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FUNCTIONAL CHARACTERISATION of *CONSTITUTIVE EXPRESSER* of *PATHOGENESIS-RELATED GENES 5*

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

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ABSTRACT

As reported previously, CPR5 negatively regulates the onset of leaf death, hypersensitive response, disease resistance and early leaf senescence. *cpr5* plants contain aberrant trichomes and higher levels of ROS, SA and JA. Cell-cycle, JA/ET, ABA and sugar signalling are also affected in *cpr5* plants. These results suggest that CPR5 is a master regulator of multiple processes. However, how CPR5 manages to exert pleiotropic effects is still poorly understood. The first objective of the current study was the purification of the CPR5 protein to solve its crystal structure. Extensive *in silico* analyses were carried out and the results showed that CPR5 is predicted to be a membrane protein with 4 or 5 transmembrane (TM) domains. Additionally, CPR5 contains intrinsically disordered regions (IDRs) at its N-terminus. Proteins containing IDRs and TM domains are often difficult to purify for crystallization studies. Therefore, the undesirable regions of CPR5 such as, IDR and TM domains were deleted and a set of 24 constructs were developed. Despite several efforts, none of the CPR5 recombinant proteins were isolated. In addition to predicting IDR and TM domains, *in silico* results also predicted three NLS-encoding clusters, casein kinase phosphorylation sites, multiple start codons, coiled-coil domains and glycine motifs. To find out the roles of these putative structural elements on CPR5 functions, firstly a *CPR5* cDNA was synthesised and termed as *SynCPR5*. Subsequently, predicted sites or motifs were mutated in *SynCPR5* through site-directed mutagenesis and a set of 25 mutated *CPR5* transgenes (cDNA constructs) were developed. Using a complementation strategy, all the constructs were transformed into *cpr5-2* plants. The results show that the complementation of *cpr5-2* plants with *SynCPR5*, fully restored HR-like lesions, wildtype-like trichomes and leaves on *SynCPR5* plants. Further physiological characterization such as, transcript abundance of *SynCPR5*, *PR1*, *PR5* and *PDF1.2*, leaf area measurements and ploidy levels showed that *CPR5* regulates some of its functions and phenotypes quantitatively as well as qualitatively. When compared with the wildtype, better growth (larger leaves) but enhanced disease susceptibility was found in *metCPR5* transgenic lines (in which putative start codons were mutated), indicating that CPR5 regulates a balance between growth and resistance. Functional characterization of NLS mutants (*nlsCPR5*) showed that NLS-encoding clusters are important for CPR5 proper

functions. However, current evidence is insufficient to relate their role in CPR5 localization. Moreover, *in silico* results show that putative NLS clusters are present in the region of CPR5 which were annotated as intrinsically disordered region (IDR). Similar phenotypes shown by both *nlsCPR5* and *Del63CPR5* (in which the first 63 amino acids of CPR5 including putative NLS were deleted), indicate that the putative NLS clusters could be part of IDR and may have dual functions. Loss-of-function phenotypes shown by coiled-coil domain mutants (*ccdCPR5*) reinforce the role of coiled-coil domains in CPR5 homo-dimerization. Moreover, in contrast to previous reports, the downregulation of *PDF1.2* in the majority of *CPR5* complementation lines proposes CPR5 to be a positive regulator of *PDF1.2*. Based on the results presented in the current study, putative CPR5 IDRs and coiled-coil domains are proposed to facilitate CPR5 dimerization in order to restrict the entry of deregulated cargos into the nucleus. Moreover, these results uncover a novel role of CPR5 in the regulation of balance between plant growth and resistance. Furthermore, this study, for the first time, reports evidence of the requirement of NLS clusters for CPR5 functions.

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MUHAMMAD FAISAL

TABLE OF CONTENTS

ABSTRACT .... **I**

ACKNOWLEDGEMENTS .... **III**

LIST OF FIGURES .... **XI**

LIST OF TABLES .... **XIV**

LIST OF ABBREVIATIONS .... **XV**

Chapter 1 CPR5 as a master regulator- review of literature **1**

1.1 INTRODUCTION 1

1.1.1 CONSTITUTIVE EXPRESSER OF PATHOGENESIS-RELATED GENES (CPR) mutants 2

1.1.2 CONSTITUTIVE EXPRESSER of PATHOGENESIS-RELATED GENES 5 (CPR5) and other CPR5 mutant alleles 2

1.1.3 CPR5 Localizes in nuclear membrane 4

1.1.4 *CPR5* is constitutively expressed in the majority of plant tissues 4

1.2 Role of CPR5 in programmed cell death, hypersensitive response and pathogen resistance 5

1.3 Leaf senescence regulation and CPR5 10

1.4 Cell cycle, cell growth and development, and CPR5 14

1.5 Potassium homeostasis in *cpr5* 17

1.6 Germination and seedling growth response in *cpr5* 18

1.7 Plant growth and resistance trade-off and *CPR5* 19

1.8 Outlook and Aims of the Research 25

Chapter 2 Materials and Methods **27**

2.1 *In silico* computer tools used for CPR5 analyses 27

2.2 Primer designing, RNA extractions and cDNA synthesis 28

2.2.1 Primer designing 28

2.2.2 RNA Extractions and cDNA synthesis 29

2.2.3 qRT-PCR data analyses 30

2.2.4 PCR reactions and profiles 30

2.3 Cloning strategy and development of *CPR5* constructs for structural studies 31

2.4 Amplification, transformation and confirmation of 8 *CPR5* transgenes 32

2.5 Cloning techniques and protocols 33

2.5.1 DNA restriction/digestions 33

2.5.2 DNA elution/extractions from agarose gels 33

2.5.3 DNA ligation 37

2.6 Bacterial transformation protocol 38

2.6.1 Preparation of LB liquid and agar media 38

2.6.2 LB agar plates for IPTG/X-Gal for white/blue Selection 39

2.7 Bacterial plasmid (DNA) isolation protocol (Alkaline lysis) 39

2.8 Competent cells preparations for *E. coli* and *Agrobacterium*-mediated transformations 40

2.9 Purification of CPR5 protein variants 40

2.9.1 Sample preparation 40

2.9.2 Resin (sepharose beads) preparation 41

2.9.3 Purification using His-tag 41

2.9.4 Purification using ion-exchange chromatography 41

2.9.5 List and recipes of buffers used for Ni^{+}-column based purifications 42

2.9.6 List and Recipes of buffers used for ion-exchange purifications 43

2.9.7 Preparation of 12% acrylamide gels 44

2.10 In-gel protein digestion for mass-spectrometry 44

2.10.1 Excision of the protein bands of interest from Gel 44

2.10.2 Solutions and suffers used for In-gel protein digestion 46

2.11 General sowing and plant growth conditions 46

2.12 Trichome counting and leaf area measurements 46

2.13 *CPR5* gene synthesis, site-directed mutagenesis and transformations 47

2.13.1 Gene Synthesis 47

2.13.2 *SynCPR5* transgenes transformation into *Agrobacterium GV3101* 48

2.13.3 *Agrobacterium*-mediated transformations of *cpr5-2* plants 48

2.13.4 Confirmation of positive transformants (genotyping) 49

2.14 *Pseudomonas syringae* infiltrations 49

2.15 Electron Scanning Microscopy (SEM) and cell size measurements 50

2.16 Ploidy level measurement using Flow Cytometer 51

2.17 Preparation of antibiotics 53

2.17.1 Ampicillin sodium salt (Sigma) 53

2.17.2 Kanamycin sulphate (Sigma) 53

2.17.3 Tetracycline hydrochloride (Sigma) 53

2.17.4 Chloramphenicol (Duchefa) 53

2.17.5 Rifampicin (Duchefa) 53

2.17.6 Gentamycin (Duchefa) 53

Chapter 3 *In silico* Characterization of CPR5 54

3.1 INTRODUCTION 54

3.2 RESULTS 55

3.2.1 *CPR5* is widely distributed throughout the plant kingdom 55

3.2.2 CPR5 *in plant* homology amongst its homologues 55

3.2.3 AtCPR5 is preditictED to have 4 or 5 transmembrane regions 56

3.2.4 AtCPR5 carries two casein kinase phosphorylation sites 62

3.2.5 AtCPR5 carries multiple transcritpion or translation initiation sites 62

3.2.6 AtCPR5 is annotated to contain intrinsically disordered regions 65

3.2.7 AtCPR5 is also predicted to contain coiled-coil domains 65

3.2.8 CPR5 is predicted to have glycine zipper 67

3.3 DISCUSSION 71

3.3.1 AtCPR5 appears to be an important membrane protein 71

Chapter 4 AtCPR5 may be one of the Intrinsically Disordered Proteins 72

4.1 INTRODUCTION 72

4.2 RESULTS 75

4.2.1 Like typical IDRs, CPR5 IDR is highly polymorphic in amino acid composition 75

4.2.2 Amino acid composition of AtCPR5 IDRs is consistent with typical IDRs 75

4.2.3 IDRs of CPR5 and CPR5-like proteins appear to have INDELs 79

4.2.4 AtCPR5 IDRs are annotated to contain MoRFs and disordered binding regions 79

4.2.5 AtCPR5 disordered regions annotate to be unfolding and flexible regions 80

4.3 DISCUSSION 81

4.3.1 AtCPR5 appears to be an intrinsically disordered protein 81

4.3.2 Having IDP characteristics, AtCPR5 could exert pleiotropic effects 81

Chapter 5 *In silico* characterisation and purification of AtCPR5 protein for structural studies 84

5.1 INTRODUCTION 84

5.2 RESULTS 86

5.2.1 Eliminations of predicted transmembrane domains 86

5.2.2 Elimination of predictied AtCPR5 IDRs 86

5.2.3 Eliminations of TM and IDR show no improvement in crystallizability of CPR5 truncated proteins 87

5.2.4 *CPR5* constructs were cloned in pET32 expression vector 87

5.2.5 Integration and orientation of *CPR5* transgenes were confirmed via PCR and restriction analyses 88

5.2.6 AtCPR5 recombinant proteins were soluble in Rosetta-gami cells 93

5.2.7 Purification of His-tagged AtCPR5 recombinant proteins 93

5.2.8 Purification of untagged versions using ion exchange chromatography 98

5.2.9 Identification of CPR5 protein by Mass Spectrometry 98

5.2.10 CPR5 protein was failed to be purified using size exclusion chromatography 99

5.3 DISCUSSION 102

5.3.1 Potential consequences of transmembrane domains eliminations 102

5.3.2 Putative impacts of IDRs eliminations on CPR5 structure 103

5.3.3 Purification of AtCPR5 protein is not trivial 104

Chapter 6 Development of CPR5 mutants based on *in silico* information........106

6.1 INTRODUCTION 106

6.2 RESULTS 107

6.2.1 Putative IDRs were deleted to study their roles in CPR5 functions 107

6.2.2 Puttive start codons were mutated to study CPR5 isoforms 108

6.2.3 NLS clusters were mutated indiviually as well as collectively 108

6.2.4 Casein kinase phosphorylation site 1 and 2 were deleted and mutated 109

6.2.5 Putative leucine resiudes of coiled-coil domains were mutated into asparagine 112

6.2.6 Putative glycine resiudes of glycine zipper were mutated alanine 112

6.2.7 CPR5 transgenes were complemented into *cpr5-2* plants 113

6.3 DISCUSSION 118

6.3.1 Mutagenesis studies are expected to identify roles of various putative motifs of *CPR5* 118

6.3.2 *CPR5* transgenes were introduced into *cpr5-2* plants using floral dipping 120

Chapter 7 AtCPR5 regulates balance between plant growth and disease resistance 122

7.1 INTRODUCTION 124

7.2 RESULTS 126

7.2.1 *CPR5* native promoter is able to drive *SynCPR5* expression 126

7.2.2 *SynCPR*5 complements aberrant trichomes, lesions and early leaf senescence 127

7.2.3 Leaf size and number of trichomes on *SynCPR5* plants is consistent with their *SynCPR5* expression levels 128

7.2.4 *SynCPR5* fails to complement upregulated levels of *PR1* in *SynCPR5* lines 130

7.2.5 *SynCPR5* and *cpr5-2* plants both show reduced *PDF1.2* levels 130

7.2.6 *SynCPR5* regulate resistance independent of *SynCPR5* and *PR1* levels 131

7.2.7 *SynCPR5* plants show wildtype-like ploidy levels 133

7.2.8 *metCPR5* complement aberrant trichomes, HR-like lesions and leaf yellowing shown by *cpr5-2* 136

7.2.9 *metCPR5b* lines show higher transcript abundance than wildtype 137

7.2.10 *metCPR5b* and *metCPR5bc* have bigger leaves than wildtype 137

7.2.11 *metCPR5b* and *metCPR5bc* have wildtype-like epidermal pavement cells 138

7.2.12 *metCPR5b* and *metCPR5bc* leaves have higher ploidy levels 144

7.2.13 *metCPR5b* and *metCPR5bc* have lower or wildtype-like *PR1* levels 145

7.2.14 *metCPR5* plants conferred enhanced susceptibility to *P syringae* 145

7.3 DISCUSSION 148

7.3.1 *SynCPR5* transgenic plants have variable *SynCPR5* expression levels 148

7.3.2 *SynCPR5* complements majority of *cpr5-2* compromised phenotypes 149

7.3.3 Phenotypes suggest quantitative as well as qualitative roles for CPR5 150

7.3.4 *PDF1.2* is expressed at lower levels in *SynCPR5* and *cpr5-2* than *CPR5* 151

7.3.5 Putative roles for nucleotide residues associated with alternative start codons 153

7.3.6 Putative roles of CPR5 in regulation of balance between growth and resistance 157

Chapter 8 AtCPR5 putative NLS clusters appear to have limited function in CPR5 nuclear localisation and may be part of a structural component of the Intrinsically Disordered Region 162

8.1 INTRODUCTION 164

8.2 RESULTS 167

8.2.1 AtCPR5 is predicted to contain 3 NLS-encoding clusters 167

8.2.2 Putative NLS clusters are highly conserved among AtCPR5 homologues 168

8.2.3 CPR5 putative NLS-encoding clusters are predicted to be flanked by CKI and CKII phosphorylation sites 173

8.2.4 *nlsCPR5* complemented lines show variation in grwoth 173

8.2.5 *nlsCPR5* plants show reduced *nlsCPR5* expression levels 174

8.2.6 *nlsCPR5* transgenic lines display smaller leaves 178

8.2.7 Epidermal pavement cells are smaller on *nlsCPR5* transgenic leaves 178

8.2.8 Cell expansion appears to be affected in leaves of *nlsCPR5* transgenic plants.................. 179

8.2.9 Degree of trichome aberration varies among *nlsCPR5* lines 183

8.2.10 *nlsCPR5* transgenic plants confer resistance to *Pseudomonas syringae* 191

8.2.11 *nlsCPR5* mutants show elevated levels for *PR1* and *PR5* genes 191

8.2.12 *nlsCPR5* transgenic plants display downregulation of *PDF1.2* 191

8.2.13 *nlsCPR5* mutant plants show reduced plant height 192

8.2.14 Collective mutations in NLS-encoding clusters results in early bolting 195

8.2.15 Putative CPR5 NLS clusters and an intrinsically disordered region belong to the same region of CPR5 protein 195

8.2.16 *Del37CPR5* and *Del63CPR5* complement HR-like lesions and early leaf yellowing 196

8.2.17 *Del63CPR5* transgenic lines display reduced transcript abundance 197

8.2.18 Typical pattern of trichomes is restored by *Del37CPR5* but not by *Del63CPR5* and *Del98CPR5* ... 197

8.2.19 *Del37CPR5* and *Del63CPR5* both produce leaves of intermediate sizes ... 201

8.2.20 Sizes of epidermal cells are consistent with leaf sizes of *DelCPR5* plants ... 201

8.2.21 Nuclei from *DelCPR5* leaf cells show reduced ploidy levels ... 201

8.2.22 *Del63CPR5* confers resistance but *Del37CPR5* is susceptible to *PstDC3000* ... 202

8.2.23 *PR1* levels are not restored in *Del63CPR5* transgenic plants ... 202

8.3 DISCUSSION ... 207

8.3.1 *In silico* studies suggest CPR5 to be a nuclear protein ... 207

8.3.2 Some CPR5 phenotypes/functions appear to be dependent on *CPR5* expression ... 208

8.3.3 Putative NLS clusters appear to have limited function ... 209

8.3.4 First 37 amino acids of CPR5 are crucial for wildtype-like leaf sizes ... 210

8.3.5 *nlsCPR5*-like phenotypes on *DelCPR5* plants suggest NLS clusters to be part of IDRs ... 211

Chapter 9 Characterization of putative CPR5 leucine and glycine motifs ... 215

9.1 INTRODUCTION ... 215

9.2 RESULTS ... 217

9.2.1 AtCPR5 protein is predicted to contain 4 coiled-coil domains ... 217

9.2.2 AtCPR5 protein is predicted to contain glycine motifs ... 217

9.2.3 *ccdCPR5* plants display *cpr5*-like phenotypes ... 220

9.2.4 Glycine residues of the glycine motif show intolerance to mutations ... 220

9.3 DISCUSSION ... 223

9.3.1 Leucine residues are crucial for CPR5 functioning ... 223

9.3.2 Glycine 452 and 459 are also crucial for CPR5 functions ... 223

Chapter 10 FINAL DISCUSSION AND FUTURE PROSPECTS ... 226

10.1 AtCPR5 is a nuclear membrane protein ... 226

10.2 CPR5 may exert pleiotropic effects by modifying NPC-mediated selectivity and entry of nuclear proteins ... 228

10.3 CPR5 IDRs and Coiled-coil-domains may be involved in CPR5-mediated NPC gating ... 230

Chapter 11 APPENDICES ... 234

11.1 List of primers 234

11.1.1 Primers for the amplification of untagged *CPR5* constructs 234

11.1.2 List of primers for the amplification of *CPR5* constructs with N-ter His-tag 234

11.1.3 List of primers for the amplification of *CPR5* constructs with C-ter His-tag 235

11.1.4 List of primers used for real-time quantifications 235

11.1.5 List of genotyping primers 236

11.2 List of accession number of CPR5-like proteins 237

11.3 Students' t-test results 238

11.3.1 Expression levels: Statistical significance at p < 0.05 (Students' t-test) 238

11.3.2 Trichomes with three appendages: Statistical significance at p < 0.05 (Students' t-test) 238

11.3.3 Area of third leaf pair: Statistical significance at p < 0.05 (Students' t-test) 239

11.3.4 Area of fourth leaf pair: Statistical significance at p < 0.05 (Students' t-test) 239

11.3.5 *PR1* transcript abundance: Statistical significance at p < 0.05 (Students' t-test) 240

11.3.6 Expression levels: Level of statistical significance at p < 0.05 (Students' t-test) 241

REFERENCES 242

LIST OF FIGURES

Figure 1.1 Annotated architecture of *CPR5* Gene 7

Figure 2.1 Positions of primers used for real-time qRT-PCR quantifications 34

Figure 2.2 Schematic representation of transgene cassette of pET32 35

Figure 2.3 pET32 sequence composition and landmark information 36

Figure 2.4 Schematic diagram for serial dilutions 52

Figure 3.1 Multiple sequence alignment of CPR5 putative homologues 61

Figure 3.2 Annotation, position and number of TMs in CPR5 protein 64

Figure 3.3 Positions of in-frame start codons and RNA stem-loop structures 66

Figure 3.4 Position and number of Intrinsically Disordered Regions 69

Figure 3.5 Position and number of annotated coiled-coil regions 70

Figure 3.6 Position of glycine residues in CPR5 protein... 70

Figure 4.1 Position of disordered and binding regions in AtCPR5 77

Figure 4.2 Presence of polymorphism in the N-terminus of CPR5 and CPR5-like proteins sequences 78

Figure 4.3 Folding and unfolding propensities of regions of CPR5.............................. 80

Figure 5.1 Positions of various selected truncations in CPR5 protein.......................... 90

Figure 5.2 Migration of *CPR5* cDNA amplified fragment 91

Figure 5.3 Amplification of *CPR5* constructs 91

Figure 5.4 Confirmation of integration of constructs in pET32 by PCR........................ 92

Figure 5.5 Confirmation of integration of constructs in pET32 by restriction analyses 92

Figure 5.6 Confirmation of expression and solubility of CPR5 tagged recombinant proteins.. 96

Figure 5.7 Confirmation of binding affinity of CPR5 recombinant protein B 97

Figure 5.8 Confirmation of expression and purification of untagged version 100

Figure 5.9 CPR5 identification by Mass-spectrometry... 101

Figure 6.1 Construct designing of *Del37CPR5*, *Del63 CPR5* and *Del98CPR5* transgenes 110

Figure 6.2 Designing of *metCPR5* transgenes... 111

Figure 6.3 Similarities and differences between *CPR5* wildtype and synthetic gene and positions of structural sites 116

Figure 7.1 *SynCPR5* complementation, transcript and trichomes quantification...... 129

Figure 7.2 Mean leaf area of third and fourth rosette leaf pairs.................................. 132

Figure 7.3 Relative expression levels of *PR1* in *SynCPR5* plants 132

Figure 7.4 Relative expression levels of *PDF1.2* in *SynCPR5* plants 134

Figure 7.5 *PstDC3000* infection growth assay 134

Figure 7.6 Ploidy levels in leaves of *SynCPR5* transgenic plants 135

Figure 7.7 Overexpression, *metCPR5*, Col-0 and *cpr5-2* plants at 17 DAS 139

Figure 7.8 *metCPR5* transgene and trichome quantification in *metCPR5* plants 140

Figure 7.9 Mean area of leaves and epidermal pavement cells of *metCPR5* plants 143

Figure 7.10 Ploidy levels in leaves of *SynCPR5* transgenic plants 146

Figure 7.11 Relative expression levels of *PR1* in *SynCPR5* plants 147

Figure 7.12 *PstDC3000* infection growth assay 147

Figure 8.1 Positions of potential nuclear localisation signal (NLS) facilitating basic residues in AtCPR5 and its homologues 170

Figure 8.2 Position of annotated NLS clusters and casein kinase phosphorylations sites among CPR5-like proteins 172

Figure 8.3 *nlsCPR5* and *DelCPR5* plants at 17 DAS 175

Figure 8.4 Transcript abundance of *CPR5* in *nlsCPR5* and *DelCPR5* transgenic lines 176

Figure 8.5 Mean leaf areas of *nlsCPR5* lines 177

Figure 8.6 Epidermal pavement cell size 182

Figure 8.7 Measurements of ploidy levels in leaves of *nlsCPR5* mutants at 24 DAS 185

Figure 8.8 Type and number of trichome appendages at 16 DAS 190

Figure 8.9 *Pseudomonas syringae* pv *DC3000* growth assay 193

Figure 8.10 Quantification of transcript abundance of *PR1, PR5*, and *PDF1.2* 194

Figure 8.11 Plant height measurement 198

Figure 8.12 Plant growth and bolting of *nlsCPR5* transgenic lines at 27 DAS 200

Figure 8.13 Type and average number of trichomes on *DelCPR5* leaves 203

Figure 8.14 Mean leaf area of *DelCPR5* 203

Figure 8.15 Mean area of epidermal pavement cells 204

Figure 8.16 Ploidy levels measurement in leaves of *DelCPR5* mutants 205

Figure 8.17 *Pseudomonas syringae* infection assay 206

Figure 8.18 Position of structural motifs present in CPR5 first 98 amino acids 214

Figure 9.1 Position of putative leucine and glycine residues of leucine and glycine motifs 219

Figure 9.2 Plant growth and early leaf senescence on *ccdCPR5* and *GlnCPR5* leaves.. 222

Figure 10.1 Proposed CPR5 schematic diagram in nuclear lemma and its working 233

LIST OF TABLES

Table 4.1 Positions of predicted MoRFs and binding regions in CPR5 77

Table 5.1 Predicted position of TM domains in CPR5 protein 89

Table 5.2 XtalPred probabilities for CPR5 recombinant proteins to be crystallized ... 89

Table 6.1 Mutations in putative NLS-encoding residues 111

Table 6.2 Designing of CK phosphorylation site mutants. 114

Table 6.3 Designing of leucine and glycine zipper mutants 114

Table 6.4 List of *SynCPR5* transgenes and their transformation efficiency 117

Table 7.1 Average number of cells per image 143

LIST OF ABBREVIATIONS

aa	Amino acid
ABA	Abscisic acid
ABI5	ABA-INSENSITIVE 5
acd6	*ACCELERATED CELL DEATH 6*
APC/C	anaphase-promoting complex/cyclosome
BRI	BRASSINOSTEROID-INSENSITIVE
BRs	Brassinosteroids
Ca	Calcium
CaM 35S promoter	Cauli Mosaic Virus 35S promoter
CC-domains	Coiled-coil domains
CDC*20*	*CELL DIVISION CYCLE 20*
CDH	*CDH HOMOLOG 1*
CDK	Cyclin-Dependant-Kinase
CK	Casein kinase
CKI	CDK inhibitor
CNGC	*Cyclic Nucleotide Gated Channels*
COI1	*CORONATINE-INSENSITIVE 1*
Col-0	*Arabidopsis* ecotype Columbia
CPR5	*CONSTITUTIVE EXPRESSER of PATHOGENESIS-RELATED GENES 5*
DAS	Days after sowing
EDR1	*ENHANCED DISEASE RESISTANCE 1*
EDS5	*ENHANCED DISEASE SUSCEPTIBILITY 5*
EIN2	*ETHYLENE INSENSITIVE 2*
ET	Ethylene
ETI	Effector-Triggered Immunity
FZR	*FIZZY-RELATED*
GA	Gibberellin
GeBP	*GL1 ENHANCER BINDING PROTEIN*
GFP	Green Fluorescent Protein
GIG1	*GIGAS cell 1*
GPLs	*GeBP*-like proteins
GST	Glutathione-S-transferases
H_2O_2	Hydrogen Peroxide
HR	Hypersensitive Response
HXK	*Hexokinase*
HYS1	*HYPERSENESCENCE1*
IDP	Intrinsically Disordered Protein
IDR	Intrinsically Disordered Region
JA	Jasmonic Acid
JAR1	*JASMONATE RESISTANT 1*
JAZ1	*JASMONATE-ZIM-DOMAIN PROTEIN 1*

K	Potassium
KRPs	*KIP-RELATED PROTEINS*
L*er*-0	*Landsberg erecta*
LOX	Lipoxygenases
LSD	*LESIONS SIMULATING DISEASE*
MeJA	Methyl jasmonate
MoRFs	Molecular Recognition Features
mRNA	Messenger RNA
NDGA	Nordihydroguaiaretic acid
NE	Nuclear Envelope
NLS	Nuclear Localization Signal
NO	Nitric oxide
NPC	Nuclear Pore Complex
NPR1	*NON-EXPRESSER of PATHOGENESIS-RELATED GENES 1*
OE lines	Overexpression lines
OLD1	*ONSET OF LEAF DEATH1*
OSD1	*OMISSION of SECOND DIVISION 1*
PAD4	*PHYTOALEXIN DEFICIENT 4*
PAMPs/ MAPMs	Pathogen- or microbe-associated molecular patterns
PCD	Programmed Cell Death
PCR	Poly Chain Reaction
PDF1.2	*PLANT DEFENSIN 1.2*
PIF	*PHYTOCHROME INTERACTING FACTOR*
PR1	*PATHOGENESIS-RELATED 1*
PRRs	Pathogen Recognition Receptors
PstDC3000	*Pseudomonas syringae* pv *DC3000*
PTI	PAMP-Triggered Immunity
qRT-PCR	Quantitative Reverse-Transcriptase Poly Chain Reaction
R proteins	Resistance proteins
RB	Retinoblastoma
ROS	Reactive Oxygen Species
RPM1	*RESISTANCE TO PSEUDOMONAS SYRINGAE PV MACULICOLA1*
RPS2	*RESISTANCE TO PSEUDOMONAS SYRINGAE*
SA	Salicylic Acid
SAGs	*SENESCENCE-ASSOCIATED GENEs*
SAR	Systemic Acquired Resistance
SIM	*SIAMESE*
SMR	*SIAMESE-RELATED*
SOD	Superoxide Dismutase
TFs	Transcription Factors
TM	Transmembrane
tRNA	Transfer RNA
UVI4	*UV INSENSITIVE 4*