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Microarray expression studies in the model plant *Arabidopsis thaliana* infected with the bacterial pathogen *Ralstonia solanacearum*

By

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DECLARATION

I, the undersigned hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria, is the result of my own investigation, unless specifically indicated to the contrary in the text, and has not been submitted for any degree at any other university or faculty.

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PREFACE

This thesis is a compilation of three microarray studies conducted over five years pertaining to the investigation of defence responses against *Ralstonia solanacearum* in the model plant *Arabidopsis thaliana*. Each research Chapter has been written in the format of publishable units.

At the outset, it should be mentioned that the transcript profiling and bioinformatics approach to find genes involved in a biological process is limited. Further characterisation of these genes, such as gene function studies, is necessary to determine whether the gene does have a role in resistance or susceptibility.

Chapter 1 is a literature review, which discusses the pathogen *R. solanacearum* and the model plant *Arabidopsis thaliana*, the defence responses within plants and the use of microarray technology to study plant-pathogen interactions.

Chapter 2 is an extension of the literature review focusing on considerations for the design of microarray experiments aimed at an audience that wishes to embark on microarray experiments for the first time. This Chapter has been published in the South African Journal of Science in 2005 (Naidoo S., Denby K.J. and Berger D.K. (2005) Microarray experiments: considerations for experimental design. South African Journal of Science 101, 347-354).

Chapter 3 represents the first microarray expression profiling experiment conducted towards optimising the technology. The results of this experiment provided interesting candidate genes that could be involved in defence against *Pst* in the Arabidopsis mutant *cir1*. This Chapter was published in the South African Journal of Botany in 2007 (Naidoo S., Murray S.L., Denby K.J. and Berger D.K. (2007). Microarray analysis of the Arabidopsis thaliana *cir1* (*c*onstitutively *i*nduced *r*esistance 1) mutant reveals candidate defence response genes against *Pseudomonas syringae* pv *tomato* DC3000. South African Journal of Botany 73, 412-421).

Chapter 4 investigates the differential expression pattern in Arabidopsis ecotype Col-5, which is susceptible to *R. solanacearum*. This Chapter has been written in the format of an article aimed at the Journal of Functional Plant Biology and will be submitted for review shortly.

Chapter 5 deals with a resistant interaction between Arabidopsis ecotype Kil-0 and a Eucalyptus isolate of *R. solanacearum*. The basis of this resistance is explored at the transcript level using whole genome micoarrays.

Finally, Chapter 6 provides a summary of the results obtained in this study and provides a comparison of susceptible and resistant interactions against *R. solanacearum*. The impact of this research in understanding the plant defence response against *R. solanacearum* is discussed and future research is considered.

SUMMARY

Ralstonia solanaearum, a soil borne pathogen infects several important crops causing wilting. In 2000-2001, two eucalyptus isolates, BCCF 401 and BCCF 402 were isolated from plantations in Kwa-Zulu Natal and the Democratic Republic of Congo, respectively. *Arabidopsis* has been recognised as a host for *R. solanacearum* and as such has been adopted as a model to understand the plant defence response against this pathogen. The aim of this study was to use microarray expression profiling techniques to elucidate the plant defence response and to identify candidate genes possibly contributing towards resistance against the pathogen. As a means to optimise microarray expression profiling, the differential expression in an *Arabidopsis* mutant, *cir1* (constitutively induced resistance 1) and wild-type plants was investigated using a custom 500-probe microarray. Several genes were found to be induced in *cir1* at a significance threshold of $-\log_{10}(p)$ equal to 3 ($p < 0.001$) using a mixed model ANOVA approach. The genes AtACP1 (sodium inducible calcium binding protein), AtP2C-HA (protein phosphatase 2C), AtGSTF7 (glutathione S transferase), tryptophan synthase beta-like and AtPAL1 (phenylalanine ammonia lyase 1), AtEREBP-4 (ethylene response element binding protein 4) and HFR1 (long hypocotyl in far-red 1) were further identified as possible candidate genes which may contribute to disease resistance in *cir1* against *Pseudomonas syringae* pv. *tomato.*

A similar transcript profiling approach, using the optimised protocols, was adopted to investigate the compatible interaction between *Arabidopsis* ecotype Col-5 and the *R. solanacearum isolate* BCCF 401. A screen of 5000 *Arabidopsis* ESTs revealed approximately 120 genes differentially regulated by *R. solanacearum* infection at a significance threshold of $p<0.03$ (Bonferroni corrected). Subsequent bioinformatic comparisons revealed that abscisic acid responses appear to be induced in Col-5 in response to the pathogen and that *R. solanacearum* induces an expression profile consistent with a necrotroph. The basal defence responses in Col-5 against *R. solanacearum* infection were investigated by comparing the expression data to that during treatment with the pathogen associated molecular patterns (PAMPs) flg22 and lipopolysaccharide, and the Type Three Secretion System deficient *Pst hrp*$^-$ mutant. Expression patterns for a subset of these genes were suggestive of host basal defences manipulated by the pathogen. It is hypothesised that genetic engineering to alter the expression of these “pathogen-manipulated” genes could contribute to resistance against *R. solanacearum* in the host.

In order to further elucidate the defence response to *R. solanacearum*, expression profiling was performed in the resistant ecotype Kil-0 challenged with isolate BCCF 402 using whole-genome *Arabidopsis* microarrays. Thirteen genes were found to be differentially expressed in Kil-0 at a p-value <0.01 and fold change greater than 1.65. Using a quantitative RT-PCR approach, it was shown that the expression of of lipid transfer protein 3 (LTP3), peroxidase (PRX34), tropinone reductase (SAG13), avirulence-induced gene (AIG), translation initiation factor (SUI1), SKP1 interacting partner 5 (SKP5) and an "expressed protein" are preferentially expressed to a higher level earlier in the resistant interaction than in the susceptible one. The role of these genes in defence against the pathogen remains to be elucidated by gene function studies. The current study has, however allowed the identification of important candidate genes that could be targeted in future to improve resistance against *R. solanacearum* in *Eucalyptus.*

ABBREVIATIONS

ABA	abscisic acid
Avr	avirulence
BCCF	Bacterial Culture Collection FABI
BGT	Bacto-agar Glucose Triphenyltetrazolium chloride
bp	base pairs
cDNA	complementary DNA
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dpi	days post inoculation
EDTA	ethylenediamine tetraacetic acid
EST	expressed sequence tag
ET	ethylene
hr	hour
HR	hypersensitive response
ISR	induced systemic resistance
JA	jasmonic acid
kb	kilobase
min	minute
mRNA	messenger ribonucleic acid
MeJA	methyljasmonate
MS	Murashige and Skoog media
ng	nanogram
NO	nitric oxide
PCR	polymerase chain reaction
pmol	picomole
PR	pathogenesis related
qRT-PCR	quantitative reverse transcriptase PCR
Rif^r	rifampicin resistant
RNA	ribonucleic acid
RNAse	ribonuclease
ROS	reactive oxygen species
rpm	revolutions per minute

RT	reverse transcriptase
SA	salicylic acid
SAR	systemic acquired resistance
SDS	sodium dodecyl sulphate
SSC	sodium chloride / sodium citrate
UV	ultraviolet
μg	microgram