Plant Signaling & Behavior 5:4, 425-427; April 2010; © 2010 Landes Bioscience

A role for chloroplast-localized *thylakoid formation 1* (*THF1*) in bacterial speck disease development

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Coronatine (COR), a jasmonate mimic produced by *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) is required for full virulence of *Pst* DC3000 in tomato and Arabidopsis. COR is shown to induce a range of physiological processes including chlorosis, root growth inhibition and anthocyanin accumulation in tomato. To elucidate the host/signaling genes involved in COR-responses, we utilized a forward genetics approach using *Nicotiana benthamiana* and virus-induced gene silencing (VIGS) and identified genes that play a role in COR-mediated chlorosis. We designated these genes as altered COR response (*ALC*). When silenced, one gene designated *ALC1* produced a hypersensitive/necrosis-like phenotype after COR application in a coronatine insensitive 1 (*COI1*)-dependent manner. In pathogenicity assays performed on Arabidopsis thylakoid formation 1 (*thf1*) knockout lines and *SlALC1*-silenced tomato plants, *Pst* DC3000 induced coalescing necrotic lesions in an accelerated manner. Furthermore, we showed that COR affects ALC1 localization in chloroplast in a *COI1*-dependent manner. In conclusion, our results show the potential of VIGS-based, forward genetic screens to identify new players in COR-mediated signal transduction.

Key words: coronatine, THF1, VIGS, *Pseudomonas syringae*, bacterial speck, chlorosis, jasmonates, tomato

Submitted: 01/20/10

Accepted: 01/20/10

Previously published online: www.landesbioscience.com/journals/psb/article/11284

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Addendum to: Wangdi T, Uppalapati SR, Nagaraj S, Ryu, C-M, Bender CL, Mysore KS. A virus-induced gene silencing screen identifies a role for *Thylakoid Formation1* in *Pseudomonas syringae* pv. tomato symptom development in tomato and Arabidopsis. Plant Physiol 2010; 152:281–92; PMID: 19915014; DOI: 10.1104/pp.109.148106.

Coronatine (COR), a phytotoxin produced by *P. syringae* pv. *tomato* (*Pst* DC3000) contributes to the virulence of *Pst* DC3000 in Arabidopsis, tomato, collard and turnip.[1-3] It has been shown that COR has structural and functional resemblance to 12-oxo-phytodienoic acid (12-OPDA), methyl jasmonate (MeJA), and derivatives related to jasmonic acid (JA).[4]

During a compatible interaction, COR-producing *Pst* DC3000 activates JA signaling, which leads to a suppression of the salicylic acid (SA) pathway.[1,5-8] One hallmark of bacterial speck disease on tomato leaves is the formation of necrotic lesions surrounded by chlorosis. COR is shown to be required for chlorosis development.[1-3,9] In addition to chlorosis, COR induces a wide array of effects in plants including anthocyanin production, alkaloid accumulation, ethylene emission, tendril coiling, inhibition of root elongation, hypertrophy and stomatal opening.[4,9-13] Despite our present understanding of COR function, it is not clear how chlorosis impacts or benefits pathogen virulence. Moreover, the molecular targets for COR and the downstream signaling cascades are not well understood.

To identify plant genes that are involved in COR signaling, we used virus-induced gene silencing (VIGS)-based forward genetic screen in *Nicotiana benthamina*. A *N. benthamiana* cDNA library cloned in tobacco rattle virus (TRV) RNA2 based VIGS vector, was used.[14] *N. benthamiana* plants were individually inoculated with *Agrobacterium tumefaciens* TRV2 cDNA clones,[14] along with an Agrobacterium

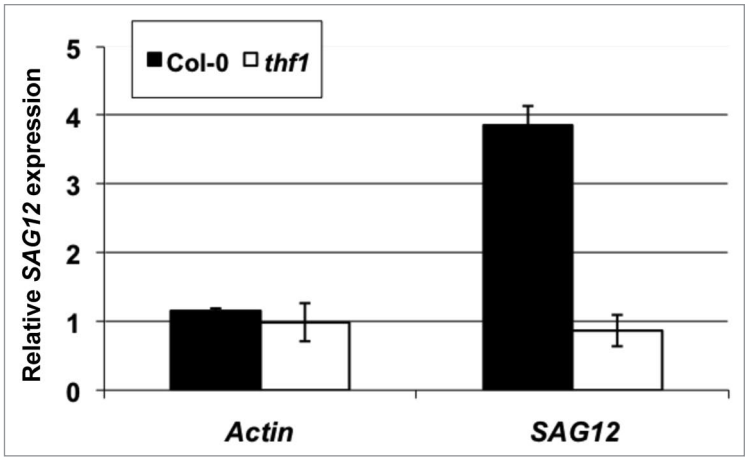

Figure 1. Senescence-associated *SAG12* expression in *thf1* mutants in response to *Pst* DC3000. Transcripts of *SAG12* were quantified by real time quantitative PCR in wild-type (Col-0, black bars) and *thf1* mutant (open bars) lines of Arabidopsis after *Pst* DC3000 inoculation. Four-week old plants of Col-0 and the *thf1* mutant were syringe-infiltrated with either *Pst* DC3000 (10^6 CFU/ml) or buffer (mock control). The transcript levels were quantified relative to the transcript levels on mock control, which was assigned a value of 1.

strain containing *pTRV1*, to silence the corresponding genes in *N. benthamiana*.[14] Two weeks after inoculation with TRV, COR (0.2 nmol) was spotted on the leaves of silenced plants, and the phenotypes were recorded 5–7 days later.

After screening ~4,000 cDNA clones, we identified five non-redundant cDNA clones that when silenced resulted in an altered COR response (ALC) phenotype upon exogenous application of COR.[15] The silencing of a gene designated *ALC1* resulted in a hypersensitive (HR)-like necrosis instead of chlorosis after COR inoculation. *ALC1* is a homologue of the Arabidopsis Thylakoid formation 1 (*THF1*) gene.[15] We also demonstrated that the loss of the *N. benthamiana* gene *NbALC1* and its orthologs, *SlALC1* in tomato and *AtTHF1* in Arabidopsis, resulted in a necrotic response to COR and *Pst* DC3000. When *ALC1*-silenced tomato plants were spray-inoculated with *Pst* DC3000, necrotic lesions without visible chlorosis developed in an accelerated manner, and the phenotype was distinctly different from typical bacterial speck symptoms with chlorotic halos.[5,13] Furthermore, necrosis spread beyond the region where COR was applied beginning at 10 days post-inoculation (dpi), which is similar to the runaway cell death phenotype reported earlier in the Arabidopsis *lsd1* mutant.[15,16]

To determine the role of *ALC1* in response to COR and *Pst* DC3000, we used Arabidopsis since it is genetically tractable and a host of *Pst* DC3000. The ortholog of *ALC1* in Arabidopsis, known as *THF1,* is a single-copy gene with no closely related sequences in the Arabidopsis genome.[17] Similar to *ALC1*-silenced tomato plants, inoculation of Arabidopsis *thf1* mutants with *Pst* DC3000 did not result in a typical bacterial speck symptoms. Interestingly, no differences in the *Pst* DC3000 populations were seen between *ALC1*-silenced tomato plants and the *thf1* mutant line when compared to wild-type plants. These results suggested that *Pst* DC3000 may tightly regulate chloroplast homeostasis during infection, and the levels of THF1 for controlled necrosis during infection may assist in pathogen dissemination and spread. We recently demonstrated that COR-induced effects on the photosynthetic machinery result in the generation of light-dependent, reactive oxygen species (ROS) in tomato seedlings.[18,19] We speculate that in COR-treated or *Pst* DC3000-inoculated *ALC1* silenced tomatoes and in the *Pst* DC3000-inoculated Arabidopsis *thf1* mutant, the necrotic/HR-like cell death phenotype may appear because the effect of ROS supersedes the detoxifying capacity of antioxidants.

The COR-induced necrotic phenotype in *ALC1*-silenced plants is COI1-dependent, and the JA-mediated defense pathway is functional but subdued in Arabidopsis *thf1* mutants.[15] Since COR is a functional mimic of JA and is shown to induce senescence in Arabidopsis,[20] we investigated whether the senescence-associated gene *SAG12* was differentially expressed in the Arabidopsis *thf1* mutant in response to *Pst* DC3000. Using qRT-PCR, we analyzed the transcript level of *SAG12* four days after infiltration with *Pst* DC3000. Infected leaves of Col-0 expressed approximately four-fold higher levels of *SAG12* (**Fig. 1**) as compared to the *thf1* mutant line. These results suggested that the typical *Pst* DC3000-induced chlorosis is associated with senescence.

Furthermore, results from GFP-tagged ALC1 suggest that COR has direct effects on ALC1 and might target ALC1 to degradation in a *COI1*-dependent manner.[15] Based on these results it is tempting to speculate that ALC1/THF1 may directly interact with COR if localized in the chloroplast membrane. Interestingly, a chloroplast protein in wheat, ToxABP1 (an ortholog of THF1), directly interacts with the *Pyrenophora tritici-repentis* protein ToxA.[21]

In conclusion, we have developed a VIGS-based forward genetic screen for identification of new targets involved in COR signaling. Although the precise role of THF1 in COR signaling pathway could be argued and needs further confirmation, our results present a new role for chloroplast-localized THF1 in bacterial speck disease development.

Acknowledgements

This work was supported by the Samuel Roberts Noble Foundation and in part by a grant to S.R. Uppalapati from Oklahoma Center for Advancement of Science and Technology (PSB09-021). C.L. Bender acknowledges financial support from National Science Foundation grant (IOB-0620469). A spinning disk confocal microscope was purchased by an equipment grant from NSF (DBI-0722635).

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