

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**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**

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

## 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 guard cell 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 guard cell 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 guard cell adaptation 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 are 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.

I am very grateful to Dr. Janet Reid, Dr. Vincent Bus and Dr. David Chagne for their guidance, insightful discussions and support. I want to thank Dr. Rosie Bradshaw and Dr. Joanna Bowen for the enormous help with thesis preparation and scientific advice. I enjoyed my time doing research and having fun weekends with the "PhD awesome foursome" of KSL lab, including Jay, Sera and Toby. I also want to thank members of KSL in Korea, including Jeongmin, Hayoung and Haseong for their help to get used to a completely different country.

I am thankful to my parents for their endless support and understanding during my ups and downs. Even from far away they made me feel that they are very close. Finally, I would like to thank my partner Sera, who coped with me through the long course of this PhD, listening to my endless ideas during the good times and my limitless moaning during the bad.

## Table of contents

<b>Abstract</b> .....	<b>2</b>
<b>Acknowledgments</b> .....	<b>4</b>
<b>Table of contents</b> .....	<b>5</b>
<b>List of figures</b> .....	<b>10</b>
<b>List of tables</b> .....	<b>14</b>
<b>Abbreviations</b> .....	<b>16</b>
<b>Chapter 1. General introduction</b> .....	<b>20</b>
<b>1.1 Apple industry in New Zealand</b> .....	<b>20</b>
<b>1.2 <i>Venturia inaequalis</i> is the causal agent of apple scab disease</b> .....	<b>21</b>
<b>1.3 <i>Erwinia amylovora</i> is the causal agent of apple fire blight disease</b> .....	<b>26</b>
<b>1.4 Plant immunity general overview</b> .....	<b>32</b>
<b>1.5 PAMP-triggered immunity</b> .....	<b>35</b>
1.5.1 PAMP recognizing receptors .....	36
1.5.2 Signaling during PTI .....	37
<b>1.6 Effectors of plant pathogens</b> .....	<b>38</b>
1.6.1 Effector delivery from bacterial pathogens .....	39
1.6.2 Effector delivery from oomycete and fungal pathogens.....	43
<b>1.7 Effectors and their recognition</b> .....	<b>46</b>
1.7.1 Effectors suppressing PTI.....	48
1.7.2 Effectors manipulating plant phytohormones.....	50
1.7.3 Effectors targeting gene expression machinery.....	52
1.7.4 Effectors interfering with the plant cell cytoskeleton.....	54
<b>1.8 Effector triggered immunity in plants and R proteins</b> .....	<b>55</b>
1.8.1 NB-LRRs.....	56
1.8.2 Cytoplasmic serine/threonine kinases.....	60
1.8.3 Downstream signaling during ETI.....	60
<b>1.9 Direct and indirect recognition of effectors</b> .....	<b>61</b>
1.9.1 Direct effector recognition .....	61
1.9.2 Indirect effector recognition and guard-guardee/decoy hypothesis ....	62
1.9.3 Integrated decoy as a strategy to trap effectors .....	66

<b>1.10 Aims of this study</b> .....	<b>67</b>
<b>Chapter 2. Materials and Methods</b> .....	<b>70</b>
<b>2.1 Materials</b> .....	<b>70</b>
2.1.1 Bacterial strains used in this study:.....	70
2.1.2 Plasmids and constructs used in this study:.....	70
2.1.3 Plant material .....	99
2.1.4 Bacterial & Plant Media.....	101
2.1.5 Antibiotics .....	102
<b>2.2 Microbiology methods</b> .....	<b>102</b>
2.2.1 Bacterial conjugation .....	102
2.2.2 Competent cell preparation & transformation.....	103
2.2.3 Glycerol stocks .....	103
<b>2.3 Plant Methods</b> .....	<b>104</b>
2.3.1 Hypersensitive response assays in <i>Arabidopsis thaliana</i> .....	104
2.3.2 <i>Agrobacterium tumefaciens</i> infiltration for transient protein expression .....	104
2.3.3 <i>N. benthamiana</i> ion leakage assay .....	104
2.3.4 Arabidopsis stable transformation.....	105
2.3.5 Arabidopsis crossing.....	106
<b>2.4 Molecular Biology Methods</b> .....	<b>106</b>
2.4.1 Enzymes used in this study.....	106
2.4.2 Bacterial genomic DNA extraction methods.....	106
2.4.3 Chelex plant genomic DNA extraction .....	106
2.4.4 Plant genomic DNA extraction methods.....	107
2.4.5 Polymerase chain reaction .....	107
2.4.6 Nested PCR.....	107
2.4.7 Colony PCR.....	108
2.4.8 Agarose gel electrophoresis.....	108
2.4.9 Agarose gel purification of DNA.....	109
2.4.10 Blunt-end <i>Sma</i> I/T4 cloning.....	109
2.4.11 Golden gate cloning.....	109
2.4.12 Plasmid DNA purification.....	111
2.4.13 Alkaline Lysis Miniprep.....	112
2.4.14 Site-directed mutagenesis.....	112

2.4.15 DNA sequencing.....	113
2.4.16 RNA extraction .....	113
2.4.17 Reverse transcription PCR (RT-PCR) .....	114
2.4.18 Semi-quantitative PCR.....	114
2.4.19 Total protein extraction.....	114
2.4.20 SDS-PAGE & Western blot.....	115
2.4.21 CoIP.....	116
<b>Chapter 3: molecular basis of AvrRpt2 recognition by the NLR MR5 from the hybrid apple <i>Malus x robusta</i> 5.....</b>	<b>117</b>
<b>3.1 Introduction .....</b>	<b>117</b>
3.1.1 AvrRpt2 homologs are important for successful plant infection.....	117
3.1.2 AvrRpt2 recognition in Arabidopsis.....	118
3.1.3 MR5 is a CC-type NLR conferring resistance to fire blight.....	120
<b>3.2 Results.....</b>	<b>121</b>
3.2.1 Transiently expressed AvrRpt2 homologs induce moderate cell death in <i>Nicotiana benthamiana</i> leaves .....	121
3.2.2 AvrRpt2 activates MR5 by elimination of RIN4 .....	122
3.2.3 AvrRpt2-mediated elimination of RIN4 homologs from apple closely-related species can activate MR5.....	124
3.2.4 EaAvrRpt2 <sup>C156S</sup> does not lose catalytic activity and can be still recognized by MR5 and RPS2.....	124
3.2.5 Mutation analysis of MR5 critical domains .....	127
3.2.6 RIN4 natural variants have differing abilities to suppress or activate NLRs.....	129
3.2.7 The presence of MdrIN4 CLV3 is necessary and sufficient to elicit MR5-mediated cell death .....	134
3.2.8 Only fully intact version of MR5 can be activated by MdrIN4 CLV3 ...	135
3.2.9 MR5 domain combinations cannot be activated by MdrIN4 CLV3 .....	137
3.2.10 Two amino acid residues in a highly conserved part of MdrIN4 CLV3 are critical for MR5 activation.....	138
3.2.11 MR5 is not able to recognize RIN4 phosphorylation by AvrRpm1 or AvrB .....	141
3.2.12 Polymorphic residues alter the suppression and activation properties of full length RIN4 .....	142



3.2.13 Polymorphic residues alter the suppression and activation properties of RIN4 CLV3.....	144
3.2.14 Polymorphic residues alter interaction of MdrIN4 with RPS2 and MR5 .....	146
<b>3.3 Discussion .....</b>	<b>148</b>
3.3.1 Cysteine to serine substitution in position 156 in <i>E. amylovora</i> AvrRpt2 does not alter its RIN4 cleavage ability but might interfere with its delivery .....	148
3.3.2 MR5 uses activation rather than de-repression to trigger immune responses upon recognition of AvrRpt2 .....	148
3.3.3 Two polymorphic residues in a conserved region of CLV3 dramatically alter RIN4 properties.....	151
<b>Chapter 4. Validating and characterization of prospective <i>Venturia inaequalis</i> effectors .....</b>	<b>153</b>
<b>4.1 Introduction .....</b>	<b>153</b>
4.1.1 <i>Venturia inaequalis</i> is the causal agent of apple scab disease .....	153
4.1.2 The <i>Venturia inaequalis</i> and <i>Malus</i> pathosystem .....	153
4.1.3 Relationship (8) of <i>V. inaequalis</i> NZ188B.2 and <i>M. sieversii</i> W193B....	162
<b>4.2 Results.....</b>	<b>165</b>
4.2.1 Cloning of AvrRvi8 effector candidate genes.....	165
4.2.2 Functional validation of AvrRvi8 gene candidates <i>in planta</i> .....	166
4.2.3 <i>Pseudomonas fluorescens</i> mediated delivery of AvrRvi8-7 into apple leaves.....	167
4.2.4 Validating <i>Venturia inaequalis</i> 120 Candidate Effectors.....	168
<b>4.3 Discussion .....</b>	<b>172</b>
4.3.1 Sequence and functional validation of <i>V. inaequalis</i> AvrRvi8.....	172
4.3.1 Sequence and functional validation of novel <i>in silico</i> predicted <i>V. inaequalis</i> effector candidates.....	173
<b>Chapter 5. General conclusions and Future directions .....</b>	<b>175</b>
<b>5.1 General conclusions from a detailed study of MR5-RIN4 system.....</b>	<b>176</b>
<b>5.2 Evidence supporting co-evolution of guarded proteins with their cognate NLRs.....</b>	<b>177</b>
<b>5.3 Significance and future directions of the MR5-RIN4 system research .....</b>	<b>180</b>

<b>5.4 General conclusions from <i>V. inaequalis</i> prospective effector genes research.....</b>	<b>181</b>
<b>5.5 Significance and future directions of the <i>V. inaequalis</i> prospective effector genes research.....</b>	<b>182</b>
<b>References .....</b>	<b>185</b>
<b>Appendices .....</b>	<b>217</b>

## List of figures

Figure 1.1: Apple scab disease on apple fruits and apple leaves.....	22
Figure 1.2: <i>Venturia inaequalis</i> life cycle.....	24
Figure 1.3: Fire blight symptoms in apple caused by <i>Erwinia amylovora</i> on blossoms, fruits and shoots. ....	27
Figure 1.4: Fire blight disease cycle.....	29
Figure 1.5: Schematic representation of plant immunity.....	33
Figure 1.6: Pattern recognition receptors (PRRs) recognize multiple PAMPs from bacteria .....	35
Figure 1.7: Bacterial pathogens deliver effectors through type 3 secretion system (T3SS).....	42
Figure 1.8: Fungal and oomycete structures for effector secretion.....	45
Figure 1.9: Schematic representation of important domains in NOD-Like Receptors (NLRs).....	57
Figure 1.10: Current understanding of NLR activation. ....	59
Figure 1.11: Recognition of AvrB and AvrRpm1 or AvrRpt2 effectors by RPM1 and RPS2 NLRs respectively. ....	64
Figure 1.12: Recognition of AvrPphB effector by RPS5.....	65
Figure 1.13: Recognition of PopP2 effector from <i>Ralstonia solanacearum</i> by RPS4-RRS1 complex.....	66
Figure 2.1. Schematic representation of Golden Gate cloning system. ....	111
Figure 3.1: Phylogenetic analysis of AvrRpt2 homologs.....	118
Figure 3.2: Model of RPS2 activation via RIN4 cleavage by AvrRpt2.....	119
Figure 3.3: Agrobacterium mediated overexpression of <i>P. syringae</i> and <i>E. amylovora</i> AvrRpt2 variants in <i>N. benthamiana</i> .....	121
Figure 3.4: AvrRpt2-directed cleavage of MdrIN4 is recognized by both RPS2 and MR5.....	123
Figure 3.5: RIN4 homologs from <i>Pyrus</i> species can activate MR5 in presence of EaAvrRpt2.....	124
Figure 3.6: EaAvrRpt2 <sup>C156S</sup> can be recognized by RPS2 and MR5 systems. ....	126

<b>Figure 3.7: Expression of MR5 variants with mutations in critical domains.</b>	<b>128</b>
<b>Figure 3.8: RIN4 natural variants have different abilities to suppress autoactive NLRs.</b>	<b>130</b>
<b>Figure 3.9: Different regions of RIN4 are required for suppression and activation of NLRs.</b>	<b>133</b>
<b>Figure 3.10: Cleavage at MdrIN4 RCS2 is required for MR5 activation.</b>	<b>134</b>
<b>Figure 3.11: MdrIN4 CLV3 can activate MR5 even in absence of AvrRpt2.</b>	<b>135</b>
<b>Figure 3.12: Only fully intact MR5 protein can initiate signaling when co-expressed with MdrIN4 CLV3.</b>	<b>136</b>
<b>Figure 3.13: Co-expression of separate domains of MR5 cannot recapitulate PCD-triggering activity in the presence of MdrIN4 CLV3.</b>	<b>137</b>
<b>Figure 3.14: Two amino acid residues in the conserved C-NOI domain of CLV3 are critical for NLR compatibility.</b>	<b>140</b>
<b>Figure 3.15: MR5 cannot activate PCD in presence of AvrRpm1 and AvrB or RIN4 phosphorylation mimic.</b>	<b>141</b>
<b>Figure 3.16: Two amino acid residues in the conserved C-NOI domain of CLV3 are critical for NLR compatibility with full length RIN4 variants.</b>	<b>143</b>
<b>Figure 3.17: MdrIN4 CLV3 carrying D186N and F193Y mutations suppresses NLR autoactivity.</b>	<b>145</b>
<b>Figure 3.18: An MdrIN4 variant showing enhanced suppression of RPS2 autoactivity forms a stronger association with RPS2.</b>	<b>147</b>
<b>Figure 3.19: Comparison of well-studied RPS2-mediated AvrRpt2 recognition and proposed model of MR5-mediated AvrRpt2 recognition.</b>	<b>150</b>
<b>Figure 4.1: Scab resistance reactions on apple leaves.</b>	<b>154</b>
<b>Figure 4.2: Global positions of the 17 <i>Rvi</i> scab resistance genes identified in the apple genome to date.</b>	<b>162</b>
<b>Figure 4.3: AvrRvi8-7 overexpression in <i>N. benthamiana</i> and <i>N. tabacum</i> does not trigger a macroscopic response.</b>	<b>167</b>
<b>Figure 4.4: AvrRvi8-7 delivery via <i>P. fluorescens</i> T3SS does not trigger a macroscopic response in susceptible or resistant apple plants.</b>	<b>168</b>

Figure 4.5: <i>V. inaequalis</i> candidate effectors do not trigger any macroscopic response when expressed in <i>N. benthamiana</i> leaves. ....	170
Figure 4.6: <i>V. inaequalis</i> candidate effectors are able to attenuate RPS2 and HopAS1-mediated response when expressed in <i>N. benthamiana</i> leaves. ....	171
Figure 6.1: Protein alignment of AvrRpt2 effector from <i>Pseudomonas syringae</i> JL1506 and <i>Erwinia amylovora</i> Fb12-027. ....	222
Figure 6.2: Protein alignment of <i>Malus domestica</i> RIN4-1 and RIN4-2 homologs.....	223
Figure 6.3: Nucleotide alignment of <i>Venturia inaequalis</i> AvrRvi8-1 and AvrRvi8-2 candidates predicted <i>in silico</i> with the sequencing results of amplification products from cDNA. ....	224
Figure 6.4: Nucleotide alignment of <i>Venturia inaequalis</i> AvrRvi8-6 and AvrRvi8-8 candidates predicted <i>in silico</i> with the sequencing results of amplification products from cDNA. ....	225
Figure 6.5: Nucleotide alignment of <i>Venturia inaequalis</i> AvrRvi8-candidate predicted <i>in silico</i> with the sequencing results of amplification products from cDNA.....	226
Figure 6.6: Schematic representation of intron mis-annotations and early STOP-codons in nucleotide alignments of <i>Venturia inaequalis</i> AvrRvi8-10 and AvrRvi8-11 candidates predicted <i>in silico</i> with the sequencing results of amplification products from cDNA. ....	227
Figure 6.7: Schematic representation of intron mis-annotations STOP-codon variations in nucleotide alignments of <i>Venturia inaequalis</i> ViCE5, 7 and 8 genes predicted <i>in silico</i> with the sequencing results of amplification products from cDNA.....	228
Figure 6.8: Plasmid map of pICH41021 .....	229
Figure 6.9: Plasmid map of pICH86966 .....	230
Figure 6.10: Plasmid map of pICH86988.....	231
Figure 6.11: Plasmid map of EpiGreenB5::Spec::GG.....	232
Figure 6.12: Plasmid map of pAGM4723 .....	233
Figure 6.13: Plasmid map of pBBR1MCS-5 with AvrRps4 promoter and fragment coding for AvrRps4 secretion signal.....	234

**Figure 6.14: Plasmid map of pBBR1MCS-5 with AvrRpm1 promoter and fragment coding for AvrRpm1 secretion signal ..... 235**

## List of tables

Table 1.1: New Zealand apple fresh fruit industry overview and dynamics. .....	20
Table 1.2: Relative susceptibility of common apple and pear cultivars to fire blight.....	31
Table 2.1 Standard constructs and vectors for Golden Gate cloning.....	72
Table 2.2. Constructs generated for <i>Venturia inaequalis</i> 120 <i>AvrRvi8</i> candidates cloning.....	75
Table 2.3. Constructs used for <i>Venturia inaequalis</i> 120 <i>AvrRvi8</i> transient protein expression and T3SS delivery.....	76
to plant cells. ....	76
Table 2.4. Constructs used for bacterial effector cloning. ....	77
Table 2.5. Constructs used for bacterial effector T3SS delivery and transient expression: .....	78
Table 2.6. Constructs used for plant genes cloning. ....	80
Table 2.7. Constructs used for plant protein transient expression and stable transformation. ....	84
Table 2.8. Constructs used for <i>Venturia inaequalis</i> predicted candidate effectors (ViCE) cloning. ....	94
Table 2.9. Constructs used for <i>Venturia inaequalis</i> predicted candidate effectors (ViCE) transient expression.....	95
without signal peptide.....	95
Table 2.10. Constructs used for <i>Venturia inaequalis</i> predicted candidate effectors (ViCE) transient expression fused.....	97
with PR1 $\alpha$ signal peptide.....	97
Table 2.11. <i>Arabidopsis thaliana</i> genotypes used in this study. ....	99
Table 2.12. <i>Arabidopsis thaliana</i> genotypes generated during this study...	99
Table 4.1: Representation of gene-for-gene relationships between <i>Venturia</i> <i>inaequalis</i> and <i>Malus</i> . ....	156
Table 4.2. Sequence analysis and validation of 12 <i>in silico</i> predicted <i>AvrRvi8</i> gene candidates.....	166

<b>Table 4.3. Sequence validation of <i>V. inaequalis</i> candidate effectors (ViCE).</b>	<b>169</b>
<b>Table 6.1: Primers used in this study for Golden Gate module generation:</b>	<b>217</b>
<b>Table 6.2: Primers used in this study for site-directed mutagenesis:</b>	<b>220</b>



## Abbreviations

aa	Amino acids
ABA	Abscisic acid
<i>At</i>	<i>Arabidopsis thaliana</i>
ABC	ATP-binding cassette
Avr	Avirulence
bp	Base pair
BAK1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1
BIC	Biotrophic interfacial complex
BIK1	BOTRYTIS 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 <i>Escherichia coli</i>
<i>Ea</i>	<i>Erwinia amylovora</i>
ET	Ethylene
ETI	Effector-triggered immunity
EV	Empty vector

FLS2	Flagellin-sensitive 2 (a sensor PRR/RLK)
flg22	Flagellin-derived epitope from <i>Pseudomonas aeruginosa</i> g gram
FOB	Free on board
GA	Gibberellic acid
h	hours
His	Histidine
<i>Hpa</i>	<i>Hyaloperonospora arabidopsidis</i>
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
<i>Md</i>	<i>Malus domestica</i>
mg	milligram
min	minutes
mL	millilitre
mM	millimolar
<i>Mr5</i>	<i>Malus x robusta</i> 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 <sub>3</sub> -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
<i>Pf</i>	<i>Pseudomonas fluorescens</i>
<i>Pp</i>	<i>Pyrus pyrifolia</i>
<i>Ps</i>	<i>Pseudomonas syringae</i>
<i>Pto</i>	<i>Pseudomonas syringae pv. tomato</i>
<i>Pu</i>	<i>Pyrus ussuriensis</i>
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 <i>Peronospora parasitica</i> (NLRs for <i>Hyaloperonospora arabidopsidis</i> )
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 <i>skp1</i> (required for most NLRs)
SID2	Salicylic acid induction deficient 2
SOBIR1	Suppressor of <i>bir1-1</i> (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)
<i>Vi</i>	<i>Venturia inaequalis</i>
ViCE	<i>Venturia inaequalis</i> candidate effector
μL	microlitre
μM	micromolar