Elucidation of defence response mechanisms in pearl millet

by

Bridget Genevieve Crampton

Submitted in partial fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

Department of Botany
in the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

September 2006

Supervisor: Prof. D.K. Berger

Declaration

I, the undersigned hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria, contains my own independent work and hitherto has not been submitted for any degree at any university or faculty.

BG Crampton
September 2006

Summary

Thesis title: Elucidation of defence response mechanisms in pearl millet
Student: Bridget Genevieve Crampton
Supervisor: Professor D.K. Berger
Department: Botany
Degree: PhD

Studies were undertaken to elucidate defence response mechanisms in pearl millet, a non-model cereal crop. This was accomplished through the construction and characterisation of a pearl millet defence response cDNA library, which was subsequently utilised in large scale gene expression studies to profile pearl millet's response to the defence signalling compounds nitric oxide (NO), methyl jasmonate (MeJA) and salicylic acid (SA), and to the rust *Puccinia substriata* var. *indica*.

A pearl millet cDNA library was constructed by treating pearl millet plants with the defence elicitors chitin and flagellin, and by wounding the plants. Suppression subtractive hybridisation (SSH) was employed to enrich the library for defence response transcripts. The SSH library was characterised using a quantitative cDNA microarray-based screening method that enabled identification of false positive transcripts and clones that represented rare or abundant transcripts. Based on this screening method, clones were selected for sequence analysis, which identified genes known to play important roles in defence response.

The pearl millet SSH defence response library was arrayed onto a glass slide and used in transcript profiling studies to examine pearl millet's response to the defence signalling molecules NO, MeJA and SA. Whilst only 45 cDNAs responded significantly to NO treatment, 279 and 224 cDNAs responded to MeJA and SA sprays, respectively. Closer examination of MeJA and SA responsive genes revealed that many of the induced transcripts were common to both signalling pathways, demonstrating that a substantial network of regulatory interactions exists between the salicylate and jasmonate pathways.

Transcript profiling of a susceptible pearl millet line in response to virulent rust infection revealed that genes common to both the jasmonate and salicylate

pathways were induced. However, pathology studies indicated that pretreatment of pearl millet with SA conferred resistance to a virulent isolate of rust, whereas MeJA application did not significantly reduce subsequent infection levels. In view of these pathology results, it is probably genes that are significantly induced in response to SA that actually confer resistance to avirulent rust isolates.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the following people and organisations for helping to make this thesis a reality:

My supervisor, Prof. Dave Berger, for his advice, enthusiasm and input into my PhD, and supervision over the past five years;

Dr Fourie Joubert and Danie Theron for their willing assistance with microarray data analyses;

Lufuno Petrus Ngomani for help with sequencing pearl millet clones and performing subsequent GenBank homology searches;

CSIR Biosciences for affording me the time and opportunity to complete my studies whilst in their employment, and for funding the project for four consecutive years;

The African Centre for Gene Technologies for project financial support over a four year period;

My fellow students from the “fruit salad” lab for helpful discussions and technical advice;

My work colleagues at CSIR Biosciences for technical assistance and continuous encouragement;

My parents, Gayle and Ernie Campbell, for always believing in my capabilities and inspiring me to follow my dreams; and

My husband Michael for his love, open mindedness and emotional support throughout the duration of my studies.

PREFACE

This thesis presents a collection of studies conducted over five years that deal with molecular based investigations into defence response mechanisms in the non-model crop plant pearl millet. The thesis is presented as a compilation of five chapters, with each chapter being introduced separately. All studies were conducted independently and have been written as separate publishable units. Thus, some repetition between parts of chapters, which contain a progression of knowledge accumulated over a period of time, has been unavoidable.

CHAPTER 1: Literature review – overview of plant defence response mechanisms.

CHAPTER 2: Construction and characterisation of a pearl millet defence response cDNA library.

CHAPTER 3: Nitric oxide mediated transcriptional changes in pearl millet.

CHAPTER 4: Evaluation of pearl millet defence signalling pathways involved in leaf rust (*Puccinia substriata)* resistance and perception.

CHAPTER 5: Concluding remarks and future prospects.

Excerpts from Chapter 2 have appeared in the publications listed below.

Berger, D. K., B. Crampton, I. Hein, and W. Vos. 2006. Screening cDNA libraries on glass slide microarrays, *In*: J. M. Walker (ed.), DNA Arrays. Humana Press, Totowa, New Jersey, USA.

Van den Berg, N., B. G. Crampton, I. Hein, P. R. J. Birch, and D. K. Berger. 2004. High-throughput screening of suppression subtractive hybridization cDNA libraries using DNA microarray analysis. Biotechniques **37**:818-824.

Chapters 3 and 4 have been written up as publishable units and will be shortly submitted to international peer reviewed journals for publication.

ABSTRACT

Pearl millet is a staple food source for millions of African families living in semi-arid regions of the continent. Yet, despite its importance and ability to provide consistent yields, very little research and resources have been directed towards understanding mechanisms governing this crop's resilience to biotic and abiotic stresses. The research outlined in this thesis therefore aimed to elucidate defence response mechanisms in pearl millet, a non-model cereal crop. This was accomplished through the construction and characterisation of a pearl millet defence response cDNA library, which was subsequently utilised in large scale gene expression studies to profile pearl millet's response to the defence signalling compounds nitric oxide (NO), methyl jasmonate (MeJA) and salicylic acid (SA), and to the biotrophic rust fungus *Puccinia substriata* var. *indica*.

A pearl millet cDNA library was constructed by treating pearl millet plants with the defence elicitors chitin and flagellin, and by wounding the plants. Suppression subtractive hybridisation (SSH) was employed to enrich the library for defence response transcripts. In order to characterise the cDNA libraries, a quantitative cDNA microarray-based screening method was developed that enabled identification of false positive transcripts, as well as clones that represented rare or abundant transcripts. Based on this screening method, a number of clones were selected for sequence analysis, and their identity ascertained through homology searches with previously sequenced genes. This revealed a number of genes known to play important roles during pathogen attack.

The pearl millet SSH defence response library, consisting of 1920 cDNAs either up- or down regulated in defence response, was spotted onto a glass slide microarray and used in transcript profiling studies to examine pearl millet's response to the defence signalling molecules NO, MeJA and SA. Whilst only 45 cDNAs responded significantly to NO treatment, 279 and 224 cDNAs responded to MeJA and SA sprays, respectively. Closer examination of MeJA and SA responsive genes revealed that many of the induced

transcripts were common to both signalling pathways, demonstrating that a substantial network of regulatory interactions exists between the salicylate and jasmonate pathways, which were previously believed to act in an antagonistic manner.

Pathology studies indicated that pretreatment of pearl millet with SA conferred resistance to a virulent isolate of *P. substriata* var. *indica*, whereas MeJA application did not significantly reduce subsequent infection levels. Transcript profiling of a susceptible pearl millet line in response to virulent rust infection revealed that genes common to both the jasmonate and salicylate pathways were induced, suggesting that the plant adopts elements from a number of defence signalling pathways in an attempt to ward off infection by the virulent rust fungus. However, in view of results obtained from pearl millet defence signalling molecule pretreatments, it is probably genes that are significantly induced in response to SA, but to a lesser extent by MeJA that actually confer resistance to an avirulent rust isolate. Treatment of pearl millet plants with an avirulent *P. substriata* strain and subsequent microarray analysis would answer this hypothesis by revealing whether an incompatible reaction elicits more elements of the salicylate defence response pathway.

ABBREVIATIONS

AFLP	amplified fragment length polymorphism
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base pairs
cDNA	complementary DNA
DIG	digoxygenin
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
EDTA	ethylenediamine tetraacetic acid
ER	expression ratio
EST	expressed sequence tag
ET	ethylene
h	hour
HR	hypersensitive response
IPTG	isopropyl-β-D-thiogalactopyranoside
ITS	internal transcribed spacer region
JA	jasmonic acid
kb	kilobase
kDa	kilodalton
LB	Luria Bertani
min	minute
mRNA	messenger ribonucleic acid
MeJA	methyl jasmonate
MS	Murashige and Skoog media
NBT	nitroblue tetrazolium chloride
ng	nanogram
NO	nitric oxide
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pg	picogram
pmol	picomole
PR	pathogenesis related

qPCR	quantitative PCR
RNA	ribonucleic acid
RNAase	ribonuclease
ROS	reactive oxygen species
rpm	revolutions per minute
RT	reverse transcription
SA	salicylic acid
SAR	systemic acquired resistance
SDS	sodium dodecyl sulphate
SNP	sodium nitroprusside
SSC	sodium chloride/sodium citrate
SSH	suppression subtractive hybridisation
TAE	Tris-acetate ethylenediamine tetraacetic acid
TCA	Trichloroacetic acid
TE	Tris ethylene diamine tetracetic acid
UV	ultraviolet
µg	microgram
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside