UTILISATION OF MOLECULAR MARKERS IN THE
SELECTION AND CHARACTERISATION OF WHEAT-ALIEN
RECOMBINANT CHROMOSOMES

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SUMMARY

Attempts to transfer useful alien genetic material into wheat initially involved substitution of a whole chromosome or chromosome arm from the related alien species into wheat genome. Most of these wheat derivatives carrying alien chromosomes / chromosome arms have had limited use in practical breeding because of the linked undesirable genes on the alien segment which resulted in loss of yield and/or quality of the recipient wheats. The amount of alien genetic material in these lines can be reduced by induction of allosyndetic recombination between the alien and homoeologous wheat chromosomes. In the earlier studies, cytological procedures based upon chromosome pairing frequencies and/or biochemical loci (seed storage proteins and isozymes) were used to detect and isolate the wheat-alien recombinant chromosomes. Because of large amount of time and effort required (crossing of the plants carrying the putative recombinant chromosomes with the tester stocks) and the limited resolving power of the technique, chromosome pairing studies though used successfully by the pioneer workers (e.g. E. R. Sears, R. Riley) have not proved very efficient for the identification and isolation of such recombinant chromosomes. Dissociation of linked biochemical loci has been used successfully to identify a limited number of wheat-alien recombinant chromosomes, but the paucity of useful biochemical marker loci over a large part of the genomes has limited the usefulness of this approach. Recent advances in recombinant DNA technology have generated a large number of molecular markers (especially co-dominant RFLP loci) and these have provided new opportunities for using marker-assisted selection of homoeologous recombination between wheat and its related alien species.

This thesis reports a comprehensive study of induced homoeologous recombination along almost the complete genetic length of two homoeologous chromosomes in the Triticeae, using co-dominant DNA markers. The studies were undertaken to
determine the patterns of homoeologous recombination along the whole length of chromosomes 7A of common wheat and 7Ai of *Agropyron intermedium*. Chromosome 7Ai was chosen as a model alien chromosome because it has been reported to carry agronomically important genes conferring resistances to stem rust and barley yellow dwarf virus on its short and long arms, respectively.

Sears' (1977) *ph1b* mutant was used to induce homoeologous pairing between chromosomes 7A of common wheat and 7Ai of *Agropyron intermedium*, in genetic stocks having single doses of chromosomes 7A and 7Ai and which were homozygous or hemizygous for the *ph1b* allele. Cytological, biochemical and molecular assays were carried out to search for useful polymorphic markers for the two chromosomes, but only RFLPs produced polymorphisms suitable for this study.

A total of 390 F3 progeny deficient for the *Ph1* locus were screened using six RFLP marker probes viz. CDO -545, -595 (short arm makers) and CDO673, WG686, PSR-117, -121 (long arm markers). A total of 62 putative recombinants showing dissociation of the RFLP markers within the arm(s) were detected, giving a crude recombination rate of 16%. Recombinants involving the short arm of the two chromosomes were obtained more frequently (40 recombinants) as compared to those involving the long arms (16 recombinants). A few recombinants (6) showed dissociation of markers for both the arms. In most cases the chromosomes showing dissociation of marker loci were detected in the presence of an intact parental homoeologous chromosome (7A or 7Ai), but in a few examples (seven short arm, four long arm recombinants) the recombinant chromosomes were directly isolated as a univalent chromosome in the F3 progeny. In 117 F3 progeny having the *Ph1* allele (control populations), only one suspected recombinant / deletion was observed.
Whenever the recombinants produced seeds either by self fertilising or by crossing with pollen from euploid or NT 7A-7B stock of wheat cv. CS, DNA from a sample of progeny were tested with the same six RFLP probes to confirm the classification of the original plant showing marker dissociation and to isolate the recombinant chromosomes in hemizygous or homozygous state. These progeny tests confirmed the recombinant status of almost all the non-parental F₃ progeny tested and also recombinant chromosomes were isolated in many cases.

The cross-over breakpoints were inferred along the length of the chromosomes. Evidence for the occurrence of more than one homoeologous cross-over involving 2 or more chromosomes were obtained but no evidence for intra-arm wheat-\textit{Agropyron} double cross-overs was obtained during present studies. During the progeny tests, new dissociations of the marker loci were detected with a low frequency presumably arising as a consequence of a second round of homoeologous recombination since the progeny plants were still deficient for \textit{Phl}.

The recombinant chromosomes were characterised using RFLP markers, genomic \textit{in situ} hybridisation and determining their reaction to stem rust and barley yellow dwarf virus diseases. Detailed analysis of recombinant chromosomes using 15 RFLP markers identified the homoeologous cross-over products having varying lengths of \textit{Agropyron} chromatin introgressed onto homoeologous group 7 chromosomes of wheat, especially the targeted chromosome (7A). It was possible to establish the likely linear order of the probe loci along the lengths of chromosomes 7Ai and 7A.

The distribution of chiasmata along chromosome arm 7AS was analysed in the homoeologous recombinants. In most cases the translocation breakpoints were concentrated around the loci which were located distally on 7AS (based upon linear order of probe loci obtained during present work and genetic and physical locations of
the loci reported in literature). The pattern of recombination between the homoeologous chromosomes observed during present study was similar to that reported in other studies for homologous recombination between the same markers on chromosome 7A of wheat.

Genomic *in situ* hybridisation was applied to the recombinant chromosomes and the presence of a small terminal segment of *Agropyron* chromatin was detected in two of the short arm recombinant chromosomes.

The reference stocks (including wheat parents, addition, substitution and ditelosomic addition lines) and the plants carrying short arm recombinant chromosomes were screened with wheat stem rust pathotype ("21-2,3,7"). The recombinants having *Agropyron* segment distal to the locus *Xcdo475* and proximal to the locus *Xpsr119* were found to be resistant to this pathotype, indicating that the stem rust resistance gene (*SrAgi*) was located on the distal part of chromosome 7Ai of *Agropyron intermedium*. Recombinant chromosomes having the *SrAgi* gene and overlapping distal and proximal segments of chromosome 7Ai were isolated which can be used to reduce the amount of alien chromatin in the resistant recombinant lines through allowing homologous chromosome pairing between the overlapping alien segments, to produce an interstitial introgressed segment.

The reference stocks and the plants carrying the long arm recombinant chromosomes were screened against barley yellow dwarf virus, but no clear differences were found between euploid wheat and the addition or substitution lines carrying whole chromosome 7Ai or the long arm of chromosome 7Ai, which suggested that BYDV resistance gene reported to be present on the long arm of chromosome 7Ai was ineffective at least against the BYDV serotype (BYDV.PAV Adel-) used during the present study.
Results of the present study have indicated new and more efficient protocols for the incorporation of alien segments from chromosome 7A1 of *Ag. intermedium* into group 7 homoeologous chromosome of wheat.