

**Isolation and characterization of *Diuraphis noxia* induced
Sequences from wheat line PI 294994**

by

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*Every experiment proves something. If it doesn't
prove what you want it to prove, it proves something
else.*

Anon.

Preface

The results presented in this thesis follow from a study, which was carried out at the Department of Genetics and the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, Pretoria, under the supervision of Prof. A-M. Oberholster (Botha) and co-supervision of Mr. E. Venter.

The results presented here are original and have not been submitted in any form to another university.

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Summary

Infestation by the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has caused large-scale damage to small-grain crops since its introduction into South Africa in 1978 and many other countries world-wide. The extreme damage caused by *D. noxia* in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) has resulted in a concerted effort by scientists to investigate the mechanisms and genes involved in the resistance response. This is done in an effort to understand and ultimately use the knowledge of resistance responses to produce aphid resistant crops. The aim of the present study was to use suppression subtractive hybridization (SSH) and degenerate oligonucleotide primers to amplify cDNA of differentially expressed genes and nucleotide-binding site (NBS)-containing sequences respectively. This served as a source for the identification and characterization of disease-resistance genes in the wheat line PI 294994 after infestation with the Russian wheat aphid. SSH is an effective approach for studying the genetic nature of many biological processes by identifying differentially expressed genes. The sequences that were obtained in this study did indeed show some similarity to the underlying mechanisms involved in lesion formation and disease resistance. At the amino acid level similarities were identified with polypeptides such as lymphoma receptors. At the nucleotide level similarities were identified to enzymes involved in fibrinogen lysis and the chloroplast regulatory system. A heat shock protein gene was also identified, which may possibly play a role in the induction of the resistance response during aphid infestation. The use of PCR with degenerate oligonucleotide primers, designed from the NBS region of cloned disease resistance genes, has led to the cloning of resistance sequences in various plant species. In this study oligonucleotide primers designed from conserved motifs in the NBS domain were used to clone disease resistance gene homologues from the wheat line PI 294994. The sequences obtained showed a homology to an NBS region in lettuce, which was linked to a resistance protein candidate gene. In the wheat line PI 294994 the cloned NBS sequence was expressed at varying levels in different wheat tissues, which requires further study. However, when the full-length cDNA sequence of the NBS-containing gene was sequenced, there was no significant homology to any disease resistance genes at the nucleotide level. At the amino acid level there was homology to a kinase type resistance protein. The degenerate NBS

PCR approach was very successful in producing a number of NBS regions, which may be used for further study even if they are not necessarily involved in disease resistance. These results have aided the process of identifying novel aphid induced transcripts after infestation and has contributed to the growing base of knowledge about the underlying mechanisms involved in lesion formation and resistance responses to insects.

Opsomming

Sedert die eerste waarneming van Russiese koringluis (*Diuraphis noxia* Mordvilko) in Suid Afrika in 1974, is gootskaalse verliese by klein-graan gewasse gerapporteer. Dit is ook die geval vir ander lande. Die ernstige skade wat deur *D. noxia* op koring (*Triticum aestivum*) en gort (*Hordeum vulgare*) veroorsaak word, het tot 'n gesamentlike poging deur wetenskaplikes gelei om die meganismes en gene betrokke by die weerstandsreaksie te ondersoek. Die poging lei tot 'n beter begrip van die meganisme en gene betrokke by die RWA weerstandsreaksie wat uiteindelik gebruik kan word om nog meer luisweerstandbiedende gewasse te kweek. Die doelwit van die huidige studie was om subtraksie-suppressie-hibridisasie (SSH) en gedegenererde oligonukleotiedvoorvoeders te gebruik om komplimentêre-DNA (cDNA) van differensieël uitgedrukte gene en nukleotiedbindingsetels- (NBS) -bevattende volgordes onderskeidelik te amplifiseer. Dit is gedoen om siekteweerstandsgene in die koringlyn PI 294994, na infestering met die Russiese koringluis, te identifiseer en te karakteriseer. SSH is 'n effektiewe nadering om die genetiese aard van baie biologiese prosesse te bestudeer deur die identifikasie van differensieël uitgedrukte gene. Die volgordes wat in hierdie studie verkry was, het wel sommige ooreenkomste met die onderliggende meganismes gewys en was dan wel funksioneel in letsselformasie en siekteweerstand. Op aminosuurvlak is ooreenkomste geïdentifiseer met onder andere limfoomreseptors. Terwyl ooreenkomste met ensieme wat by fibrinogeenoplossing en die chloroplasreguleeringsstelsel betrokke is, op nukleotiedvlak geïdentifiseer is. 'n Hitte-skok-proteïen is ook geïdentifiseer wat dalk 'n rol in die induksie van die weerstandsreaksie gedurende plantluisinfestering mag speel. Die gebruik van die polimerasekettingreaksie (PCR) met gedegenererde oligonukleotiedvoorvoeders van die NBS-gebied, het tot die klonering van weerstandvolgordes in verskeie plantsoorte gelei. In dié studie is oligonukleotiedvoorvoeders vanaf gekonserveerde motiewe in die NBS-gebied ontwerp en gebruik om die siekteweerstandgeen-homoloë van die koringlyn PI 294994 te kloner. Die volgordes wat verkry is, het homologie met 'n NBS-gebied in blaarslaai getoon wat aan 'n weerstandproteïenkandidaatgeen gekoppel is. In die koringlyn PI 294994 is die gekloneerde NBS-volgorde in variërende vlakke, in verskillende koringweefsel uitgedruk. Hierdie aspek regverdig verdere studie. Die volledige cDNA-basisvolgorde van die NBS-bevattendegeen het geen betekenisvolle

homologie aan enige siekteweerstandsgene op nukleotiedvlak getoon nie. Op aminosuurvlak was daar wel homologie met 'n kinase-tipe weerstandsproteïen. Die gedegenererde NBS-PCR-nadering was baie suksesvol om 'n aantal NBS-bevattende fragmente te produseer, al hou dit nie noodwendig met siekteweerstand verband nie. Hierdie fragment kan vir verdere studie gebruik word. Hierdie resultate het die proses van identifikasie van nuwe plantluis-geïnduseerde transkripte na infestasië geondersteun en bygedra tot die groeiende basis van kennis oor die onderliggende meganismes wat by letsselformasie en die weerstandsreaksie teen insekte betrokke is.