

**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

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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 | - | <i>Triticum urartu</i> genome |
| AABBDD | - | <i>Triticum aestivum</i> genome |
| AFLP | - | amplified length polymorphism |
| ATP | - | adenosine triphosphate |
| <i>avr</i> | - | avirulence gene |
| BB | - | <i>Aegilops speltoides</i> 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 |
| μ g | - | microgram |
| mg | - | milligram |
| MgCl ₂ | - | magnesium chloride |
| mM | - | millimolar |
| μ l | - | 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 | - | salicylic 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-propanediol |
| 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.