Identification and functional analysis of *Pseudomonas syringae* pv. *actinidiae* effector-triggered immunity in *Nicotiana spp.* and *Arabidopsis thaliana.*

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Abstract

Pseudomonas syringae pv. *actinidiae* (*Psa)* is the causal agent of bacterial canker in commercially important cultivars of kiwifruit (*Actinidia delicosa and A. chinensis*) worldwide, including New Zealand. Like many gram-negative pathogens, *Psa* is expected to utilise type III effectors to promote virulence in host plants. In order to better understand *Psa* effector-triggered immunity and susceptibility, we aimed to investigate multiple molecular characteristics of *Psa* type III effectors and their recognition mechanisms in model plants, *Nicotiana* spp. and *Arabidopsis thaliana.*

Nicotiana tabacum and *N. benthamiana* are widely-used model plants for *Agrobacterium*-mediated transient expression (agroinfiltration) of effectors for functional characterization. Firstly, we screened multiple characteristics of effectors from two *Psa* strains, *Psa* NZ V13 and *Psa* NZ LV5. The former is a strongly virulent and the latter is a weakly virulent strain in kiwifruit. By using agroinfiltration in *Nicotiana* spp. to express individual effector proteins*,* we observed diverse subcellular localisation for *Psa* effectors. Additionally, we identified multiple *Psa* effectors that can trigger HR-like cell death (HCD) in both *N. tabacum* and *N. benthamiana.* Using virus-induced gene silencing (VIGS), we identified that some *Psa* effector-triggered HCD requires the immunity regulator *SGT1*, suggesting that the *Psa* effector-triggered HCD could be a result of immunity activation.

We focused on one *Psa* NZ V13 effector, HopZ5, which belongs to the YopJ-like acetyltransferase family. HopZ5 triggers hypersensitive response (HR) in *Arabidopsis* accession, Ct-1. Another Arabidopsis accession, Col-0, does not develop an HR but shows immunity in response to HopZ5. The gene that confers HopZ5-triggered HR in Ct-1 was identified as *SOBER1 (SUPPRESSOR OF AVRBST-ELICITED RESISTANCE 1)* by using recombinant inbred lines derived from two parental accessions, Ct-1 and Col-0. *SOBER1* is a known suppressor of *Xanthomonas* effector AvrBsT-triggered immunity. Interestingly, AvrBsT also belongs to YopJ family. Uniquely, SOBER1 specifically suppressed HCD triggered by several YopJ-like acetyltransferase effectors in *N. benthamiana*, including HopZ5 and HopZ3 from *Psa*. This suggests a common mechanism shared between a subset of YopJ-like acetyltransferase effectors is suppressed by SOBER1.

Finally, we identified one *Arabidopsis* accession, Ga-0, which carries a truncated *SOBER1* variant but does not develop an HR upon HopZ5 delivery. Using bulked- segregant analysis of an F_2 population derived from a cross between Ct-1 and Ga-0, we mapped the locus conferring HopZ5-recognition in Ct-1 to the upper arm of Chromosome 3.

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