixelius C. (2008) RLM3, a TIR domain encoding gene involved in broad-range immunity of *Arabidopsis* to necrotrophic fungal pathogens. *The Plant Journal* **55**, 188–200.
- Todesco M., Balasubramanian S., Hu T.T., et al. (2010) Natural allelic variation underlying a major fitness trade-off in *Arabidopsis thaliana*. *Nature* **465**, 632–636.
- Ton J. & Mauch-Mani B. (2004) Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *The Plant Journal* **38**, 119–130.
- Ton J., Pieterse C.M.J. & Van Loon L.C. (1999) Identification of a locus in *Arabidopsis* controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against *Pseudomonas syringae* pv. *tomato*. *Molecular Plant–Microbe Interactions* **12**, 911–918.
- Ton J., Davison S., Van Wees S.C.M., Van Loon L. & Pieterse C.M.J. (2001) The *Arabidopsis* ISR1 locus controlling rhizobacteria-mediated induced systemic resistance is involved in ethylene signaling. *Plant Physiology* **125**, 652–661.
- Ton J., De Vos M., Robben C., Buchala A., Métraux J.-P., Van Loon L.C. & Pieterse C.M.J. (2002a) Characterization of *Arabidopsis* enhanced disease susceptibility mutants that are affected in systemically induced resistance. *The Plant Journal* **29**, 11–21.
- Ton J., Van Pelt J.A., Van Loon L.C. & Pieterse C.M. (2002b) Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. *Molecular Plant–Microbe Interactions* **15**, 27–34.
- Ton J., Jakab G., Toquin V., Flors V., Iavicoli A., Maeder M.N., Métraux J.-P. & Mauch-Mani B. (2005) Dissecting the β -aminobutyric acid-induced priming phenomenon in *Arabidopsis*. *The Plant Cell* **17**, 987–999.
- Ton J., D'Alessandro M., Jourdie V., Jakab G., Karlen D., Held M., Mauch-Mani B. & Turlings T.C. (2007) Priming by airborne signals boosts direct and indirect resistance in maize. *The Plant Journal* **49**, 16–26.
- Ton J., Flors V. & Mauch-Mani B. (2009) The multifaceted role of ABA in disease resistance. *Trends in Plant Science* **14**, 310–317.
- Torres M.A., Jones J.D. & Dangl J.L. (2006) Reactive oxygen species signaling in response to pathogens. *Plant Physiology* **141**, 373–378.
- Van der Biezen E.A., Freddie C.T., Kahn K., Parker J.E. & Jones J.D. (2002) *Arabidopsis* *RPP4* is a member of the *RPP5* multi-gene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. *The Plant Journal* **29**, 439–451.
- Van der Ent S., Van Hulten M., Pozo M.J., Czechowski T., Udvardi M.K., Pieterse C.M.J. & Ton J. (2009) Priming of plant innate immunity by rhizobacteria and beta-aminobutyric acid: differences and similarities in regulation. *The New Phytologist* **183**, 419–431.
- Van Hulten M., Pelser M., van Loon L.C., Pieterse C.M. & Ton J. (2006) Costs and benefits of priming for defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 5602–5607.
- Van Leeuwen H., Kliebenstein D.J., West M.A., Kim K., van Poecke R., Katagiri F., Michelmore R.W., Doerge R.W. & St Clair D.A. (2007) Natural variation among *Arabidopsis thaliana* accessions for transcriptome response to exogenous salicylic acid. *The Plant Cell* **19**, 2099–2110.
- Van Oosten V.R., Bodenhausen N., Reymond P., Van Pelt J.A., Van Loon L.C., Dicke M. & Pieterse C.M.J. (2008) Differential effectiveness of microbially induced resistance against herbivorous insects in *Arabidopsis*. *Molecular Plant–Microbe Interactions* **21**, 919–930.
- Van Poecke R.M.P., Sato M., Lenarz-Wyatt L., Weisberg S. & Katagiri F. (2007) Natural variation in RPS2-mediated resistance among *Arabidopsis* accessions: correlation between gene expression profiles and phenotypic responses. *The Plant Cell* **19**, 4046–4060.
- Van Wees S.C.M., Luijendijk M., Smoorenburg I., Van Loon L.C. & Pieterse C.M.J. (1999) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. *Plant Molecular Biology* **41**, 537–549.
- Van Wees S.C.M., De Swart E.A.M., Van Pelt J.A., Van Loon L.C. & Pieterse C.M.J. (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 8711–8716.

- Verhagen B.W.M., Glazebrook J., Zhu T., Chang H.S., Van Loon L.C. & Pieterse C.M.J. (2004) The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant-Microbe Interactions* **17**, 895–908.
- Von Dahl C. & Baldwin I. (2007) Deciphering the role of ethylene in plant-herbivore interactions. *Journal of Plant Growth Regulation* **26**, 201–209.
- Walters D.R., Paterson L., Walsh D.J. & Havis N.D. (2009) Priming for plant defense in barley provides benefits only under high disease pressure. *Physiological and Molecular Plant Pathology* **73**, 95–100.
- Whalen M.C., Innes R.W., Bent A.F. & Staskawicz B.J. (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *The Plant Cell* **3**, 49–59.
- Xiang T., Zong N., Zhang J., Chen J., Chen M. & Zhou J. (2010) FLS2, but not BAK1, is a target of the *Pseudomonas syringae* effector AvrPto. *Molecular Plant-Microbe Interactions* **24**, 100–107.
- Zhang J., Shao F., Li Y., et al. (2007) A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants. *Cell Host & Microbe* **1**, 175–185.
- Zhang J., Lu H., Li X., Li Y., Cui H., Wen C.K., Tang X., Su Z. & Zhou J.M. (2010) Effector-triggered and pathogen-associated molecular pattern-triggered immunity differentially contribute to basal resistance to *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **23**, 940–948.
- Zhang Y.L., Goritschnig S., Dong X.N. & Li X. (2003) A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1-1, constitutive 1. *The Plant Cell* **15**, 2636–2346.
- Zipfel C., Robatzek S., Navarro L., Oakeley E.J., Jones J.D., Felix G. & Boller T. (2004) Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature* **428**, 764–767.

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SUPPORTING INFORMATION

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

Figure S1. Transcriptional expression of *ORA59*, *ERF1* and *MYC2*, and their genomic polymorphisms between accessions Bur-0 and Col-0. (a) RT-qPCR analysis of basal *ORA59*, *ERF1* and *MYC2* gene expression in 5-week-old

Col-0 and Bur-0 plants. Transcription of these transcription factor (TF) genes was normalized to the expression of *GAPDH*. Values shown are average expression values from biologically replicate samples ($n = 6$), which were analysed for statistically significant differences with a Student's *t*-test. (b) Single nucleotide polymorphisms between Col-0 and Bur-0 in the 3000 bp regions covering the genomic sequences of *MYC2*, *ERF1* and *ORA59*. Black and red letters indicate polymorphic nucleotides between Col-0 and Bur-0, respectively. Yellow bars represent open reading frames; blue bars indicate untranslated gene regions.

Figure S2. Natural variation in basal resistance to *Pst* DC3000 over a 3 d time interval after pressure infiltration of the leaves with a bacterial suspension. Shown are average values (\pm SEM; $n = 5$ –10). Asterisks indicate statistically significant differences compared to reference accession Col-0 (Student's *t*-test; $\alpha = 0.05$).

Figure S3. Cluster analysis of transcription profiles of 28 transcription factor (TF) genes and two reference genes (*GAPDH* and *At1g13220*) to compare basal transcription profiles in Bur-0, Can-0, Col-0, Sf-2 and Ws-2 with the transcription profiles from β -aminobutyric acid (BABA)-treated Col-0. Leaf material from BABA-primed Col-0 plants was collected at 2 d after soil-drench treatment with 80 μ M BABA. Colour intensity of induced (yellow) or repressed genes (blue) is proportional to their level of expression. Data represent log-transformed fold-change values relative to the average expression value in (untreated) Col-0. Values were subjected to average linkage clustering (Euclidean distance).

Table S1. *Arabidopsis thaliana* accessions used in this study, their geographical origin and their basal resistance to virulent *Pseudomonas syringae* pv. *tomato* DC3000.

Table S2. Synonymous single nucleotide polymorphisms in the *ACD6* gene and the *RPP4/SNC1/RPP5* cluster of resistance genes between accession Col-0 and Bur-0.

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