Molecular analysis of plant innate immunity triggered by secreted effectors from bacterial and fungal pathogens of apple

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Abstract

In comparison to animals, plants do not have a dedicated immune system with mobile immune cells to protect themselves. Instead they rely on the innate immunity of each cell. Plant immunity branches into two classical layers: PTI (PAMP-triggered immunity) and ETI (Effector-triggered immunity). PTI detects the conserved molecular patterns (PAMPs) associated with pathogens and often can be overcome by pathogens translocating effector molecules into plant cells to inhibit the PTI. ETI, in turn, relies on intracellular receptors that can specifically recognize effectors or their activity and activate a rapid and robust response.

The research presented in this thesis is focused on two pathogens of apple plants: the bacterial pathogen *Erwinia amylovora* (the causal agent of fire blight) and fungal pathogen *Venturia inaequalis* (the causal agent of apple scab disease). As both bacterial and fungal pathogens deliver effector molecules in order to promote their virulence, ETI engineering is a promising universal strategy to control these pathogens.

In Chapter 3, the main aim was to elucidate the requirements and precise mechanism of how an important effector of *E. amylovora*, AvrRpt2, is recognized by the MR5 disease resistance (R) protein, derived from a hybrid apple *Malus* x *robusta* 5. I identified that a fragment of the guardee apple protein RIN4 was required and sufficient and required for MR5 activation. I further identified crucial amino acid residues responsible for this activation. Interestingly, cognate residues in RIN4 guardee homolog from *Arabidopsis thaliana* are responsible for suppression of the autoactivity of R protein RPS2. These findings led to the proposal of a novel hypothesis for evolutionary guardee adaption to the pool of R proteins present in plants.

In Chapter 4, the main focus was to apply newly acquired whole-genome sequencing data of *V. inaequalis* for identifying the previously mapped AvrRvi8 effector, as well as several novel effectors predicted *in silico*. The sequences of these effectors were validated by amplification and resequencing of candidate genes from *V. inaequalis* cDNA. Further functional analysis of the selected gene candidates was performed. In addition, a library of constructs for generating *V. inaequalis* knock-out strains was prepared for future work.

The findings from this thesis expected to be useful for breeders of apple to battle two economically important pathogens devastating the industry.

Deployment of the MR5 system in apples should facilitate fire blight resistance in pipfruit and offers the opportunity for further engineering of MR5 to detect other pathogens.

Furthermore, the effector library developed for *V. inaequalis* offers a novel tool for studying both virulence and avirulence mechanisms present in the apple-scab pathosystem. It is envisaged that further effector research will elucidate authentic targets critical for resistance development in apple.

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Table of contents

List of figures

List of tables

Abbreviations

aa	Amino acids
ABA	Abscisic acid
At	Arabidopsis thaliana
ABC	ATP-binding cassette
Avr	Avirulence
bp	Base pair
BAK1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1
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
CEBiP	CHITIN OLIGOSACCHARIDE ELICITOR BINDING PROTEIN
CIN1	Cellophane induced protein 1
CIN3	Cellophane induced protein 3
CK	Cytokinin
CNL	Coiled-coil nucleotide-binding leucine-rich-repeat receptor
CSP	Cold-shock protein
CWDE	Cell wall-degrading enzymes
DNA	Deoxyribonucleic acid
dpi	Days post inoculation
DTT	dithiothreitol
EDS1	Enhanced disease susceptibility 1
EDTA	ethylenediamine tetraacetic acid
EFR	EF-Tu receptor (a sensor PRR/RLK)
EF-Tu	Elongation factor thermo unstable
EIX	ETHYLENE-INDUCING XYLANASES
elf18	EF Tu-derived epitope from Escherichia coli
Ea	*Erwinia amylovora*
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
FOB	Free on board
GA	Gibberellic acid
h	hours
His	Histidine
Hpa	*Hyaloperonospora arabidopsidis*
hpi	Hours post infiltration
HR	Hypersensitive response
HSP90	Heat shock protein 90
IFP	Integrated fruit production
IP	Invasion pattern
IPR	Invasion pattern receptor
IPTR	Invasion pattern triggered response
JA	Jasmonic acid
kb	kilobase
kDa	kilodaltons
LPS	Lipopolysaccharide
LRD	leucine-rich domain
LRR	Leucine rich repeat
Leu	Leucine
M	molar
MAPK	Mitogen-activated protein kinase
Md	*Malus domestica*
mg	milligram
min	minutes
mL	millilitre
mM	millimolar
Mr5	*Malus* x *robusta* 5
NBS	Nucleotide binding site (domain of NB-LRR)
NLR	Nod-like receptors
NLS	Nuclear localization signal
NDR1	Nonrace-specific disease resistance 1
NOI	NO_3-induce domain

OD	Optical density of bacterial suspension with 600nm wavelength light
PAD4	Phytoalexin deficient 4
PAMP	Pathogen-associated molecular pattern
PDA	Potato dextrose agar
PCD	Programmed cell death
PCR	Polymerase chain reaction
PG	polygalacturonase
PGN	Peptidoglycan
PR	Pathogenesis-related
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
Pf	*Pseudomonas fluorescens*
Pp	*Pyrus pyrifolia*
Ps	*Pseudomonas syringae*
Pto	*Pseudomonas syringae pv. tomato*
Pu	*Pyrus ussuriensis*
qPCR	Quantitative polymerase chain reaction
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
RPS2	RESISTANCE TO PSEUDOMONAS SYRINGAE 2
RPS4	RESISTANCE TO PSEUDOMONAS SYRINGAE 4
RPS5	RESISTANCE TO PSEUDOMONAS SYRINGAE 5
RRS1	RESISTANCE TO RALSTONIA SOLANACEARUM 1
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)
TAE	tris acetate EDTA
TAL	Transcriptional activator-like (effector)
TCE	Tray equivalent (18 kg sale weight)
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
TEV	Tobacco etch virus
TIR	Toll-interleukin-1 receptor (a domain in NB-LRRs)
TNL	Toll-interleukin-1 receptor nucleotide-binding leucine-rich-repeat receptor
Tris	tris(hydroxymethyl)aminomethane
Trp	Tryptophan
T1SS	Type I secretion system
T3SS	Type-three secretion system
T3E	Type-three secreted effector (bacterial)
Vi	*Venturia inaequalis*
ViCE	*Venturia inaequalis* candidate effector
µL	microlitre
µM	micromolar