Insight into three putative *Cercospora zeina* effector genes and the role they play in virulence

By

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DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree *Magister Scientiae* to the University of Pretoria, contains my own independent work and has not been submitted by me for a degree at this or any other tertiary institution.

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Date

PREFACE

Maize is an important crop grown in many regions worldwide and is considered as one of the major staple food sources globally. Alternatively, maize can also be used in bio-fuel production, as raw material for industrial products, as fodder, and many more. Many factors influence the production of maize, but one of the most important diseases influencing maize production is Grey Leaf Spot (GLS) disease. GLS disease has been associated with major maize yield losses in many maize growing regions. Two sibling fungal species have been identified as the casual agents for GLS disease, namely *Cercospora zeae-maydis* and *Cercospora zeina*. *C. zeina* is associated with GLS affecting African maize production. As no effective and sustainable management strategy for GLS disease has been identified, research needs to be focussed on the establishment of effective control mechanisms.

Pathogens such as fungi make use of effector molecules to be able to evade detection by the host or to manipulate the host defence system. Effectors therefore enable a pathogen to cause disease in the absence of a putative host resistance gene. Nothing is known about the mechanisms of maize infection of *C. zeina* on a molecular level. Identification of *C. zeina* effectors would aid in our understanding of host-pathogen interactions to be able to develop effective control strategies.

The **aim of this MSc study** was therefore to determine if homologs of the previously identified effectors *Avr4*, *Ecp2*, and *Ecp6* were present in the draft genome of an African isolate of *C. zeina*. The identified *C. zeina* effector homologs were annotated and the expression profiles of these effectors were determined and correlated with fungal quantities *in planta*.

All work presented in this dissertation is based on experiments conducted in the Cereal Foliar Pathogen Research Laboratory at the University of Pretoria, South Africa. The dissertation is presented in the form of four separate chapters. Due to the nature of this style, some repetition between chapters and the literature review was unavoidable.

Chapter 1 provides a comprehensive overview of GLS disease of maize and plant-fungal interactions. The chapter focussed on the disease cycle, symptoms, and management strategies of GLS as well as plant-fungal interactions and resistance, defence signalling in plants, and the function and evolution of Dothideomycete effectors. **Chapter 2** describes the identification and annotation process of the *C. zeina Avr4*, *Ecp2*, and *Ecp6* effectors. The predicted sequences of these effectors were also analysed through protein alignments and

phylogenetics. In **Chapter 3**, *in planta* expression profiles of the *C. zeina Avr4*, *Ecp2*, and *Ecp6* effectors were elucidated across eight time points. Two *C. zeina* reference genes (*GAPDH* and *Cyt III*) were also identified for effective normalisation of *in planta* expression levels. Fungal quantity at each time point was determined and correlated with the relative expression levels of each effector. **Chapter 4** consists of a general discussion on the results and conclusions obtained from the two research chapters as well as future prospects based on the results.

Research done in this dissertation have been presented at FABI (Forestry and Agricultural Biotechnology Institute) seminar meetings, a post-graduate symposium held at the University of Pretoria in 2012, and at the GRI (Genome Research Institute) symposium at the University of Pretoria in 2013. Research findings from chapter two and three will be written up as a publication and will be submitted to an international peer-reviewed journal for publication.

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LIST OF ABBREVIATIONS

2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
aa	amino acids
ABA	absissic acid
Avr	avirulence gene
BIC	biotrophic interfacial complex
BLAST	basic local alignment search tool
BLASTn	search a nucleotide database using a nucleotide query
BLASTp	search a protein database using a protein query
BME	β-mercaptoethanol
bp	base pair
CBM14	chitin-binding Peritrophin-A
cDNA	complimentary DNA
Ct	threshold cycle
CTAB	cetyl trimethylammonium bromide
CV	pair wise variation
d	rate of change
DAMP	damage-associated molecular pattern
DEPC	diethylpyrocarbonate
dNTP	deoxynucleoside triphosphates
dpi	days post inoculation
dsRNA	double stranded RNA
Ecp	extracellular protein
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
ETI	effector-triggered immunity
e-value	expected value
g	gravitational force
gDNA	genomic DNA
GFP	green fluorescent protein
GlcNac	N-acetyl-D-glusamine
GLS	grey leaf spot
Hce2	homologs of *C. fulvum Ecp2*
HGT	horizontal gene transfer
HIGS	host induced gene silencing

HR	hypersensitive response
IPTG	isopropyl-β-D-thiogalactopyranoside
JA	jasmonic acid
Kb	kilobyte
kDa	kilodalton
LRR	leucine-rich repeat
LysM	lysin motif
M	gene stability
MIQE	minimum information for publication of RT-qPCR experiments
ML	maximum likelihood
mM	millimolar
MOPS	N-morpholinopropanesufonic acid
mRNA	messenger RNA
NB-LRR	nucleotide binding leucine-rich repeat
NCBI	National Centre for Biotechnology Institute
ng	nanogram
NJ	neighbourhood-joining
NRQ	normalised relative expression
nt	nucleotide
NTC	non-template control
PAMP	pathogen associated molecular pattern
PCR	polymerase chain reaction
PDA	potato dextrose agar
PRR	pathogen recognition receptor
PTI	PAMP- triggered immunity
P-value	statistical significance
PVP	polyvinylpyrroilidone
PVX	potato virus X
QDR	quantitative disease resistance
qPCR	quantitative PCR
QTL	quantitative trait loci
R^2	correlation coefficient
SEM	standard error of the mean
RFU	relative fluorescence units
R-gene	resistance gene
RLP	receptor-like protein
RNAseq	RNA sequence

ROS	reactive oxygen species
rpm	revolutions per minute
rRNA	ribosomal RNA
RT-PCR	reverse transcription PCR
RT-qPCR	real-time quantitative PCR
R-value	Pearson's correlation value
SA	salicylic acid
SAR	systemic acquired resistance
SDS	sodium dodecyl sulphate
SP	signal peptide
T	temperature
TAE	Tris-acetate-EDTA
tBLASTx	search a translated nt database using a translated nt query
T_m	melting temperature
U	enzyme units
µg	microgram
µl	microliter
µM	micromolar
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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