CYTOGENETIC AND MOLECULAR GENETIC MARKERS FOR CHROMOSOME 6R
OF RYE LINKED TO CCN RESISTANCE

by

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Thesis submitted to The University of Adelaide for the degree of Doctor of Philosophy

December, 1996
This thesis reports on the generation of molecular tools for the analysis of chromosome 6R of rye and the application of these tools in a structural analysis of 6RL. Results presented include physical and genetic maps of chromosome 6RL incorporating RFLP and PCR markers and CreR, the locus conferring resistance to cereal cyst nematode (CCN).

An important tool for both the physical and genetic mapping has been a clone containing an almost complete member of the R173 family of dispersed, repetitive rye-specific DNA sequences. This clone was used to identify rye chromosomes in wheat-rye hybrids via non-isotopic in situ hybridisation (ISH). Hybridisation of this clone to metaphase chromosomes of triticale cv. Carman and various Chinese Spring-Imperial disomic chromosome addition lines indicates that the R173 family is evenly dispersed on all seven rye chromosomes with the exception of some telomeres and centromeric regions. The ability to detect small introgressions of rye chromatin in wheat is demonstrated.

Molecular markers suitable for the development of physical and genetic linkage maps were generated. RFLP markers mapping to the long arm of chromosome 6R of rye were identified. Complementary and genomic DNA probes previously mapped to wheat and barley homoeologous group 6 chromosomes were obtained and screened across the Chinese Spring-Imperial 6R disomic addition line and Chinese Spring-T701-4-6 6R(-6D) disomic substitution line. A high proportion of clones were found to map to both chromosome 6R_{imp} and chromosome 6R^{T701}. Importantly, high levels of polymorphism were detected between chromosome 6R_{imp} and chromosome 6R^{T701} for both cDNA and gDNA clones. In spite of the success in developing RFLP markers for the long arm of chromosome 6R, the ever-present difficulties associated with RFLP technology led to the development of PCR-based molecular markers.
Three approaches to the generation of polymerase chain reaction (PCR)-based markers were utilised. An attempt was made to generate a PCR-based assay to replace RFLP analysis using a clone mapped to chromosome 6RL. Partial sequencing of this clone allowed the generation of primers for use in PCR studies on wheat, rye and barley genomic DNA. The second approach utilised primers derived from the flanking regions of cloned members of rye-specific dispersed, repetitive DNA sequences. PCR amplification products generated were mapped to all seven rye chromosomes. This study has provided some information regarding the local genomic organisation of the families of DNA sequences utilised. Finally, the 442 bp insert present in the clone pAW173 was sequenced. Primers were generated from this sequence in an attempt to develop markers from the internal regions of R173 elements. Southern analysis of amplification products generated in wheat and rye has provided information regarding the evolution of sequences related to the R173 family of repetitive DNA sequences.

A physical map of the long arm of chromosome 6R was generated. RFLP and PCR markers were subchromosomally localised to 6RL using the chromosome 6RL<sup>T701</sup> terminal deletion lines described by Dundas <i>et al.</i> (1992). Comparative mapping indicates that an internal inversion is present in rye 6RL relative to the long arm of wheat group 6 chromosomes. Results suggest that segments of 3RL and 7RL known to be present at the distal end of 6RL constitute approximately 30% of the physical length of the arm. Significantly, the use of homoeologous group 6 RFLP probes indicates that at least 90% of the ancestral 6RL arm remains on the present day chromosome arm. The implications of these findings on the prospects of inducing homoeologous recombination between rye chromosome 6R and wheat group 6 chromosomes are discussed.

The generation of a mapping population for chromosome 6R of rye is described. Individuals demonstrating recombination around the <i>CreR</i> locus were identified through the use of flanking markers. Twelve individuals were selected and progeny tested to ascertain
their response to challenge with CCN. Unambiguous genotypic assignment for the CreR locus was possible for eight individuals.

A genetic linkage map comprising eight RFLP loci and five PCR loci has been constructed for the long arm of chromosome 6R. The CreR locus was mapped via the inclusion of progeny testing results and the assignment of genotypes to individuals upon visual inspection of the data set. This map spans approximately 60 cM and the order of loci established conforms to the order established via physical mapping. The RFLP locus XksuF37 was mapped 3.5 cM proximal to CreR and represents a potentially useful marker for future introgression studies. Ten loci were found to form two clusters within a small internal region of the linkage map. Recombination was observed between these groups but not within groups. These observations indicate reduced levels of recombination between chromosome 6R$^{T701}$ and chromosome 6R$^{imp}$. These results are discussed with respect to the limitations of the TC-F$_1$ mapping population and the likely effects of structural differences between chromosomes 6R$^{T701}$ and 6R$^{imp}$. 
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