SGT1b is required for HopZ3-mediated suppression of the epiphytic growth of Pseudomonas syringae on N. benthamiana

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Type III secreted effectors shape the potential of bacterial pathogens to cause disease on plants. Some effectors affect pathogen growth only in specific niches. For example, HopZ3 causes reduced epiphytic growth of Pseudomonas syringae strain B728a on Nicotiana benthamiana. This raises the question of whether genes important for effector-triggered disease resistance are needed for responses to effectors whose major effect is in the epiphytic niche. We report that SGT1b, a protein known to be important for defense activation, is essential for HopZ3-mediated suppression ofPsyB728a epiphytic growth. SGT1b is required for HopZ3- and AvrB3-induced cell death in N. benthamiana plants that express the Pto resistance gene from tomato. We suggest that HopZ3 activates R gene mediated responses in N. benthamiana.

Plants possess many resistance (R) proteins that directly or indirectly interact with effector proteins to detect pathogen infection. The result of such detection is activation of defenses that suppress pathogen growth. Sometimes detection of an effector can also lead to cell death (the hypersensitive response, HR). Effectors that elicit a resistance response (also called “effector triggered immunity”) are considered avirulence (Avr) effectors, because they render the pathogen avirulent.1,2 Plant R genes mostly encode proteins that belong to a superfamily that contain an NB-LRR (nucleotide binding-leucine rich repeat) domain and also possess an N-terminus with either a coiled-coil or a Toll interleukin-1 receptor domain.1 SGT1b (suppressor of the G2 allele of Skp1), RAR1 (required for Mla 2 resistance 1) and/or HSP90 (heat shock protein 90) are required for the stability of R proteins; reduction of SGT1b, RAR1 and HSP90 using virus-induced gene silencing (VIGS) compromises some resistance responses mediated by R genes.3,7

Pathogenic Pseudomonas syringae, the causal agent of bacterial leaf speck, can exist in both epiphytic populations on leaf surfaces and also in endophytic populations that neighbor mesophyll cells.8 In general, epiphytic bacteria population sizes and diversity are influenced by environmental conditions, plant species, plant cultivar, and stage of growth. Differences in temperature, rainfall and UV exposure, which typically fluctuate with season, are associated with changes in the total cultivable bacteria on leaves. Survival and/or growth on leaf surfaces can influence the potential of pathogenic P. syringae to invade leaves and grow endophytically. P. syringae pv. syringae B728a (PsyB728a) lacking a type III secretion system does not survive well on Nicotiana benthamiana leaf surfaces.9 Activation of salicylic acid signaling also results in poor survival of PsyB728a on leaf surfaces. Finally, two effectors, HopZ3 and HopAA1, specifically restrict the epiphytic growth of PsyB728a on N. benthamiana, but promote bacterial survival on tomato.9 Together these findings indicate that defenses are active in restricting epiphytic PsyB728a growth and that some effectors may activate defenses in the epiphytic niche of N. benthamiana, but not tomato.

HopZ3 does not elicit cell death when transiently expressed on wild-type N. benthamiana. However, HopZ3 elicits mild cell death on transgenic N. benthamiana that ectopically expresses the tomato Pto gene under the CaMV 35S promoter (N. benthamiana/Pto) or when HopZ3 and Pto are transiently co-expressed.9 Like HopZ3, AvrB3 also elicits cell death on N. benthamiana/Pto. The presence of Pto causes constitutive defenses,10 which may lower the threshold for HopZ3 and AvrB3 to activate HR-like responses. We hypothesize that HopZ3 and possibly AvrB3 can activate resistance responses through their actions as avirulence proteins and may require known defense components.

We first sought to determine whether cell death observed in N. benthamiana/Pto after HopZ3 effector production involves any known defense signaling components. We chose to test the roles of three different genes (NPR1, RAR1 and SGT1b) known to be required for the cell death induced by some Avr effector-R protein recognition events.5,6 We silenced these genes individually using VIGS as described by Dinesh-Kumar et al.7 Two weeks after introducing the constructs, silencing was confirmed by reverse transcription polymerase chain reaction (RT-PCR) (Fig. 1A). SGT1b-silenced plants showed the expected curled leaf morphology (Fig. 1B) that was previously reported.7 HopZ3 was transiently expressed under dexamethasone control in NPR1-,
investigate the role of SGT1b in epiphytic bacterial growth on plant leaves, we silenced SGT1b in *N. benthamiana* plants. At two weeks after introducing the constructs, the silenced leaves were spray-inoculated with *PsyB728a* and *HopZ3*- bacteria. At three days after spray-inoculation, the average fluorescence area of *PsyB728a* bacteria in SGT1b-silenced plant was greatly increased relative to TRV:00 control plants (*p* = 0.0099, Mann-Whitney test, *n* = 43–49, Fig. 2A).

When epiphytic bacteria populations were quantified using a leaf wash assay, SGT1b-silenced leaves supported significantly more *PsyB728a* growth (Fig. 2B) compared with growth on the TRV:00 control plant leaves. In contrast, silencing SGT1b did not affect the growth of *HopZ3*- bacteria. Together, these finding suggest that SGT1b-mediated host resistance is important for epiphytic bacterial growth on leaf surfaces of *N. benthamiana*.

Recently, SGT1b was shown to be required for cell death that occurs during disease in addition to its role in resistance responses. We have found that even when *PsyB728a* infections of *N. benthamiana* result in disease, some effectors can quantitatively restrict *PsyB728a* growth. Thus, it seems possible that cell death associated with disease and high growth of bacteria may be due in part to defense responses that are quantitative and thus only partially successful. Wang et al. showed there was no difference in the growth of *PsyB728a* bacteria between SGT1b-silenced and non-silenced control *N. benthamiana* plants. In that study, the authors used vacuum inoculation with surfactant, conditions that promote immediate bacterial growth of *PsyB728a* into the mesophyll area without the 48 h growth lag period that usually occurs with more natural routes of infection mimicked by spraying without additives. In contrast, we used spray-inoculation on leaf surfaces without surfactant. We observed that *NbsGT1b*-silenced plants supported more growth of epiphytic *PsyB728a* compared with growth on the TRV:00 control plant leaves (Fig. 2A and 2B). Our finding that HopZ3-dependent suppression epiphytic bacterial growth requires SGT1b suggests that SGT1b is likely acting through its function in stabilizing defense components in epidermal cells in contact with epiphytic bacteria.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Figure 1.** The cell death induced by HopZ3 and AvrB3 on *N. benthamiana*/*Pto* plants is dependent on SGT1b mediated defense signaling. One-week-old *N. benthamiana*/*Pto* overexpressing *N. benthamiana* plants were inoculated with *Agrobacterium tumefaciens* containing TRV::NbsGT1b or TRV:00 (vector control). Relative expression of NbsGT1b determined by the semi-quantitative RT-PCR in *N. benthamiana*/*Pto* plants 2 weeks after *A. tumefaciens* inoculation. (A) PCR bands representing NbsGT1b and actin (internal control) after 25 cycles are shown for TRV:00-vector control and TRV-SGT1b samples. (B) Cell death phenotype in control *N. benthamiana*/*Pto* plants was induced by HopZ3 or AvrB3, whereas cell death did not occur in SGT1b silenced plants. Photographs were taken at 3 d after 30 µM dexamethasone-treatment.

### Table 1

| Treatment          | Cell Death |
|--------------------|------------|
| TRV:00             | No Cell Death |
| TRV::sgt1b         | Cell Death |

### Diagram

#### A

| SGT1b | Actin |
|-------|-------|
| TRV:00 | TRV::sgt1b |

#### B

| HopZ3 | AvrB3 | Vector |
|-------|-------|--------|
| TRV:00 | TRV::sgt1b |

**RAR1- and SGT1b-silenced N. benthamiana/Pto.** In *N. benthamiana/Pto* plants with the TRV:00 vector control or silenced for NPR1 or RAR1, cell death usually occurred 48 h after dexamethasone application (data not shown). In contrast, in SGT1b-silenced plants, cell death either did not develop or was greatly diminished compared with control plants (Fig. 1B). AvrB3 induced-cell death also did not develop on SGT1b-silenced *N. benthamiana/Pto* plants. These data are consistent with the hypothesis that the cell death events induced by HopZ3 and AvrB3 on *N. benthamiana* plants were due to their avirulence and HR-inducing activities.

In previous our study, a detailed analysis of the role of effectors in epiphytic bacterial growth on leaf surfaces using quantitative microscopy proved to be very useful. Therefore, we directly visualized green fluorescent protein (GFP)-labeled *PsyB728a* carrying Ptrp-GFP (Ptrp drives constitutive expression of GFP) and analyzed the bacteria on the surfaces of leaves of *N. benthamiana* plants silenced for SGT1b using epifluorescence microscopy.
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