Effector-triggered immunity against *Pseudomonas syringae* pv. *actinidiae* in nonhost plants

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Contents

Abstract

Pseudomonas syringae pv. *actinidiae* (*Psa*) is a virulent and highly damaging pathogen causing bacterial canker in all currently commercially important cultivars of kiwifruit (*Actinidia* spp.). Arabidopsis and *Nicotiana* spp. plants, however, are nonhosts to *Psa*. In our course of investigating the various nonhost resistance mechanisms in play against *Psa*, we identified several sources of resistance against several *Psa* strains as well as a possible novel virulence mechanism used by *Psa* and *Hyaloperonospora arabidopsidis* (*Hpa*), a biotrophic pathogen of Arabidopsis.

Firstly, we discovered that the highly virulent strain, *Psa* V13, triggers hypersensitive response (HR) in Arabidopsis in an accession-specific manner and that HopZ5$_{Psa\text{V13}}$, a member of the YopJ family of putative acetyltransferases, confers this bacterial avirulence. We also show that the immunity triggered by HopZ5 is independent from HR in the Arabidopsis accession Col-0. Through mutagenesis, we show that key amino acid residues predicted for acetyltransferase activity are vital to HopZ5-triggered immunity and HR, phenotypes reproduced in *Nicotiana* spp.

Secondly, we identified multiple sources of avirulence for the kiwifruit low-virulence strain, *Psa* LV5, in Arabidopsis and *Nicotiana benthamiana*, namely homologs of previously characterized effectors, HopAR1 and HopAB3, respectively. We additionally show that HopAB3 can trigger resistance in cultivated tomato putatively due to a novel recognition by a cultivated tomato homolog (*Sl*PtoB) of the resistance gene *Fen*.

Finally, we identified several nuclear-localized effectors from *Psa* and *Hpa* that interact with Arabidopsis WRKY transcription factors, different to WRKYs targeted by previously identified AvrRps4 and PopP2. We show that some WRKYs can trigger a cell death response in *N. benthamiana* when overexpressed and that coexpression of AvrRps4 or PopP2 is able to suppress this cell death response for the WRKYs they interact with. We show that this suppression is associated with suppression of transcriptional activation ability of the WRKY and

propose that this mechanism of transcription suppression may be utilized by other *Psa* and *Hpa* effectors identified in this study.

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Contributions to this thesis

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These include:

Amandine Spiandore – for assistance with cloning of *Psa* V13 effector modules described in Chapter 3 (Appendix 2).

Sera Choi – for cloning of *Psa* V13 effector modules described in Chapter 3 (Appendix 2), assembly of GoldenGate constructs of HopZ5 and its variants (Appendix 4), and production of results used for Figure 3.12 (VIGS) and Figure 3.15 (immunoblots).

Jun Zhou – for cloning of WRKY transcription factor modules from Arabidopsis in Chapter 5 (resulting in matierials in Appendix 6).

Without these significant contributions, this thesis would not have been able to achieve the level of results produced herein. All other work described in this thesis is mine alone conducted under the guidance of Prof. Kee Hoon Sohn or Prof. Cecile Segonzac.

Abbreviations

aa	Amino acids
ABA	Abscisic acid
Avr	Avirulence
bp	Base pair
BAK1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (a helper RLK)
BIC	Biotrophic interfacial complex
BIK1	BOTRYITIS INDUCED KINASE 1 (a cytoplasmic kinase)
BR	Brassinosteroid
CC	Coiled-coil (a domain in NB-LRRs)
cDNA	Complementary deoxyribonucleic acid
CDPK	Calcium-dependent protein kinase (Also abbreviated to CPK)
CEL	Conserved effector locus
CFU	Colony forming unit
CK	Cytokinin
CNL	Coiled-coil nucleotide-binding leucine-rich-repeat receptor (a class of NLR)
DNA	Deoxyribonucleic acid
dpi	Days post inoculation
DTT	dithiothreitol
EDS1	Enhanced disease susceptibility 1 (required for most TNLs)
EDTA	ethylenediamine tetraacetic acid
EFR	EF-Tu receptor (a sensor PRR/RLK)
EF-Tu	Elongation factor thermo unstable
elf18	EF Tu-derived epitope from *Escherichia coli*
ET	Ethylene
ETI	Effector-triggered immunity
EV	Empty vector
FLS2	Flagellin-sensitive 2 (a sensor PRR/RLK)
flg22	Flagellin-derived epitope from *Pseudomonas aeruginosa*
g	gram
GA	Gibberellic acid

h	hours
HCD	Hypersensitive response-like cell death
His	Histidine
Hpa	*Hyaloperonospora arabidopsidis*
hpi	Hours post infiltration
HR	Hypersensitive response
HSP90	Heat shock protein 90
ICE	Integrated conjugative element
JA	Jasmonic acid
kb	kilobase
kDa	kilodaltons
LPS	Lipopolysaccharide
LRR	Leucine rich repeat (domain common in PRRs and NB-LRRs)
Leu	Leucine
M	molar
MAPK	Mitogen-activated protein kinase
mg	milligram
min	minutes
mL	millilitre
mM	millimolar
NAC	NAM, ATAF, and CUC (stress-related plant transcription factor family)
NB-LRR	Nucelotide-binding leucine-rich-repeat receptor (intracellular)
NBS	Nucleotide binding site (domain of NB-LRR)
NLR	Nod-like receptors
NLS	Nuclear localization signal
NDR1	Nonrace-specific disease resistance 1 (required for many CNLs)
OD	Optical density of bacterial suspension with 600nm wavelength light
PAD4	Phytoalexin deficient 4
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PGN	Peptidoglycan
PPHGI	*Pseudomonas phaseolicola* genomic island (a type of ICE)
PR	Pathogenesis-related

PRR	Pattern recognition receptor (cell plasma membrane)
PTI	PAMP-triggered immunity
Pf	*Pseudomonas fluorescens*
Pgy	*Pseudomonas syringae* pv. *glycinea*
Pma	*Pseudomonas syringae* pv. *maculicola*
Pph	*Pseudomonas syringae* pv. *phaseolicola*
Psa	*Pseudomonas syringae* pv. *actinidiae*
Psy	*Pseudomonas syringae* pv. *syringae*
Pto	*Pseudomonas syringae* pv. *tomato*
qPCR	Quantitative polymerase chain reaction
RAC	Resistance to *Albugo candida* (NLRs for *A. candida* effectors)
RAR1	Required for Mla12 resistance
RBOHD	RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D
RIN4	RPM1-interacting 4
RLCK	Receptor-like cytoplasmic kinase (intracellular)
RLK	Receptor-like kinase (a class of PRR)
RLP	Receptor-like protein (a class of PRR)
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPP	Resistance to *Peronospora parasitica* (NLRs for *Hyaloperonospora arabidopsidis*)
RPM1	RESISTANCE TO *PSEUDOMONAS SYRINGAE* PV. *MACULICOLA* 1 – detects AvrRpm1
RPS2	RESISTANCE TO *PSEUDOMONAS SYRINGAE* 2 – NLR detects AvrRpt2
RPS4	RESISTANCE TO *PSEUDOMONAS SYRINGAE* 4 – NLR detects AvrRps4 (and PopP2)
RPS5	RESISTANCE TO *PSEUDOMONAS SYRINGAE* 5 – NLR detects AvrPphB (HopAR1)
RPS6	RESISTANCE TO *PSEUDOMONAS SYRINGAE* 6 – NLR detects HopPsyA (HopA1)
RRS1	RESISTANCE TO *RALSTONIA SOLANACEARUM* 1 – NLR detects PopP2 (and AvrRps4)
Rso	*Ralstonia solanacearum*

s	seconds
SA	Salicylic acid
SAG101	Senescence associated gene 101
SAR	Systemic acquired resistance
SDS	Sodium dodecyl sulphate
SGT1	Suppressor of G2 allele of *skp1* (required for most NLRs)
SID2	Salicylic acid induction deficient 2
SOBIR1	Suppressor of *bir1-1* (a helper RLK)
STR	Strigolactone
SUMM2	SUPPRESSOR OF MKK1 MKK2 2– NLR detects HopAI1
TAE	tris acetate EDTA
TAL	Transcriptional activator-like (effector)
TEMED	N,N,N',N'-teramethyl-ethylenediamine
TIR	Toll-interleukin-1 receptor (a domain in NB-LRRs)
TNL	Toll-interleukin-1 receptor nucleotide-binding leucine-rich-repeat receptor (a class of NLR)
Tris	tris(hydroxymethyl)aminomethane
Trp	Tryptophan
T3SS	Type-three secretion system
T3E	Type-three secreted effector (bacterial)
Xcv	*Xanthomonas campestris* pv. *vesicatoria*
ZAR1	HopZ activated resistance 1 – NLR detects HopZ1a
µg	microgram
µL	microlitre
µM	micromolar