

## BIOLOGICAL AND GENETIC STUDIES OF WHEAT RESISTANCE TO HETERODERA AVENAE

**by** 

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A thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Agricultural and Natural Resource Sciences at The University of Adelaide.

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October 1994

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The cereal cyst nematode (CCN) Heterodera avenae (Woll.) is an economically damaging root pathogen of cereals worldwide. This thesis presents the results of studies into the biology and genetics of resistance to CCN in wheat conferred by the gene Cre.

Following the development of a synchronised infection system, growth of *H.avenae* juveniles within the roots of resistant and susceptible near-isogenic lines (NILs) was investigated for evidence of resistance gene expression. Female nematodes developed similarly in both cultivars up till 15 days, when development was halted in the resistant line. This correlated with the appearance of the nematode-induced feeding site, a syncytium, in the resistant line. It became vacuolate at about this time, eventually degenerating totally while the syncytia of the susceptible line appeared to have high metabolic activity. This study indicates that the CCN resistance response occurs late in the infection process, and is not a hypersensitive-type reaction.

Protein profiles of syncytium-enriched root sections from the NILs were examined for resistance-related differences. Micro-dissection of roots was used to obtain stele sections containing syncytia. No specifically resistance-related proteins were observed. A major 15 kD protein accumulated in sections from 27-35 day old roots of susceptible lines. This may be a syncytium-specific protein, as syncytia in resistant roots are generally degenerate by this time. Protein differences were observed between sections from syncytium-containing roots and those from uninfected roots, indicating nematode-directed alteration of host gene transcription and/or translation.

A directed search was made for *Cre*-linked RFLP markers using the NILs. Two out of fifty-eight probes produced a RFLP between the lines. These were mapped using aneuploid stocks of "Chinese Spring" and two F2 populations segregating for CCN

resistance. Cre was mapped to the proximal region of the long arm of chromosome 2B in the line "AUS10894". A linkage group containing Cre and four RFLP loci was identified.

Molecular techniques were employed to develop the RFLP marker closest to *Cre* into a PCR-based assay. The clone was sequenced and PCR primers designed. A dominant PCR marker was produced when amplification products from resistant and susceptible lines were digested with a restriction enzyme. These PCR products were cloned and a single base substitution was found in the resistant line. Allele-specific primers were designed with respect to the mutation, and a co-dominant *Cre*-linked PCR marker was produced using a dual-PCR strategy.