

**Fine Mapping of Nematode Resistance Genes  
*Rlnn1* and *Cre8* in Wheat (*Triticum aestivum*)**

A thesis submitted in fulfillment of the requirements for the  
degree of the Doctor of Philosophy at the University of  
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# List of Abbreviations

7AL - long arm of chromosome 7A

6BL - long arm of chromosome 6B

RLN - root lesion nematodes

CCN - cereal cyst nematodes

QTL - quantitative trait loci

MAS - marker-assisted selection

AFLP - amplified fragment length polymorphism

DH - doubled haploid

RFLP - restriction fragment length polymorphism

STS - sequence tagged site

SSR - simple sequence repeat

BAC - bacterial artificial chromosome

NBS - nucleotide-binding-site

LRR - leucine-rich-repeat

DAI - days after inoculation

qPCR - quantitative real-time polymerase chain reaction

DArT™ - Diversity Arrays Technology

SNP - single nucleotide polymorphism

ISBP - insertion site based polymorphism

KASP™ - Kompetitive allele-specific polymerase chain reaction

GBS - genotyping-by-sequencing

PCR - polymerase chain reaction

LOD - likelihood of odds

SM - single marker analysis

SIM - simple interval mapping

CIM - composite interval mapping

MIM - multiple interval mapping

WGAIM - whole genome average interval mapping

LRS - likelihood ratio statistics

EST - expressed sequence tags

Pt - *Puccinia triticina*

Pgt - *Puccinia graminis*

BLUPs - best linear unbiased predictions

RI - recombinant inbred

HRM - high resolution melting technology

BLUEs - best linear unbiased estimates

FISH - fluorescent *in-situ* hybridisation

BS - bootstrap support

TILLING - targeting induced local lesions in genomes

FISHIS - fluorescent *in-situ* hybridization in suspension

# Table of contents

List of Tables .....	viii
List of Figures .....	x
List of Appendices .....	xiv
Abstract.....	xvi
Thesis declaration .....	xviii
Acknowledgements.....	xix
Chapter 1: Introduction.....	1
Chapter 2: Literature review .....	6
2.1 Root lesion nematodes .....	6
2.1.1 Resistance against <i>Pratylenchus neglectus</i> in wheat .....	8
2.1.2 <i>Pratylenchus neglectus</i> resistance locus <i>Rlnn1</i> .....	9
2.1.3. Other important genes mapped near <i>Rlnn1</i> .....	10
2.1.3a Phytoene synthase gene <i>Psy-A1</i> and the catalase gene <i>Cat3-A1</i> .....	10
2.1.3b Rust resistance genes on 7AL.....	13
2.2 Cereal cyst nematodes .....	14
2.2.1 Resistance against <i>Heterodera avenae</i> in wheat.....	15
2.2.2 <i>Heterodera avenae</i> resistance locus <i>Cre8</i> .....	16
2.2.3. Plant defence responses to cereal cyst nematode infection .....	18
2.3 Evaluation of resistance against <i>H. avenae</i> and <i>P. neglectus</i> .....	21

2.4 Molecular markers, linkage and QTL mapping.....	22
2.4.1 Molecular markers .....	22
2.4.2 Linkage mapping .....	25
2.4.3 QTL mapping.....	26
2.5 Research gaps .....	28
2.6 Overall research goal .....	29
Chapter 3: Genetic mapping and marker development for resistance of wheat against the root lesion nematode <i>Pratylenchus neglectus</i> .....	31
3.1 Statement of Authorship .....	33
3.2 Abstract.....	35
3.3 Keywords.....	36
3.4 Background.....	36
3.5 Results.....	38
3.6 Discussion.....	49
3.7 Conclusions.....	52
3.8 Methods .....	53
Chapter 4: Suppressed recombination in a region of wheat chromosome 7A that carries loci affecting resistance against a root lesion nematode and fungal pathogens.....	62
4.1 Statement of Authorship .....	64
4.1 Introduction.....	66
4.2 Part A – Improving the Excalibur/Kukri chromosome 7A linkage map and refining the map position of the <i>Rlnn1</i> locus .....	67
4.2.1 Objectives .....	67

4.2.2 Methods .....	68
4.2.3 Results.....	71
4.3 Part - B: Is recombination suppressed in the distal end of chromosome 7AL?.....	83
4.3.1 Objective.....	83
4.3.2 Method.....	83
4.3.3 Results.....	87
4.4 Part - C: What causes the suppressed recombination? .....	97
4.4.1 Objective.....	97
4.4.2 Method.....	97
4.4.3 Results.....	100
4.5 Discussion.....	107
Chapter 5: Genetic mapping of the <i>Cre8</i> locus for resistance against cereal cyst nematode ( <i>Heterodera avenae</i> Woll.) in wheat .....	116
5.1 Statement of Authorship .....	118
5.2 Abstract.....	120
5.3 Keywords.....	120
5.4 Introduction.....	120
5.5 Materials and methods .....	122
5.6 Results.....	127
5.7 Discussion.....	135
Chapter 6: Development of materials for fine mapping <i>Cre8</i> .....	139
6.1 Introduction.....	139

6.2 Methods .....	139
6.3 Results and Discussion .....	141
Chapter 7: General discussion .....	150
7.1 Genetic mapping of the nematode resistance loci <i>Rlnn1</i> and <i>Cre8</i> .....	150
7.2 Towards cloning of <i>Cre8</i> .....	155
7.3 Suppressed recombination at the distal end of chromosome 7AL in Excalibur/Kukri genetic material.....	158
7.4 A translocation at the distal end of 7AL? .....	160
7.5 Other possibilities .....	165
7.6 Future prospects for <i>Rlnn1</i> .....	165
7.7 Resistance responses of <i>Cre8</i> and <i>Rlnn1</i> resistance locus .....	170
Chapter 8: Contributions to knowledge .....	172
References.....	174
Appendix 1: Supplementary material of Chapter 3 .....	199
Appendix 2: Supplementary material of Chapter 4 .....	232
Appendix 3: Supplementary material of Chapter 5 .....	264
Appendix 4: A first look at the infection of cereal cyst nematode ( <i>Heterodera avenae</i> Woll.) and establishment of syncytia in wheat carrying the <i>Cre8</i> resistance allele.....	290
S4.1 Introduction .....	290
S4.2 Methods .....	291
S4.3 Results .....	293
S4.4 Discussion.....	298



# List of Tables

Table 3.1: PCR markers designed based on five wheat expressed sequence tags (ESTs). .....	58
Table 4.1: Primer sequences of newly designed molecular markers based on the 9K-iSelect genotyping array .....	75
Table 4.2: Primer sequences of newly designed insertion site-based polymorphism markers.	77
Table 4.3: Primer sequences of molecular markers designed based on single nucleotide polymorphisms between Excalibur and Kukri sequences .....	78
Table 5.1: Composite interval mapping (CIM) statistics of major quantitative trait loci (QTL) for resistance against cereal cyst nematode in the Trident/Molineux doubled haploid population as assessed in experiments conducted in 2004 and 2005.....	130
Table S1.1: Rice BLASTN results using query sequences for ESTs from wheat deletion bins 7AL16-0.86-0.90 and 7AL18-0.90-1.00. ....	212
Table S1.2: Primer sequences of markers <i>schfc3</i> , <i>sts638</i> , <i>csPSY</i> and <i>PSY7A5</i> .....	220
Table S2.1 Polymorphism of 53 KASP™ markers between Excalibur and Kukri .....	232
Table S2.2: BLAST hit table of amplicon/EST/contig sequences representing molecular markers from the terminal cluster of Excalibur/Kukri chromosome 7A linkage map against chromosome 7A syntenic build .....	242
Table S2.3: BLAST hit table of amplicon/EST/contig sequences representing molecular markers from the terminal cluster of Excalibur/ Kukri chromosome 7A linkage map against chromosome 7B syntenic build .....	245
Table S2.4: BLAST hit table of amplicon/EST/contig sequences representing molecular markers from the terminal cluster of Excalibur/Kukri chromosome 7A linkage map against chromosome 7D syntenic build .....	247

Table S2.5: Graphical representation of the genotypes and estimated <i>P. neglectus</i> DNA/pot of Excalibur, Kukri and 14 informative Excalibur/Kukri recombinant inbred lines at the distal end of chromosome 7AL. ....	248
Table S2.6: Details about the <i>PsyI</i> allele sequences used in the phylogenetic analysis.....	253
Table S3.1: Primer sequences of KASP™ assays .....	264
Table S3.2: Primer sequences of HRM and gel-based assays .....	266
Table S4.1: Analysis of variance (ANOVA) table. ....	295

# List of Figures

Figure 2.1: A schematic representation of the carotenoid biosynthesis pathway of plants (based on Lu and Li [94]). .....	12
Figure 3.1: <i>Pratylenchus neglectus</i> resistance of rust-resistant and rust-susceptible Excalibur/Kukri doubled haploid lines. ....	39
Figure 3.2: Genetic map of chromosome 7A showing <i>Rlnn1</i> , <i>Lr20/Sr15</i> , <i>Psy-A1</i> and molecular marker loci. ....	41
Figure 3.3: Amplicons of markers <i>wri1</i> , <i>wri2</i> , <i>wri3</i> , <i>wri4</i> and <i>wri5</i> separated by agarose gel electrophoresis. ....	45
Figure 3.4: High-resolution melting curves for amplicons of markers <i>wri1</i> (A) and <i>wri3</i> (B). ....	46
Figure 3.5: High-resolution melting curves for amplicons of the co-dominant marker <i>wri1</i> ... ..	48
Figure 4.1: Amplicons of marker <i>AWW5L7</i> separated by agarose gel-electrophoresis.....	73
Figure 4.2: Amplicons of markers <i>Cat3-A1</i> (A) and <i>AWW622</i> (B) separated by agarose gel- electrophoresis. ....	74
Figure 4.3: High-resolution melting curves of insertion site based polymorphism marker <i>wri41</i> and <i>wri34</i> and 9K-iSelect array derived markers <i>wri30</i> and <i>wri28</i> .....	80
Figure 4.4: Genetic map of chromosome 7A showing <i>Rlnn1</i> , <i>Lr20</i> , <i>Sr15</i> , <i>Psy-A1</i> , <i>Cat3-A1</i> and molecular marker loci. ....	81
Figure 4.5: Schematic representation of the map positions of common markers between Excalibur/Kukri and other published linkage maps.....	88
Figure 4.6: A graphical illustration of the anchored positions of markers from the ‘terminal cluster’ on the Chinese Spring syntenic builds 7A (A), 7B (B) and 7D (C).....	89

Figure 4.7: A graphical representation of the separation and re-ordering of molecular markers from the distal-end of chromosome 7AL. ....	91
Figure 4.8: Frequency histogram showing the distribution of 94 Excalibur/Kukri recombinant lines carrying the Excalibur allele at the marker <i>wri2</i> . ....	92
Figure 4.9: Frequency histogram showing the distribution of 626 Excalibur/Kukri recombinant inbred lines carrying Excalibur allele at the marker <i>wri2</i> . ....	93
Figure 4.10: Re-evaluation of <i>P. neglectus</i> DNA/pot in Excalibur, Kukri and six Excalibur/Kukri recombinant inbred lines carrying Excalibur allele at the marker <i>wri2</i> . ....	94
Figure 4.11: Best linear unbiased estimates of <i>P. neglectus</i> DNA/pot of Excalibur, Kukri and 14 Excalibur/Kukri recombinant lines and 6 Excalibur/Kukri recombinant inbred line progeny carrying informative recombinations at the distal end of 7AL. ....	95
Figure 4.12: Estimated <i>P. neglectus</i> nematode DNA/pot in Excalibur, Kukri and 25 pairs of Excalibur/Kukri recombinant inbred 'sister-lines'. ....	96
Figure 4.13: Alignment of sequences of <i>PSY7A5_F/R</i> amplicons from cultivar Schomburgk to an extraction of the <i>Psy-Alt</i> allele sequence of the breeding line WAWHT2074 [GenBank:HM006895] and the <i>Psy-Als</i> allele sequence of the cultivar Schomburgk [GenBank:EU649795]. ....	102
Figure 4.14: An illustration of the gene tree of <i>Psy1</i> alleles of wheat and related species.....	103
Figure 4.15: Alignment of trimmed partial sequence of <i>AWW5L7-Left3/Right</i> from cultivar Excalibur and Raven to the <i>AWW5L7-Left3/Right</i> sequence of cultivar Halberd [Genbank:BV693737].....	105
Figure 4.16: Fluorescence <i>in-situ</i> hybridization in Excalibur and Kukri. ....	107
Figure 5.1: A) Genetic linkage map and LRS test-statistic scans for cereal cyst nematode resistance on chromosome 6B based on composite interval mapping using data	

from 161 Trident/Molineux doubled haploid lines. B) A comparison of the Trident/Molineux chromosome 6B linkage map of Williams et al. [197] (left) with the corresponding region of the current map (right).....	132
Figure 5.2: Mean nematode counts for eight genotypic classes of Trident/Molineux lines with different combinations of alleles at markers linked with the <i>H. avenae</i> resistance loci <i>Cre5</i> ( <i>gwm359-wmc177</i> ), <i>QCre.srd-1B</i> ( <i>wmc367-gwm140</i> ) and <i>Cre8</i> ( <i>BS00011603</i> ). .....	133
Figure 5.3: Marker haplotypes for Molineux, Trident and 13 other wheat cultivars. ....	134
Figure 6.1: An illustration of the crossing procedure in wheat. ....	141
Figure 6.2: A graphical representation of the chromosome 1BG2, 2A and 6B of TMDH006 and TMDH082. ....	142
Figure 6.3: Line means of nematode counts in 2004 <i>versus</i> 2005 of 121 Trident/Molineux doubled haploid lines. ....	144
Figure 6.4: <i>wri15</i> assay on 101 F <sub>1</sub> progeny of a cross between TMDH082 and TMDH006. ....	145
Figure 6.5: A flow diagram illustrating the population development and screening for informative recombinants. ....	146
Figure S1.1: Pairwise recombination fraction and LOD linkage plot of chromosome 7A. ...	199
Figure S1.2: Alignment of sequences of <i>PSY7A5_F/R</i> amplicons from Excalibur and 11 other cultivars to the <i>Psy-Alt</i> allele sequence of the breeding line WAWHT2074 [GenBank:HM006895] and the <i>Psy-Alt</i> s allele sequence of the cultivar Schomburgk [GenBank:EU649795]......	211
Figure S1.3: Alignment of sequences of <i>wri2_F/R</i> amplicons from Excalibur, Kukri and 10 other cultivars.....	219
Figure S2.1: Phylogeny of <i>Psy1</i> alleles of wheat and close relatives.....	252
Figure S2.2: Sequence alignment of 13 <i>Psy1</i> alleles in the ‘Mixed clade’ .....	262

Figure S3.1 High-resolution melting curves for markers <i>wri6</i> (A), <i>wri7</i> (B), <i>wri8</i> (C) and <i>wri9</i> (D).....	278
Figure S3.2: End-point genotyping clusters for markers <i>wri10</i> (A), <i>wri11</i> (B), <i>wri12</i> (C) and <i>wri13</i> (D).....	280
Figure S3.3: Agarose gel electrophoresis of amplicons of markers <i>wri14</i> (A), and <i>wri15</i> (B) and end-point genotyping scatter plot of amplicons of marker <i>wri16</i> (C). ...	281
Figure S3.4: Trident/Molineux linkage map.....	288
Figure S3.5 Distributions of <i>Heterodera avenae</i> nematode counts among 38 Trident/Molineux doubled haploid lines evaluated in pot tests. ....	289
Figure S4.1: Cereal cyst nematode development in wheat roots. ....	294
Figure S4.2: Nematode count per seminal root in TMDH006 and TMDH082 at 11, 18, 21 and 25 days after inoculation. ....	296
Figure S4.3: Development of syncytia in TMDH006 and TMDH082 in longitudinal root sections from 4, 7, 11 and 18 days after inoculation (DAI) with <i>H. avenae</i> . ....	297

# List of Appendices

Appendix 1: Supplementary material of Chapter 3 .....	199
Additional file S1.1 .....	199
Additional file S1.2 .....	200
Additional file S1.3 .....	212
Additional file S1.4 .....	218
Additional file S1.5 .....	220
Appendix 2: Supplementary material of Chapter 4 .....	232
Additional file S2.1 .....	232
Additional file S2.2 .....	242
Additional file S2.3 .....	248
Additional file S2.4 .....	252
Additional file S2.5 .....	263
Appendix 3: Supplementary material of Chapter 5 .....	264
Online Resource S3.1 .....	264
Online Resource S3.2 .....	277
Online Resource S3.3 .....	282
Online Resource S3.4 .....	289

Appendix 4: A first look at the infection of cereal cyst nematode (*Heterodera avenae* Woll.) and establishment of syncytia in wheat carrying the *Cre8* resistance allele .....299



# Abstract

The root lesion nematode *Pratylenchus neglectus* and the cereal cyst nematode *Heterodera avenae* cause significant yield damage to wheat (*Triticum aestivum* L.) and crops that are grown in rotation with wheat. The focus of this thesis is on two loci in wheat, *Rlnn1* and *Cre8*, which confer resistance against *P. neglectus* and *H. avenae*, respectively, with an overall scientific goal of characterizing these two resistance loci as an initiative towards isolation of the causal gene(s) and identification of diagnostic molecular markers for the use in marker-assisted selection in wheat breeding programmes.

The thesis presents improvements to an existing Excalibur/Kukri linkage map of chromosome 7A by adding *Lr20* (a gene for resistance against leaf rust caused by *Puccinia triticina*), *Sr15* (a gene for resistance against stem rust caused by *P. graminis*), *Psy-A1* (a phytoene synthase gene), *Cat3-A1* (a catalase gene) and 59 new molecular markers. The genomic location of the *Rlnn1* quantitative trait locus (QTL) was confirmed as the distal end of long arm of chromosome 7A (7AL). It coincides with the position of *Lr20/Sr15*, *Psy-A1*, *Cat3-A1* and 34 molecular markers.

Based on the findings that 1) some markers that collocate with the resistance genes *Lr20/Sr15* and *Rlnn1* are widely separated in mapping populations that do not segregate for these genes; 2) when anchored to a chromosome 7A syntenic build, these markers spanned a 0.9-Mb region; and 3) no recombinants were found in a large population of recombinant inbred lines, it is suggested that the clustering of molecular markers/genes/QTL at the distal end of 7AL is due to suppressed recombination. The suppressed recombination in Excalibur may be a result of a translocation. This suggestion is based on 1) phylogenetic analysis of *Psy-A1* alleles; 2) marker amplification patterns that suggested that sequences at the distal end of 7AL in Excalibur are very different from those in Kukri and Chinese

Spring; 3) amplicons observed for a normally 7B-specific marker that collocates with *Rlnn1* on 7AL, and 4) FISH images that revealed an unknown putative translocation in Excalibur that is absent in Kukri. It seems likely that the *Rlnn1*-containing segment of 7AL may have been translocated from a 7B-like chromosome arm with an unknown ancestry. Such a translocation could have pre-dated hexaploidisation and occurred in a tetraploid or diploid ancestor.

The thesis also presents a high-resolution genetic linkage map for a Trident/Molineux population. This map was used to confirm the locations of three previously reported QTL for *H. avenae*, including the *Cre8* locus mapped as a large-effect QTL at the distal end of the long arm of chromosome 6B (6BL), with an estimated position 0.9 cM from the closest markers. A cross was designed and made to develop a population for future use in fine mapping. With these materials and with the closely-linked molecular markers developed here, *Cre8* seems amenable to positional cloning.

In the research conducted for this thesis, the *Rlnn1* and *Cre8* resistance loci were mapped at the distal ends of 7AL and 6BL, respectively and diagnostic markers were identified for the use in marker-assisted selection. A suppressed recombination at the end of 7AL impedes the prospects of cloning *Rlnn1*, while the research reported here have identified suitable markers and genetic resources for cloning the *Cre8* gene with a forward genetics approach.

# Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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