MOLECULAR ANALYSIS OF STRUCTURE OF CHROMOSOME 6R OF TRITICALE T701-4-6

by

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Summary

Cereal cyst nematode (CCN), *Heterodera avenae* Woll., is a most devastating pathogen of cereals causing serious crop losses. Chemical control of the disease is costly both to the environment and to the farmer. Resistant cultivars are considered the most economical component of a nematode management system. Wheat is susceptible to CCN attack whereas the hybrid triticale T701-4-6 possesses a useful resistance gene on the long arm of rye chromosome 6R. Earlier attempts using a whole chromosome $6R^{T701}$ as a starting material have failed to introgress the CCN resistance gene into wheat. Although pairing was induced in the absence of the *Ph1* locus, the structural differences between the rye chromosomes and its wheat homoeologues might have prevent the recombination. Thus it has become necessary to understand the complexities in the structure of rye 6R which caused major differences between rye and wheat chromosomes.

The recent advent in recombinant DNA technology provides an array of useful techniques to understand the complex genomes structure and evolutionary chromosomal rearrangements in related species. Employing conventional hybridisation methods and molecular tools, this thesis reports the evidence for the diverse structure of 6R relative to wheat homoeologous chromosomes thus minimising its exploitation in a wheat breeding program.

Using RFLP and PCR techniques, DNA based markers were developed and mapped to rye 6R. A PCR based marker C1 derived from R173 (a dispersed repeat from the rye genome) was useful in determining the sub microscopic deletion in the structure of Imperial rye 6R, proved the efficiency of PCR technique as a fast screening assay for breeders seeds stock. Five new RFLP markers CDO534, CDO1158, PSR113, Tam-6 and Tam-24 were assigned to the short arm of rye 6R. Since only few markers have been mapped to 6RS, the new rye markers mapped in this study will be useful in mapping genes on the short arm of rye 6R. However the map position of new rye markers is not known as a suitable mapping population was not available. The order of the marker loci was inferred by comparing their locations on wheat and other cereals maps.

The comparative genome analysis using RFLP markers provided an opportunity to understand the level of homoeology between homoeologous group 6 wheat and rye chromosomes. Analysis revealed that the short arm of rye 6R contains a putative inversion in proximal region and a deletion of the distal region might have been translocated to 4RL via a 2L interchange during speciation. The presence of PSR148 (groups 2 and 7) on the intact 6RL confirmed a non-group 6 translocation at distal regions of 6RL. However, the assignment of a large number of group 6 probes on 6RL using deletion lines, indicated that a fragment of 6RL present in these deletion lines (in variable length) may maintain a complete synteny with wheat homoeologues and assumed to be useful in introgressing CCN resistance gene into wheat.

An important aspect of this study was the use of five variable 6RL length mutant lines to introgress the 6RL segment into wheat via homoeologous recombination. The Sear's *ph1b* mutant (1977) was used in test crosses to induce recombination between 6RL and wheat homoeologous chromosomes in genetic stocks having monosomes of translocated 6RL and 6D in a homozygous *ph1b* background. The successful screening of Sears *ph1b* mutant plants and test-cross progenies with the RFLP probe PSR128 (mapped in the deletion of region containing the *Ph1* locus) confirmed the homozygous status of *ph1b* in the plants. Isozymes markers, α-amylase and GOT were used to establish dosage of 6R and 6D and select plants monosomic for 6R and 6D. The isozyme markers in conjunction with RFLP markers, provided an opportunity to identify the recombination pattern along the homoeologous chromosomes.

A total of five hundred TC-F2 progeny from the five different 6RL mutant lines and deficient for the *Ph1* locus, was screened using two isozyme markers and ten to twelve RFLP probes depending on the length of deleted fragment of 6RL. Initial screening used the isozyme markers α-amylase and GOT, and no dissociation was observed. Since these two markers are very close to each other and proximal to CCN resistance gene, RFLP markers which are distributed along the length of chromosome flanking the CCN resistance gene were used in secondary screening. However, no dissociation in RFLP markers was observed on 6RL and 6D chromosomes, although a total of eleven putative recombinants were isolated. These recombinants showed deletion of RFLP markers on chromosomes other than 6R and 6D. A putative cross-over point was identified between loci *Xpsr915* and *Xpsr149*. In most cases (7 of 11), the chromosomes

showing dissociation of marker loci were detected in the presence of two doses of 6R and 6D. In few cases a single dose of 6D was present (e.g. G7-17 and I5-24) or absent (e.g. I5-10); and a single dose of 6R was present (e.g. H1-10). The abnormal plants were isolated in four different families of 1411-54 line which possesses 6RL fragment containing *Got-R2* and *Cre-R* loci. The recombination status of these plants is not yet known and it was assumed that these plants involved the recombination between homoeologous wheat chromosomes. This study has also shown that RFLPs are superior and more reliable for the identification of recombinants than isozyme markers because of their increased ability to detect polymorphism. Although the pairing conditions were maximised using Sears *ph1b* mutant and 6RL deletion stock, the failure to isolate any wheat-rye recombinant chromosomes reconfirms the complex structure of 6R preventing its involvement in homoeologous recombination.

A molecular approach was taken to directly analyse the structure of 6R by constructing a cDNA library from ten days old roots of the wheat-$6R^{T701}$(-6D) substitution line. Differential screening of this library generated two hundred clones differentially expressed in wheat-$6R^{T701}$(-6D) substitution line. One hundred and five clones fall into four families, whereas relationships of the remaining 95 clones are still unknown. Southern hybridisation of representative clones from each family with two wheat varieties (Schomburgk and Chinese Spring), two rye varieties (Imperial rye and South Australian rye) and triticale T701-4-6 and its derived 6R lines identified homoeoloci on rye and wheat chromosomes. The results suggest that clones identified in this study represent genes expressed differentially in wheat-$6R^{T701}$(-6D) substitution line by repression of expression of homoeoalleles in Schomburgk.

In summary, this study shows that chromosome $6R^{T701}$ possesses a complex structure relative to its wheat homoeologues. This complexity suggests that introgression of the CCN resistance gene from $6R^{T701}$ into wheat may not be practicable.

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