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A novel Arabidopsis–oomycete pathosystem: differential interactions with *Phytophthora capsici* reveal a role for camalexin, indole glucosinolates and salicylic acid in defence

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ABSTRACT

***Phytophthora capsici* causes devastating diseases on a broad range of plant species. To better understand the interaction with its host plants, knowledge obtained from a model pathosystem can be instrumental. Here, we describe the interaction between *P. capsici* and Arabidopsis and the exploitation of this novel pathosystem to assign metabolic pathways involved in defence against *P. capsici*. Inoculation assays on Arabidopsis accessions with different *P. capsici* isolates revealed interaction specificity among accession-isolate combinations. In a compatible interaction, appressorium-mediated penetration was followed by the formation of invasive hyphae, haustoria and sporangia in leaves and roots. In contrast, in an incompatible interaction, *P. capsici* infection elicited callose deposition, accumulation of active oxygen species and cell death, resulting in early pathogen encasement in leaves. Moreover, Arabidopsis mutants with defects in salicylic acid signalling, camalexin or indole glucosinolates biosynthesis pathways displayed severely compromised resistance to *P. capsici*. It is anticipated that this model pathosystem will facilitate the genetic dissection of complex traits responsible for resistance against *P. capsici*.**

Key-words: defence signalling; host–pathogen interaction; model system; natural variation; resistance.

INTRODUCTION

In nature, plants are being endangered by a broad range of microbial pathogens. The development of durable strategies to control plant diseases is therefore a major challenge when striving for successful plant protection. To this end, increased knowledge on the mechanisms underlying plant–pathogen interactions would be helpful and this can greatly benefit from information obtained from model pathosystems. *Arabidopsis thaliana* has been put forward as a model plant to study plant–pathogen interactions due to its unique attributes such as the enriched genetic and genomic resources (Schlaich 2011). New insights gained from Arabidopsis research can be exploited to generate pathogen resistance in crop plants (Lacombe *et al*. 2010; Zhang *et al*. 2010).

Successful defence in plants against pathogen attack relies on the initiation of a range of inducible responses, including cell wall reinforcement, accumulation of reactive oxygen species (ROS), programmed cell death, transcriptional activation of defence-related genes and synthesis of biologically active secondary metabolites. The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play important roles in resistance to various pathogens as induction of defence responses in many plant species is coordinated by SA-, JA- and ET-dependent signalling pathways (Robert-Seilaniantz, Grant & Jones 2011). In the case of Arabidopsis and other Brassicaceae species, accumulation of indolic secondary metabolites, such as camalexin and indole glucosinolates (iGS), is also of central importance to limit pathogen infection (Thomma *et al*. 1999; Ferrari *et al*. 2003; Schlaeppi *et al*. 2010). Defence strategies, however, vary between host–pathogen interactions, as shown in a number of studies on Arabidopsis, in which mutants impaired in defence-related pathways and collections of accessions were tested for differential responses to various pathogens (Thomma *et al*. 2001; Kover & Schaal 2002; Bohman *et al*. 2004; Glazebrook 2005; Spoel, Johnson & Dong 2007). For example, disease resistance in Arabidopsis to the fungal pathogen *Alternaria brassicicola* requires camalexin and JA-dependent pathways (Van Wees *et al*. 2003), while the bacterial pathogen *Pseudomonas syringae* and the oomycete pathogen *Hyaloperonospora arabidopsidis* can be inhibited by SA-dependent defence responses (Liu *et al*. 2010). Moreover, closely related pathogens not necessarily activate the same defence signalling pathways. This is, for example, illustrated in studies on interactions of Arabidopsis with *Phytophthora.* In the three *Phytophthora*–Arabidopsis pathosytems that have been described so far, the role of canonical defence signalling pathways has been investigated and there seems to be no overlap (Roetschi *et al*. 2001; Rookes, Wright & Cahill 2008; Attard *et al*. 2010; Schlaeppi *et al*. 2010). In this study, we describe a novel *Phytophthora*–Arabidopsis pathosystem in which yet another distinct defence network is activated in order to prevent pathogen invasion.

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The novel pathosystem presented here is the interaction between Arabidopsis and *Phytophthora capsici*, a renowned plant pathogen that causes severe diseases on a broad range of economically important crops, such as tomato, eggplant, cucurbits and pepper, and is responsible for the $1 billion loss in worldwide vegetable production every year (Lamour *et al*. 2012b). The *P. capsici*–Arabidopsis system has attractive features that make it an ideal model system for dissecting broad-host-range oomycete–plant interactions, as it not only benefits from the genetic and genomic resources and tools available for the host but also those available for the pathogen. Compared to the other *Phytophthora* species that interact with Arabidopsis, *P. capsici* has several advantages that will greatly facilitate the functional analyses of candidate effectors and pathogenicity factors. It is one of the few (heterothallic) species for which genetic crosses and backcrosses can be generated fairly easy, molecular markers (including numerous single nucleotide variations) and high-density genetic linkage maps are available; it is amenable to DNA transformation; and its genome has been sequenced (Huitema, Smoker & Kamoun 2011; Lamour *et al*. 2012a,b).

The aims of this study were to analyse whether or not Arabidopsis can function as a host for *P. capsici* and to gain more insight into the defence pathways that are activated in *P. capsici*–Arabidopsis interactions. Firstly, we inoculated different Arabidopsis accessions with a set of *P. capsici* isolates in order to analyse natural variation in resistance towards *P. capsici*. Subsequently, we analysed the cellular defence responses in Arabidopsis by microscopy during compatible and incompatible interactions. Finally, we tested mutants with specific defects in defence for enhanced susceptibility to *P. capsici* and found that in Arabidopsis, SA signalling, as well as camalexin and iGS, are required for resistance to *P. capsici.*

MATERIALS AND METHODS

P. capsici culture conditions and inoculum preparation

P. capsici isolates (Table 1) were routinely cultured in the dark at 25 °C on 20% (v/v) V8 juice agar plates (Erwin & Ribeiro 1996). Zoospores for plant infection assays were obtained by incubating mycelial plugs (Ø 1.0 cm) from the margin of a 4-day-old growing colony in 10% (v/v) cleared V8 broth in the dark at 25 °C for 2 days. Hereafter, the V8 medium was replaced with sterilized mineral solution (Wang *et al*. 2011) and refreshed once every hour up to four times. After 2 days of incubation under continuous light, numerous sporangia developed. Zoospore release was induced with a cold shock by flooding the plates with cold water (4 °C) for 30 min. Zoospores were filtered through one layer of Miracloth (Merck, Rahway, NJ, USA) and adjusted to the desired concentrations.

Table 1. *Phytophthora capsici* isolates used in this study

	Origin			
Isolate	Year	Country/state	Collected from	MT
LT51	1997	USA, Michigan	Cucumber	A1[a]
LT62	1998	USA, Michigan	Squash	A2[b]
LT123	1998	USA, Michigan	Cucumber	A1[a]
LT263	2004	USA, Tennessee	Pumpkin	A2[a]
LT3112	2006	USA, Tennessee	Pumpkin	A1[a]
LT3145	2006	USA, Tennessee	Pumpkin	A2[b]
LT3239	2006	USA, Tennessee	Pumpkin	A2[b]
LT3241	2006	USA, Tennessee	Pumpkin	A2[a]

[a]As previously reported (Donahoo & Lamour 2008).
[b]Determined as described by Harutyunyan *et al*. (2008).

Plant material and growth conditions

Arabidopsis seeds were sown on soil, or *in vitro* on 0.5 MS medium (Murashige and Skoog; Duchefa, Haarlem, The Netherlands) supplemented with 1% (w/v) sucrose and 1.2% (w/v) plant agar (Duchefa), and were stratified by placing them in the dark at 4 °C for 3 d. Subsequently, Arabidopsis was grown in a conditioned growth chamber at 19–21 °C, with a 16 h photoperiod and a relative humidity (RH) of 75–80%. Tomato plants of cultivar Moneymaker were grown under standard greenhouse conditions.

Plant inoculation procedures and SA treatment

Four-week-old Arabidopsis or tomato leaves were sprayed with water, and subsequently inoculated on the abaxial leaf side with fresh mycelial plugs (Ø 0.5 cm). Mock-inoculation was conducted with blank V8 plugs. Mycelial plugs were removed from Arabidopsis leaves 2 d post-inoculation (dpi) and from tomato leaves 1 dpi. Zoospore inoculation of Arabidopsis leaves was performed by inoculating 10 μL droplets containing 10^5 zoospores mL^{-1} on each leaf. For root inoculation, roots of the 16-day-old Arabidopsis seedlings were dipped in water or a zoospore suspension (10^5 zoospores mL^{-1}) for 5 s and then transplanted to soil. During the first 24 h after inoculation, plants were kept in the dark at 22 °C in trays covered with lids to maintain a high humidity. Subsequently, plants were incubated at a 75–80% RH and a 10 h photoperiod.

For SA treatment, plants were exposed to SA (Merck Schuchardt OHG, Hohenbrunn, Germany) 1 d prior to inoculation with *P. capsici* by spraying the leaves with a solution containing 2.5 mM SA, 0.1% ethanol (v/v) and 0.02% Silwet L-77 (v/v). Control plants were sprayed with water supplemented with 0.1% ethanol (v/v) and 0.02% Silwet L-77 (v/v).

Disease severity index

Disease development on Arabidopsis leaves was evaluated using a disease severity index (DSI) on a scale of 0–4. A score of '0' indicates no visible disease symptoms or small necrotic flecks; '1', a lesion diameter less than 0.5 cm; '2', a lesion covering less than 50% of the leaf surface; '3', a lesion covering 50–75% of the leaf surface; and a score of '4' refers to 75–100% collapse of the leaf. The mean DSI was calculated according to the equation:

$$\text{Mean DSI} = \frac{\left\{\sum_{\text{index no.}}[(\text{index no.}) \times (\text{amount of leaves in each index})]\right\}}{(\text{total amount of leaves})}.$$

Tomato disease severity was monitored by measuring lesion sizes at 3 dpi. All experiments were repeated at least three times.

Quantification of *P. capsici* biomass in infected Arabidopsis leaves

Six leaves inoculated with *P. capsici* collected at 3 dpi were used for genomic DNA isolation with a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Real-time PCR was performed for quantifying the *P. capsici* biomass using an ABI 7300 PCR machine (Applied Biosystems, Foster City, CA, USA). The Arabidopsis *Rubisco* primer pair AtRub-F4/AtRub-R4 was used as endogenous control, while the primer pair CAP-Fw/CAP-Rv1 was used to target *P. capsici* internal transcribed spacer regions (Silvar *et al.* 2005; Yadeta *et al.* 2011; Supporting Information Table S1). The ratio of *P. capsici* genomic DNA to Arabidopsis DNA was calculated using a ΔΔCT method. Two biological replicates were analysed, each with a technical triplicate.

Histological staining and microscopy

Staining with trypan blue (Sigma, St Louis, MO, USA) visualizing infection hyphae and plant cell death, and aniline blue (Acros Organics, Fair Lawn, NJ, USA) visualizing callose deposition, was carried out as described by Bouwmeester *et al.* (2011). Detection of H_2O_2 and O_2^- was performed as described by Thordal-Christensen *et al.* (1997) and Dunand, Crevecoeur & Penel (2007). Microscopy was performed with a Nikon 90i microscope (Nikon, Amstelveen, the Netherlands) using differential interference contrast and epifluorescence settings (i.e. DAPI filter, EX 340-380, DM 400 and BA 435-4850).

RESULTS AND DISCUSSION

Arabidopsis is a host for *P. capsici*

To determine whether *P. capsici* is capable of infecting Arabidopsis, eight *P. capsici* isolates (Table 1) were tested using mycelial plugs or zoospores on mature Col-0 leaves. In most cases, infection was observed, but disease development varied from complete wilting to no visible symptoms at 3 dpi (Fig. 1a). Based on these observations, a DSI was adopted to evaluate disease development at 3 dpi, in which the lowest score ('0') represents no infection and the highest score ('4') represents collapse of the inoculated leaf (Fig. 1b). As shown in Fig. 1c, plug inoculation revealed that five of eight isolates (i.e. LT263, LT3241, LT3112, LT51 and LT3239) were capable of producing rapid spreading lesions on Col-0, and more than 90% of the inoculated leaves showed a DSI ≥ 3 at 3 dpi, resulting in a mean DSI ≥ 3.6. Isolate LT3145 was less virulent on Col-0 as the proportion of infected leaves with a DSI ≥ 3 was approximately 60%, with a mean DSI of 3.0. In contrast, isolates LT123 and LT62 were not virulent on Col-0 as they produced no visible disease symptoms (mean DSI = 0.2 and 0, respectively). Quantification of the *P. capsici* biomass in Col-0 leaves at 3 dpi by real-time PCR revealed similar levels in leaves inoculated with LT263, LT3241, LT3112, LT51 and LT3239, whereas *P. capsici* was hardly detectable in leaves inoculated with isolates LT123 and LT62 (Supporting Information Fig. S1). These results show that disease severity is highly correlated with pathogen development. Upon zoospore inoculation, disease symptoms on Col-0 developed at a slower rate when compared with the plug inoculation, but the outcomes were similar. At 7 dpi, the mean DSI of LT123 and LT3145 infected leaves was 0 and 3, respectively, whereas the DSI of leaves infected by the remaining isolates, with the exception of LT62, was ≥3. LT62 was not included in this assay because this isolate could not produce enough zoospores for inoculation.

Since *P. capsici* is known as a soil-borne pathogen, inoculation assays with various isolates were also performed on Arabidopsis roots by dipping roots of the 16-day-old seedlings into the zoospore suspensions. Consistent with the leaf inoculation assays, disease phenotypes to individual isolates varied from no symptoms to 100% mortality of the inoculated seedlings. Inoculation of Col-0 roots with the six isolates that were found to infect Col-0 leaves resulted in wilting of the lower leaves within 3 dpi and a complete collapse of the seedlings at 4 dpi (Fig. 1d). In contrast, no apparent disease symptoms were observed on seedlings inoculated with LT123 (Fig. 1d).

To know whether the behaviour of *P. capsici* isolates on Arabidopsis is comparable to that of host plants of *P. capsici*, all eight isolates were tested for their ability to infect tomato. Notably, isolates LT123 and LT62 that were not capable of infecting Arabidopsis Col-0 could infect tomato leaves (Supporting Information Fig. S2). Both isolates caused lesions that were visible at 3 dpi, but, as on Arabidopsis Col-0, the lesions caused by the other six isolates were much larger.

In summary, pathogenicity tests on Arabidopsis and tomato revealed striking differences among *P. capsici* isolates. Since five out of eight isolates (i.e. LT263, LT3241, LT3112, LT51 and LT3239) behaved similarly on Arabidopsis and tomato, isolate LT263 was selected for further analysis, together with isolate LT123, which caused no disease symptoms on Arabidopsis Col-0.

Natural variation of Arabidopsis accessions in response to *P. capsici*

Different responses of Arabidopsis Col-0 to *P. capsici* isolates point to interaction specificity between Arabidopsis and *P. capsici*. To obtain a further understanding of the interaction specificity, another 34 Arabidopsis accessions originating from different geographic locations were analysed for their response to *P. capsici* isolates LT263 and LT123 (Fig. 2).

All accessions could be infected by isolate LT263 but the disease severity on the accessions varied significantly (Fig. 2).

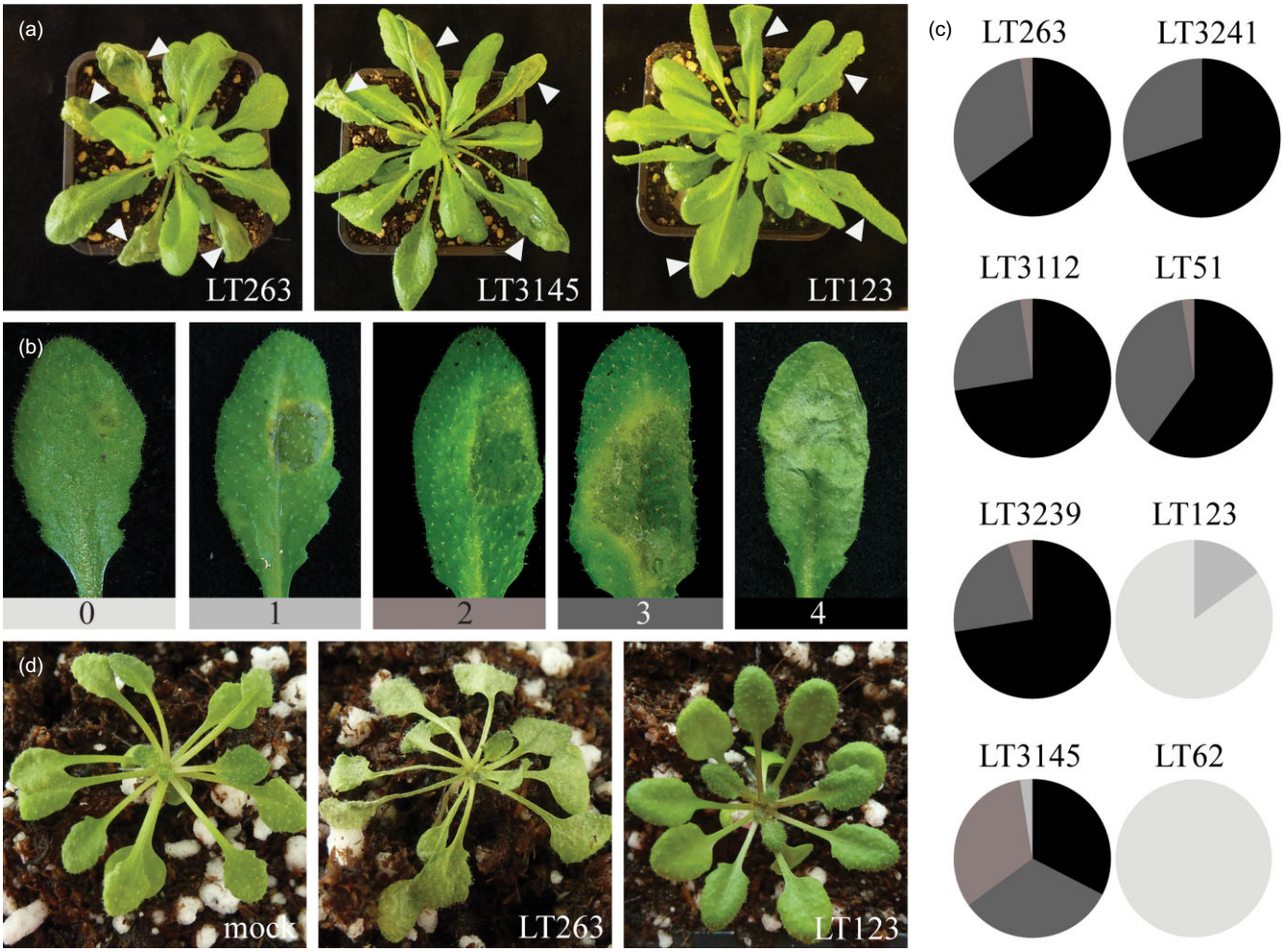

Figure 1. *Phytophthora capsici* isolates vary in virulence on Arabidopsis Col-0. (a) Lesions on plug-inoculated Arabidopsis Col-0 leaves 3 d after inoculation (dpi) with three isolates of *P. capsici*. White arrowheads point to the inoculated leaf. (b) Disease severity index (DSI) presented by numbers from '0' to '4' and increasing levels of grey shading. '0' no infection; '1' lesion diameter less than 0.5 cm; '2' lesion covering less than 50% of the leaf; '3' lesion covering between 50 and 75% of the leaf; and '4' collapse of the inoculated leaf. (c) Disease severity on leaves of Arabidopsis Col-0 inoculated with eight *P. capsici* isolates at 3 dpi. Each circle represents the DSI [shown from light to dark grey as in (b)] caused by the indicated *P. capsici* isolate on 40 leaves collected in three independent experiments. (d) Disease symptoms on Col-0 seedlings at 4 dpi. Roots of 16-day-old seedlings were dipped into water (mock) or *P. capsici* zoospore suspensions (10^5 zoospores mL^{-1}) (LT263; LT123).

Of the 35 accessions, 29 including Col-0 were classified as susceptible. On these accessions, lesions extended quickly across the leaves, resulting in a mean DSI ≥ 2.5 at 3 dpi. The remaining six accessions (i.e. An-1, Can-0, Est-0, Mt-0, Rsch-4 and Ty-0) were moderately tolerant to LT263; there was restricted lesion development, with around 70% of the inoculated leaves showing a DSI ≤ 2. Furthermore, the variations in disease severity were consistent with the level of colonization by *P. capsici* and the amount of sporangia present on the lesions (data not shown).

After inoculation with LT123, only two of the tested accessions, Wei-0 and L*er*-0, developed obvious disease symptoms at 3 dpi (Fig. 2). Lesions spreading from the inoculation spots, as well as formation of sporangia, confirmed the susceptibility of Wei-0 and L*er*-0. Nevertheless, infection of Wei-0 and L*er*-0 leaves by LT123 resulted in lesions with a mean DSI of 2.2 and 2.1 at 3 dpi, respectively. This was not comparable to the lesions caused by isolate LT263, with a mean DSI of 4.0 and 3.9, respectively.

Compatible interaction between Arabidopsis and *P. capsici*

To further confirm the compatible interaction between Arabidopsis and *P. capsici*, we analysed the infection process of Arabidopsis Col-0 with isolate LT263 by microscopy. Col-0 plants were inoculated by applying LT263 zoospores on the upper side of the leaves. Initially, zoospores attached to the leaf surface and readily germinated, and approximately 96% of cysts developed appressoria at the tips of the germ tubes within 3 h (Fig. 3a). Three hours post-inoculation (hpi), penetration pegs emerged beneath the appressoria. Unlike

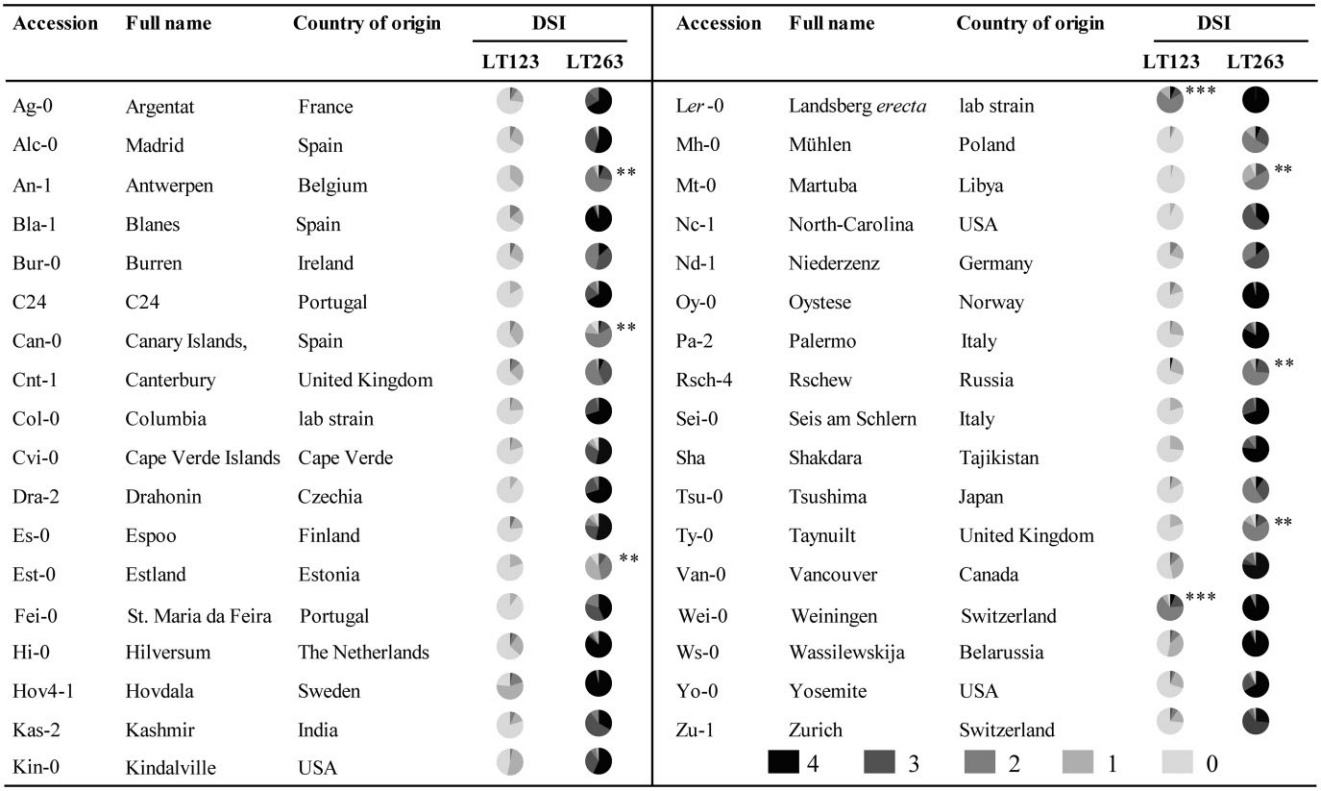

Figure 2. Disease severity on Arabidopsis accessions inoculated with *Phytophthora capsici*. Disease severity was assessed at 3 dpi according to the disease severity index (DSI) shown in Fig. 1b. Each circle represents the DSI (from light to dark grey as shown at the bottom on the right) caused by the indicated *P. capsici* isolate on at least 30 leaves collected from three independent experiments. ***Accessions that show significant susceptibility to isolate LT123 compared with Col-0 based on a *t*-test ($P < 0.001$). **Accessions that show significant resistance to isolate LT263 compared with Col-0 based on a *t*-test ($P < 0.005$).

Phytophthora brassicae (Roetschi *et al*. 2001) and *Phytophthora parasitica* (Attard *et al*. 2010; Wang *et al*. 2011), *P. capsici* did not only enter the leaf tissue at the junction between epidermal cell walls (Fig. 3b) but was occasionally observed penetrating epidermal cells directly (Fig. 3c), or via stomatal cavities (Fig. 3d). Upon penetration, haustoria differentiated from the penetration hyphae adjacent to the epidermal cell. Subsequently, the infection hyphae progressed to the adjacent epidermal cells or mesophyll cell layers and more haustoria were observed. From 1 dpi onwards, intercellular hyphae with haustoria increased markedly and massive ramifying mycelia were evident in the leaf at 2 dpi (Fig. 3e). After that, hyphae started to emerge from the leaf through stomatal openings or via the intercellular space between epidermal cells (Fig. 3f,g) and eventually developed sporangia on the leaf surface (Fig. 3h).

To evaluate cellular responses of Arabidopsis upon infection, aniline blue staining was used to detect the callose deposition, while 3,3′-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) were used as marker dyes for H_2O_2 and O^{2-} production, respectively. The first day after inoculation, neither microscopic detectable callose deposition nor NBT-stained cells were observed. Only some faint brown-coloured cells were found at a few infection sites after DAB staining (data not shown). Furthermore, infected cells did not show recognizable trypan blue staining within 2 dpi. This indicates that Arabidopsis cells remain viable after penetration, which is likely a prerequisite for the initial biotrophic relationship between *P. capsici* and Arabidopsis.

P. capsici development in Col-0 roots followed a pattern similar to that observed in leaves. After penetration into the roots, invading hyphae progressed rapidly along the cortex cells with hardly any branches but formed haustoria within 6 hpi. Hereafter, infection hyphae started to branch, developed abundant haustoria and perturbed into the vascular system (Fig. 3i). Sporangia were observed at the root surface within 2 dpi (Fig. 3j). No recognizable host responses were observed during the course of infection, that is, no callose deposition or DAB- and NBT-stained cells (data not shown).

Incompatible interaction between Arabidopsis and *P. capsici*

For microscopic analysis of an incompatible interaction between Arabidopsis and *P. capsici*, Col-0 leaves were drop-inoculated with LT123 zoospore suspensions. Approximately 90% of the LT123 zoospores germinated and formed appressoria on the leaf surface within 6 hpi, but were unable to successfully penetrate plant cells. In most cases, failure of the first penetration led to the formation of secondary germ

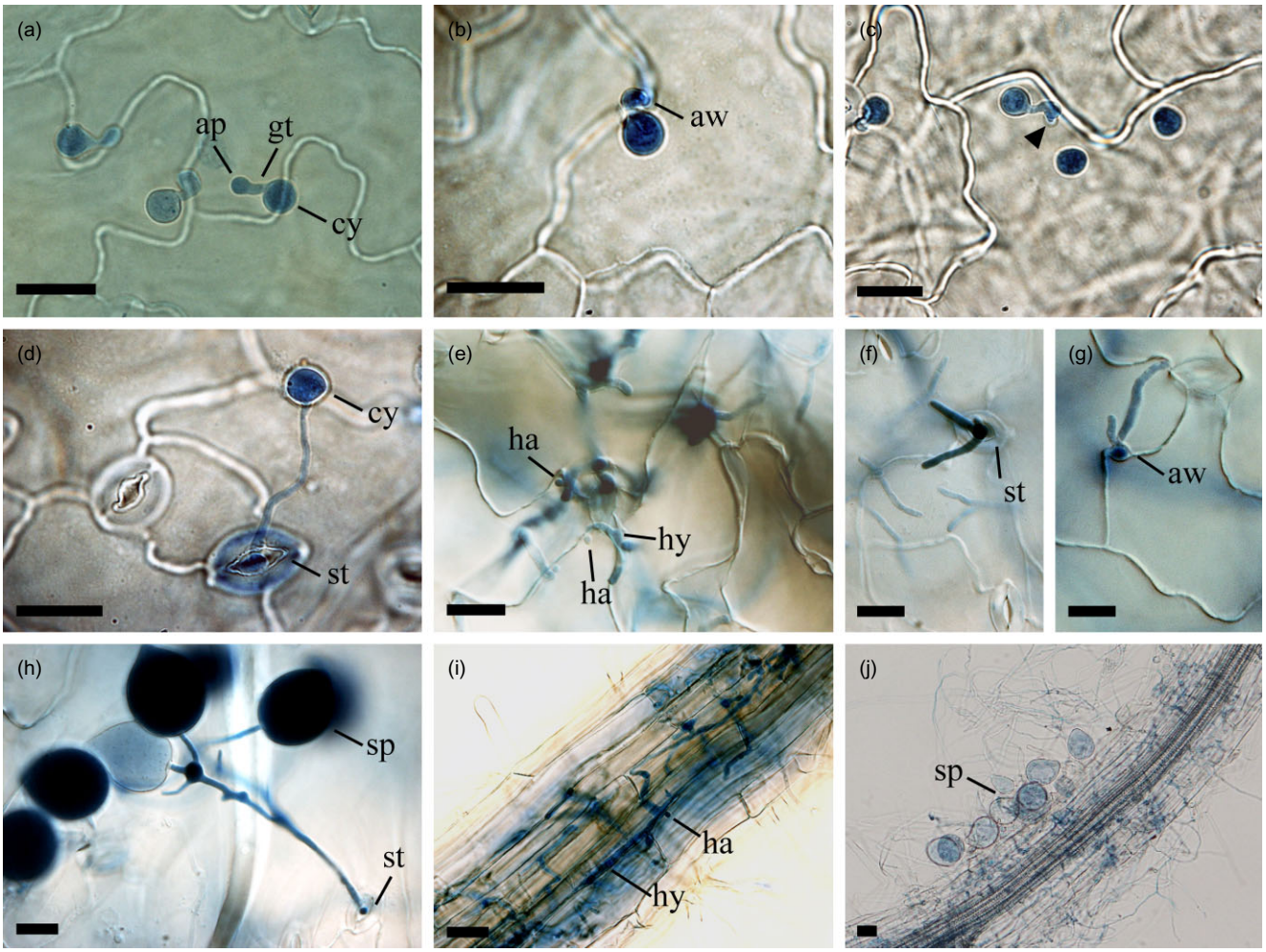

Figure 3. Cytology of a compatible *Phytophthora capsici*–Arabidopsis interaction. Arabidopsis Col-0 leaves and roots inoculated with *P. capsici* LT263 zoospores (10^5 zoospores mL^{-1}) were harvested and stained with trypan blue. (a) Zoospores attached to the leaf surface, germinated and formed appressoria within 3 hpi. (b–d) Appressorium-mediated penetration of the leaf through the anticlinal cell wall junction (b), directly through the epidermis (► in c) or via stomata (d). (e) Massive invasive hyphae with haustoria in leaf tissue at 2 dpi. (f–g) Infection hyphae emerging from the leaves via stomata (f) or the space between epidermal anticlinal cell walls (g). (h) Sporangia development on the leaf surface. (i) Invasive hyphae with haustoria in root cells at 1 dpi. (j) Sporangia on the root surface at 2 dpi. ap, appressorium; aw, anticlinal cell wall junction; cy, cyst; gt, germ tube; ha, haustorium; hy, invasive hyphae; sp, sporangium; st, stomata. Bars represent 20 μm.

tubes and appressoria (Fig. 4a). However, this did not lead to successful infection as cell death response, visualized by trypan blue-stained zones, was observed at each penetration site (Fig. 4b). In addition, infection induced the accumulation of H_2O_2 and O^{2-}, which was revealed by the DAB- and NBT-stained cells, respectively, at every attempted infection site (Fig. 4c,d). Local accumulation of ROS, that is, H_2O_2 and O^{2-}, is often linked to cell wall-based defence responses, such as the production of callose, a phenomenon considered to be an active resistance response to invading pathogens (Hückelhoven 2007). Consistent with this, localized callose deposition, as shown in Fig. 4e, was detected at nearly every attempted penetration site during the early phase of infection. In a few instances, invasive hyphae were detected in the mesophyll cell layer at the inoculation sites in samples collected after 1 dpi; however, infection hyphae were accompanied by extensive cell death, which was observed not only in mesophyll cells in direct contact with the infection hyphae but also in the neighbouring mesophyll cells (Fig. 4g). No haustoria or sporangia were detected in inoculated Col-0 leaves. Apparently, during an incompatible interaction, ROS burst, callose deposition and cell death, phenomena not commonly observed in the compatible interaction, contributed to restricting the infection.

In order to examine whether the resistance phenotype observed on Arabidopsis Col-0 leaves upon dip-inoculation of roots with LT123 zoospores (Fig. 1d, right panel) is due to failed infection of root tissue, we cytologically examined these inoculated roots. LT123 zoospores encysted and germinated, and infection of the root tissue resulted in proliferation of infection hyphae and sporulation (Fig. 4i,j). This disease development in Col-0 roots was similar to that observed in roots in the compatible interaction between Col-0 and LT263. However, root colonization by LT123 did not lead to systemic

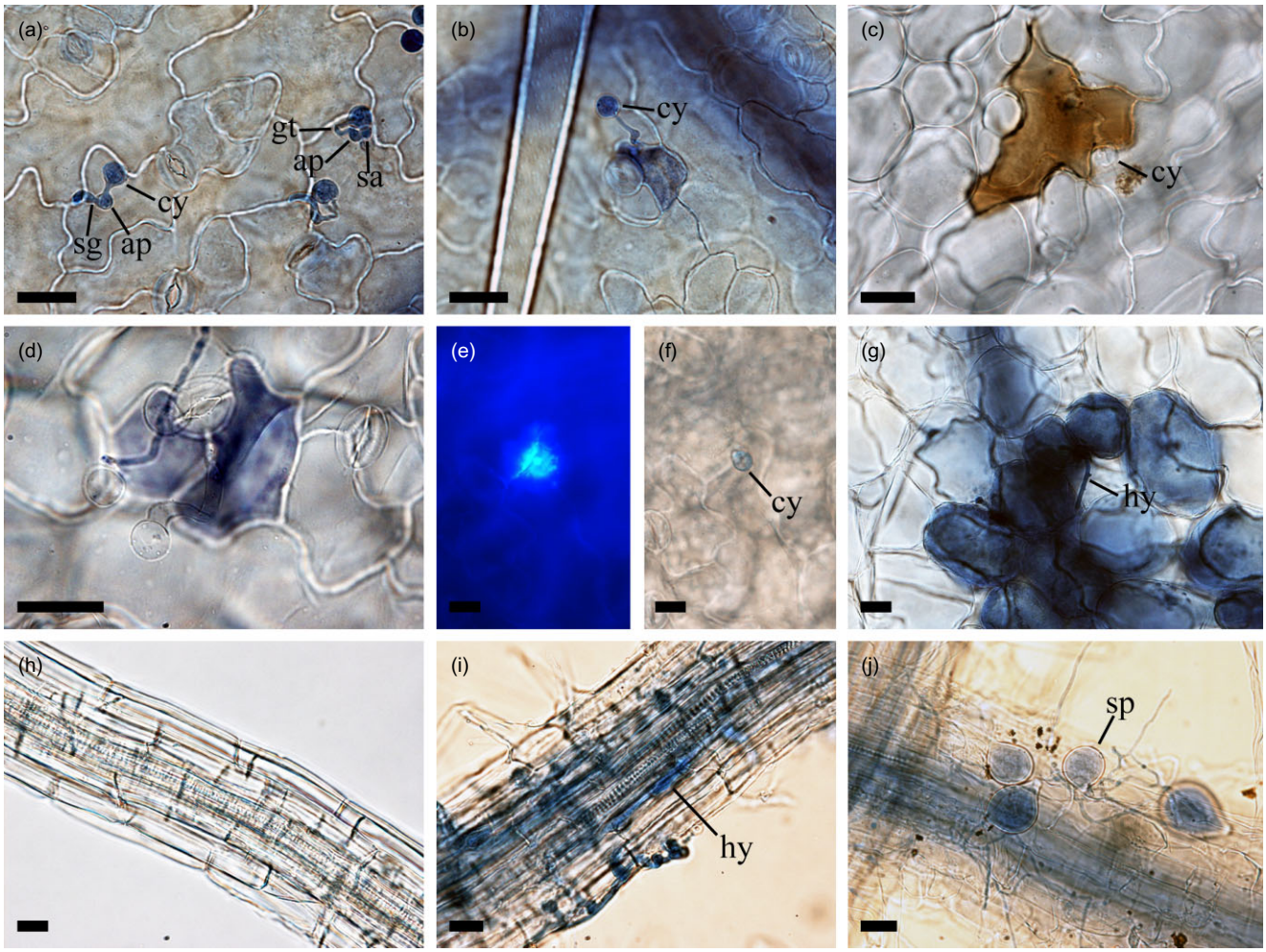

Figure 4. Cytology of an incompatible *Phytophthora capsici*–Arabidopsis interaction. Arabidopsis Col-0 leaves and roots were inoculated with *P. capsici* LT123 zoospores. (a) Abortive penetration by LT123 led to the formation of secondary germ tubes and appressoria on the leaf surface as was observed at 6 hpi. (b) Cell death of epidermal cells indicated by the dark blue-stained Arabidopsis cells. (c, d) 3,3′-Diaminobenzidine (DAB)-reacting H_2O_2 accumulation (c) and nitroblue tetrazolium (NBT) staining of O^{2-} (d) in Arabidopsis epidermal cells at 12 hpi. (e) Cell wall deposition at the penetration site along the junction between epidermal cell walls, revealed by aniline blue staining under UV light. (f) Bright-field image of (e) showing the germinated cyst at the penetration site. (g) Invasive hyphae surrounded by extensive trypan blue-stained plant cells observed at 1 dpi. (h–j) Arabidopsis roots were dipped in water (h) or in a zoospore suspension of LT123 (i, j) and stained with trypan blue. Invasive hyphae of LT123 colonizing Col-0 roots (i) and sporulation on the root surface (j). ap, appressorium; cy, cyst; gt, germ tube; hy, invasive hyphae; sa, secondary appressorium; sg, secondary germ tube; sp, sporangium. Bars represent 20 μm.

invasion as no classical disease symptoms were observed on the aerial parts of the seedlings (Fig. 1d). This tolerance response was also observed on Arabidopsis Col-0 when challenged with *Phytophthora cinnamomi* (Rookes *et al.* 2008) and on cucurbits inoculated with a pathogenicity mutant of the anthracnose fungus *Colletotrichum magna* (Freeman & Rodriguez 1993). Apparently, in these pathosystems, the ability to establish infection and the ability to further colonize host tissues are separate events.

Phenotypic analysis of JA and ET signalling mutants

To further investigate the relevance of various defence signalling pathways and secondary metabolites in resistance of Arabidopsis to *P. capsici*, a set of Col-0 mutants defective in SA, JA and ET signalling pathways, or in camalexin and iGS biosynthesis, was selected (as shown in Fig. 5 and listed in Supporting Information Table S2) and screened for loss of resistance to *P. capsici* LT123, one of the isolates that is unable to infect Col-0 (Fig. 1). Disease severity on the selected mutants was determined according to the DSI as described above and is shown by circles in Fig. 5. Quantitative values are included in Supporting Information Table S2.

The analysis included seven mutants that are positioned in JA/ET pathways. Of these seven, four retained Col-0-like resistance towards LT123, that is, *jin1* and *jar1* in which JA signalling is blocked (Berger, Bell & Mullet 1996; Devoto & Turner 2003), and *ein2-1* and *ein3-1* which are mutated in ET signalling (Chao *et al.* 1997; Alonso *et al.* 1999). In contrast,

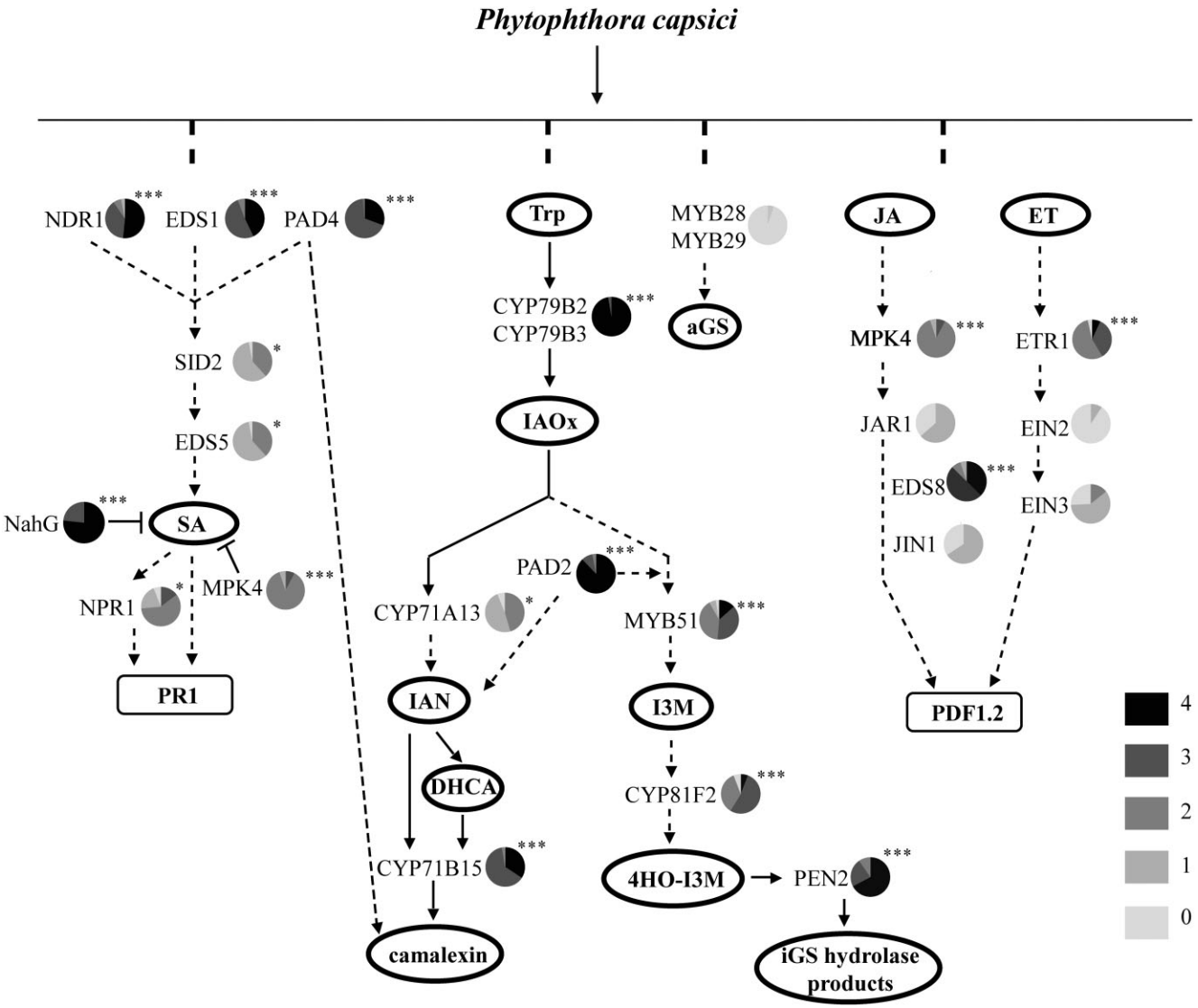

Figure 5. Disease severity on Arabidopsis mutants impaired in defence related pathways after inoculation with *Phytophthora capsici*. A schematic model illustrating the network of defence-related signalling pathways and secondary metabolic biosynthesis routes, and the genes mutated in the selected Arabidopsis mutants (as listed in Supporting Information Table S2). Note that *NahG* is not a mutant but a transgenic line producing an enzyme that breaks down SA. *PR1* and *PDF1.2* are defense related genes of which the expression is governed by the SA and JA/ET signaling pathway, respectively. In the network, the undotted arrows represent established relationships between substrates, the enzymes encoded by the genes and the products, whereas dotted arrows represent relationships that are deduced from genetic studies and mutant analysis. The disease severity on the inoculated mutants at 3 dpi with *P. capsici* isolate LT123 is shown by circles. Each circle represents the disease severity index (DSI) (from light to dark grey as shown on the right) on 30–40 leaves collected in three independent experiments. Mutants that, according to a *t*-test, show significant gain of susceptibility when compared with Col-0 are marked by *** ($P < 0.001$) or * ($P < 0.05$). Quantitative values are included in Supporting Information Table S2. SA, salicylic acid; JA, jasmonic acid; ET, ethylene; iGS, indole glucosinolates; aGS, aliphatic glucosinolates; Trp, tryptophan; IAOx, indole-3-acetaldoxime; IAN, indole-3-acetonitrile; DHCA, dihydrocamalexic acid; I3M, indole-3-yl-methyl glucosinolates; 4HO-I3M, 4-hydroxy-indole-3-yl-methyl glucosinolate.

resistance was attenuated in the JA-insensitive mutants *mpk4* and *eds8-1* (Petersen *et al*. 2000; Brodersen *et al*. 2006) and in *etr1-1* that has a mutation in the ET receptor gene *ETR1* (Bleecker *et al*. 1988). These three mutants all exhibited severe disease symptoms at 3 dpi, with 90% of the inoculated leaves having a DSI ≥ 2. The JA pathway mutants *jin1* and *jar1* are known to be relatively weak alleles (Lorenzo *et al*. 2004), which may explain the difference in phenotype with *mpk4* and *eds8-1*. Because of the discrepancy in the four JA signalling mutants, a role for JA in *P. capsici* resistance cannot be ruled out. In the case of ET signalling mutants, differences in disease susceptibility have been reported before, for example, by Geraats, Bakker & Van Loon (2002), who tested susceptibility of these mutants to various *Pythium* species, and by Johansson, Staal & Dixelius (2006) and Pantelides, Tjamos & Paplomatas (2010), who analysed the response to *Verticillium dahliae*. EIN2 and EIN3 act downstream of *ETR1* and the *ein2-1* mutant has a stronger

ethylene-insensitive phenotype than *etr1-1* (Geraats *et al*. 2002). Nevertheless, upon *P. capsici* inoculation, *etr1-1* is much more susceptible than *ein2-1*, and hence, there is no positive correlation between the *P. capsici* susceptibility phenotype and the ethylene-insensitive phenotype. Since *ein3-1* shows a slight gain of susceptibility compared to *ein2-1* and Col-0, we cannot rule out that ET has a role in resistance to *P. capsici.* However, since *etr1-1* is affected not only in the sensitivity to ET but also in hydrogen peroxide signalling and C6-aldehyde-induced defence responses (Desikan *et al*. 2005; Kishimoto *et al*. 2006), we speculate that *ETR1* is involved in an ethylene-independent pathway that affects disease resistance to *P. capsici*.

SA, camalexin or iGS deficiency compromises resistance of Arabidopsis to *P. capsici*

In the SA signalling pathway, seven mutants were tested as well as the SA-deficient line *NahG* (Delaney *et al*. 1994). *NahG*, *pad4*, *eds1-2* and *ndr1* (Century *et al*. 1997; Zhou *et al*. 1998; Falk *et al*. 1999) displayed enhanced susceptibility towards *P. capsici* LT123, with fast spreading lesions accompanied by sporulation at 3 dpi, and resulting in mean DSIs significantly higher than 3. In contrast, inoculation of *sid2-2*, *npr1-1*, *npr1-3* and *eds5* (Cao *et al*. 1994, 1997; Glazebrook, Rogers & Ausubel 1996; Nawrath & Métraux 1999; Ton *et al*. 2002) resulted in visible lesions with mean DSIs smaller than 2. The difference in susceptibility of, on one hand, the *NahG, eds1-2*, *pad4* and *ndr1*, and, on the other hand, *sid2-2, npr1-1*, *npr1-3* and *eds5* could be due to the functional redundancy of genes involved in the SA pathway; it is known that SA synthesis is dependent not solely on isochorismate synthase (ICS/SID) activity but also on phenylalanine ammonium lyase (PAL) (Wildermuth *et al*. 2001; Ferrari *et al*. 2003). Alternatively, the reduced resistance anticipated in the SA signalling mutants *sid2-2* and *eds5* might be recovered by the enhanced accumulation of camalexin observed in these mutants (Nawrath & Métraux 1999; Nawrath *et al*. 2002). Last but not least, mutations in *EDS1*, *PAD4* and *NDR1* suppress not only SA-mediated defence responses but also resistance controlled by a subset of resistance (*R*) genes (Coppinger *et al*. 2004; Zhu *et al*. 2011). Thus, the enhanced susceptibility of these mutants could also point to a potential role of *R* genes in the interaction between Col-0 and LT123. Because of the pleiotropic effects encountered in the SA signalling mutants, the results on the role of SA were not conclusive. To address the question of whether loss of resistance to *P. capsici* in the *NahG* plants is due to deficiency of SA, we tested the effect of exogenous application of SA on lesion development. On *NahG* plants sprayed with 2.5 mM SA 1 d prior to inoculation with *P. capsici* LT123, lesions were smaller than on *NahG* plants sprayed with water (Supporting Information Fig. S3), confirming that SA plays a role in defence against *P. capsici*.

In the pathways leading to the production of indolic secondary metabolites, nine mutants were considered, including *pad4* mentioned above. The phytoalexin-deficient mutants *pad3* (*cyp71b15*) and *pad4* (Glazebrook & Ausubel 1994; Glazebrook *et al*. 1997) displayed a high level of susceptibility to *P. capsici* LT123 (Fig. 5). Lesions became clearly visible as early as 2 dpi, and these fast advancing lesions led to a mean DSI of 3.3 at 3 dpi. CYP71B15 (PAD3) catalyses the last step of camalexin biosynthesis (Schuhegger *et al*. 2006); PAD4 is a lipase-like protein with a role in camalexin biosynthesis as well as SA/*R* gene-mediated defence (Glazebrook *et al*. 1997; Zhou *et al*. 1998; Zhu *et al*. 2011). Unlike *pad4*, the mutant *pad3* has no other deficiencies apart from camalexin synthesis, and therefore, the observed susceptibility of *pad3* plants to LT123 revealed an important role for camalexin in disease resistance against *P. capsici*. Compared to *pad3*, the *cyp71a13* mutant showed much smaller lesion sizes. This difference could be due to different levels of camalexin. In *pad3*, camalexin production is completely abolished; even upon pathogen attack or during abiotic stress, there is no camalexin accumulation (Glazebrook & Ausubel 1994; Schuhegger *et al*. 2006). In contrast, in *cyp71a13*, which has a mutation in the pathway upstream of CYP71B15, camalexin is still produced albeit at a reduced level (Nafisi *et al*. 2007). Moreover, the cytochrome P450 enzyme CYP71A12, which shows 89% identity with CYP71A13 at the amino acid level, was reported to function in the camalexin biosynthesis pathway (Nafisi *et al*. 2007; Millet *et al*. 2010). Hence, in the *cyp71a13* mutant, the absence of CYP71A13 might be compensated by the activity of CYP71A12. Different phenotypes for *cyp71a13* and *pad3* were also reported by Van de Mortel *et al*. (2012), who tested the response to non-pathogenic, root-colonizing *Pseudomonas fluorescens* bacteria.

PAD2 is a γ-glutamylcysteine synthetase that is required for the synthesis of glutathione (Parisy *et al*. 2007). The *pad2* mutant, which is deficient in both iGS and camalexin, showed increased susceptibility to insect herbivory and *P. brassicae* (Schlaeppi *et al*. 2008, 2010). Consistently, in our study, *pad2* was found to be very susceptible to *P. capsici* LT123 with a mean DSI of 3.9 at 3 dpi, thus indicating that disease resistance to *P. capsici* is also largely dependent on camalexin and iGS. Furthermore, the double mutant *cyp79b2cyp79b3*, which fails to convert tryptophan into indole-3-acetaldoxime (IAOx), the precursor of camalexin and iGS (Zhao *et al*. 2002), showed a similar high DSI (i.e. 4) as *pad2*. Schlaeppi *et al*. (2010) reported that the mutation in *CYP79B2* and *CYP79B3* does not affect the level of other defence-associated responses, and hence, the susceptibility of the double mutant *cyp79b2cyp79b3* to *P. capsici* could be attributed to the deficiency of iGS and camalexin.

To further confirm the potential function of iGS in disease resistance, the iGS-related mutants *pen2*, *myb51* and *cyp81f2* were included in the screening. In the iGS metabolic pathway, MYB51 is a positive regulator of indole-3-yl-methyl glucosinolate (I3M) biosynthesis (Gigolashvili *et al*. 2007). I3M can be converted to 4-hydroxy-indole-3-yl-methyl glucosinolate (4HO-I3M) by the cytochrome P450 monooxygenase CYP81F2 (Pfalz, Vogel & Kroymann 2009). Subsequently, PEN2 myrosinase catalyses the hydrolysis of both I3M and 4HO-I3M (Bednarek *et al*. 2009). Consequently, the compromised resistance in *myb51*, *cyp81f2* and *pen2*

indicates that iGS, as well as iGS hydrolysis products, are important for *P. capsici* resistance in Arabidopsis. Arabidopsis produces besides iGS, also aliphatic glucosinolates (aGS), which have been shown to function in defence towards pest insects (Beekweelder *et al*. 2008). Biosynthesis of aGS is dependent on the transcription factors MYB28 and MYB29, and the synthesis of aGS is completely abolished in the double mutant *myb28myb29* (Beekweelder *et al*. 2008). Inoculation of *myb28myb29* with *P. capsici* did not result in any changes in disease susceptibility when compared with Col-0, indicating that blocking aGS synthesis by mutation in MYB28 and MYB29 is not sufficient to confer Arabidopsis susceptibility to *P. capsici*.

Arabidopsis exploits a different basal defence network for each *Phytophthora* species

Collectively, the results described here indicate that basal resistance to *P. capsici* in Arabidopsis involves the activation of SA signalling and the biosynthesis of camalexin and iGS. Blocking the accumulation of SA, camalexin or iGS results in susceptibility to *P. capsici*. In comparison, resistance to *P. parasitica* depends on JA/ET signalling in addition to SA signalling, as the mutants *etr1-3*, *ein2-1* and *jar1-1* were found to be more susceptible to this pathogen (Attard *et al*. 2010). In contrast, Arabidopsis resistance to *P. brassicae* was shown to be independent of SA and JA/ET signalling pathways; *NahG* plants, nor *etr1*, *ein2* and *jar1* mutants showed a change in phenotype demonstrating that blocking SA or JA/ET signalling had no significant effect on resistance. Moreover, mutants with defects in the accumulation of either camalexin or iGS, like *pad3* or *myb51*, showed no significant change in resistance to *P. brassicae.* However, the combined deficiency of iGS and camalexin as manifested in either *pad2* or *cyp79b2cyp79b3* resulted in susceptibility, demonstrating that the resistance to *P. brassicae* requires the sequential action of iGS and camalexin (Roetschi *et al*. 2001; Schlaeppi *et al*. 2010). In the case of *P. cinnamomi*, resistance in Arabidopsis seems to be independent of any of above-mentioned pathways. All mutants including *pad2*, *pad3*, *pad4*, *ein2* and *jar1* as well as *NahG* plants tested by Rookes *et al*. (2008) retained wild-type resistance. Taken together, it is evident that every attacking pathogen elicits the activation of specific signalling networks and that *Phytophthora* species vary in their ability to tolerate different defence components.

CONCLUSION

In this study, we demonstrated that *P. capsici* is capable of infecting Arabidopsis and that there is natural variation among Arabidopsis accessions in response to different *P. capsici* isolates. Cell death, callose deposition and ROS accumulation were induced in Arabidopsis during the early infection stages in an incompatible interaction. Blocking SA signalling, camalexin or iGS biosynthesis pathways in Arabidopsis conferred susceptibility to *P. capsici*, demonstrating that SA signalling and biosynthesis of camalexin and iGS are essential for basal resistance to this *Phytophthora* species. This newly established pathosystem will facilitate the identification of novel traits responsible for resistance to the broad-host-range oomycete pathogen *P. capsici*.

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REFERENCES

Alonso J.M., Hirayama T., Roman G., Nourizadeh S. & Ecker J.R. (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. *Science* **284,** 2148–2152.

Attard A., Gourgues M., Callemeyn-Torre N. & Keller H. (2010) The immediate activation of defense responses in Arabidopsis roots is not sufficient to prevent *Phytophthora parasitica* infection. *New Phytologist* **187,** 449–460.

Bednarek P., Piślewska-Bednarek M., Svatoš A., *et al*. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science* **323,** 101–106.

Beekweelder J., van Leeuwen W., van Dam N.M., *et al*. (2008) The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. *PLoS One* **3,** e2068.

Berger S., Bell E. & Mullet J.E. (1996) Two methyl jasmonate-insensitive mutants show altered expression of *AtVsp* in response to methyl jasmonate and wounding. *Plant Physiology* **111,** 525–531.

Bleecker A.B., Estelle M.A., Somerville C. & Kende H. (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241,** 1086–1089.

Bohman S., Staal J., Thomma B.P.H., Wang M. & Dixelius C. (2004) Characterisation of an Arabidopsis–*Leptosphaeria maculans* pathosystem: resistance partially requires camalexin biosynthesis and is independent of salicylic acid, ethylene and jasmonic acid signalling. *The Plant Journal* **37,** 9–20.

Bouwmeester K., de Sain M., Weide R., Gouget A., Klamer S., Canut H. & Govers F. (2011) The lectin receptor kinase LecRK-I.9 is a novel *Phytophthora* resistance component and a potential host target for a RXLR effector. *PLoS Pathogens* **7,** e1001327.

Brodersen P., Petersen M., Nielsen H.B., Zhu S., Newman M.A., Shokat K.M., Rietz S., Parker J. & Mundy J. (2006) Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *The Plant Journal* **47,** 532–546.

Cao H., Bowling S.A., Gordon A.S. & Dong X. (1994) Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell* **6,** 1583–1592.

Cao H., Glazebrook J., Clarke J.D., Volko S. & Dong X. (1997) The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **88,** 57–63.

Century K.S., Shapiro A.D., Repetti P.P., Dahlbeck D., Holub E. & Staskawicz B.J. (1997) *NDR1*, a pathogen-induced component required for Arabidopsis disease resistance. *Science* **278,** 1963–1965.

Chao Q., Rothenberg M., Solano R., Roman G., Terzaghi W. & Ecker J.R. (1997) Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* **89,** 1133–1144.

Coppinger P., Repetti P.P., Day B., Dahlbeck D., Mehlert A. & Staskawicz B.J. (2004) Overexpression of the plasma membrane-localized NDR1 protein results in enhanced bacterial disease resistance in *Arabidopsis thaliana*. *The Plant Journal* **40,** 225–237.

Delaney T.P., Uknes S., Vernooij B., *et al*. (1994) A central role of salicylic acid in plant disease resistance. *Science* **266,** 1247–1250.

Desikan R., Hancock J.T., Bright J., Harrison J., Weir L., Hooley R. & Neill S.J. (2005) A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. *Plant Physiology* **137,** 831–834.

Devoto A. & Turner J.G. (2003) Regulation of jasmonate-mediated plant responses in Arabidopsis. *Annals of Botany* **92,** 329–337.

Donahoo R.S. & Lamour K.H. (2008) Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* **100,** 911–920.

Dunand C., Crevecoeur M. & Penel C. (2007) Distribution of superoxide and hydrogen peroxide in Arabidopsis root and their influence on root development: possible interaction with peroxidases. *New Phytologist* **174,** 332–341.

Erwin D.C. & Ribeiro O.K. (1996) *Phytophthora Diseases Worldwide*. The American Phytopathological Society, St. Paul, MN.

Falk A., Feys B.J., Frost L.N., Jones J.D.G., Daniels M.J. & Parker J.E. (1999) EDS1, an essential component of *R* gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 3292–3297.

Ferrari S., Plotnikova J.M., De Lorenzo G. & Ausubel F.M. (2003) Arabidopsis local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires *EDS4* and *PAD2*, but not *SID2*, *EDS5* or *PAD4*. *The Plant Journal* **35,** 193–205.

Freeman S. & Rodriguez R.J. (1993) Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. *Science* **259,** 75–78.

Geraats B.P.J., Bakker P.A.H.M. & Van Loon L.C. (2002) Ethylene insensitivity impairs resistance to soilborne pathogens in tobacco and *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* **15,** 1078–1085.

Gigolashvili T., Berger B., Mock H.P., Müller C., Weisshaar B. & Flügge U.I. (2007) The transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. *The Plant Journal* **50,** 886–901.

Glazebrook J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43,** 205–227.

Glazebrook J. & Ausubel F.M. (1994) Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. *Proceedings of the National Academy of Sciences of the United States of America* **91,** 8955–8959.

Glazebrook J., Rogers E.E. & Ausubel F.M. (1996) Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. *Genetics* **143,** 973–982.

Glazebrook J., Zook M., Mert F., Kagan I., Rogers E.E., Crute I.R., Holub E.B., Hammerschmidt R. & Ausubel F.M. (1997) Phytoalexin-deficient mutants of Arabidopsis reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics* **146,** 381–392.

Harutyunyan S.R., Zhao Z., Den Hartog T., Bouwmeester K., Minnaard A.J., Feringa B.L. & Govers F. (2008) Biologically active *Phytophthora* mating hormone prepared by catalytic asymmetric total synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **105,** 8507–8512.

Hückelhoven R. (2007) Cell wall-associated mechanisms of disease resistance and susceptibility. *Annual Review of Phytopathology* **45,** 101–127.

Huitema E., Smoker M. & Kamoun S. (2011) A straightforward protocol for electro-transformation of *Phytophthora capsici* zoospores. *Methods in Molecular Biology* **712,** 129–135.

Johansson A., Staal J. & Dixelius C. (2006) Early responses in the Arabidopsis-*Verticillium longisporum* pathosystem are dependent on NDR1, JA- and ET-associated signals via cytosolic NPR1 and RFO1. *Molecular Plant-Microbe Interactions* **19,** 958–969.

Kishimoto K., Matsui K., Ozawa R. & Takabayashi J. (2006) ETR1-, JAR1- and PAD2-dependent signaling pathways are involved in C6-aldehyde-induced defense responses of Arabidopsis. *Plant Science* **171,** 415–423.

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.

Lacombe S., Rougon-Cardoso A., Sherwood E., *et al.* (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nature Biotechnology* **28,** 365–394.

Lamour K.H., Mudge J., Gobena D., *et al.* (2012a) Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. *Molecular Plant-Microbe Interactions* **25,** 1350–1360.

Lamour K.H., Stam R., Jupe J. & Huitema E. (2012b) The oomycete broad-host-range pathogen *Phytophthora capsici*. *Molecular Plant Pathology* **13,** 329–430.

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

Lorenzo O., Chico J.M., Sanchez-Serrano J.J. & Solano R. (2004) Jasmonate-insensitive 1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. *The Plant Cell* **16,** 1938–1950.

Millet Y.A., Danna C.H., Clay N.K., Songnuan W., Simon M.D., Werck-Reichhart D. & Ausubel F.M. (2010) Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *The Plant Cell* **22,** 973–990.

Nafisi M., Goregaoker S., Botanga C.J., Glawischnig E., Olsen C.E., Halkier B.A. & Glazebrook J. (2007) Arabidopsis cytochrome P450 monooxygenase 71A13 catalyzes the conversion of indole-3-acetaldoxime in camalexin synthesis. *The Plant Cell* **19,** 2039–2052.

Nawrath C. & Métraux J.P. (1999) Salicylic acid induction-deficient mutants of Arabidopsis express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. *The Plant Cell* **11,** 1393–1404.

Nawrath C., Heck S., Parinthawong N. & Metraux J.P. (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. *The Plant Cell* **14,** 275–286.

Pantelides I.S., Tjamos S.E. & Paplomatas E.J. (2010) Ethylene perception via ETR1 is required in Arabidopsis infection by *Verticillium dahliae*. *Molecular Plant Pathology* **11,** 191–202.

Parisy V., Poinssot B., Owsianowski L., Buchala A., Glazebrook J. & Mauch F. (2007) Identification of *PAD2* as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. *The Plant Journal* **49,** 159–172.

Petersen M., Brodersen P., Naested H., *et al.* (2000) Arabidopsis MAP kinase 4 negatively regulates systemic acquired resistance. *Cell* **103,** 1111–1120.

Pfalz M., Vogel H. & Kroymann J. (2009) The gene controlling the *indole glucosinolate modifier1* quantitative trait locus alters indole glucosinolate structures and aphid resistance in Arabidopsis. *The Plant Cell* **21,** 985–999.

Robert-Seilaniantz A., Grant M. & Jones J.D.G. (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* **49,** 317–343.

Roetschi A., Si-Ammour A., Belbahri L., Mauch F. & Mauch-Mani B. (2001) Characterization of an Arabidopsis-*Phytophthora* pathosystem: resistance requires a functional *PAD2* gene and is independent of salicylic acid, ethylene and jasmonic acid signalling. *The Plant Journal* **28,** 293–305.

Rookes J.E., Wright M.L. & Cahill D.M. (2008) Elucidation of defence responses and signalling pathways induced in *Arabidopsis thaliana* following challenge with *Phytophthora cinnamomi*. *Physiological and Molecular Plant Pathology* **72,** 151–161.

Schlaeppi K., Bodenhausen N., Buchala A., Mauch F. & Reymond P. (2008) The glutathione-deficient mutant *pad2-1* accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. *The Plant Journal* **55,** 774–786.

Schlaeppi K., Abou-Mansour E., Buchala A. & Mauch F. (2010) Disease resistance of Arabidopsis to *Phytophthora brassicae* is established by the sequential action of indole glucosinolates and camalexin. *The Plant Journal* **62,** 840–851.

Schlaich N.L. (2011) *Arabidopsis thaliana* – the model plant to study host-pathogen interactions. *Current Drug Targets* **12,** 955–966.

Schuhegger R., Nafisi M., Mansourova M., Petersen B.L., Olsen C.E., Svatoš A., Halkier B.A. & Glawischnig E. (2006) CYP71B15 (PAD3) catalyzes the final step in camalexin biosynthesis. *Plant Physiology* **141,** 1248–1254.

Silvar C., Duncan J.M., Cooke D.E.L., Williams N.A., Diaz J. & Merino F. (2005) Development of specific PCR primers for identification and detection of *Phytophthora capsici Leon*. *European Journal of Plant Pathology* **112,** 43–52.

Spoel S.H., Johnson J.S. & Dong X. (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 18842–18847.

Thomma B.P.H., Nelissen I., Eggermont K. & Broekaert W.F. (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *The Plant Journal* **19,** 163–171.

Thomma B.P.H., Tierens K.F.M., Penninckx I.A.M.A., Mauch-Mani B., Broekaert W.F. & Cammue B.P.A. (2001) Different micro-organisms differentially induce Arabidopsis disease response pathways. *Plant Physiology and Biochemistry* **39,** 673–680.

Thordal-Christensen H., Zhang Z., Wei Y. & Collinge D.B. (1997) Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *The Plant Journal* **11,** 1187–1194.

Ton J., De Vos M., Robben C., Buchala A., Metraux J.P., Van Loon L.C. & Pieterse C.M.J. (2002) Characterization of Arabidopsis enhanced disease susceptibility mutants that are affected in systemically induced resistance. *The Plant Journal* **29,** 11–21.

Van de Mortel J.E., De Vos R.C.H., Dekkers E., Pineda A., Guillod L., Bouwmeester K., van Loon J.J.A., Dicke M. & Raaijmakers J.M. (2012) Metabolic and transcriptomic changes induced in Arabidopsis by the rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiology* **160,** 2173–2188.

Van Wees S.C.M., Chang H.S., Zhu T. & Glazebrook J. (2003) Characterization of the early response of Arabidopsis to *Alternaria brassicicola* infection using expression profiling. *Plant Physiology* **132,** 606–617.

Wang Y., Meng Y., Zhang M., Tong X., Wang Q., Sun Y., Quan J., Govers F. & Shan W. (2011) Infection of *Arabidopsis thaliana* by *Phytophthora parasitica* and identification of variation in host specificity. *Molecular Plant Pathology* **12,** 187–201.

Wildermuth M.C., Dewdney J., Wu G. & Ausubel F.M. (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* **414,** 562–565.

Yadeta K.A., Hanemian M., Smit P., Hiemstra J.A., Pereira A., Marco Y. & Thomma B.P.H. (2011) The *Arabidopsis thaliana* DNA-binding protein AHL19 mediates *Verticillium* wilt resistance. *Molecular Plant-Microbe Interactions* **24,** 1582–1591.

Zhang X., Francis M.I., Dawson W.O., Graham J.H., Orbovic V., Triplett E.W. & Mou Z. (2010) Over-expression of the Arabidopsis *NPR1* gene in citrus increases resistance to citrus canker. *European Journal of Plant Pathology* **128,** 91–100.

Zhao Y., Hull A.K., Gupta N.R., Goss K.A., Alonso J., Ecker J.R., Normanly J., Chory J. & Celenza J.L. (2002) Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes and Development* **16,** 3100–3112.

Zhou N., Tootle T.L., Tsui F., Klessig D.F. & Glazebrook J. (1998) *PAD4* functions upstream from salicylic acid to control defense responses in Arabidopsis. *The Plant Cell* **10,** 1021–1030.

Zhu S., Jeong R.-D., Venugopal S.C., Lapchyk L., Navarre D., Kachroo A. & Kachroo P. (2011) SAG101 forms a ternary complex with EDS1 and PAD4 and is required for resistance signaling against turnip crinkle virus. *PLoS Pathogens* **7,** e1002318.

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SUPPORTING INFORMATION

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

Figure S1. Relative quantification (RQ) of *P. capsici* biomass in Arabidopsis leaves at 3 dpi by real-time PCR.

Figure S2. Disease severity on tomato leaves inoculated with different *P. capsici* isolates.

Figure S3. SA treatment of Arabidopsis *NahG* plants decreases susceptibility to *P. capsici* isolate LT123.

Table S1. Primers used in this study.

Table S2. Arabidopsis defence-related mutants and transgenic lines used in this study and the disease severity at 3 d after inoculation with *P. capsici* isolate LT123. The information on the pathways or metabolites that are affected and the pathogens that were studied in the interaction was collected from the references listed.