# 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.

Weich

Johanna Petronel Weich

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# Summary

*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 r) and non-virulent (hrcS -) 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.

# Opsomming

*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 siektesisteem 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 geoptimiseer. 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 vinning identifisering van *R. solanacearum* bewerkstellig.

Verskeie ekotipes (Col-5, Nd-1, Kil-O, Be-O, Sf-2, Laer en Cvi) van *A. thaliana* is geinokuleer 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*$^-$ or *hrcS*$^-$) in die *hrp* geen bevat het.

Nadat die plante met die bepaalde virulente (CK Rif $^{r}$) en nie-virulente (*hrcS* $^{-}$) isolate geinokuleer 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 analises 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 geinduseer 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/microarry analise. Dit sal verder bydra tot die kennis van bakteriese 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

| | |
|---|---|
| aa | Amino acids |
| *Avr* | Avirulence |
| B | Bouchers' liquid media |
| BGT | Bacto-agar Glucose Triphenyltetrazolium chloride |
| bp | Base pairs |
| CFU | Colony Forming Units |
| CTAB | Hexadecyl trimethyl ammonium bromide |
| dATP | Deoxyadenosine triphosphate |
| dCTP | Deoxycytosine triphosphate |
| dGTP | Deoxyguanidine 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) |
| *hrc* | *hrp*-conserved |
| *hrp* | 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 |
| $Rif^r$ | 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 |

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