Analysis of gene expression in *Triticum aestivum* L. cv. 'Tugela DN' after Russian wheat aphid (*Diuraphis noxia* Mordvilko) infestation.

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, contains my own independent work. This work has hitherto not been submitted for any degree at any other University faculty.

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Publications resulting from this thesis

Botha A-M, Lacock L., Van Niekerk C., Matsioloko M.T., Du Preez F.B., Myburg A.A., Kunert K. & Cullis C.A. 2003. *Transcriptome changes in response to Diuraphis noxia feeding in Triticum aestivum utilizing a custom array.* (To be submitted)

Botha A-M, Lacock L., Van Niekerk C., Matsioloko M.T., Du Preez F.B., Myburg A.A., Kunert K. & Cullis C.A. 2003. *Gene expression profiling during Diuraphis noxia infestation of Triticum aestivum cv. 'Tugela DN' using microarrays.* (Proceedings of the 10th international wheat genetics symposium, Paestum, Italy. 1-6 September 2003. Vol. 1 p. 334-338)

Lacock L., Van Niekerk C., Loots S., Du Preez F. & Botha A-M. 2003. *Functional and comparative analysis of expressed sequences from Diuraphis noxia infested wheat obtained utilizing the conserved Nucleotide Binding Site.* African Journal of Biotechnology 2(4): 75-81.

Van Niekerk C. & Botha A-M. 2003. *Using Suppression subtractive hybridization (SSH) to screen for novel sequences expressed in response to Russian wheat aphid feeding.* (Proceedings of the 10th international wheat genetics symposium, Paestum, Italy. 1-6 September 2003. Vol. 1 p. 1281-1283)

Van Niekerk C. & Botha A-M. 2003. *Isolation and Characterization of cDNA sequences from Russian wheat aphid induced 'Tugela DN' (Dn1) libraries.*
(GenBank accession numbers: BU808656, BU808657, BU808658, BU808659, BU808660, CA407984, CA407985, CB412196, CB412197, CB412198, CB412199, CB412200, CB412201, CB412202, CB412203, CB412204, CB412205, CB412206, CB412207, CB412208, CB412209, CB412210, CB412211, CB412212, CB412213, CB412214, CB412215, CB412216, CB412217, CB412218, CB412219, CB412220, CB412221, CB412222, CB412223, CB412224, CB412225, CB412226, CB412227, CB412228, CB412229, CB412230, CB412231, CB412232, CB412233, CB412234, CB412235, CB412236, CB412237, CB412238, CB412239, CB412240, CB412241, CB412242, CB412243, CB412244, CB412245, CB412246, CB412247, CB412248, CB412249, CB412250, CB412251, CB412252, CB412253, CB412254, CB412255, CB412256, CB412257, CB412258; www.ncbi.nlm.nih.gov/BLAST)

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"For when the road you're travelling on seems difficult at best, you only have to trust in God – He will do the rest"

List of Abbreviations

AA	-	*Triticum urartu* genome
AABBDD	-	*Triticum aestivum* genome
AFLP	-	amplified length polymorphism
ATP	-	adenosine triphosphate
avr	-	avirulence gene
BB	-	*Aegilops speltoides* genome
BC	-	before Christ
BLAST	-	Basic Local Alignment Search Tool
bp	-	base pair
BSA	-	bovine serum albumin
°C	-	degree centigrade
CC	-	coiled coil
cDNA	-	complimentary DNA
cfu	-	colony forming units
cm	-	centimetre
CNL	-	coiled-coil –NBS-LRR
DDRT	-	differential display reverse transcriptase
DEB	-	DNA extraction buffer
DEPC	-	diethyl pyrocarbonate
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxy nucleotide triphosphate
EDTA	-	ethylenediamine tetra acetic acid
EST	-	expressed sequence tag
EtOH	-	ethanol
e-value	-	expected value
g	-	gram
GTP	-	guanosine triphosphate
HCl	-	hydrochloric acid
HR	-	hypersensitive response

HSP	-	heat shock protein
IPTG	-	isopropylthio-β-D-galactoside
JA	-	jasmonic acid
KCl	-	potassium chloride
LB	-	Luria Bertani
LRR	-	leucine-rich repeat
LZ	-	leucine zipper
m	-	meter
M	-	molar
µg	-	microgram
mg	-	milligram
$MgCl_2$	-	magnesium chloride
mM	-	millimolar
µl	-	microlitre
ml	-	millilitre
mm	-	millimeter
mRNA	-	messenger RNA
MS	-	multiple sclerosis
NaCl	-	sodium chloride
NaOAc	-	sodium acetate
NCBI	-	National Center for Biotechnological Information
NBS	-	nucleotide binding site
ng	-	nanogram
nm	-	nanometer
PCD	-	programmed cell death
PCR	-	polymerase chain reaction
PEG	-	polyethylene glycol
pg	-	picogram
pmol	-	picomol
PR	-	pathenogenesis-related
r	-	regression coefficient
RACE	-	rapid amplification of 5' and 3' cDNA ends

RAPD	-	random amplified polymorphic DNA
RDA	-	representational difference analysis
RFLP	-	random fragment length polymorphism
RGA	-	resistance gene analogue
R gene	-	resistance gene
RNA	-	ribonucleic acid
rRNA	-	ribosomal RNA
ROS	-	reactive oxygen species
rpm	-	revolutions per minute
RWA	-	Russian wheat aphid
SA	-	salicyclic acid
SAR	-	systemic acquired resistance
Sp6 primer	-	5'-ATT-TAG-GTG-ACA-CTA-TAG-AA-3'
SDS	-	sodium dodecyl sulphate
SSC	-	sodium chloride-sodium citrate solution
SSH	-	suppression subtractive hybridization
T7 primer	-	5'-TAA-TAC-GAC-TCA-CTA-TAG-GG-3'
TIR	-	toll-interleukin receptor
TNL	-	toll-interleukin receptor-NBS-LRR
tRNA	-	transfer RNA
Tris	-	2-amino-2-(hydroxymethyl)-1,3-propandiol
U	-	unit
USA	-	United States of America
UV	-	ultra violet
V	-	volt
v/v	-	volume to volume
w/v	-	weight to volume
X-Gal	-	5-bromo-4-chloro-3-indolyl-β-D-galactoside
YAC	-	yeast artificial chromosome

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Thesis Composition

Chapter 1 of this thesis is an introduction to wheat and the Russian wheat aphid (RWA). This chapter also describes plant resistance, especially resistance genes and NBS-LRRs and a brief summary is given on the primary molecular techniques employed to study R genes.

Chapter 2 focuses on the use of a PCR-based method to isolate ESTs. The results are discussed that were obtained after degenerate primers that target nucleotide binding sites (NBS) were used to amplify ESTs from RWA-infested wheat. The viability of such an approach to isolate specific genes i.e. resistance genes is discussed.

Chapter 3 concentrates on the use of suppression subtractive hybridization (SSH) on RWA infested and uninfested wheat. The results obtained after applying SSH to two RWA infested near-isogenic wheat lines, as well as to RWA infested and uninfested resistant wheat, are discussed. Further results from Southern and Northern hybridization as well as Real-Time PCR are also outlined. The suitability of this approach to isolate genes is discussed.

Chapter 4 details the results obtained from a microarray assay that consisted of NBS-amplified ESTs (generated as described in Chapter 2), probed with cDNA synthesized from material obtained from RWA resistant wheat ('Tugela DN'). Southern and Northern hybridizations, as well as Real-Time PCR results are discussed.

Chapter 5 is an outline of the significance of the techniques applied in this thesis and the scientific achievements made. Possibilities for future studies are mentioned.

Appendices are included, containing a summary and published articles.

Research Aim and Objectives

The Russian wheat aphid (*Diuraphis noxia*, Mordvilko; RWA) is a serious pest on wheat in many countries. To my knowledge, there is no detailed report yet of isolated and characterised genes, including nucleotide binding site-leucine-rich repeats (NBS-LRRs), involved in the RWA resistance response. There is considerable evidence from other plant species that NBS-LRRs are actively involved in protein-protein interaction. They are further indirectly involved in signal transduction during resistance responses of plants due to wounding or pathogen attack. In this PhD project, the first question asked was which genes are affected in their expression due to RWA infestation and secondly, if NBS-LRRs are among these genes. To answer these questions the techniques of suppression subtractive hybridization (SSH), the microarray-technology, Real-Time PCR and traditional Northern blotting were been applied after RWA infestation in RWA susceptible and resistant wheat cultivars.

To achieve this aim, the project had the following objectives:

(1) Isolate NBS-LRRs from wheat by using degenerate primers for conserved NBS regions. This would allow determining if NBS-LRRs are present in wheat comparable to other plant species. It would further allow determining the degree of homology to already reported NBS-LRRs.

(2) Create a cDNA library from ESTs created by amplification with degenerated NBS primers, which would enable random screening for NBS-LRRs.

(3) Identify and characterise ESTs for their function and distribution in the wheat genome by carrying out a database search and grouping of ESTs into functional categories. This would allow carrying out a comparison study with reported data on ESTs from other plant species.

(4) Identify and characterise a range of genes possibly involved in the response to RWA infestation by using the technique of SSH. This would enable setting up a library for genes involved in the response of wheat to RWA infestation.

(5) Confirm results obtained by using the microarray technology. This would allow determining the reliability of the microarray technology and general applicability to determine regulation of genes involved in the response of wheat to RWA infestation.