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

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)
in Plant Science

Institute of Agriculture and Environment
Massey University, New Zealand.

Maxim Prokchorchik

December 2017

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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.
Acknowledgments

I would like to thank my supervisor, Prof. Kee Hoon Sohn, for his support, scientific guidance and overall help while undertaking my PhD. His excellent knowledge of the field and creativity always served as a beacon, helping me navigate a sea of data and failed experiments. His support and enthusiasm encouraged me to never give up or stop the journey of my PhD. I also want to thank Dr. Cecile Segonzac for the invaluable guidance in the lab, as well as in the world of Western Blotting and CoIPs.

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>aa</td>
<td>Amino acids</td>
</tr>
<tr>
<td>ABA</td>
<td>Abscisic acid</td>
</tr>
<tr>
<td>At</td>
<td>Arabidopsis thaliana</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>Avr</td>
<td>Avirulence</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BAK1</td>
<td>BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1</td>
</tr>
<tr>
<td>BIC</td>
<td>Biotrophic interfacial complex</td>
</tr>
<tr>
<td>BIK1</td>
<td>BOTRYTIS INDUCED KINASE 1 (a cytoplasmic kinase)</td>
</tr>
<tr>
<td>BR</td>
<td>Brassinosteroid</td>
</tr>
<tr>
<td>CC</td>
<td>Coiled-coil (a domain in NB-LRRs)</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CEBiP</td>
<td>CHITIN OLIGOSACCHARIDE ELICITOR BINDING PROTEIN</td>
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</tr>
<tr>
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<td>CK</td>
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<td>CNL</td>
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<td>ETHYLENE-INDUCING Xylanases</td>
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<tr>
<td>ET</td>
<td>Ethylene</td>
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<tr>
<td>ETI</td>
<td>Effector-triggered immunity</td>
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<td>Empty vector</td>
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<td>Abbreviation</td>
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<td>FLS2</td>
<td>Flagellin-sensitive 2 (a sensor PRR/RLK)</td>
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<td>molar</td>
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<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
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<td>mg</td>
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<td><em>Malus x robusta</em> 5</td>
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</tr>
<tr>
<td>NLS</td>
<td>Nuclear localization signal</td>
</tr>
<tr>
<td>NDR1</td>
<td>Nonrace-specific disease resistance 1</td>
</tr>
<tr>
<td>NOI</td>
<td>NO_3-induce domain</td>
</tr>
</tbody>
</table>
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  
\( \mu \text{L} \) microlitre  
\( \mu \text{M} \) micromolar