Genetic and Biological Characterisation of Resistance to Root Lesion Nematode *Pratylenchus thornei* in Wheat

by

Katherine Joanne Linsell

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Discipline of Plant Breeding & Genetics
School of Agriculture, Food & Wine
Faculty of Sciences
The University of Adelaide
Waite Campus
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This thesis is submitted in a combination of conventional and publication format. The main body of work contains three manuscripts (Chapter 2, 3 and 4) and a conventional thesis chapter (Chapter 5). A literature review establishes the field of knowledge and provides a link between publications. A general conclusion shows the overall significance of the work and future directions. The manuscripts have been prepared in the formats outlined by the journals to which they are to be submitted (excluding line numbering, Americanised spellings, table and figure lists after literature cited and font size) and thus chapters contain different formatting.

The manuscript - Characterisation of Resistance to *Pratylenchus thornei* in Wheat; Attraction, Penetration and Maturation

This manuscript will be submitted to the journal of Phytopathology. Outlined is the contributions made by each author in development of the manuscipt in terms of conceptual, experimental and documentation. The co authors give permission for this manuscript to be included in the thesis of Linsell, Katherine J. to be submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy at the University of Adelaide.

First author
Linsell, Katherine J.

- Conceptualisation of the work
- Performed all experimental work
- Drafting text and composition of all tables and figures within manuscipt

Signature:……..

Second author
Riley, I.T.

- Support with statistical analysis of data
- Assisted in manuscript editing

Signature:……..

Third author
Davies, K.A.

- Advisory on nematology techniques
- Assisted in manuscript editing

Signature:……..

Last author.
Oldach, K.H.

- Conceptualisation of the work
- Drafting and editing of the manuscript

Signature:……..

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First author
Linsell, Katherine J.

- Conceptualisation of the work
- Performed all experimental work
- Drafting text and composition of all tables and figures within manuscipt

Signature:……..

Second author
Riley, I.T.

- Support with statistical analysis of data
- Assisted in manuscript editing

Signature:……..

Third author
Davies, K.A.

- Advisory on nematology techniques
- Assisted in manuscript editing

Signature:……..

Last author
Oldach, K.H.

- Conceptualisation of the work
- Drafting and editing of the manuscript

Signature:……..

The manuscript - Quantitative Trait Loci for Resistance to Root Lesion Nematode, *Pratylenchus thornei*, from a Synthetic Hexaploid Wheat Source

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Firrt author
Linsell, Katherine J.

- Conceptualisation of the work
- Performed all experimental work (except first set of phenotypic data)
- Drafting text and composition of all tables and figures within manuscipt

Signature:……..

Second author
Taylor, S.P.

- Responsible for the first phenotypic analysis of the population for genetic analysis
- Assisted in manuscript editing

Signature:……..

Third author
Wallwork, H.

- Responsible for the generation of the mapping population
- Assisted in manuscript editing

Signature:……..

Last author
Oldach , K.H.

- Conceptualisation of the work
- Drafting and editing of the manuscript

Signature:……..

Table of Contents

Glossary of Abbreviations

°c	Degrees Celsius
µg	Microgram
µL	Microlitre
µm	Micrometre
a.i.	After Inoculation
AUD	Australian dollars
AFLP	Amplified Fragment Length Polymorphism
BAC	Bacterial Artificial Chromosome
CCN	Cereal Cyst Nematode
cM	CentiMorgan
CR	Crushed Root
d	Days
DArT	Diversity Array Technology
df	Degrees of Freedom
DH	Doubled Haploid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
EST	Expression Sequence Tag
F1	First Filial Generation
F2	Second Filial Generation
g	Grams
h	Hours
H2	Heritability
HF	Hatching Factor
HI	Hatching Inhibitor
ITS	Internal Transcribed Spacer
J2	Juvenile Stage Two
J3	Juvenile Stage Three
J4	Juvenile Stage Four
kbp	Kilobase pair
kg	Kilogram
L	Litre
LOD	Logarithm of Odds
LRS	Likelihood Ratio Statistic
LSD	Least Significant Difference
M	Molar
MAS	Marker Assisted Select

mg	Milligram
mL	Millilitre
mm	Milimetre
mM	Millimolar
mRNA	Messenger Ribonucleic Acid
NIL	Near Isogenic Line
PCR	Polymerase Chain Reaction
Pg	Picogram
QTL	Quantitative Trait Loci
R^2	Correlation Coefficient
RAPD	Random Amplified Length Polymorphism
RE	Root Exudate
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
RIP	Ribosome Inactivating Protein
RLN	Root Lesion Nematode
RNAi	Ribonucleic acid interference
RNase	Ribonuclease
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
RT	Room Temperature
s	Second
s.e.	Standard Error
SARDI	South Australian Research & Development Institute
SCAR	Sequence Characterised Amplified Region
SDS	Sodium dodecyl sulfate
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
Taq	Thermus aquaticus DNA Polymerase
TE	Tris-EDTA
Tris HCl	Tris (hydroxymethyl) aminomethane hydrochloride
UV	Ultraviolet
w	Week
χ^2	Chi-squared

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Abstract

Root lesion nematodes of the genus *Pratylenchus* feed and reproduce in the root cortex of many plant species, including wheat. Migration through root tissue causes extensive root damage, and in turn severe reductions in growth and yield. In Australia, one of the most prevalent and widespread species affecting wheat is *Pratylenchus thornei*. Due to the wide host range of *Pratylenchus* spp. and the restrictions and inefficiency of chemical pesticides, the development of resistant cultivars has become increasingly important. Despite the identification and investigation of several resistance sources and resistance quantitative trait loci (QTL), no *P. thornei* resistance has been integrated into commercial cultivars. In addition, prior to this study, the biological resistance mechanisms of wheat against *P. thornei* were not well characterised. The identification of novel sources of genetic resistance in wheat and understanding of the biological mechanisms will allow effective combinations of genes either to be used alternatively or pyramided to generate effective and stable *Pratylenchus* resistance. The major objectives of the study were to identify genetic loci associated with *P. thornei* resistance and to investigate the associated biological mechanisms in a double haploid wheat population developed from a cross between the synthetically derived Sokoll and the Australian adapted Krichauff parental lines.

The resistance to *P. thornei* observed in the Sokoll x Krichauff wheat population is complex and under the control of several loci which suppress all nematode developmental stages. The four main components of the root invasion process by *Pratylenchus*: root attraction, penetration, endoparasitic feeding and reproduction, were investigated to determine the location, timing and role of resistance against *P. thornei*. Through analysing root invasion by each nematode life stage, it was shown that resistance in the Sokoll x Krichauff population occurs post penetration to suppress *P. thornei* motility/migration and juvenile development causing reduced reproduction (egg deposition and hatch).

Attraction and penetration assays were conducted on seedlings grown both in sand and on agar. There was no significant difference in the rate at which *P. thornei* was attracted towards resistant or susceptible roots in sand. However on agar, when both genotypes were present, there was a significantly higher attraction towards the susceptible roots indicating resistant roots may secrete repellent or toxic compounds during pre-penetration or that susceptible roots secrete more attractants. The penetration rates of *P. thornei* in resistant and susceptible roots, both on agar and in sand, did not significantly differ. No preferred root penetration zone was observed with *P. thornei*, but penetration was not random as nematodes were attracted to root regions previously invaded. In concordance with other *Pratylenchus* studies, resistance to *P. thornei* in this Sokoll x Krichauff population acts post penetration.

Analysis of *P. thornei* development in the resistant and susceptible genotypes showed that significantly fewer *P. thornei* nematodes of all stages occurred in the resistant compared to the susceptible roots. Juvenile development was suppressed as no juvenile stage two nematodes (J2) were present 35 days after inoculation in resistant genotypes. At 45 days after inoculation, forty times more *P. thornei* juvenile stage three (J3) were present in the susceptible than the resistant parent. Unlike other studies where resistance against *Pratylenchus* caused nematodes to exit roots, in this study, similar numbers of *P. thornei* J2 were still present within the resistant roots 10 days after inoculation, indicating that resistance suppresses nematode development rather than causing nematodes to leave resistant roots.

The inhibition of juvenile development resulted due to the suppression of nematode migration/motility which suppressed feeding but also due to reduced egg deposition and hatch. Simple and inexpensive assays were designed to investigate *P. thornei* motility, egg hatch and deposition in root exudates/extracts and roots grown on agar. Significantly higher numbers of *P. thornei* nematodes became non-motile when exposed to root exudates from resistant (65%) versus susceptible (30%) roots after exposure for 3 days. The effects of these compounds were found to be reversible and to specifically affect *P. thornei* but not *Pratylenchus neglectus*. In migration assays, *P. thornei* only migrated a small distance through the resistant root cortex from the point of inoculation (10 mm), but further in the susceptible roots (70 mm). *Pratylenchus thornei* reproduction was also affected by resistance. Egg deposition was up to 30% less within resistant than in the susceptible lines. About 40% less hatch occurred from eggs within and adjacent to roots of resistant versus susceptible seedlings. Similarly, hatching was decreased by 10% in resistant root exudate compared to the susceptible after 10 days of exposure. An increased hatch after dilution of root exudates and a lower hatch in resistant versus the absence of roots, indicates the presence of hatching inhibitor compounds. As these root exudates were derived from plants not exposed to *Pratylenchus*/other pathogens, this indicates resistant genotypes constitutively produce compounds that inhibit motility and reproduction.

In order to identify QTL and develop molecular markers accounting for the observed resistance, a genetic map was constructed from the Sokoll x Krichauff doubled haploid population comprising 150 lines. A total of 860 Diversity Array Technology markers and 111 microsatellite markers were used to assemble the genetic map. Two highly significant *P. thornei* resistance QTL were identified on chromosomes 2BS and 6DS, *QRlnt.sk-2B.1 and QRlnt.sk-6D*, explaining 24 and 43% of the phenotypic variation, respectively. These QTL mapped to chromosome regions previously identified to be associated with *Pratylenchus* resistance, based on common marker locations. Two significant QTL were also identified on chromosomes 4A and 5A, explaining 6 and 9% of the phenotypic variation. The population was fixed for the effects of the highly significant QTL on 2BS and 6DS and further QTL were identified on chromosomes 2B, 2D, 3A, 5B and 6B. The *QRlnt.sk-2B.1* and *QRlnt.sk-6D*

account for a large portion of the observed resistance, showing that in this population the Sokoll derived resistance to *P. thornei* is very strong and is controlled by a few loci with large effects.

There are considerable financial and labour costs associated with *Pratylenchus* phenotypic screening methods. Molecular markers employed through marker assisted selection will eliminate the need for large scale phenotyping in breeding programs and thus accelerate the development and availability of resistant cultivars. The microsatellite marker *barc183* linked to *QRlnt.sk-6D* is also associated with *P. thornei* resistance in other mapping studies in different genetic backgrounds and thus highlights the potential benefit of this marker for use in marker assisted selection. However, the highly significant QTL on 2BS and 6DS currently span large chromosomal regions, thus fine mapping is required to delimit the QTL interval to establish more closely linked markers before they can be utilised in breeding programs.

The ultimate aim of this project was to correlate a biological role with an identified *P. thornei* resistance QTL. Thus, in order to identify whether the QTL linked to *P. thornei* were associated with the observed motility and hatch inhibition, a subset of the population was phenotyped using the motility and hatching assays designed in this study. Suggestive QTL were identified on chromosomes 2B, 5B, 6B and 6D linked to hatching and motility suppression, which co-located to the *P. thornei* resistance QTL identified in this and previous studies. Although only suggestive, alignment with other QTL indicates that these resistance QTL may play a role in inhibiting *P. thornei* motility or juvenile hatching. To further define and confirm these QTL, phenotypic analysis needs to be performed on the entire population.

The biochemical characteristics of the preformed resistant root compounds causing motility and hatching suppression were investigated. Root exudates that were subjected to heat/cold treatments caused less motility suppression than compared to the untreated control, indicating these resistant root compounds are water soluble and fairly stable in nature. Flavonoids, oxidised phenols and peroxidases associated with insect resistance genes that co-located with the hatching and motility suppression QTL and the *P. thornei* resistance QTL regions have been implicated in other *Pratylenchus*-plant resistance interactions. These results indicate a potential role for these compounds in the *P. thornei* resistance observed in Sokoll x Krichauff. Further investigation is required to define the chemical nature and specific roles of resistant root compounds in the suppression of nematode development.

The results of this study show that the resistance observed in the Sokoll x Krichauff wheat population to *P. thornei* is complex and under the control of two highly significant and several minor loci, which do not affect penetration but suppress nematode feeding, development and reproduction.