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

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By

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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

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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

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