

Induced expression of defense-related genes in *Arabidopsis* upon infection with *Phytophthora capsici*

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Keywords: *Phytophthora capsici*, *Arabidopsis*, defense signaling, salicylic acid, jasmonic acid, ethylene, camalexin, indole glucosinolates

Abbreviations: SA, salicylic acid; JA, jasmonic acid; ET, ethylene; iGS, indole glucosinolates; DSI, disease severity index

Recognition of pathogens by plants initiates defense responses including activation of defense-related genes and production of antimicrobial compounds. Recently, we reported that *Phytophthora capsici* can successfully infect *Arabidopsis* and revealed interaction specificity among various accession-isolate combinations. We used this novel pathosystem to demonstrate that camalexin, indole glucosinolates (iGS) and salicylic acid (SA) have a role in defense against *P. capsici*. To further investigate the role of camalexin-, iGS- and SA-related pathways in the differential interaction between *Arabidopsis* and *P. capsici*, we monitored expression of marker genes over time during infection. In both compatible and incompatible interactions, induction of expression was detected, but in compatible interactions transcript levels of camalexin and iGS marker genes were higher.

Phytophthora capsici is a pathogen with a broad host range and is particularly devastating in vegetable crops worldwide. Recently, we showed that *P. capsici* can also infect *Arabidopsis* and we anticipate that such a model pathosystem will facilitate the genetic dissection of complex traits responsible for *P. capsici* resistance in crops.¹ Microscopic investigation of the infection process revealed that inoculation of *Arabidopsis* Col-0 with zoospores of the highly virulent *P. capsici* isolate LT263 resulted in colonization of leaf and root tissue and formation of haustoria followed by sporulation. In contrast, inoculation of Col-0 with zoospores of the intermediate virulent isolate LT123 resulted in an incompatible interaction characterized by callose deposition, accumulation of active oxygen species and cell death.¹ We also observed natural variation among *Arabidopsis* accessions in response to the two isolates; LT263 caused severe disease symptoms on 29 out of 35 *Arabidopsis* accessions tested, while LT123 could infect only 2 out of the 35, i.e., *Ler-0* and *Wei-0*. Moreover, *Arabidopsis* mutants deficient in SA signaling and the biosynthesis of camalexin and iGS showed compromised resistance against *P. capsici*, indicating a role for these components in defense. The aim of this study was to find additional support for the involvement of these secondary metabolites in defense against *P. capsici*. Therefore we monitored the expression of several defense-related genes in *Arabidopsis* upon inoculation with *P. capsici* over time using real-time PCR.

For inoculation we used mycelial plugs instead of zoospores; the outcome is similar but disease development is faster thus shortening the time course.¹ Based on previous work,¹ we selected two *P. capsici* isolates and two *Arabidopsis* accessions. This allowed us to compare an incompatible and compatible interaction on either the same accession (*Col-0*) inoculated with different isolates (LT123 and LT263, respectively) or different accessions (*Col-0* and *Ler-0*, respectively) inoculated with the same isolate (LT123). In these interactions the disease severity index (DSI) as defined previously,¹ ranged from 0.2 with hardly any visible symptoms, to 3.9 showing complete collapse of the inoculated leaves (Fig. 1).

The cytochrome P450 monooxygenase (CYP) encoding genes *CYP71B15* (*PAD3*) and *CYP71A13* were selected as marker genes for the biosynthesis of camalexin.^{2,3} Another CYP gene, *CYP81F2* and the gene encoding the transcription factor MYB51 were used as markers for the iGS biosynthesis pathway.^{4,5} In *Col-0*, inoculation with either *P. capsici* LT123 or LT263 leads to an increase in transcript levels of these four genes over time with a peak at 36 h post inoculation (hpi), regardless of the compatible and incompatible nature of the interaction. In particular for *CYP71B15* and *CYP71A13*, drastic increases in transcript levels were observed (Fig. 2) whereas this increase did not occur in mock-inoculated leaves. As shown previously¹ loss-of-function mutants of these

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Submitted: 03/04/13; Revised: 04/09/13; Accepted: 04/09/13

Citation: Wang Y, Bouwmeester K, van de Mortel JE, Shan W, Govers F. Induced expression of defense-related genes in *Arabidopsis* upon infection with *Phytophthora capsici*. Plant Signal Behav 2013; 8: e24618; <http://dx.doi.org/10.4161/psb.24618>

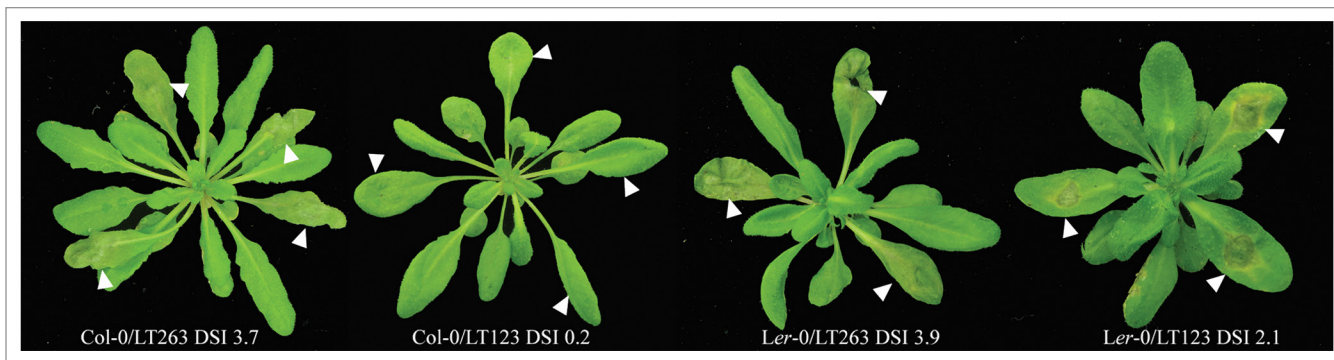


Figure 1. Symptoms on *Arabidopsis* accessions Col-0 and Ler-0 after plug-inoculation with *P. capsici* isolate LT263 or LT123 at 3 dpi. The disease severity index (DSI) determined according to Wang et al.³ is indicated. The white arrowheads point to inoculated leaves.

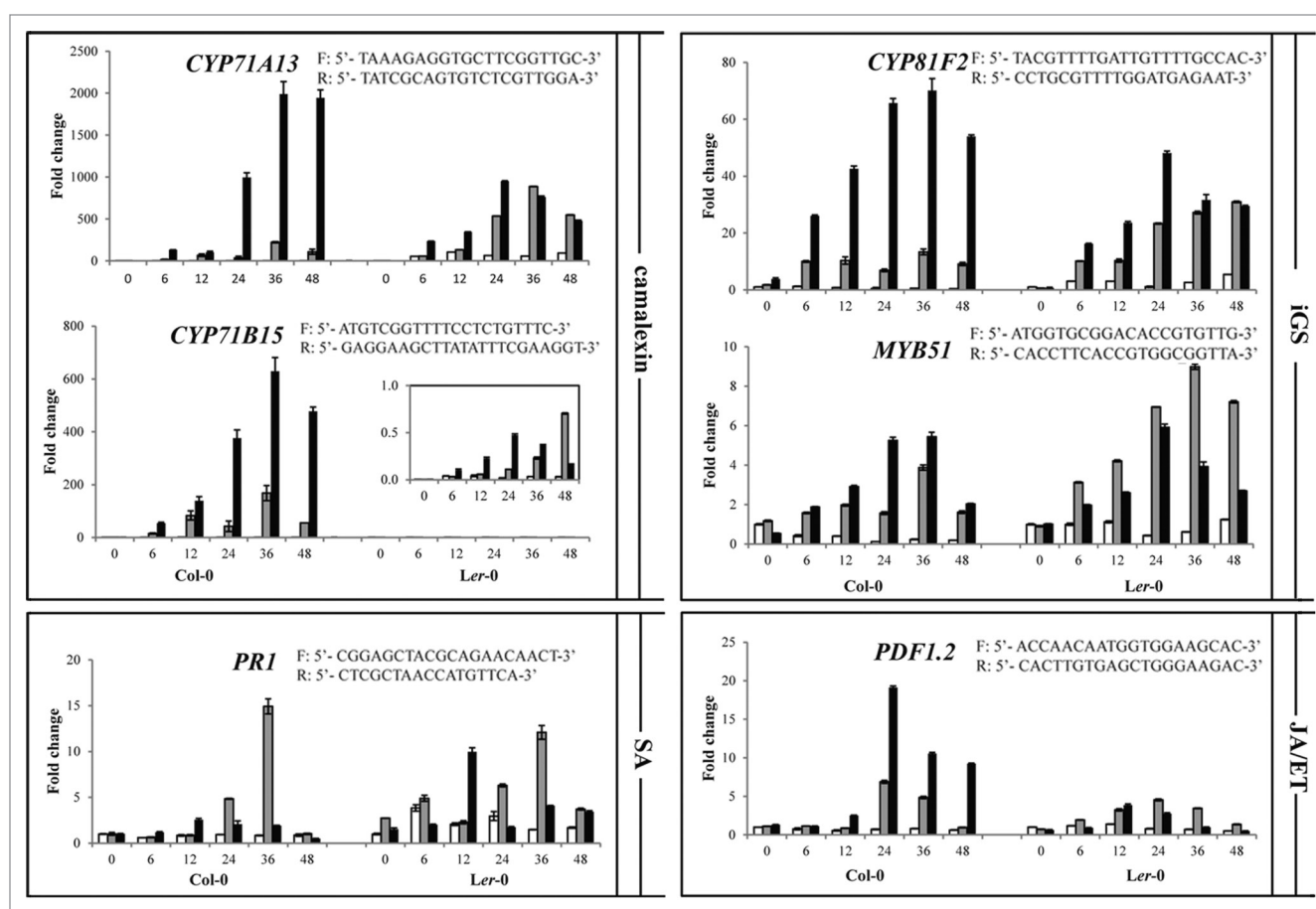


Figure 2. Transcript levels of *CYP71A13*, *CYP71B15*, *CYP81F2*, *MYB51*, *PR1* and *PDF1.2* genes during compatible and incompatible *Arabidopsis*-*P. capsici* interactions. Four-to-five-week-old *Arabidopsis* leaves were plug-inoculated with *P. capsici* LT123 (gray bars) and LT263 (black bars). Six circular leaf discs (\varnothing 1 cm), with the inoculation spot in the center, were collected from three plants at the time points indicated on the X-axes (hours post inoculation). Subsequently, total RNA was isolated and used as template for cDNA synthesis. Transcript levels were quantified by real-time PCR using gene-specific primers of which the sequences are shown in the figure and were normalized with *Arabidopsis Actin 2* (F: 5'-TAA CTC TCC CGC TAT GTA TGT CGC-3'; R: 5'-GAG AGA AAC CCT CGT AGA TTG GC-3'). Values on the Y-axes are expressed as mean fold changes (\pm SD) relative to the transcript level in mock-inoculated Col-0 leaves (white bars) at time point 0 which was arbitrary set as 1. Experiments were repeated twice with similar results.

four genes showed gain of susceptibility upon infection with *P. capsici* LT123, suggesting a role in resistance and thus in incompatible interactions. Remarkably, at all time points the transcript

levels were significantly higher in the compatible interaction than in the incompatible interaction (Fig. 2). This might be due to the fact that in LT263-infected Col-0 leaves, many more cells are

colonized thus resulting in a stronger response to infection, especially for the camalexin-related marker genes. In several pathosystems, camalexin accumulation as well as transcripts of relevant biosynthesis genes were found to be strictly limited to the infection sites and correlated with the spatial expansion of the pathogen.^{6,7} To investigate if this holds for the *P. capsici*-*Arabidopsis* pathosystem, we checked expression of these marker genes during infection of *Arabidopsis Ler-0*. This accession is susceptible to LT123 (DSI 2.1), but even more susceptible to LT263 (DSI 3.9). In these compatible interactions transcript levels of *CYP71A13* were higher than in the incompatible interaction of Col-0 with LT123 (DSI 0.2) but overall lower than in the compatible interaction of Col-0 with LT263 (DSI 3.7). Remarkably, in *Ler-0* basal expression of *CYP71B15* is extremely low when compared with Col-0. Nevertheless, also in infected *Ler-0* leaves the *CYP71B15* transcript levels increased dramatically (more than 100-fold after 24 hpi), as shown in the inset in **Figure 2**. During time, the peak in expression in LT263-infected *Ler-0* leaves (with a higher DSI) was at 24 hpi, so slightly earlier than in LT123-infected *Ler-0* leaves (with a lower DSI) and in Col-0, but overall the transcript changes followed a similar pattern in all interactions. Taken together we found no evidence for a strict correlation between transcript levels and DSI. We do however, observe a consistent pattern for the two camalexin marker genes, namely induction of expression upon inoculation and overall higher transcript levels in compatible compared with incompatible interactions. A similar strong increase in the compatible interaction was found for one of the iGS biosynthesis marker genes, i.e., *CYP81F2*. For the other one, *MYB51*, the differences between transcript levels in the Col-0 compatible and incompatible interaction were less pronounced and in *Ler-0*, *MYB51* transcript levels in leaves with the lower DSI (2.1) were overall higher than in those with a higher DSI (3.9).

PRI, the gene that encodes a pathogenesis-related protein, was selected as marker gene for the SA pathway.⁸ In Col-0, *PRI* expression was found to be induced during the incompatible interaction with *P. capsici* isolate LT123 with a maximum at 36 hpi followed by a rapid decline to the basal level. In the compatible interaction with isolate LT263, *PRI* transcript accumulation was triggered faster, but to a lesser extent. Also in the compatible

interactions of *Ler-0* with LT123 and LT263, *PRI* expression was triggered rather fast and the fold increase approached the levels observed in the incompatible interaction in Col-0. As a marker gene for the JA/ET-pathway we used *PDFI.2*.⁸ In Col-0, the *PDFI.2* transcript levels started to increase from 12 hpi, reaching a peak at 24 hpi and then decreasing steadily in both the compatible and incompatible interaction. Transcript levels in the compatible interaction, however, are significantly higher than in the incompatible interaction. Also in *Ler-0* *PDFI.2* transcript levels increase upon inoculation but to a much lesser extent than in Col-0.

In summary and in line with our recent report¹ in which we screened the response of *Arabidopsis* mutants to *P. capsici* and revealed requirement of SA, camalexin and iGS for resistance to this pathogen, we found induced expression of relevant marker genes in *Arabidopsis* challenged with *P. capsici*. Variations in expression levels were detected in different accession-isolate combinations and for nearly all marker genes expression was higher in compatible interactions as compared with incompatible interaction. The expression profiles, however, do not reveal any obvious correlation between expression levels and disease phenotype. These results raise the question why in the absence of proteins encoded by the camalexin and iGS marker genes, so in the mutants, the plants are susceptible and can no longer stop the pathogen while in wild-type plants these genes are highly induced upon successful infection. When assuming that expression indeed leads to protein production, one would expect that in the compatible interaction camalexin and iGS are produced but apparently not in time or at sufficient levels to block disease development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This project was financially supported by a Wageningen University sandwich PhD fellowship and a Huygens scholarship (Y.W.) and by the Centre for BioSystems Genomics (CBSG) (K.B., F.G.), which is part of the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research.

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