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**Studies on the interaction between  
*Arabidopsis thaliana*  
and  
African isolates of *Ralstonia solanacearum***

by

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**Courage doesn't always roar.  
Sometimes courage is the  
little voice at the end  
of the day that says:  
"I'll try again tomorrow"**

Anonymous



## DECLARATION

I hereby certify that this research, unless specifically indicated to the contrary in the text, is the result of my own investigation and that no part of this thesis has been submitted to any other university.

A handwritten signature in blue ink that reads "Weich".

Johanna Petronel Weich

# TABLE OF CONTENTS

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SUMMARY.....	VII
OPSOMMING.....	IX
ACKNOWLEDGEMENTS .....	XI
LIST OF ABBREVIATIONS .....	XII
INDEX OF FIGURES.....	XIV
INDEX OF TABLES.....	XVII
<b>CHAPTER 1: LITERATURE REVIEW</b>	
1.1. INTRODUCTION .....	2
1.2. THE PATHOGEN.....	3
1.2.1. Genome sequence.....	3
1.2.2. Exopolysaccharide and extracellular enzyme production .....	4
1.3. HOSTS.....	5
1.3.1. Symptom expression .....	5
1.3.2. Epidemiology .....	6
1.3.2.1. <i>The influence of the environment on epidemiology</i> .....	7
1.3.3. Regulatory systems of <i>R. solanacearum</i> : Hrp (hypersensitive response and pathogenicity) genes .....	8
1.3.4. Regulation of virulence and phenotypic conversion .....	9
1.4. CLASSIFICATION OF <i>R. SOLANACEARUM</i> .....	10



<b>1.5. DETECTION OF <i>R. SOLANACEARUM</i></b> .....	10
(A) Serological detection of <i>R. solanacearum</i> by ELISA.....	12
(B) Fatty acid profiling.....	13
(C) Metabolic profiles.....	13
(D) Nucleic acid-based technology, paving the pathway to new discoveries.....	14
• POLYMERASE CHAIN REACTION (PCR).....	14
• RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP).....	15
• PHYLOGENY OF BIOVARS BASED ON 16S RRNA SEQUENCING.....	15
• GENOMIC FINGERPRINTING.....	16
<b>1.6. CONTROL STRATEGIES</b> .....	16
A) Host-plant resistance.....	17
B) Cropping systems.....	17
C) Soil amendment.....	17
D) Disease avoidance.....	18
E) Integrated control.....	18
F) Biological control.....	18
<b>1.7 THE IMPORTANCE OF <i>ARABIDOPSIS THALIANA</i> IN PHYTOPATHOLOGY</b> .....	19
1.7.1 Understanding plant-pathogen interactions.....	20
1.7.2 How do plants protect themselves?.....	21
1.7.3 What is resistance?.....	22
1.7.4 The costs of resistance.....	23
1.7.5 Gene-for-gene interactions.....	24
1.7.6 Hypersensitive Response (HR).....	27
1.7.7 Signal transduction pathways.....	27
1.7.8 Pathogenesis-related (PR) proteins.....	30
<b>1.8 AIM OF THIS STUDY</b> .....	31



**CHAPTER 2: IDENTIFICATION AND CHARACTERIZATION OF AFRICAN STRAINS OF *RALSTONIA SOLANACEARUM***

<b>2.1 INTRODUCTION</b> .....	<b>33</b>
<b>2.2 MATERIALS AND METHODS</b> .....	<b>34</b>
<b>2.2.1 Bacterial strains and media</b> .....	<b>34</b>
<b>2.2.2 PHENOTYPIC METHODS</b> .....	<b>35</b>
2.2.2.1 <i>Morphological characteristics</i> .....	35
2.2.2.2 <i>Biovar differentiation</i> .....	36
2.2.2.3 <i>Physiological tests</i> .....	36
<b>2.2.3 MOLECULAR METHODS</b> .....	<b>37</b>
2.2.3.1 <i>DNA extraction</i> .....	37
2.2.3.2 <i>Polymerase Chain Reaction (PCR) of the 16S rRNA gene</i> .....	38
2.2.3.3 <i>PCR-RFLP</i> .....	38
2.2.3.4 <i>Amplified Fragment Length Polymorphism (AFLP) analysis</i> .....	40
<b>2.3 RESULTS</b> .....	<b>41</b>
<b>2.3.1. PHENOTYPIC METHODS</b> .....	<b>41</b>
2.3.1.1. <i>Morphological characteristics</i> .....	42
2.3.1.2. <i>Biovar differentiation</i> .....	42
<b>2.3.2. MOLECULAR METHODS</b> .....	<b>43</b>
2.3.2.1. <i>DNA extraction</i> .....	43
2.3.2.2. <i>PCR of the 16S rRNA gene</i> .....	44
2.3.2.3. <i>PCR-RFLP</i> .....	45
2.3.2.4. <i>AFLP analysis</i> .....	48
<b>2.4. DISCUSSION</b> .....	<b>50</b>

**CHAPTER 3: THE SUSCEPTIBILITY AND RESISTANCE OF DIFFERENT ECOTYPES OF *ARABIDOPSIS THALIANA* TO THE AFRICAN *EUCALYPTUS* STRAINS OF *RALSTONIA SOLANACEARUM***

<b>3.1. INTRODUCTION</b> .....	<b>54</b>
<b>3.2. MATERIALS AND METHODS</b> .....	<b>56</b>
<b>3.2.1. BACTERIAL ISOLATES</b> .....	<b>56</b>
<b>3.2.2. PLANT MATERIALS</b> .....	<b>56</b>
3.2.2.1. <i>Arabidopsis thaliana</i> ecotypes challenged in this study .....	56
3.2.2.2. <i>Seed preparation</i> .....	57
3.2.2.3. <i>Plant growth conditions</i> .....	58
<b>3.2.3. BACTERIAL INOCULATIONS</b> .....	<b>58</b>
3.2.3.1. <i>Preparation of the inoculum</i> .....	58
3.2.3.2. <i>Preparation of the plants</i> .....	58
<b>3.2.4. RATING OF DISEASE PROGRESS ON <i>A. THALIANA</i></b> .....	<b>59</b>
<b>3.2.5. EXPERIMENTAL PLANNING</b> .....	<b>59</b>
<b>3.2.6. MOLECULAR ANALYSIS BETWEEN THE ECOTYPES</b> .....	<b>60</b>
3.2.6.1. <i>Extraction of genomic DNA from <i>A. thaliana</i></i> .....	61
3.2.6.2. <i>Sequence analysis of the C-terminal region of the RRS1 locus in different <i>A. thaliana</i> ecotypes</i> .....	61
<b>3.2.7. MOLECULAR ANALYSIS OF THE POP P2 GENE IN <i>R. SOLANACEARUM</i></b> .....	<b>63</b>
3.2.7.1. <i>Pop P2 PCR</i> .....	63
<b>3.3. RESULTS</b> .....	<b>64</b>
<b>3.3.1. SCREENING OF THE ECOTYPES AGAINST THE STRAINS</b> .....	<b>64</b>



3.3.2. MOLECULAR ANALYSIS BETWEEN THE ECOTYPES.....	71
3.3.2.1. <i>Extraction of genomic DNA from A. thaliana</i> .....	71
3.3.2.2. <i>Sequence analysis of the C-terminal region of the RRS1 locus in different A. thaliana ecotypes</i> .....	72
3.3.3. MOLECULAR ANALYSIS OF THE POP P2 GENE IN <i>R. SOLANACEARUM</i> ...74	
3.3.3.1. <i>Pop P2 PCR</i> .....	74
3.4. DISCUSSION .....	75
CHAPTER 4: CHARACTERIZATION OF A NEW PATHOSYSTEM BETWEEN <i>ARABIDOPSIS THALLANA</i> AND AN AFRICAN ISOLATE OF <i>RALSTONIA SOLANACEARUM</i> FROM <i>EUCALYPTUS</i>	
4.1. INTRODUCTION .....	80
4.2. MATERIALS AND METHODS.....	83
4.2.1. BACTERIAL GROWTH CURVES.....	83
4.2.1.1. <i>Preparing rifampicin resistant (Rifr) mutants</i> .....	84
4.2.1.2. <i>Preparing hrp<sup>-</sup> mutants</i> .....	85
4.2.1.3. <i>Inoculations and dilution plating</i> .....	86
4.2.2. NORTHERN ANALYSIS.....	86
4.2.2.1. <i>Plant material harvested for Northern analysis</i> .....	86
4.2.2.2. <i>RNA isolation</i> .....	87
4.2.2.3. <i>RNA evaluation</i> .....	88
4.2.2.4. <i>Probe generation by PCR and purification</i> .....	88
4.2.2.5. <i>Probe labelling</i> .....	89
4.2.2.6. <i>Northern hybridisation</i> .....	90
4.2.2.7. <i>Autoradiography</i> .....	90
4.3. RESULTS.....	91
4.3.1. BACTERIAL GROWTH CURVES.....	91
4.3.1.1. <i>Rifampicin resistant (Rif) strains</i> .....	91





4.3.1.2. <i>HrpB</i> <sup>-</sup> / <i>HrcS</i> <sup>-</sup> mutants .....	91
4.3.1.3. <i>A. thaliana</i> analysis .....	93
4.3.2. NORTHERN ANALYSIS OF THE <i>A. THALIANA</i> ECOTYPES .....	97
4.3.2.1. RNA extraction and gel .....	97
4.3.2.2. Probe generation and labelling .....	97
4.3.2.3. Northern hybridisation .....	98
4.4. DISCUSSION .....	101
CHAPTER 5: CONCLUDING DISCUSSION .....	106
LITERATURE CITED .....	112
Appendices	
APPENDIX A : <i>Culture media</i> .....	124
APPENDIX B : <i>Primers used in this study</i> .....	126

## Summary

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*Ralstonia solanacearum* is the causal agent of bacterial wilt resulting in world-wide economic crop losses. The chief objective of this study was to develop a new pathosystem between *R. solanacearum* and *Arabidopsis thaliana*. The results obtained would enable researchers in Africa to limit disease spread due to a better knowledge of the pathogen as well as provide them with a better understanding of the mechanisms involved in plant defence.

The host plant used in the study was *A. thaliana* of which the whole genome has been sequenced. Growth conditions for *A. thaliana* plants in plant growth chambers in South Africa were investigated and subsequently optimized. Various *R. solanacearum* strains were characterized. This was achieved by implementing metabolic profiling and the polymerase chain reaction (PCR) of the hypersensitive response and pathogenicity (*hrp*) gene region. PCR-RFLP (restriction fragment length polymorphism) together with amplified fragment length polymorphism (AFLP) data grouped the *Eucalyptus* isolates into Biovar 3. This data showed that the PCR-RFLP enabled Biovar classification while the *hrp* PCR method was reliable for diagnosis and enables rapid identification of *R. solanacearum*.

Several ecotypes (Col-5, Nd-1, Kil-O, Be-O, Sf-2, Laer and Cvi) of *A. thaliana* were inoculated and disease development recorded, scoring wilt symptoms on a scale of 0-4. All strains were virulent on at least one ecotype. The Uganda isolate, BCC 0327 (27B), was the most pathogenic. BCC 0302 (CK) from the Congo, revealed a clear differential between the susceptible ecotype, Be-O and resistant ecotype, Kil-O. This was selected for further analysis. Non-virulent strains of *R. solanacearum* were obtained by direct transformation with genomic DNA from a strain carrying the desired knockout insertion (*hrpB* - or *hrcS* -) in the *hrp* gene.

After inoculating the plants with the respective virulent (CK Rif<sup>r</sup>) and non-virulent (hrcS<sup>-</sup>) strain, growth of the bacterial populations *in planta* was determined by dilution plating on selective media. A difference of one order of magnitude was present between the resistant and the susceptible ecotypes. Ten days after inoculation Be-O was completely wilted, while no symptoms had developed on Kil-O.

Northern analyses were performed using the Pathogenesis Related (PR)-genes. The data obtained revealed the absence of PR-1, PR-2 and PR-5 expression in Be-O, possibly explaining the rapid onset of disease development. These markers of the salicylic acid pathway were, however, induced in Kil-O conferring the absence of wilt symptoms and thus resistance.

Finally, a new *A. thaliana* - *R. solanacearum* pathosystem was developed, fit for transcriptome analysis. This will aid in the understanding of bacterial wilt, ultimately limiting further disease spread and conservation of vital nutritional food sources in Africa and other developing countries.

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## Opsomming

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*Ralstonia solanacearum* is die oorsaak van bakteriese verwelk wat wêreldwye ekonomiese verliese veroorsaak. Die hoof doel van hierdie studie was die ontwikkeling en daarstelling van 'n nuwe siektesisteen tussen *R. solanacearum* en *Arabidopsis thaliana*. Die uitkoms bemagtig dan die navorsers in Afrika om verdere verspreiding van die siekte hok te slaan asook die uitbreiding van hul kennis aangaande die weerstands meganismis betrokke by plant beskerming.

*Arabidopsis thaliana* was die gasheerplant wat in hierdie studie gebruik is, en die hele genoom is bekend. Groeitoestande van *A. thaliana* in plantgroeikabinette in Suid-Afrika is ondersoek en geoptimeer. Verskeie *R. solanacearum* isolate is ook gekarakteriseer deur gebruik te maak van metaboliese profiele en die polimerase ketting reaksie (PKR) van die hipersensitiewe reaksie and patogeniese (*hrp*) geen area. PKR-RFLP (Restriksie fragment lengte polimorfisme) tesame met AFLP (amplifiseerde fragment lengte polimorfisme) data het die *Eucalyptus* isolate gegroepeer in Biovar 3. Hierdie data bewys dan dat die PKR-RFLP tegniek Biovar klassifisering moontlik maak en die *hrp* PKR metode betroubaar is vir diagnostiese gebruik en dus winning identifisering van *R. solanacearum* bewerkstellig.

Verskeie ekotipes (Col-5, Nd-1, Kil-O, Be-O, Sf-2, Laer en Cvi) van *A. thaliana* is geïnokuleer en siekte ontwikkeling is waargeneem, verwelk simptome is geëvalueer op 'n skaal van 0-4. Alle bakteriese isolate was patogenies op ten minste een ekotipe. Die Uganda isolaat BCC 0327 (27B) was die virulentste. BCC 0302 (CK) van die Kongo, het 'n duidelike verskil getoon tussen die vatbare ekotipe, Be-O en die weerstandbiedende ekotipe, Kil-O. Dit is gekies vir verder navorsing. Nie-virulente isolate van *R. solanacearum* is bekom deur middel van direkte transformasie met genomiese DNS van 'n isolaat wat die verlangde uitklop invoeging (*hrpB* of *hrcS*) in die *hrp* geen bevat het.

Nadat die plante met die bepaalde virulente (CK Rif<sup>+</sup>) en nie-virulente (*hrcS*<sup>-</sup>) isolate geïnkuleer is, is die groei van die patogeen populasie *in planta* bepaal deur middel van verdunnings uitplating op selektiewe media. 'n Verskil van 1 orde van betekenis is gevind tussen die weerstandbiedende en vatbare ekotipes. Tien dae na inokulasie was Be-O heeltemal verlep, terwyl Kil-O geen simptome getoon het nie.

Northern analyses is ook uitgevoer en die Patogeen Verwante (PR)-genes is gebruik. Die data het die afwesigheid and die PR-1, PR-2 en PR-5 uitdrukking in Be-O getoon, dit verklaar moontlik die vinnig aanvang van verwelk en siekte ontwikkeling. Hierdie merkers vir die Salisielsuur padweg is egter geïnduseer in Kil-O wat moontlik verantwoordelik is vir die weerstandbiedenheid van die ekotipe.

Uiteindelik is 'n nuwe *A. thaliana* - *R. solanacearum* ontwikkel, geskik vir transkriptoom/microarray analise. Dit sal verder bydra tot die kennis van bakteriële verwelk, met 'n beslissende inperking op die verspreiding van die siekte asook beskerming van noodsaaklike voedsel bronne in Afrika en ander ontwikkelende lande

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## List of Abbreviations

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aa	Amino acids
<i>Avr</i>	Avirulence
B	Bouchers' liquid media
BGT	Bacto-agar <u>G</u> lucose <u>T</u> riphenyltetrazolium chloride
bp	Base pairs
CFU	Colony Forming Units
CTAB	Hexadecyl trimethyl ammonium bromide
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanine triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Ethylenediamine tetraacetic acid
EtBr	Ethidium Bromide
EtOH	Ethanol
HR	Hypersensitive response
hr	hour(s)
<i>hrc</i>	<i>hrp</i> -conserved
<i>hrp</i>	hypersensitive response and pathogenicity
kb	Kilobase pairs
Mb	Megabase pairs
min	Minutes
MM	Molecular marker
MS	Murashige and Skoog
NaAc	Sodium acetate
OD	Optical density



PCR	Polymerase chain reaction
RE	Restriction Enzyme
Rif <sup>r</sup>	Rifampicin resistant
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
s	seconds
SDS	Sodium dodecyl sulfate
TAE	Tris-acetate ethylenediamine tetraacetic acid
TE	Tris-ethylenediamine tetraacetic acid
Tris	Tris hydroxy methyl aminoethane
TTC	Triphenyltetrazolium chloride



## Index of Figures

	Page
<b>Figure 1.1.</b> Overview of local and systemic signaling in <i>A. thaliana</i> disease resistance.	29
<b>Figure 2.1.</b> Location of the three selective primer pairs (RS20-RS201, RS600-RS61, RS80-RS81), within the <i>hrp</i> genes of <i>R. solanacearum</i> reference strain GMI 1000 (Poussier <i>et al.</i> , 1999)	39
<b>Figure 2.2.</b> Characteristic mucoid colonies of virulent <i>R. solanacearum</i> isolates on BGT medium.	42
<b>Figure 2.3.</b> Genomic DNA of <i>R. solanacearum</i> extracted using the method of Chen and Kuo (1993).	44
<b>Figure 2.4.</b> PCR amplification of the 16S rRNA region of the bacterial isolates	44
<b>Figure 2.5.</b> Selective amplification of <i>hrpC/B</i> gene of <i>R. solanacearum</i> using primers RS600-RS61.	45
<b>Figure 2.6.</b> Three PCR fragments of the <i>hrp</i> cluster amplified from <i>R. solanacearum</i> .	46
<b>Figure 2.7.</b> Restriction patterns: A) Fragment 1 digested with <i>Ava</i> I (AAv), B) Fragment 1 digested with <i>Pvu</i> II (APv), C) Fragment 2 digested with <i>Hae</i> II (BHa) and D) Fragment 3 digested with <i>Bss</i> HIII (CBs).	47
<b>Figure 2.8.</b> AFLP data viewed as an image on high performance autoradiogram film.	49
<b>Figure 2.9.</b> AFLP data viewed as a dendrogram	49
<b>Figure 3.1.</b> Illustrating the different wilt symptoms (ws) on Col-5 inoculated with the control strain BCC 0300 (GMI 1000).	60
<b>Figure 3.2.</b> Nucleotide sequence of a section of the C-terminal end of the <i>RRS1</i> allelic structure of Col-0.	62
<b>Figure 3.3.</b> Disease symptoms after 18 days in the various <i>A. thaliana</i> ecotypes upon inoculation with the control strain, (A) BCC 0300 (GMI 1000), and strain (B) BCC 0302 (CK).	65
<b>Figure 3.4.</b> Disease symptoms in various <i>A. thaliana</i> ecotypes after root inoculation by different strains of <i>R. solanacearum</i> .	67

<b>Figure 3.5.</b>	Progression of disease symptoms in the different <i>A. thaliana</i> ecotypes over 18 days after root inoculation by the <i>R. solanacearum</i> control strain, BCC 0300 (GMI 1000) during the three replicate experiments (A, B and C).	68
<b>Figure 3.6.</b>	Progression of disease symptoms in the different <i>A. thaliana</i> ecotypes over 18 days after root inoculation by the <i>R. solanacearum</i> isolate BCC 0302 (CK) during the three experiments (A, B and C).	69
<b>Figure 3.7.</b>	A comparison between the most susceptible ecotypes, Be-O and Col-5, with the more resistant ecotypes, Kil-O and Sf-2, over 19 days after inoculated with isolate BCC 0302 (CK).	70
<b>Figure 3.8.</b>	Illustration of wilt symptoms of selected <i>A. thaliana</i> ecotypes, Be-O, Col-5 and Kil-O, one week after inoculation with <i>R. solanacearum</i> strains BCC 0302 (CK) and BCC 0300 (GMI 1000) compared to the water control.	71
<b>Figure 3.9.</b>	DNA extracted from selected <i>A. thaliana</i> ecotypes	72
<b>Figure 3.10.</b>	<i>A. thaliana</i> DNA amplified with RRS1 primers	73
<b>Figure 3.11.</b>	PCR amplification of the PopP2 gene of <i>R. solanacearum</i> using primer pair, Pop P2-1 and Pop P2-7.	74
<b>Figure 4.1.</b>	Genetic organization of the <i>R. solanacearum</i> <i>hrp</i> gene cluster	82
<b>Figure 4.2.</b>	Model for the role of the different Hrp proteins in the assembly of <i>R. solanacearum</i> type III secretion apparatus	82
<b>Figure 4.3.</b>	The presence and absence of HR respectively, on the nonhost tobacco cv. Bottom Special generated with Rif <sup>r</sup> strains	92
<b>Figure 4.4.</b>	The absence of HR when the <i>hrcS</i> / <i>hrpB</i> virulence gene has been disrupted	92

- Figure 4.5.** Wilt symptoms observed 12 days after inoculation on Be-O and Col-5 inoculated with BCC 0352 (CK Rif<sup>r</sup>) and the absence of wilt symptoms after inoculation with BCC 0372 (CK *hrcS*<sup>-</sup>). 94
- Figure 4.6.** Internal bacterial growth curves of BCC 0352 (CK Rif<sup>r</sup>) and BCC 0372 (*hrcS*<sup>-</sup>) strains of *R. solanacearum* in leaves of *A. thaliana* Be-O, Col-5 and Kil-O plants after root inoculation. 94
- Figure 4.7.** The changes in humidity (A), temperature (B) and light (C) during the 14 days after inoculation. 96
- Figure 4.8.** Formaldehyde-agarose electrophoresis of total RNA isolated from *A. thaliana* ecotypes 97
- Figure 4.9.** Agarose gel electrophoresis of the PCR probes products prior to probe labelling 98
- Figure 4.10.** (A) Progression of disease symptoms and (B) northern analysis of PR proteins in three *A. thaliana* ecotypes inoculated with *R. solanacearum* strain BCC 0302 (CK). 98

## Index of Tables

---

	<b>Page</b>
<b>Table 1.1.</b> Characteristics of races and their relationship to Biovars and RFLP subdivisions of <i>R. solanacearum</i>	11
<b>Table 2.1.</b> Isolates of <i>R. solanacearum</i> used in this study	35
<b>Table 2.2.</b> Differentiation of <i>Ralstonia solanacearum</i> isolates into Biovars based on the ability to metabolise hexose alcohols and disaccharides	43
<b>Table 2.3.</b> Classification of the PCR-RFLP patterns identified between the eight strains of <i>R. solanacearum</i> into Biovars	48
<b>Table 3.1.</b> Phenotypic description of Ecotypes used in this study	57
<b>Table 3.2.</b> Rating scale of wilted <i>A. thaliana</i> plants inoculated with <i>R. solanacearum</i> .	59
<b>Table 3.3.</b> Presence of stop codon in the <i>RRS1</i> gene of the <i>A. thaliana</i> ecotypes	73
<b>Table 4.1.</b> Isolates of <i>R. solanacearum</i> used in this study	94
<b>Table 4.2.</b> Characteristics of Northern probes	98