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.
Identification and characterization of *Dothistroma septosporum* effectors

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Genetics

Yanan Guo

2015
Abstract

*Dothistroma septosporum* is the main causal agent of Dothistroma needle blight of pines. However little is known about mechanisms of pine resistance against *D. septosporum*, or whether there is any classical gene-for-gene resistance involved. The molecular basis of how fungal effector proteins can trigger plant host resistance in a gene-for-gene manner was determined partly by work with the model fungus *Cladosporium fulvum* and its tomato host. Comparative genome analysis of *C. fulvum* and *D. septosporum* genomes identified nine putative effector genes (*DsAvr4, DsEcp2-1, DsEcp2-2, DsEcp2-3, DsEcp4, DsEcp5, DsEcp6, DsEcp13* and *DsEcp14*) in *D. septosporum* that are homologous to well-characterized *C. fulvum* effector genes. Other effector candidates were identified as small cysteine rich proteins that are highly expressed *in planta*, including *DsHdp1* which is a hydrophobin gene and *Ds69335* which belongs to the sperm-coating protein-like extracellular protein SCP/Tpx-1/Ag5/PR-1/Sc7 (SCP/TAPS) superfamily.

Transcriptome analysis showed that, except for *DsEcp2-1* and *DsEcp6*, the *in planta* expression of *D. septosporum* effectors was low. Targeted gene replacement of *DsAvr4, DsEcp2-1, DsEcp6* and *DsHdp1* caused no observed changes in fungal physiology *in vitro* compared to wild type (WT) and also showed that *DsAvr4, DsEcp6* and *DsHdp1* are not virulence factors when infecting *Pinus radiata*. However deletion of *DsEcp2-1* caused larger lesions compared to WT, suggesting that *DsEcp2-1* may act to suppress a host target which is involved in necrosis induction during the biotrophic infection stage.

A domain swap experiment in this study showed that swapping the region between cysteine residues C6 (Cys102) to C7 (Cys114), which contains the chitin binding domain, caused loss of resistance (R) protein Cf-4 recognition of *DsAvr4* (with *CbAvr4*) and gain of Cf-4 recognition of *CbAv4* (with *DsAvr4* or *CfAvr4*). Further experiments carried out in Wageningen University showed that a Pro residue located in the chitin binding domain in *DsAvr4* is important for Cf-4 recognition, and may have a role in *DsAvr4* stability. In this study, effector candidates *DsEcp2-1* and *DsEcp2-3* were able to trigger a non-host necrotic response in *N. tabacum* suggesting possible interaction with a *N. tabacum* protein. Polymorphism analysis showed that *DsEcp4* and *DsEcp5* have internal stop codons and encode pseudogenes in all the *D. septosporum* strains tested,
except for DsEcp4 in strains from Guatemala and Columbia in which a functional gene is predicted. DsEcp4 and DsEcp5 are the only D. septosporum effectors tested that showed evidence of positive selection. Those results lead to the suggestion that R proteins that recognise DsEcp4 and DsEcp5 may be present in pine species. DsEcp13 appears to be absent from ten D. septosporum strains, suggesting that DsEcp13 is not important for virulence and can also be deleted to avoid an R protein mediated defence response such as a hypersensitive response. Infiltration of DsAvr4, DsEcp2-1 and DsEcp6 P. pastoris expression culture filtrates triggered necrosis in P. radiata needles suggesting that R proteins that directly or indirectly recognise those effectors may also be present in P. radiata.

The finding that D. septosporum has homologues of C. fulvum effectors allowed the first study of molecular pathogen-host interactions in this pathosystem. Targeted gene replacement studies identified genes that may have a virulence function and resistance against these effectors may be durable in the field. The pine needle infiltration assay provides a basic screening method to identify pine genotypes that carry resistance proteins and future work in this area is expected to impact on breeding strategies in the forest industry.
Acknowledgements

I would like to gratefully thank my supervisor Dr Rosie Bradshaw for her continuous encouragement, guidance and support during the course of my PhD. Thanks for your kindness and patience when I have questions. You are a great supervisor and I could not have imagined having a better advisor and mentor for my PhD study. I would also like to express my gratitude to my co-supervisors Dr Rebecca Ganley and Dr Kee Sohn for their valuable advice and support.

To my lab colleagues, thanks for Carol for her technical support and advice, you are always there to help whenever I knock on your door. Thanks for Pranav for his willingness to listen and sharing his knowledge with me. Thanks for Kabir, Yanfei, Kutay, Simren and Lukas for technical assistance and advice. It is a pleasure to work with all of you.

Most important I would like to thank my parents, although it is far from home, you have always been so supportive, encouraging and understanding over the duration of my studies. To my loving husband Xiaoxiao, thanks for believing in me, without your support I would not have achieved my goals.

Finally I would like to thank Scion for providing me with a fellowship for three and half years to pursue my PhD studies in New Zealand. Thanks to Massey University for providing six month financial support at the end of my PhD and travel fund.
Table of contents

Abstract .............................................................................................................................. i

Acknowledgements .......................................................................................................... iii

Table of contents .............................................................................................................. iv

List of Figures .................................................................................................................. xi

List of Tables ................................................................................................................... xiii

Abbreviations .................................................................................................................. xiv

Chapter 1. Introduction ..................................................................................................... 1

1.1 Dothistroma septosporum: A foliar pathogen that causes Dothistroma needle blight in Pinus spp...1

1.1.1 Dothistroma needle blight ....................................................................................... 1

1.1.2 Causal organisms Dothistroma septosporum and Dothistroma pini ......................... 2

1.1.3 Dothistroma septosporum is a hemibiotrophic fungus ........................................ 3

1.2 Plant Immunity .......................................................................................................... 3

1.2.1 Plant Immunity system ........................................................................................... 3

1.2.1.1 A two layered innate immune system ............................................................... 3

1.2.2 Resistance Protein .................................................................................................. 5

1.2.2.1 Molecular models of pathogen recognition ....................................................... 5

1.2.2.2 Structure and function of resistance proteins ..................................................... 7

1.2.2.3 Resistance proteins in Pinus spp. ..................................................................... 9

1.3 Effectors ................................................................................................................... 10

1.3.1 Functions of effectors ........................................................................................... 10

1.3.1.1 Apoplastic effectors ........................................................................................... 10

1.3.1.2 Cytoplasmic effectors ....................................................................................... 12

1.3.1.3 Necrotrophic effectors ....................................................................................... 13

1.3.1.4 Other types of virulence factors ........................................................................ 14

1.3.2 Translocation of effectors ...................................................................................... 14

1.3.2.1 Bacterial type III secretion system .................................................................... 14

1.3.2.2 Biotrophic interfacial complex ......................................................................... 15

1.3.2.3 Endocytosis mediated entry ............................................................................. 15

1.4 Evolutionary arms race between tomato resistance proteins and C. fulvum effectors 16
1.4.1 Tomato Cf resistance protein variation ................................................................. 17
1.4.2 Tomato guardee protein variation ........................................................................ 18
1.4.3 *C. fulvum* effector variation ............................................................................... 19
1.5 Hypothesis, aims and objectives ............................................................................. 19

Chapter 2. Materials and Methods ............................................................................. 23
2.1 Biological material ................................................................................................. 23
2.1.1 Fungal strains .................................................................................................... 23
2.1.2 *Escherichia coli* strains .................................................................................. 24
2.1.3 *Agrobacterium tumefaciens* strain ................................................................... 25
2.1.4 *Pichia pastoris* strain ...................................................................................... 25
2.1.5 Plant material ................................................................................................... 25
2.2 Growth and maintenance of cultures .................................................................... 25
2.2.1 Growth and maintenance of bacterial cultures .................................................. 25
2.2.2 Growth, maintenance and harvest of *D. septosporum* culture and mycelia ...... 26
2.2.3 Growth and maintenance of *Pichia pastoris* cultures ....................................... 26
2.3 DNA extraction, quantification and analysis ......................................................... 27
2.3.1 Genomic DNA isolation from *D. septosporum* by CTAB method .................... 27
2.3.2 Isolation of plasmid DNA ................................................................................ 27
2.3.3 Nucleic acid quantification .............................................................................. 28
2.3.4 Agarose gel electrophoresis ............................................................................. 28
2.3.5 Agarose gel purification of DNA .................................................................... 28
2.3.6 Restriction endonuclease digestion of DNA ..................................................... 29
2.3.7 Ligation reaction .............................................................................................. 29
2.3.8 Vector construction .......................................................................................... 29
2.3.8.1 Vector construction by restriction endonuclease digestion and ligation ........ 29
2.3.8.2 Gateway three-fragment construction of gene replacement vector .............. 30
2.3.9 Transformation of *E. coli* .............................................................................. 32
2.3.9.1 Making competent *E. coli* cells ............................................................... 32
2.3.9.2 Transformation of *E. coli* by electroporation ............................................ 32
2.3.10 DNA sequencing ........................................................................................... 33
2.4 Polymerase chain reaction .................................................................................... 33
2.4.1 Standard PCR reactions .................................................................................. 33
2.4.2 *E. coli* and *A. tumefaciens* colony PCR ....................................................... 34
2.4.3 P. pastoris colony PCR ................................................................. 34
2.4.4 Overlapping PCR to construct DsAvr4 domain swap plasmids .......... 34
2.5 Quantitative real time PCR (qPCR) .................................................. 36
2.5.1 PCR conditions ........................................................................... 36
2.5.2 Gene expression analyses in gene replacement strains ................. 36
2.5.3 Copy number determination in complemented strains .................. 37
2.6 RNA extraction and manipulation .................................................. 37
2.6.1 RNA extraction and DNase treatment ......................................... 37
2.6.2 Formaldehyde gel electrophoresis ................................................. 38
2.6.3 DNA removal and cDNA synthesis .............................................. 39
2.7 Southern blotting and hybridization .............................................. 39
2.7.1 DIG-labelling of the probes (PCR based) .................................... 39
2.7.2 Probe concentration determination .............................................. 39
2.7.3 Southern blot ............................................................................... 39
2.7.4 Hybridization of DIG labelled probe .......................................... 40
2.7.5 Immunological detection ............................................................. 40
2.7.6 Stripping the blot ........................................................................ 41
2.8 Transformation of D. septosporum .................................................. 41
2.8.1 Preparation of protoplasts ............................................................ 41
2.8.2 Transformation of D. septosporum .............................................. 42
2.8.3 Screen for positive transformants ................................................. 42
2.8.4 Purification of the positive candidates ........................................ 42
2.9 Phenotypic characterization and pathogenicity assay of D. septosporum mutants .......... 43
2.9.1 Radial growth rate ..................................................................... 43
2.9.2 Sporulation assay ....................................................................... 43
2.9.3 Spore germination rate ............................................................... 43
2.9.4 Hydrophobicity assay ................................................................. 43
2.9.5 Spore adhesion test ................................................................... 44
2.9.6 Pathogenicity assay .................................................................. 44
2.10 Agro-infiltration on Nicotiana tabacum Plants ................................. 44
2.10.1 Transformation of A. tumefaciens .............................................. 44
2.10.1.1 Make chemically competent A. tumefaciens cells .................. 44
2.10.1.2 Transformation of A. tumefaciens ......................................... 45
2.10.2 Agro-infiltration on Nicotiana tabacum Plants ........................... 45
Chapter 2. Heterologous production of *D. septosporum* effector protein by *P. pastoris* ........................................... 46

2.11 Transformation of *P. pastoris* ................................................................................................. 46

2.11.1 Making competent *P. pastoris* cells .................................................................................. 46

2.11.2 Expression of recombinant proteins in *P. pastoris* strain .............................................. 47

2.12 Western blotting ....................................................................................................................... 48

2.12.1 SDS-polyacrylamide gel electrophoresis .......................................................................... 48

2.12.2 Western blot ....................................................................................................................... 48

2.13 Transcriptome analysis ............................................................................................................ 50

Chapter 3. Putative *Cladosporium fulvum* homologous effectors in the New Zealand strain of *Dothistroma septosporum* .......................................................................................... 53

3.1 Introduction .................................................................................................................................. 53

3.2 Results ....................................................................................................................................... 54

3.2.1 Search for putative *C. fulvum* homologous effectors in the *D. septosporum* NZE10 genome .......................................................................................................................... 54

3.2.2 *In planta* transcriptome analysis of putative effectors in *D. septosporum* ...................... 56

3.2.3 Core effectors identified in the *D. septosporum* NZE10 genome ................................... 57

3.2.3.1 *D. septosporum Avr4* (*DsAvr4*) .................................................................................. 58

3.2.3.2 *D. septosporum Ecp6* (*DsEcp6*) ................................................................................ 60

3.2.3.3 *D. septosporum Ecp2* (*DsEcp2*) ................................................................................ 62

3.2.4 Non-core effectors identified in the *D. septosporum* NZE10 genome ......................... 63

3.3 Discussion .................................................................................................................................... 65

3.3.1 Putative effectors identified in the *D. septosporum* NZE10 genome ............................. 65

3.3.2 *In planta* transcriptome analysis of putative *D. septosporum* effectors .................... 66

3.3.3 Identification of core putative effectors in the *D. septosporum* NZE10 genome .......... 67

Chapter 4. Characterization of a highly-expressed hydrophobin gene in *D. septosporum* ........................................................................................................................................... 71

4.1 Introduction .................................................................................................................................. 71
4.1.1 Class I and Class II hydrophobins ................................................................. 71
4.1.2 Interfacial self-assembly of hydrophobins......................................................... 72
4.1.3 Role of hydrophobins in filamentous fungi....................................................... 73
4.2 Results ................................................................................................................... 74
  4.2.1 Characterization of D. septosporum hydrophobins............................................ 74
  4.2.2 Expression profile of D. septosporum hydrophobins........................................ 78
  4.2.3 Targeted gene replacement and complementation of D. septosporum hydrophobin DsHdp1 ......................................................................................................................... 78
  4.2.4 D. septosporum ΔDsHdp1 showed reduced colony surface hydrophobicity ...... 80
4.3 Discussion ............................................................................................................. 83

Chapter 5. Functional characterization of putative effectors in Dothistroma septosporum NZE10 strain ................................................................................................................... 87

  5.1 Introduction ........................................................................................................... 87
  5.2 Results ................................................................................................................... 88
  5.2.1 Functional characterization of D. septosporum effectors .................................. 88
    5.2.1.1 Targeted gene replacement and complementation of D. septosporum effectors DsAvr4, DsEcp2-1 and DsEcp6 .............................................................. 88
    5.2.1.2. Virulence testing of DsAvr4, DsEcp2-1 and DsEcp6 in planta ...................... 94
  5.2.2 One or more amino acid residues in the conserved chitin binding domain of DsAvr4 is required for Cf-4 mediated hypersensitive response....................................................... 98
  5.2.3 Screening for necrosis inducing activity of D. septosporum effectors in P. radiata . 102
    5.2.3.1 P. pastoris heterologous protein production ............................................. 102
    5.2.3.2 DsEcp2-1 and DsEcp2-3 are able to trigger a necrosis response in N. tabacum... 104
    5.2.3.3 Screening for necrosis-inducing activity of D. septosporum effectors in P. radiata .............................................................................................................................. 105
  5.3 Discussion ............................................................................................................. 107
    5.3.1 DsEcp2-1 mutants showed increased lesion size compared to wild-type ............ 107
    5.3.2 One or more amino acid residues in the conserved chitin binding domain of DsAvr4 are required for Cf-4 mediated hypersensitive response......................................................... 109
    5.3.3 Screening for necrosis inducing activity of D. septosporum effectors .............. 112
      5.3.3.1 D. septosporum contains a DsNLP pseudogene......................................... 112
      5.3.3.2 Pichia pastoris heterologous protein production ..................................... 113
      5.3.3.3 DsEcp2-1 and DsEcp2-3 are able to trigger necrosis in N. tabacum .......... 113
      5.3.3.4 Screening for necrosis inducing activity on P. radiata ............................... 114
Chapter 6. Allelic variation of candidate effector genes in *Dothistroma septosporum* 119

6.1 Introduction .......................................................................................................... 119
6.2 Results................................................................................................................... 122
6.2.1 Presence/absence of effectors in a global collection of *D. septosporum* strains..... 122
6.2.2 Allelic variation of candidate effector genes .................................................... 123
6.3 Discussion.............................................................................................................. 135
6.3.1 The geographic distribution of *D. septosporum* strains is related to the number of amino acid variations in *D. septosporum* effectors.................................................. 135
6.3.2 Mutations in *D. septosporum* effector protein domains .................................. 137
6.3.3 *D. septosporum* effectors under positive selection ......................................... 138

Chapter 7. General conclusions and future work .......................................................... 141

7.1 General conclusions ............................................................................................... 141
7.2 Future work........................................................................................................... 143

Appendix 1. Media, buffers and solutions .................................................................... 145

Appendix 1.1 Growth media ........................................................................................ 145
Appendix 1.2 Buffers and reagents for DNA extraction, quantification and analysis ...... 147
Appendix 1.3 RNA extraction and manipulation solutions.......................................... 149
Appendix 1.4 Southern blotting and hybridization solutions ...................................... 150
Appendix 1.5 Transformation of *D. septosporum*...................................................... 151
Appendix 1.6 Agro-infiltration on *Nicotiana tabacum* Plant .................................... 152
Appendix 1.7 Heterologous production of *D. septosporum* effector protein by *P. pastoris* .............................................................................................................. 153
Appendix 1.8 Western blotting .................................................................................... 155

Appendix 2. Schematic diagrams for materials and methods ........................................ 156

Appendix 2.1 Schematic diagram of primer positions for *DsAvr4* used in this study ...... 156
Appendix 2.2 *DsAvr4* WT and chimeric nucleotide sequences ................................... 158
Appendix 2.3 *P. pastoris* heterologous protein expression plasmid ............................. 160
Appendix 3. Figures

Appendix figure 3.1 Predicted translation of core *D. septosporum* effectors .......... 161
Appendix figure 3.2 Phylogenetic analysis and amino acid alignment of Ecp6 homologues ...................................................................................................................... 165
Appendix figure 3.3 Predicted translation of *DsEcp4, DsEcp5, DsEcp13, DsEcp14* and hydrophobin genes ........................................................................................................ 167
Appendix figure 3.4 gDNA of complemented *DsAvr4, DsEcp2-1* and *DsEcp6* mutants .... 172

Appendix 4. Tables

Appendix table 4.1 Copy number estimation in *DsAvr4, DsEcp2-1* and *DsEcp6* complementation strains by real-time PCR ........................................................................ 173
Appendix table 4.2 *DsAvr4* and *DsEcp6* expression in *DsLaeA* mutant strain .............. 175
Appendix table 4.3 Percentage of amino acid identities of nine effectors from *D. septosporum* strains ................................................................................................. 176
Appendix 4.4 Phenotypic and pathogenicity assay ................................................................ 185
Number of germinated spores ..................................................................................... 187

Appendix 5. Primers used in this study

Appendix 5.1 Construction of gene replacement plasmids ............................................ 190
Appendix 5.2 Construction of plasmids by restriction endonuclease digestion and ligation .................................................................................................................. 191
Appendix 5.3 Construction of *DsAvr4* and *CbAvr4* domain swap chimeras ................. 192
Appendix 5.4 real-time PCR primer sequences .................................................................. 193
Appendix 5.5 DIG-labelling of the probes (PCR based) ................................................... 194
Appendix 5.6 Primers used for polymorphism analysis .................................................. 195

References ..................................................................................................................... 196
List of Figures

Figure 1.1 Dothistroma needle blight symptoms .............................................................. 2
Figure 1.2 Arms race between pathogen and plant ........................................................... 5
Figure 1.3 Models of plant pathogen recognition ............................................................. 7
Figure 1.4 Structural organization of Hcr9 and Hcr2 Locus derived from various Lycopersicon species ....................................................................................................... 18
Figure 2.1 MultiSite Gateway three-fragment recombination reaction scheme ............. 31
Figure 2.2 Scheme for overlapping PCR used to swap domains between DsAvr4 and CbAvr4 proteins with an example of a chimeric protein in which the domain between C1-C2 has been exchanged. ........................................................................................................ 35
Figure 3.1 Phylogenetic analysis and amino acid alignment of Avr4 homologues from Dothideomycete fungi ..................................................................................................... 59
Figure 3.2 Predicted D. septosporum Ecp6 protein structure ......................................... 61
Figure 3.3 Phylogenetic analysis of Ecp2-1 Ecp2-2 and Ecp2-3 from fungi ...................... 62
Figure 3.4 Amino acid alignments of Ecp4, Ecp5, Ecp13 and Ecp14 .............................. 64
Figure 4.1 Phylogenetic tree and amino acid identity of hydrophobin genes .......... 76
Figure 4.2 Partial amino acid alignments of hydrophobin ............................................. 77
Figure 4.3 Southern hybridization of targeted DsHdp1 replacement candidates in D. septosporum ................................................................................................................ 79
Figure 4.4 PCR screening and copy determination of DsHdp1 complementation strains ....................................................................................................................................... 80
Figure 4.5 Hydrophobicity assay ............................................................................... 83
Figure 5.1 PCR screening of D. septosporum transformants for targeted gene replacement of DsAvr4, DsEcp2-1 and DsEcp6 ............................................................... 89
Figure 5.2 Southern hybridization confirmation of targeted gene replacement of DsAvr4, DsEcp2-1 and DsEcp6 in D. septosporum ................................................................. 90
Figure 5.3 PCR screening for DsAvr4, DsEcp2-1 and DsEcp6 complementation strains ..................................................................................................................................... 93
Figure 5.4 Amino acid alignment of mature Avr4 .......................................................... 98
Figure 5.5 PCR screening for positive agrobacteria colonies for Avr4 chimeras ........... 99
Figure 5.6 Co-infiltration of Cf-4 with Avr4 chimeras on N. tabacum leaves ............. 101
Figure 5.7 Western blot confirmation of heterologous D. septosporum effector protein production .................................................................................................................. 103
Figure 5.8 Infiltration of expressed protein culture filtrates on *N. tabacum* leaves...... 104
Figure 5.9 Vacuum infiltration of pine needles using 0.1% neutral red dye .......... 105
Figure 5.10 Infiltration of *P. pastoris* culture filtrates on detached susceptible *P. radiata* needles................................................................. 107
Figure 5.11 Proposed function of DsEcp2-1 when infecting *P. radiata*.............. 118
Figure 6.1 DsAvr4 amino acid changes summary ............................................... 126
Figure 6.2 DsEcp2-1 amino acid changes summary ............................................ 127
Figure 6.3 DsEcp2-3 amino acid changes summary ............................................ 129
Figure 6.4 DsEcp4 amino acid changes summary ............................................... 131
Figure 6.5 DsEcp5 amino acid changes summary ............................................... 132
Figure 6.6 Amino acid changes summary for DsEcp6, DsEcp13, Ds69335 and DsEcp14 ....................................................................................................................... 134
List of Tables

Table 2.1 Wild-type *D. septosporum* strains ................................................................. 23
Table 2.2 Mutant *D. septosporum* strains ................................................................. 24
Table 3.1 Characterization of putative effectors in *D. septosporum* NZE10 genome .... 55
Table 3.2 *In planta* transcriptome analysis of putative *D. septosporum* effectors ........ 57
Table 4.1 Characterization of hydrophobins in *D. septosporum* NZE10 genome ........ 75
Table 4.2 Transcriptome analysis of four *D. septosporum* hydrophobin genes .......... 78
Table 4.3 Phenotypic analysis and virulence assay using *DsHdp1* mutant and complemented strains ...................................................................................................... 82
Table 5.1 Phenotypic and pathogenicity assay ............................................................. 96
Table 6.1 Global collection of *D. septosporum* strains and sequencing results .......... 121
Table 6.2 Summary of allelic variations of *D. septosporum* effectors ...................... 124
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avr</td>
<td>avirulence protein</td>
</tr>
<tr>
<td>CC:</td>
<td>coiled-coil domain</td>
</tr>
<tr>
<td>ChBD</td>
<td>chitin-binding domain</td>
</tr>
<tr>
<td>Cys</td>
<td>cysteine residue</td>
</tr>
<tr>
<td>Drase</td>
<td>deoxyribonuclease</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>Ecp</td>
<td>extra cellular protein</td>
</tr>
<tr>
<td>eLRR</td>
<td>extracellular LRR</td>
</tr>
<tr>
<td>G</td>
<td>gram</td>
</tr>
<tr>
<td>HR</td>
<td>Hypersensitive response</td>
</tr>
<tr>
<td>IPTG</td>
<td>isopropyl-β-d-thiogalactoside</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase pair</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LRR</td>
<td>leucine-rich repeat domain</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>Mb</td>
<td>megabase</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>NBS</td>
<td>nucleotide-binding site</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NLP</td>
<td>necrosis and ethylene-inducing like protein</td>
</tr>
<tr>
<td>NLS</td>
<td>nuclear localization signal</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>PAMPs</td>
<td>pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PCD</td>
<td>programed cell death</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PEST</td>
<td>Pro-Glu-Ser-Thr domain</td>
</tr>
<tr>
<td>PRRs</td>
<td>plant PAMP-recognition receptors</td>
</tr>
<tr>
<td>PTI</td>
<td>PAMP triggered immunity</td>
</tr>
<tr>
<td>qPCR</td>
<td>real-time PCR</td>
</tr>
<tr>
<td>R protein</td>
<td>resistant protein</td>
</tr>
<tr>
<td>RGAs</td>
<td>Rprotein analogs</td>
</tr>
<tr>
<td>RLKs</td>
<td>receptor-like kinase proteins</td>
</tr>
<tr>
<td>RLPs</td>
<td>receptor like protein</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SSCP</td>
<td>small secreted cysteine rich protein</td>
</tr>
<tr>
<td>T3SS</td>
<td>bacterial type III secretion system</td>
</tr>
<tr>
<td>TIR:</td>
<td>toll-interleukin 1 receptors</td>
</tr>
<tr>
<td>TM</td>
<td>transmembrane domain</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>WRKY</td>
<td>DNA binding domain</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
<tr>
<td>X-Gal</td>
<td>5- bromo-4-chloro-3-indolyl-β-D-galactopyranoside</td>
</tr>
<tr>
<td>μg</td>
<td>microgram</td>
</tr>
<tr>
<td>μl</td>
<td>microlitre</td>
</tr>
<tr>
<td>μM</td>
<td>micromolar</td>
</tr>
</tbody>
</table>