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

BY

IMTIAZ AHMED KHAN
M.Sc. (Genetics) University of Karachi
Pakistan

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

Department of Plant Science
Waite Agricultural Research Institute
University of Adelaide

October 1996

Dedicated to my parents
wife and daughter

Table of contents

Statement of originality

and consent to photocopy or loan

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference is made in the text.

I give consent to the librarian of the Barr-Smith Library, The University of Adelaide, or his/her appointed agent to make this thesis, or any part thereof, available for photocopying or loan.

Imtiaz Ahmed Khan

October, 1996

Acknowledgments

I wish to express my gratitude to Dr. K. W. Shepherd for his constant supervision and constructive criticism throughout the present study and during the preparation of this thesis.

I am thankful to my co-supervisor Dr. AKMR Islam for his help and interest in present work. Thanks are also due to Dr. Ian S. Dundas for his help, encouragement and interest in present work and for a very friendly company.

Thanks are also due to the late Mr. Peter Ellis, Nerida Hunt, Steven Szarvas and Naomi MacMillan for their help in glasshouse and laboratory work. I thank Dr. Peter Langridge and his group for providing facilities for RFLP work and Mr. Nick Collins and Brendon King for help in BYDV assays.

I acknowledge the financial support of the University of Adelaide Scholarship for postgraduate Research and AUSAID for EMS scholarship.

Finally, I express my gratitude to my parents, parents-in-law, wife and daughter for their patience and understanding which made it possible for me to work long hours throughout this study.

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 F$_3$ 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 F$_3$ progeny. In 117 F$_3$ 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-*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 *Ph1*.

The recombinant chromosomes were characterised using RFLP markers, genomic *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 *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 7Ai of *Ag. intermedium* into group 7 homoeologous chromosome of wheat.

CHAPTER - 1
GENERAL INTRODUCTION

Bread wheat (*Triticum aestivum* (L.) em. Thell., 2n = 6x = 42, genomically ABD) is the world's most important cereal crop and has been cultivated for over 8000 years (Helbaek 1959). It is generally believed that the natural genetic variability of this crop is decreasing mainly because of the replacement of highly variable landraces with the high yielding pure line varieties (Frankel 1970; Sears 1981). On the other hand, wild relatives of wheat (including *Aegilops*, *Agropyron*, *Secale*, *Hordeum*) are rich sources of many useful genes, especially those conferring resistance to biotic (pests and diseases) and abiotic (cold, drought and salt) stresses.

A number of alien chromosomes or chromosome segments carrying useful genes have been transferred to wheat (for a review, see Gale and Miller 1987). Addition and/or substitution lines (involving whole chromosome or chromosome arms) have been produced utilising a wide range of wheat relatives, including even barley (*Hordeum vulgare*) which belongs to a different taxonomic subtribe than wheat (Islam *et al.* 1981). But with few exceptions [e.g. the 1R (1B) substitution and 1BL-1RS centric fusion products, present in many European wheat cultivars (Zeller 1973)] these wheat-alien chromosome addition/substitution lines have not been used in commercial agriculture because the alien chromatin introduced also carries undesirable genes or in the case of substitution lines, cannot compensate fully for the missing wheat chromatin. The chances of obtaining a commercially acceptable transfer would be improved by genetic recombination, but such recombination between wheat and alien chromatin cannot be obtained through conventional hybridisation and selection procedures. The inability of wheat chromosomes to pair and ultimately exchange genetic material with the homoeologous chromosomes from related species is mainly due to the presence of the *Ph1* gene on the long arm of chromosome 5B of wheat, which allows pairing between

homologous chromosomes only and inhibits pairing between homoeologous chromosomes (Okamoto 1957), making this hexaploid species functionally diploid (Riley and Chapman 1958).

During the past 30 years, various cytological methods have been developed to overcome the problems posed by the *Ph1* gene. These methods include (1) removal of the *Ph1* gene either by utilising stocks deficient for chromosome 5B or by the use of a *ph1b* mutant (Sears 1977) and (2) suppression of the *Ph1* gene using epistatic genes from *T. speltoides* (Tausch) Gren. ex Richter (syn. *Aegilops speltoides*) or *T. tripsacoides* (Jaub. & Spach) (syn. *Aegilops mutica*) (Riley *et al.* 1968a). X-ray induced chromosome translocations (for a review, see Knott 1971) have also been successfully applied to promote introgression of alien genetic material into wheat chromosomes.

The use of Sears' *ph1b* mutant offers some advantages over other available methods for the incorporation of alien genetic material into wheat chromosomes. X-ray induced transfers involve random breakage and rejoining of the wheat and alien chromosome segments mostly involving non-homoeologous exchanges which will usually be of a non-compensatory type and hence would likely to be more disruptive for gene balance in the plant (for a review, see Islam and Shepherd 1991). Sears (1981, 1993) concluded that radiation induced transfer is "at best a very laborious process with a low probability of yielding an acceptable transfer". More recently, tissue culture methods have been claimed to be highly successful for incorporating alien genetic material into wheat (Larkin *et al.* 1984; Banks *et al.* 1995), but earlier Lapitan *et al.* (1984) and recently Hohmann *et al.* (1996) detected many gross chromosomal changes in the regenerated plants including non-homoeologous translocations in wheat-rye and wheat-*Agropyron* introgressions, respectively, which may have drastic effects on plant phenotype and physiology and hence have very limited practical value for wheat improvement. In contrast to these methods, wheat-alien chromosome translocations induced by the *ph1b* mutant, in the

plant monosomic for the alien chromosome and a homoeologous wheat chromosome $(20_W"+1_W'+1_A')$ are more likely to be a compensating type as pairing would occur between homoeologous chromosomes. Use of Sears' *ph1b* mutant also avoids complex aneuploidy which occurs when stocks deficient for chromosome 5B are used to induce homoeologous chromosome pairing.

In practice, induction of homoeologous recombination is only the first step in achieving the required introgression. In the progeny, it is necessary to distinguish plants carrying the required recombinant products from among the more numerous non-recombinant progeny. Initially cytogenetic procedures were used to verify suspected recombinants by identifying chromosomes which carried the desired alien gene and which were also capable of pairing with normal wheat chromosomes (Sears 1972; Riley *et al.* 1968b). Later Sears (1981) improved the efficiency of this selection process by utilising a telosomic of the alien chromosome as the source of the alien gene, instead of the whole alien chromosome. In this case proximal crossovers between the alien gene and a wheat homoeologue resulted in progeny having the alien gene associated with a complete chromosome instead of a telosome, and the recombinants could be identified directly. Because of the large amount of time involved in cytological screening to identify potential recombinants, these procedures were not considered suitable for the isolation of wheat-alien recombinant chromosomes especially where the level of homoeologous pairing was expected to be low.

Attempts to overcome this problem with those alien species showing reduced levels of homoeologous pairing have been made by employing marker genes to facilitate the selection of the wheat-alien recombinant chromosomes, expected to occur only rarely. For example, Koebner and Shepherd (1985) and later Rogowsky *et al.* (1991) showed that by screening progeny from appropriate stocks deficient for *Ph1* for evidence of dissociation between two linked co-dominant seed protein markers on wheat

chromosome arm 1DS, it was possible to identify rare recombinants carrying segments of rye 1RS translocated onto 1DS. Also Islam and Shepherd (1992) screened large numbers of progeny from triple monosomics possessing monosomic 5B (having *ph1b* allele), a barley telosome (3HL or 6HL) and a homoeologous wheat monosome, for dissociation between two co-dominant isozyme markers present on barley chromosome arms 3HL or 6HL, and were successful in obtaining homoeologous wheat-barley recombinants involving each of these barley chromosome arms. Although these studies showed the value of co-dominant seed protein or isozyme markers in selecting for rare homoeologous cross-overs between wheat and alien chromosomes, this approach lacked wide applicability because of the scarcity of such polymorphic markers across a large part of the wheat genome.

The recent introduction of molecular biology to generate a large number of molecular markers, principally co-dominant RFLPs (Paterson *et al.* 1991), over almost the whole genome of wheat and its relatives has transformed the opportunities for utilising marker-assisted selection of homoeologous recombination between wheat and its related alien species. In this thesis the use of these DNA markers to select homoeologous recombinants involving almost the complete length of chromosome 7Ai of *Agropyron intermedium* (Host) P. B., Syn. *Thinopyrum intermedium* (Host) Barkworth & Dewey (2n = 6x = 42, genomically E_1E_2X) and related wheat chromosomes will be reported. This alien chromosome, originally added to wheat by Cauderon (1966) is homoeologous to group 7 chromosomes of wheat (The and Baker 1970; Forster *et al.* 1987) and was chosen for study because it has been reported to carry economically important genes controlling resistances to barley yellow dwarf virus (Brettell *et al.* 1988) and wheat stem rust (Cauderon *et al.* 1973; McIntosh *et al.* 1995). The wheat-*Agropyron* recombinant chromosomes produced during the present study would result in the transfer of these desirable genes into wheat. Use of these genes, especially the one conferring resistance to stem rust could provide additional resources for gene pyramiding but also offers a

better chance for gene tagging by increasing the level of polymorphism which is very limited within wheats (Chao *et al.* 1989). Also a number of genes conferring rust resistance have been located in equivalent positions on homoeologous chromosomes (McIntosh 1988) on wheat and close relatives, suggesting that they might represent allelic variants at different homoeologous loci (Autrique *et al.* 1995), hence molecular tagging of one of these genes will help in locating the other genes on homoeologous chromosomes over a wide range of wheat relatives. This study will also contribute to establish the gene order along group 7 homoeologous chromosome of the Triticeae and the better understanding of allosyndetic recombination through the entire length of Triticeae chromosomes which will assist researchers to devise better strategies for transferring useful genetic variability present in the wild relatives of wheat. The question of whether these induced homoeologous exchanges are site specific or randomly distributed through the entire length of chromosomes will also be addressed by studying detailed structure of the recombinant chromosomes through RFLPs and *in situ* hybridisations.

CHAPTER - 2
REVIEW OF LITERATURE

2.1. Genomes of wheat and homoeologous chromosomes of Triticeae:

Bread wheat (*Triticum aestivum* L.) is one of the world's principal food crops and taxonomically belongs to the family *Gramineae*, tribe *Triticeae* dum, subtribe *Triticinae* Holm, genus *Triticum* L. and section *Speltoidea* Flaks (for a review, see Kerby and Kuspira 1987).

Bread wheat is a hexaploid species with 2n = 6x = 42 chromosomes which can be assigned to three different genomes, A, B and D. According to Morris and Sears (1967), the donors of the A and D genomes are likely to be *Triticum monococcum* L. and *Triticum tauschii* (Coss.) Schmal. (Syn. *Aegilops squarrosa* L.)[*], respectively. More recently, Dvorak *et al.* (1993) using repeated nucleotide sequences concluded that *T. urartu* is more likely to be the donor species of the A genomes in *T. turgidum*, *T. timopheevii* and *T. aestivum*. The donor species of the B genome is not yet known with certainty. Riley *et al.* (1958) and Konarev (1983) proposed that B genome might have been donated by a diploid species of the genus *Triticum* (Syn. *Aegilops*). Fernandez-Calvin and Orellana (1994) using the C-banding technique concluded that the genomes of *T. speltoides* (Tausch) Gren. ex Richter (Syn. *Ae. speltoides*), and *T. longissimum* (Schweinf. & Muschli in Muschli) Bowden (Syn. *Ae. longissima)* are equally related to the B genome of wheat and that at present there is no strong reason for believing that

[*] = The genus *Aegilops* was revised and incorporated in *Triticum* by Bowden (1959) and amended by Morris and Sears (1967) and the revised designations will be used in this thesis. However, the old designations which are still widely used in the literature will be given in parentheses when a species is first mentioned.

any one of them is the sole diploid ancestor of the B genome. It has been suggested that the B genome in wheat has been highly fragmented (Larsen 1973) probably through introgressive hybridisation with related taxa (Gill and Chen 1987) and it is no longer possible to detect its donor species.

These 3 genomes of wheat are believed originally to have been derived from a single ancestral diploid species having 7 pairs of chromosomes. Chromosomes of these three genomes have also retained much similarity of gene content (for a review, see Hart 1987). This triplication of the genetic material enabled Sears (1952, 1966) to show that abnormal phenotypes caused by nullisomy of any chromosome can be corrected (at least partially) by tetrasomy of certain other chromosomes. He also concluded that the haploid chromosome complement of bread wheat is made up of 7 groups (1-7) of three related chromosomes. These genetically related chromosomes from the different genomes are referred to as "homoeologous" chromosomes (Huskins 1932).

Similar tests of genetic compensation (as those provided by wheat nullisomic-tetrasomic lines) using wheat-alien chromosome substitution lines (where a pair of wheat chromosomes is replaced by a specific pair of alien chromosomes) have shown that the chromosomes within the homoeologous groups of wheat are related to particular chromosomes in other species within the Triticeae, e.g. *Secale cereale*, *T. umbellulatum* (Zhuk.) Bowden) (Syn. *Ae. umbellulata)*, *Agropyron*, and *T. comosum* (Sibth. & Sm.) Richter (Syn. *Ae. comosa)* (O'Mara 1940; Sears 1956, 1972, 1973; Riley *et al.* 1968b). Later a number of studies on the relationships between different species in the tribe Triticeae, based upon genetic and biochemical studies of enzymes and seed proteins (e.g. Hart 1973, 1979; Shepherd 1973; Hart and Tuleen 1983; Chojecki and Gale 1982, Ainsworth *et al.* 1984) indicated that the gene synteny

relationships that existed in the ancestral Triticeae genome are largely conserved in the Triticeae genomes that exist today (for a review, see Hart 1987). [Note: the term "synteny (Gk. *syn* = together; *taenia* = ribbon) was first used by Renwick (1971). He defined the term synteny as "the two loci present together on the same chromosome, whether or not the loci have been shown to be linked". "Asynteny" is therefore the state of being on different pairs of chromosomes]. Naranjo *et al.* (1987) using the C-banding technique demonstrated that chromosomes 1R, 2R, 3R, 5R and 6R of rye can reasonably be assigned to the homoeologous chromosomes of groups 1, 2, 3, 5 and 6 of wheat, while chromosomes 4R and 7R showed partial reciprocal homoeology with homoeologous chromosomes of groups 4 and 7 of wheat. These cytological evidences further supported the notion of the conservation of gene synteny groups within the Triticeae species.

The linear order of three loci, *per-D1* (leaf peroxidase), *Gpi-D1* (glucose phosphate isomerase) and *Gli-D1* (gliadin storage protein) on the short arm of chromosome 1D of wheat, obtained by Koebner *et al.* (1986) was found to be the same as that found by Ainsworth *et al.* (1984) for the short arm of chromosome 1B of wheat. Hence the phenomenon of gene synteny which relates to similar chromosomal and arm locations could be further extended to conservation of gene order or collinearity along the chromosomes.

Homoeology between the chromosomes within the Triticeae at DNA level is now being used extensively to compare genetical and physical maps of the homoeologous chromosomes and to obtain an integrated molecular marker based map of the tribe. These studies have shown a high degree of collinearity of the molecular markers (mainly RFLPs) between barley chromosome 2 and wheat group 2 homoeologous

chromosomes (Devos *et al.* 1993a) and similarly between barley chromosome 3 and wheat group 3 homoeologous chromosomes (Devos and Gale 1993). Barley chromosome 5 was also found to show collinearity with most of the markers previously mapped on chromosome 1R of rye in a comparative mapping study (Wang *et al.* 1992) and 1R of rye is known to show gene collinearity with wheat group 1 chromosomes (Devos *et al.* 1993b). Also genetic maps of barley chromosome 1 (Heun *et al.* 1991; Graner *et al.* 1991; Kleinhofs *et al.* 1993) were found to show a high degree of collinearity with the consensus physical map of group 7 homoeologous chromosomes of wheat (Hohmann *et al.* 1995). Homoeology also has been reported to exist among the species belonging to different tribes within the family *Gramineae*, such as wheat, rice and maize (Ahn and Tanksley 1993; Ahn *et al.* 1993). Devos *et al.* (1994) found a high degree of collinearity between chromosome 9 of maize and homoeologous chromosomes of groups 4 and 7 of wheat, indicating a close genetic relationship between species in widely different genera of the *Gramineae* family. Such a high degree of linkage conservation is expected to help in integrative mapping studies and in elucidating present day structure and evolutionary relationship of *Gramineae* chromosomes.

2.2. Mechanism of chromosome pairing and recognition of homology:

The basic pathway of meiosis is pairing ---- exchange ---- disjunction. During meiosis, chromosome pairing takes place which is followed by crossing over and ultimately in the creation of recombinant chromosomes from pieces of parental chromosomes. Pairing also ensures that paired chromosomes separate from each other in an orderly manner and hence each daughter cell has one complete haploid chromosome set (n). To quote Maguire (1974) "Persistent chiasmata normally serve the vitally important function of assuring the regular disjunction of homologous centromeres".

The course of events during meiotic prophase have been studied by light and electron microscopy (for reviews, see Darlington 1931; Moses 1968; Moens 1973; Gillies 1975). In brief, during meiosis chromosomes start to condense. In some animal organisms they are attached to the nuclear envelope with their telomeres, creating a bouquet arrangement by projection of intercalary chromosome regions as a bundle of loops into the nuclear membrane. In plants a similar arrangement, the "Synezetic knot" is formed which does not involve the nuclear membrane. Chromosomes then form an "axial element" (protein axis) and these elements of homologous chromosomes become close to each other at a distance of about 100 nm. A third longitudinal axis is formed at about the same time and it runs half way between the two chromosomal axes. This whole arrangement of longitudinal and transverse fibres is referred to as the "Synaptonemal complex" (SC). The majority of the chromatin of the two parental chromosomes composing the bivalent is located exterior to the "SC" and not much is known about this small amount of DNA which is directly associated with "SC". But it is assumed that this fraction of DNA consists of specific sets of pairing initiation sites which promote synapsis (Giroux 1988). The "SC" does not establish primary contact between the homologous chromosomes, rather it provides the mechanical framework within which the molecular process of DNA sequence matching and breakage and reunion of the DNA strands takes place (Gillies 1984).

When and how the process of homology testing and the approach of homologous chromosomes takes place and how meiotic chromosomes undergo recombinogenetic pairing is not known despite much research on this topic over the past fifty years.

Faberge (1942) proposed that chromosomes oscillate at a characteristic frequency and those which are attached, oscillate in phase. Alternatively, Riley and Flavell (1977) suggested that homology is tested by differential coiling and condensation patterns along the chromosomes. It has also been suggested that testing of homology works via formation of a biparental duplex of complementary non sister DNA molecules and it involves certain specific DNA sequences (for a review, see Loidl 1990).

For some species of grasses it has been claimed that the order of chromosomes within the cell is non random (Feldman and Avivi 1973, 1984; Bennett 1982, 1983). Schwarzacher *et al.* (1989) using *in situ* hybridisations reported that in a hybrid between *Hordeum chilense* and *Secale africanum* the different genomes remain in different domains throughout the cell cycle. In contrast, in many other species such somatic association has never been observed. For example Bhambani *et al.* (1984) found that the spatial distribution of chromosomes at mitosis within the single chromosome set of haploid barley was random. However, it has been postulated that in some organisms homologous chromosomes establish contact prior to meiosis and this somatic association is not strong in all tissues but it is increased in the cells of the germline (Loidl 1990).

More recently, some ribosomal rRNA repeats (McKee and Karpen 1990; McKee *et al.* 1992) and heterochromatic regions (Irick 1994) have been shown to be involved in the proper recognition of homologous chromosomes in *Drosophila melanogaster.*

None of the above models have yet been proved or found suitable as a general explanation of the phenomenon of chromosome recognition and pairing, but it is generally accepted that the homologous recognition mechanism operates at two levels:

(1) preselection of homologous chromosomes and (2) precise matching of DNA base pairs (Maguire 1988; Loidl 1990).

2.3. Genes controlling chromosome pairing:

A number of organisms are being studied to characterise the environmental and genetical factors responsible for chromosome pairing during meiosis. There are reports on the inhibition of chromosome pairing by heat in wheat (Kato and Yamagata 1980), locust (Henderson 1988) and *Allium.* (Loidl 1989) based on observations of chiasma reduction at the diplotene stage of prophase I. In lilies, it has been shown that 0.1 - 0.2 % of the DNA is not replicated until zygotene stage. This so-called "Zyg DNA" is believed to be related to the events of homologous chromosome pairing (Hotta and Stern 1971; Stern and Hotta 1987). In yeast, different genes e.g. *SPO11*, *HPO1* have been identified, which may encode for structural components of the synaptonemal complex and *RAD50*, which may be involved in homologous search (Alani *et al.* 1990). A number of recombination deficient mutations [e.g. *T(2;3)C49*, *T(2;3)C230*] have been studied in *Drosophila melanogaster* (e.g. Roberts 1972). In maize, spontaneous desynaptic mutants *dy1* (Nelson and Clary 1952) and *dys1* (Maguire *et al.* 1993) have been used to study chiasma maintenance mechanisms. Maguire *et al.* (1993) found disjunctive irregularities, pachytene synaptic errors and synaptic failures in these mutants which resulted in the mutants being completely or nearly sterile. Desynaptic mutants (*sy7*, *sy10*) have also been identified in rye (*Secale cereale*) (Fedotova *et al.* 1994). They suggested that the process of recognition of homology is impaired in these mutants which leads to indiscriminate synapsis and prevents chiasma formation, as a result of which, incompletely synapsed, non homologous and multivalent "SCs" are formed.

2.3.1. *Ph* genes of wheat:

In wheat a very complex control system operates which allows pairing between homologous chromosomes only and not between homoeologous chromosomes. A number of wheat chromosomes (particularly those of homoeologous groups 3 and 5) have been reported to carry the genes responsible for controlled pairing of chromosomes during meiosis.

A high number of trivalents and some quadrivalents (in contrast to bivalents only in euploid wheat) were observed by Okamoto (1957) and Riley and Chapman (1958) in the wheat stocks deficient for chromosome V (later designated as chromosome 5B), indicating that the gene which allows pairing between homologous chromosomes only, is located on this chromosome. Soon after, Riley (1960) demonstrated that the controlling gene is present on the long arm of this chromosome. This dominant gene, regulating the chromosome pairing in wheat is now known as *Ph1* [the name *Ph* (pairing homoeologous) was first used by Wall *et al.* 1971].

It is known that *Ph1* is not the only gene controlling meiotic chromosome pairing in wheat. A number of pairing promoter genes [on short arms of homoeologous chromosomes of group 5 (Riley and Chapman 1967; Feldman 1966, 1968; Feldman and Mello-Sampayo 1967) and long arms of homoeologous chromosomes of group 3 (Miller *et al.* 1983)] and pairing suppressor genes on short arms of chromosomes 3A, 3B and 3D (Mello-Sampayo 1971; Driscoll 1973; Miller *et al.* 1983)] have been discovered.

Although chromosome pairing in wheat is controlled by a complex balance between several suppressor and promoter genes, among all of these loci, the gene present on the

long arm of chromosome 5B (*Ph1*) plays the most important role in allowing homologous pairing and inhibiting homoeologous pairing (for a review, see Sears 1976).

Since the discovery of *Ph1*, several attempts have been made to mutate the gene (e.g. Riley *et al.* 1966; Wall *et al.* 1971). These attempts were either only partially successful or produced mutations at loci other than *Ph1*. A true mutant plant (for the *Ph1* locus) of wheat cv. Chinese Spring was finally obtained by Sears (1977) which is called *ph1b* mutant (or Sears' *ph1b* mutant) and is evidently a deficiency for the *Ph1* locus. This mutant allows homoeologous chromosomes to pair and its effect at the cytological level is much better known than the other genes responsible for chromosome pairing in different organisms. Some other less effective pairing mutants, including *ph1a* obtained by Wall *et al.* (1971) and Sears' *ph2* mutants on chromosome 3DS (cited in Sears 1981), are also available. Giorgi and Cuozzo (1980) also obtained a radiation-induced deletion mutant (for the *Ph1* locus) of tetraploid wheat cv. Cappelli, which exhibit high homoeologous pairing. Jampates and Dvorak (1986) designated this mutant as *ph1c*, to differentiate it from *ph1b*.

2.3.2. Location of *Ph1* locus:

Because of the importance of the *Ph1* gene in introgressing useful alien genes from wild relatives into wheat, various studies have been made to locate and tag it with appropriate molecular markers. Sharp *et al.* (1988) reported a cDNA probe (PSR128), specific for the homoeologous group 5 chromosomes of Triticeae, which identifies the deleted segment in Sears' *ph1b* mutant (Dr. RMD Koebner, personal communication). Gill and Gill (1991) and Gill *et al.* (1993) described RFLP probes KsuS1 and Ksu75 which detects the missing segment in the two independent mutants *ph1b* and *ph1c* and

a higher intensity fragment in a duplication of the *Ph1* gene. They also reported the physical location of the *Ph1* gene to a submicroscopic chromosome region (*Ph1* gene region) marked by a DNA probe (*XKsuS1*). Clarke *et al.* (1992) isolated a clone, "a 39" (PSR1201) from a library produced by the phenol emulsion reassociation technique (PERT) driven by *ph1b* genomic DNA. Xie *et al.* (1993) showed that the *Xpsr1201* locus is located close to the centromere on the long arm of chromosome 5B. This supported an earlier finding by Sears, (as cited in Feldman 1993), who reported that the map distance between the *Ph1* locus and the centromere was 1 cM. Dvorak *et al.* (1984), found that the deletion in the *ph1c* mutant is associated with a specific C-banding pattern in the distal part of the long arm of chromosome 5B. The discrepancy between the genetic location of the *Ph1* locus near the centromere and its physical location near the middle of the long arm of chromosome 5B is attributed to the distal localisation of chiasmata in wheat chromosomes (Xie *et al.* 1993).

2.3.3. Mode of action of *Ph1* gene:

In root tip metaphases of common wheat (having two copies of *Ph1* allele) homologous chromosomes were found to be situated closer to each other than non homologous chromosomes and this somatic (mitotic) association was considered to be continued into meiotic prophase (Feldman 1966; Feldman and Avivi 1973). Partial synapsis of homologous and some pairing of homoeologous chromosomes in the presence of extra doses of *Ph1* in diisosomic and triisomic 5BL plants was also observed. In view of these findings, it was proposed that *Ph1* effects the pre meiotic alignment (before the commencement of synapsis) of both homologous and homoeologous chromosomes, and thereby controls the regulation and pattern of pairing (for a review, see Feldman 1993).

Hobolth (1981) and Holm (1986) proposed a different model for the action of the *Ph1* gene. They observed that multivalents and interlocking bivalents were common at the zygotene stage of meiosis in euploid wheat (*Ph1Ph1*), but these configurations were corrected in early pachytene and resulted in only bivalents at the end of pachytene, while in the *ph1b* mutant, the chiasmata were formed before pairing correction took place. Hence they proposed that the *Ph1* gene delays the crossing over until multivalents are corrected into bivalents. It supported an earlier conclusion of Driscoll *et al.* (1979) that the *Ph1* gene affects the process of chiasma formation rather than the association of chromosomes.

Gualandi *et al.* (1984) proposed that *ph1b* codes for a modified tubulin (a subunit of micro tubules) or for a protein which modifies tubulin structure.

Holm *et al.* (1988) summarised the available evidences and concluded that the *Ph1* gene ensures (1) high stringency of chromosome pairing at zygotene stage, (2) correction of chromosome pairing at zygotene and pachytene where multivalents are corrected into bivalents and (3) suppression of crossing over between paired homoeologous segments.

2.3.4. Origin and interaction of *Ph* genes with other loci in Triticeae:

The exact origin of the *Ph* genes of wheat is not known but it is likely that at least some pairing control genes were present in the ancestral diploid species (Dvorak 1987). Dover and Riley (1972) reported that activity similar to *Ph1* is exerted by the accessory chromosomes of diploid species *T. tripsacoides* (Jaub. et Spach) Bowden (*Ae. mutica* Boiss) and *T. speltoides*.

Interaction of the *Ph1* gene of wheat with other loci on different Triticeae chromosomes has also been studied. Schlegel *et al.* (1991) showed that dominant *Ph1* allele of chromosome 5B of wheat affects the homologous pairing of rye chromosomes. They found reduction in chiasmata frequency and an increase in the number of univalents and rod bivalents in a telocentric addition line having 14 chromosomes of rye plus the long arm of chromosome 5B of wheat. Jouve *et al.* (1980) using the C-banding technique demonstrated that a single allele *Ph1* on a 5B hemizygote of wheat can be inhibited by two doses of chromosome 5R of rye which has a pairing promoter gene on the short arm.

Genes controlling meiotic chromosome pairing have been discovered in other species of Triticeae as well. These include homoeologous chromosome pairing promoter genes present on chromosomes 5U of *T. umbellulatum* (Zhuk.) Bowden, 5R of *Secale cereale* (Riley *et al.* 1973), 3E, 5E and 6E of *Elytrigia elongata* (Syn. *Agropyron elongatum*) (Dvorak 1981, 1987) and homoeologous chromosome pairing suppressor genes present on chromosomes 4E and 7E of *E. elongata* (Dvorak 1987).

2.4. Introgression of alien gene(s) into wheat:

To help support the world's ever increasing population, production and quality of wheat has to be increased continuously. Although scientific breeding has produced high yielding uniform varieties of wheat, it has also narrowed the germplasm available within the species. Wheat breeders are now looking towards immediate progenitors or even to the more distantly related genera to derive the required genetic variability and desired genes.

Sears (1981) placed the available relatives of wheat into 2 broad groups made up of (i) species having one or more homologous genomes such as *T. turgidum* L., *T. monococcum* L., *T. tauschii* (Coss.) Schmal. (Syn. *Ae. squarrosa*), *T. timophevii*, *T. crassum* (Boiss.) Aitch.&Hensl. (Syn. *Ae. crassa*), *T. ventricum* Ces. (Syn. *Ae. ventricosa*), *T. cylindricum* Ces. (*Ae. cylindrica*), *T. juvenale* Thell.(Syn. *Ae. juvenalis*), *T. syriacum* Bowden (Syn. *Ae. crassa* ssp. *vavilovii*) and (ii) species with homoeologous genome(s) such as *T. searsii*(Feldman & Kislev) Feldman, comb. nov. (Syn. *Ae. searsii*), *T. longissimum* (Schweinf. & Muschli in Muschli) Bowden (Syn. *Ae. longissima*), *T. bicorne* Forsk (Syn. *Ae. bicornis*), *T. speltoides*, *T. kotschyi* (Boiss) Bowden (Syn. *Ae. kotschyi*) and several species of *Secale, Haynaldia, Agropyron, Hordeum* etc. Riley and Kimber (1966) published a comprehensive review of alien genetic transfer from these relatives into wheat. More recent reviews are those of Sharma and Gill (1983a), Knott (1987), Gale and Miller (1987) and Islam and Shepherd (1991). The gene transfer from these relatives into bread wheat can be achieved by four different methods viz; (i) homologous transfers (ii) homoeologous transfers (iii) radiation induced transfers and (iv) transfers induced by homoeologous chromosome pairing.

2.4.1. Homologous transfers:

Homologous transfers are comparatively easy and have been used to transfer the genes from diploid and tetraploid species having at least one genome in common with bread wheat. Stem rust resistance genes *Sr21* and *Sr22* were transferred from *T. monococcum* (genome AA) into bread wheat by Gerechter-Amitai *et al.*(1971) and The (1973). Some accessions of *T. tauschii* (genome DD) have also been used to transfer leaf rust resistance genes *Lr21* and *Lr22* into hexaploid wheat (Rowland and Kerber 1974). Several stem and leaf rust resistance genes including *Sr2* (Hare and McIntosh 1979),

Sr9d, Sr9e, Sr9g, Sr17, Lr14a, Lr23 (R. A. McIntosh, cited in Islam and Shepherd 1991) and *Sr11, Sr12, Sr13, Sr14* (Knott 1962) have been transferred from tetraploid species, *T. turgidum* (genomes AABB), into hexaploid wheat. Another tetraploid species, *T. timopheevii* Zhuk (genomes AAGG) has also been used to transfer *Sr36* and *Sr37* genes into bread wheat (for a review, see Knott 1989).

2.4.2 Homoeologous transfers:

Unlike homologous transfers, homoeologous transfers cannot be achieved through conventional hybridisation and backcrossing procedures, rather it requires special stocks and cytogenetic procedures. The first step in the introgression of alien genetic material into wheat is to add the alien chromatin to the wheat genome either by addition of the whole genome followed by chromosome doubling (e.g. triticale) or to use this amphiploid to add individual chromosomes to wheat, through the production of addition lines. In this way, a number of alien chromosomes have been added to wheat from the related species (for details, see the compendium of Shepherd and Islam 1988). However, wheat-alien addition lines could not be used directly in commercial agriculture because such lines have an unbalanced genotype and also the alien chromosome is often eliminated mainly through pollen selection and hence the addition lines tend to return to the euploid condition (Sears 1981; Islam and Shepherd 1991).

A somewhat better alternative to these addition lines is the production of substitution lines, where the alien chromosome / chromosome arm is substituted for the homoeologous wheat chromatin. A further improvement over substitution is where a whole arm of the alien chromosome has been translocated to a wheat chromosome by centric fusion which has been found to occur after simultaneous misdivision of two univalents and the spontaneous rejoining of arms from different chromosomes (Sears

1972). The best examples of such lines are 1R(1B) substitution and 1BL-1RS centric fusion lines, which are present in many European wheat cultivars (Mettin *et al.* 1973; Zeller 1973) and carry useful genes for disease resistance. These lines, although being cytologically stable, still carry a large amount of undesirable alien genetic material and/or do not compensate well for the loss of wheat chromatin. This usually leads to low yields and/or poor quality of the recipient wheat, for example, the poor quality of dough in the wheat-rye 1R substitution / translocation lines, which are otherwise high yielding and rust resistant. The larger than necessary amount of alien genetic material present in wheat-alien chromosome addition / substitution / translocation lines may be reduced either using X-rays or by manipulating the meiotic pairing genes, especially the *Ph1* locus.

2.4.3 Irradiation induced transfers:

Ionising radiations (X-rays) have been used successfully to transfer leaf (*Lr19*) and stem (*Sr26*) rust resistance genes (Sears 1956; Knott 1971) from *T. umbellulatum* and *Ag. elongatum* into bread wheat, respectively. Despite these successes, radiation induced transfers are not considered to be widely applicable mainly because of the large amount of work involved and the low probability of obtaining useful transfers. Sears (1956, 1972) obtained 17 wheat-*T. umbellulatum* recombinant chromosomes having a leaf rust resistance gene from the alien chromosome and found that only one of these translocations was not deleterious. Secondly, since the breakage and rejoining of the chromosomes is expected to involve random non-homoeologous chromosomes, most of the translocations are likely to have deleterious effects on the viability of gametes carrying them, particularly pollen, resulting in reduced transmission of the translocated chromosome to the progeny (Islam and Shepherd 1991).

2.4.4 Transfers using induced homoeologous pairing:

After the discovery of the effect of the *Ph1* gene on chromosome pairing, cytogenetic methods (principally based upon either removal or suppression of *Ph1* gene) have opened a whole new area of wheat improvement through induction of allosyndetic recombination between wheat and homoeologous alien chromosomes. For the removal of the *Ph1* gene, nullisomy for chromosome 5B has been utilised in two different ways. The simplest way was to pollinate monosomic 5B plant with the desired alien species and to select the recombinant chromosomes in the progeny after backcrossing with wheat F_1 plants deficient for chromosome 5B. Riley (1966) and Joshi and Singh (1978) used this technique and transferred genetic material from *T. bicornis* and rye into wheat, respectively. More efficiently, Sears (1972) used an alien chromosome substitution line and nullisomic 5B-tetrasomic 5D line to generate plants nullisomic for 5B and double monosomic for the alien chromosome and a wheat homoeologue. This method improved the prospects of obtaining directional transfer from a known alien chromosome carrying the required gene to a specific wheat chromosome. Sears (1972, 1981) transferred the leaf rust resistance gene *Lr24* from *Ag. elongatum* into wheat using this approach.

To avoid the complex aneuploidy, resulting from the use of nullisomic 5B procedures, deletion mutants for the *Ph1* locus can be used for the induction of allosyndesis in wheat-alien introgressions. Sears' *ph1b* mutant has been successfully used by Liang *et al.* (1979) for wheat-*Agropyron*, Koebner and Shepherd (1986) and Dundas *et al.* (1988) for wheat-rye, Islam and Shepherd (1992) for wheat-barley and Khan and Shepherd (1994, 1995) for wheat-*Ag. intermedium* introgressions. Marais (1992) used *ph1b, ph2b* and NT 5B,5D stocks to induce homoeologous recombination between a segment of *Thinopyrum distichum* chromosome and chromosome 7D of bread wheat.

Dvorak and Gorham (1992) used the *ph1c* mutant to transfer K^+/Na^+ discrimination from chromosome 4D of *T. aestivum* to chromosome 4B in the genetic background of *T. turgidum*.

Rather than deleting the *Ph1* gene, it is also possible to suppress its inhibitory action by adding epistatic genes from *T. speltoides* or *T. tripsacoides* genomes into wheat. The genetic material from these two species can be transferred directly to wheat by first making a hybrid and then backcrossing either the hybrid or a derived amphiploid to wheat (Riley 1966; Sears 1981). Furthermore, the wheat - *T. speltoides* amphiploid can be crossed with another alien species having desirable genes. The problem with this approach is that the *T. speltoides* chromosomes can pair with the wheat chromosomes or with the alien chromosome, thereby adding to the difficulty of isolating the desired recombinant chromosomes (Sears 1981). Nevertheless, Riley *et al.* (1968b) successfully used these epistatic genes from *T. speltoides* and transferred the stripe rust resistance gene *Yr8* from *T. comosum* into wheat. Chen *et al.* (1994a) transferred these epistatic (*Ph1*) genes from *T. speltoides* to *Triticum aestivum* and identified two duplicate and independent *Ph1* genes. As *Ph1* genes show dominant epistasis, homoeologous pairing between wheat and alien chromosomes is observed in the F_1 hybrids allowing easier transfer of alien genetic material to wheat as compared to recessive *ph1b* or nulli 5B induced introgressions. But the pairing level using *Ph1* system was reportedly not as high as that induced by the *ph1b* mutant (Chen *et al.* 1994a).

In addition to these methods, cell culture of wheat-alien chromosome addition lines has also been reported to be successful for subchromosomal introgression of barley yellow

dwarf virus (Banks *et al.* 1995) and cereal cyst nematode resistances (Larkin *et al.* 1990) from *Ag. intermedium* and cereal rye, respectively, into bread wheat.

2.5. Isolation of wheat-alien recombinant chromosomes:

After the induction of homoeologous pairing between wheat and alien chromosomes, the next vital step is to identify and isolate the recombinant chromosome. So far meiotic chromosome pairing frequencies, the target gene itself (in most cases alien genes conferring disease resistance), morphological characters, seed storage proteins and isozymes have been used as genetic markers to identify the alien chromatin and to isolate the recombinant chromosomes carrying introgressed alien chromatin.

2.5.1. Isolation of recombinant chromosomes using cytological techniques:

To transfer stripe rust resistance gene (*Yr8*) from chromosome 2M of *T. comosum* (Sibth. & Sm.) Richter (Syn. *Ae. comosa*) into wheat, Riley *et al.* (1968b) crossed and backcrossed the line of wheat (having chromosome 2M of *T. comosum* plus the *T. speltoides* genome) to euploid wheat until upon selfing a backcross derivative, a 42 chromosome rust resistant plant having 21 bivalents at meiosis, was obtained. Riley et al. (1968b) studied the cytology of diagnostic crosses involving the recombinant line with wheat monosomics and showed that the segment of chromosome 2M carrying the *Yr8* gene was actually transferred to chromosome 2D of wheat.

Sears (1972) transferred the leaf rust resistance gene (*Lr24*) from chromosome 3Ag of *Ag. elongatum* into common wheat. He used the *Lr24* gene together with the lack of any univalent in some pollen mother cells (PMCs) of hybrids between putative recombinant and normal wheat as a criterion to isolate the recombinant chromosomes. Sears (1972, 1973, 1981) used standard methods of monosomic analysis and crosses with appropriate

wheat telocentrics (Sears and Sears 1978) to identify the wheat chromosomes involved in these transfer lines and to study the structure of the translocated chromosomes. Low pairing with the critical wheat arm was taken as evidence for the presence of a complete or near complete alien arm whereas high pairing suggested the presence of a substantial proximal portion of the wheat arm. These ingenious methods developed by Sears for chromosome engineering, have proved successful but lengthy and involve a large amount of cytological work and crossing with tester stocks.

2.5.2. Isolation of recombinant chromosomes using biochemical markers:

For the more efficient detection of wheat-alien chromosome recombination, Koebner and Shepherd (1986) for the first time, used co-dominant seed storage protein loci as genetic markers. The markers loci *Tri-1* (triplet protein) and *Gli-D1* (ω glidin storage protein) on wheat chromosome arm 1DS, *Gli-B1* on 1BS and *Sec-1* (Secalin) and stem rust resistance gene (*SrR*) on rye chromosome arm 1RS, were used to detect cross-over products between homeologous group 1 chromosomes of common wheat and cereal rye, under *Ph1* deficient conditions. Following induction of allosyndesis and testing the backcross or F_2 progeny for dissociation of these markers, they were able to isolate nineteen recombinant lines, where thirteen lines were derived from wheat-wheat homoeologous recombination and six lines involved wheat-rye translocations. Koebner and Shepherd (1987) also induced pairing between chromosome 1U of *T. umbellulatum* and homoeologous wheat chromosomes and obtained 8% recombination by observing the dissociation of *Glu-1* (low molecular weight glutelin subunit)-*Gpi-1* (glucose phosphate isomerase)-*Gli-1* linkage on both the alien chromosome as well as along the wheat chromosomes 1B and 1D. Dundas *et al.* (1988) isolated several translocations between rye chromosome arm 6RL and wheat chromosome 6D, using dissociation of GOT (glutamic oxaloacetic transaminase), 6PGD (6 phospho-gluconate dehydrogenase)

and α-amylase-1 isozyme markers located on the long arms of these chromosomes. Larkin *et al.* (1990) and Banks *et al.* (1995) used Cauderon's L1 source of resistance and mostly using cell culture techniques, derived families which carry resistance to barley yellow dwarf virus [gene present on the long arm of chromosome 7Ai of *Ag. intermedium* (Brettell *et al.* 1988)] but were missing the short arm marker for 7Ai chromosome (red coleoptile). They also reported that after 3 or more generations of selections in some families with 2n = 42 and normal meiosis (21 bivalents), the resistance was no longer associated with a cytologically identifiable alien chromosomal segment. Marais (1992) isolated 7 wheat-*Thinopyrum distichum* recombinant chromosomes using leaf (a homoeoallele of *Lr19*) and stem (*Sr25*) rust resistance genes and genes for yellow flour pigmentation (all three genes present on the alien chromosome segment) as genetic markers. Islam and Shepherd (1992) isolated wheat-barley recombinant chromosomes from among the progeny obtained from self fertilisation of the triple monosomic stocks ($38_W+6HL'+6A'+5B'_{ph1b}$, $38_W+3HL'+3A'+5B'_{ph1b}$) by screening for dissociation of biochemical markers on the barley arms. GOT, ACO (aconitase hydratase) and DIP (dipeptidase) isozymes were used to select recombinants involving the 6HL arm, and EST (esterase) and MDH (malate dehydrogenase) were used for the 3HL arm. Although these biochemical markers (seed storage proteins and isozymes) are reliably reproducible and less costly to assay (as compared to RFLPs) they are limited in number and hence do not allow selection for homoeologous recombination along the entire length of the chromosome.

2.5.3. Use of molecular markers in characterising wheat-alien recombinant chromosomes:

Earlier, chromosome banding techniques along with the cytological investigations of diagnostic crosses had been used to characterise the wheat-alien recombinant chromosomes. Eizenga (1987) used acetocarmine-N-banding and chromosome pairing between the line having a translocated chromosome and telosomics 7AL, 7AS, 7BL, 7BS, 7DL, 7DS, to determine the location of the leaf rust resistance gene *Lr19* transferred from *Ag. elongatum* into wheat by Sears (1972). He found that in one transfer (#12) the *Lr19* gene was carried on the long arm of wheat chromosome 7A rather than the targeted chromosome 7D. Yamamori (1994) identified a specific terminal N-band on chromosome arm 5BL of common wheat, which was introduced from *T. timophevii* together with the leaf rust resistance gene *Lr18*. These banding techniques especially C-banding (Gill *et al.* 1991a) allow identification of most of the wheat chromosomes, but cannot be used if the chromosome involved is mostly composed of euchromatin (e.g. chromosome 7Ai of *Ag. intermedium*), and therefore lacks diagnostic C-bands.

With the rapid development in molecular biology during the last 10 years, molecular markers have emerged as more powerful tools than isozymes or seed protein variants (Gale and Sharp 1988), mainly because these molecular markers can occur in almost all parts of the genome. There are now increasing number of reports where recombinant DNA technology specially RFLPs and *in situ* hybridisations have been used to characterise the wheat-alien recombinant chromosomes. Koebner *et al.* (1986) used three molecular markers, rDNA, 5SRNA and a 350-family telomeric heterochromatin sequence, for the characterisations of wheat-rye recombinant chromosomes. Autrique *et al.* (1995) used RFLPs to identify molecular markers linked to four leaf rust resistance

genes (*Lr9, Lr19, Lr24* and *Lr32*) introgressed from wild relatives into wheat. Using RFLPs and isozymes, Larkin *et al.* (1995) studied the homoeology of *Ag. intermedium* chromosomes with that of wheat chromosomes, in the lines derived from "Zhong 5", a partial amphiploid (2n=56) between common wheat and *Ag. intermedium.* Sharma *et al.* (1995) using chromosome pairing and RFLPs, showed that 42-chromosome resistant Purdue wheat lines are substitution lines in which chromosome 7D of wheat has been replaced by a chromosome from *Ag. intermedium* which carries gene(s) for barley yellow dwarf virus (BYDV) resistance. Lelley *et al.* (1995) demonstrated that RFLPs can be used successfully as an alternative to C-banding for the identification of the chromosome composition of tetraploid triticale. Using RFLPs for the characterisation of wheat-*Ag. intermedium* recombinant chromosomes, Banks *et al.* (1995) found that all of the recombinant lines they obtained, involved chromosome 7D of wheat except one where recombination occurred between chromosome 7Ai of *Ag. intermedium* and an unidentified wheat chromosome.

In situ hybridisation using cloned DNA, species-specific sequences or total genomic DNA as labelled probes have been used successfully to look directly at alien chromosomes and chromosome segments in a wheat background (Rayburn and Gill 1985; Heslop-Harrison 1992; Gill 1993). Genomic *in situ* hybridisation is widely applicable because it does not involve screening and characterisation of markers and is fast, sensitive, accurate and gives unique information about alien chromatin introgressed into wheat. This technique has identified the parental origin of each chromosome in hybrids of *Hordeum chilense* Roem. and Schult and *H. vulgare* L. with *S. africanum* Stapf. (Schwarzacher *et al.* 1989) as well as alien chromosomes and chromosome segments from *S. cereale* (Reader *et al.* 1994) and *H. vulgare* in hexaploid wheat cultivars (Mukai and Gill 1991) and triticale (Le and Armstrong 1991). Schwarzacher *et*

al. (1992) used this technique to identify the alien chromatin in chromosome spreads of wheat lines incorporating chromosomes from *Leymus multicauli*, *Thinopyrum bessarabicum* and chromosome arms from *H. chilense*, *H. vulgare* and *S. cereale*. Labelled genomic DNA has also been used as a probe on Southern blots of genomic DNA digests to distinguish related species and to identify wheat lines with alien chromosomes (Anamthawat-Jonsson *et al.* 1990).

A combination of these recently developed techniques is also being used to obtain high resolution structure of wheat-alien recombinant chromosomes. Kim *et al.* (1993) have used *in situ* hybridisation and restriction fragment length polymorphisms to determine the relative location of the translocation breakpoints and the size of the integrated chromatin segment in hexaploid wheat-*Lophopyrum* (Syn. *Agropyron*) translocation stocks carrying leaf rust resistance gene *Lr19*, introgressed into bread wheat from *L. ponticum*. Banks *et al.* (1995) used the chromosome 7Ai of *Ag. intermedium* and by cell-culture techniques isolated fourteen putative wheat-*Agropyron* recombinant lines carrying resistance to BYDV (gene for BYDV resistance is present on long arm of this alien chromosome, Brettell *et al.* 1988) but missing the short arm 7Ai marker (red coleoptile). Later, Hohmann *et al.* (1996) characterised nine of these lines using C-banding, RFLP, deletion mapping and GISH techniques and found some non-reciprocal translocations for the most proximal regions of the chromosome arm 7DL which resulted in small duplications.

Although molecular markers are now being used widely to characterise alien segments introgressed into the wheat genome, there is no report so far on the direct use of these markers for the detection and isolation of wheat-alien chromosome cross-overs. The work presented in this thesis describes such a use of co-dominant DNA markers

(RFLPs) for the isolation of wheat-alien recombinant chromosomes, using chromosome 7Ai of *Ag. intermedium* and 7A of wheat as a model system.

2.6. Utilisation of *Agropyron* species in wheat improvement:

Agropyron is a large genus comprising about 150 species, and genomes S, E, J, C, H, X and Y have been identified. The degree of meiotic pairing between chromosomes of the A, B and D genomes of wheat and the different genomes of *Agropyron* has been reported to be low (Sharma and Gill 1983b). However, the *Triticum* and *Agropyron* genera are thought to be very closely related and for a long time were grouped together in *Triticum* (L.). The generic names *Thinopyrum* (Dewey), *Lophopyrum* (Love) and *Elytrigia* (Host) Nevski have been used by different authors as synonyms for *Agropyron*.

The genus *Agropyron* has been an important source of many disease resistance genes for wheat improvement and a number of commercially useful genes have been transferred to bread wheat, including the genes conferring resistance to rusts (Sharma and Knott 1966; Smith *et al.* 1968; Knott 1971; Sears 1972), wheat streak mosaic virus (Larson and Atkinson 1973), and wheat curl mite (Whelan and Hart 1988; Kim *et al.* 1992).

The perennial intermediate wheat grass, *Agropyron intermedium* (Host) P.B. Syn. *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey, is considered to be an important source for a number of characters of agronomic importance including stress tolerance (cold and drought) and resistance to diseases including barley yellow dwarf virus (Sharma *et al.* 1984, 1989; Xin *et al.* 1988; Brettell *et al.* 1988; Larkin *et al.* 1995; Banks *et al.* 1995; Sharma *et al.* 1995)), wheat streak mosaic virus and greenbug

resistance (Lay *et al.* 1971; Stoddard *et al.* 1987), rust resistance (Sharma and Knott 1966; Cauderon *et al.* 1973; Wienhues 1966, 1973), common bunt resistance (Sinigovets 1974), low temperature and drought tolerance (Fedak 1985). The genome designations assigned to this segmental allohexaploid species (Dewey 1962) varies between authors but it is generally agreed that it consists of 2 similar genomes (E & J or E1 & E2 or J1 & J2) and a third genome (X or Z or N). The species and genomes designations widely used in the literature are *Agropyron intermedium* and E1E2X, respectively, and the same nomenclature will be used throughout this thesis.

In an attempt to transfer the rust resistance from *Ag. intermedium* into wheat, Cauderon (1966) and Cauderon *et al.* (1973) crossed a French winter wheat cv. Vilmorin 27 with *Ag. intermedium* strain No. 57 and after several backcrosses to wheat isolated a series of addition and ditelosomic addition lines. They studied the location of genes controlling resistance to rusts in *Ag. intermedium* and found that the addition line L1 (2n = 44, 21_W"+Ag") was resistant to stem rust.

In another independent attempt to transfer resistance to leaf, stripe and stem rusts, Wienhues (1966, 1973) selected wheat - *Ag. intermedium* derivatives designated W44, W49 and W52. All these lines were derived from a partial amphiploid wheat - *Ag. intermedium* (2n = 8x = 56) carrying 7 pairs of *Ag. intermedium* chromosomes added to the wheat genome. By backcrossing this amphiploid with euploid wheat cv. Heine IV, wheat - *Ag. intermedium* chromosome addition lines were obtained and these were then either crossed with monosomics of wheat cv. CS or were subjected to X-ray treatment.

The and Baker (1970) using chromosome pairing and substitution studies and later, Figueiras *et al.* (1986) and Forster *et al.* (1987) using isozyme analyses showed that the

Ag. intermedium chromosome present in L1 addition line (produced by Dr. Y. Cauderon) is homoeologous to group 7 chromosomes of common wheat. Brettell *et al.* (1988) showed that the *Agropyron* chromosome present in addition line L1 also carries genes responsible for resistance to barley yellow dwarf virus (BYDV).

More recently, Friebe *et al.* (1992) characterised the lines W44, W49 and W52 (produced by Dr. A. Wienhues) using C-banding, *in situ* hybridisation and isozyme analysis and identified the lines W44 and W52 as 7Ai-2 (7D) and 7Ai-2 (7A) substitution lines, respectively, while the line W49 was identified as a wheat - *Ag. intermedium* chromosome tranaslocation line. They also studied the C-banding pattern of the *Ag. intermedium* chromosome present in addition L1 (produced by Cauderon 1966) and concluded that the *Ag. intermedium* chromosome present in the L1 addition line is different from that present in the W44 and W52 lines produced by Wienhues (1966). Friebe *et al.* (1992) named the *Agropyron* chromosomes present in the material produced by Cauderon (1966) and Wienhues (1966) as 7Ai-1 and 7Ai-2, respectively. To simplify the description of the genotypes used in the present study, the designation 7Ai-1 has been shortened to 7Ai and whenever used implies the 7Ai-1 chromosome, unless stated otherwise.

As chromosome 7Ai is approximately metacentric with an arm ratio of 1.1:1.0 (Friebe *et al.* 1992), there is some confusion in the literature about the designation of its short and long arms. The physically shorter arm has been shown to be equivalent genetically to the long arms of chromosomes 7A and 7B of wheat and vise versa (Hohmann *et al.* 1996). During the present study the arm present in the ditelosomic addition line TAF2d, produced by Cauderon *et al.* (1973) has been referred to as the short arm (α arm of Cauderon *et al.* 1973) of chromosome 7Ai and it carries the molecular markers which have been mapped to

the short arms of group 7 homoeologous chromosomes of wheat (Chao *et al.* 1989) and the short arm of chromosome 1 (7H) of barley (Heun *et al.* 1991; Kleinhofs *et al.* 1993). The *Agropyron* telosome present in the ditelosomic addition line F-17 (produced by Banks and Larkin, as cited in Hohmann *et al.* 1996) is referred to as the long arm and carries the RFLP loci which have been mapped on the long arms of homoeologous group 7 chromosomes of Triticeae (See chapter 4 for further discussion).

In the present study, chromosomes 7Ai of *Ag. intermedium* and chromosome 7A of common wheat have been used as a model system to study the process of homoeologous recombination along almost the entire length of two Triticeae chromosomes, using recently developed co-dominant molecular markers (RFLPs). The practical aim of the research was to introgress genes conferring stem rust and BYDV resistance on the short and long arms, respectively, of this alien chromosome into homoeologous wheat chromosomes.

CHAPTER - 3
MATERIALS AND METHODS

3.1. Plant material:

Seeds of Dr. Y. Cauderon's Vilmorin 27 wheat - *Agropyron intermedium* whole chromosome addition (L1, 2n = 44, $21_W"+7Ai"$) and ditelosomic addition (TAF2d, 2n = 42+2t, $21_W"+t"S$) lines (Cauderon 1966; Cauderon *et al.* 1973), the substitution (in Chinese Spring background) lines 7Ai (7A), 7Ai (7B), 7Ai (7D) (The and Baker, 1970) and long arm addition (F-17, $21_W"+t"L$) lines (produced by Banks and Larkin, as cited in Hohmann *et al.* 1996), Vilmorin wheat and a plant of *Agropyron intermedium* were kindly provided by Dr. P. Banks, Queensland Wheat Research Institute, Toowoomba.

Seeds of 7Ai-2 (7D) substitution line W44 produced by Dr. A. Wienhues (Wienhues 1966, 1973) were kindly provided by Dr. R. A. McIntosh, Plant Breeding Institute, Cobbitty, University of Sydney.

Genetic stocks of wheat cv. Chinese Spring (CS) including double monosomics (DM-5B,7A; -5B,7B; -5B,7D), the *ph1b* mutant line and nullisomic-tetrasomic (NT) for group 7 homoeologous chromosomes (NT 7A-7B, 7B-7A, 7D-7A) were originally obtained from the late Dr. E. R. Sears and then maintained by Dr. K. W. Shepherd at the Waite Agricultural Research Institute. Seeds of ditelosomic lines of wheat cv. CS including ditelosomic 7AS (dit. 7AS), dit. 7BS and dit. 7DS were kindly provided by Dr. A. J. Lukaszewski, Department of Botany and Plant Science, Riverside, California, USA. Seeds of wheat-barley addition line 7H (2n = 44, $21_W"+7H"$, Islam *et al.* 1981) were kindly provided by Dr. A.K.M.R. Islam, Waite Agricultural Research Institute.

Because of their strong winter habit, freshly germinated seedlings of Vilmorin 27, L1 and TAF2d were vernalised for 4 weeks at 4^0C. All the plants were grown under glasshouse conditions at the Waite Institute using standard cultural practices.

3.2. Cytological analyses:

To ascertain the somatic chromosome number of the genotypes, seeds were germinated in petri dishes on moist filter paper at 4^0C for 24 hours and then at $23-25^0C$ for 3-4 days. When the roots were 3-4 cm long, 1-2 mm of the root tips were excised and placed in pre-chilled reverse-osmosis (R.O.)water at 4^0C for 18-24 hours in order to increase the mitotic index and arrest the dividing cells at metaphase stage. Root tips were then fixed in 3:1 ethanol : glacial acetic acid at room temperature for 2 hours and then transferred to 4^0C in the same fixative solution until used. Before making the slides, root tips were washed in R.O. water for one hour at room temperature, hydrolysed in 1N HCl at 60^0C for 12 minutes and then stained with Feulgen reagent at room temperature for 30-45 minutes. Root tip squashes were made under a drop of 45 % acetic acid.

For meiotic studies, individual anthers were collected at the metaphase I stage, fixed and stained as described above, except that the hydrolysis time in 1N HCl was reduced to 8 minutes.

The C-banding technique used was adapted from Gill *et al.* (1991a) with minor modifications. Root tips were first treated as described above. The fixed root tips were squashed in a drop of 45 % acetic acid and the quality of the slide was checked using phase contrast microscopy. Coverslips were flicked off after freezing in liquid nitrogen and the slides were dehydrated in 98 % ethanol for 10-15 minutes at room temperature followed by air drying. The dried slides were hydrolysed in 0.2 N HCl at 60^0C for 3 minutes, rinsed in R.O. water, and then transferred to a saturated solution of $Ba(OH)_2$ for 20 minutes at room temperature followed by washing under running tap water for 2-3

minutes. Subsequently, the slides were transferred to 2XSSC [0.3M NaOH, 0.03 M tri sodium citrate (pH 6.8)] at 60^{0}C for one hour and then stained with 1% Giemsa solution in phosphate buffer (0.5 M Na$_2$HPO$_4$, 0.5 M KH$_2$PO$_4$). Finally the slides were rinsed in R.O. water, dried on a hot plate and a coverslip was mounted with Depex (Gurr) mounting medium. Photographs were taken with an Olympus microscope with camera attachment (model BH2) using "Kodak Technical Pan" film.

3.3. Isozyme and water soluble protein-1 analyses:

The isozymes of enzyme systems - α-amylase-2 (α-AMY-2) and endopeptidase-1 (EP-1) and water soluble protein-1 (WSP-1) were separated by flat bed isoelectric focusing. The genes α-Amy-2 (Nishikawa and Nobuhara 1971; Gale *et al.* 1983), *Ep-1* (Hart and Langston 1977) and *Wsp-1* (Liu *et al.* 1989) are located on the long arms of homoeologous group 7 chromosomes of wheat. The gels comprised 5% w/v polyacrylamide, 0.1% bis (N,N'-methylene-bis-acrylamide) and 2% carrier ampholyte [polymerised by addition of 5μl TEMED (N,N,N',N'-tetra methyl ethanediamine) and 0.5 mg/ml amonium per sulphate] were 12 cm wide and 0.5 mm thick and were run on a Multiphor II electrophoresis system (Pharmacia). The protocols used were essentially the same as described by Gale *et al.* (1983) for α-AMY-2, by Koebner *et al.* (1988) for EP-1 and Liu *et al.* (1989) for WSP-1, respectively.

3.4. DNA isolation:

For small scale total genomic DNA isolation, about 10 cm of fresh leaf material from 3-4 weeks-old seedlings was ground to a fine powder in a 2 ml eppendorf tube under liquid nitrogen. The powder was homogenised with 750 μl of DNA extraction buffer (4% sarkosyl, 0.1 M Tris-HCl, 10 mM EDTA, pH 8.0, Weining and Langridge 1991) and then an equal volume of phenol:chloroform:isoamylalcohol (25:24:1) was added to it. The whole mixture was vigorously shaken for 20-30 seconds and the aqueous phase was recovered by centrifugation at 12000 rpm for 3 minutes. The supernatant was transferred

to a fresh tube and mixed with an equal volume of ice cold chloroform, mixed gently by inverting the tubes and centrifuged for 1 minute. The DNA was precipitated from the supernatant by adding 1/10 volume of 3M sodium acetate (pH 5.5) and equal volume of isopropanol. The DNA was pelleted by centrifugation for 7 minutes. The pellet was washed twice with ice cold 70% ethanol, dried at 37^0C and dissolved in 40-50 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 40 µg/ml RNAase (Sigma). The concentration of DNA was estimated by comparing its intensity with that of the λ DNA of known concentration (Promega) on a 0.8% agarose/TAE gel.

(N.B. All the aqueous solutions used in DNA isolation, PCR, RFLPs and *in situ* hybridisations were made in nanopure water)

3.5. Polymerase chain reaction (PCR):

The primers used during the present studies including A1, A2 (α-amylase primers), and intron splice junction (ISJ) primers (R1, R2, E3, E4), (Weining and Langridge 1991) were kindly provided by Dr. P. Langridge, Waite Agricultural Research Institute. Twenty three "sequence-tagged site" (STS) primers, whereby PCR primers were designed from mapped low copy number sequences (Talbert *et al.* 1994) were kindly provided by Dr. Tom Blake. The polymerase chain reactions were carried out in 25 µl volume containing 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$ and 0.2 units of Taq DNA Polymerase (Advanced Biotechnologies), overlayed with a drop of mineral oil. The amplification conditions using A1, A2 primers were as follows: An initial denaturation step of 4 minutes at 94^0C, followed by 45 cycles each consisting of a denaturation step of 1 min at 94^0C, followed by an annealing step of 2 min at 55^0C, and an extension step of 2 min at 72^0C. The last cycle was followed by 10 minutes extension at 72^0C, to ensure that primer extension reaction proceeded to completion. While using "ISJ" primers, for the first 6 cycles, the annealing temperature was 40^0C, while for the rest of the cycles annealing was carried out at 55^0C. For "STS"

primers, the annealing temperature used was 49°C. The rest of the reaction conditions including denaturation and primer extension were same. All the amplification reactions were performed using a "PTC-100 programmable thermal controler" (Bresatec). The amplification products were electrophoresed on 2.6% agarose/TBE gels and visualised by staining with ethidium bromide and viewing under UV light.

3.6. Restriction fragment length polymorphisms (RFLPs):

For the different RFLP probes, used during present studies, bacterial clones (kindly provided by Dr. P. Langridge), were grown on Lauria Bertaini (LB) medium (Sambrook *et al.* 1989) at 37°C for 18-24 hours with constant agitation. Plasmid miniprep DNA was prepared using the alkali lysis method as described by Sambrook *et al.*(1989). The inserts were amplified by PCR using M13 forward and reverse primers (Molecular Resources, Colorado State University), separated on 1.5% Agarose/TAE gels and cleaned using Progenius DNA purification kit (Progen).

About 10-15 micrograms of total genomic DNA was digested with restriction enzymes following the instructions of manufacturers. The DNA fragments were separated on 0.8% agarose/TAE gels and transferred to Hybond N+ membrane (Amersham) using 20 XSSC as the transfer buffer. The DNA on the membrane was fixed with 0.4 M NaOH.

The membranes were prehybridised for 3-4 hours at 65°C (Hybaid hybridisation oven, Hybaid) in a solution containing 10% Dextran Sulphate, 5X Denhardt's III, 2XSSC and 100 µg/ml autoclaved salmon sperm DNA. 50-100 ng of the probe DNA was labelled with ^{32}p (dCTP) using 9mer random primers (Molecular Resources, Colorado State University) according to the method described by Sambrook *et al.* (1989) and cleaned through a Sephadex G-100 column. The labelled probe was denatured in boiling water for 10 minutes, chilled on ice for 5 minutes and then was added to the prehybridisaton solution. Hybridisation was carried out overnight at 65°C. Membranes were washed at

65^0C for 20 minutes each at 2XSSC, 1XSSC, 0.5XSSC, 0.2XSSC with 0.1% SDS. They were then wrapped in plastic sheets and exposed to X-Ray film at -80^0C using intensifying screen, for 4-5 days or as needed. The membranes were reused after stripping in filter stripping solution.

3.7. Stem rust testing:

For the test entries (including short arm recombinants and the parents used in the present study) 6-12 seedlings/genotype were grown under glasshouse conditions. At the two leaf stage, the seedlings were inoculated with pathotype '21-2,3,7' (nomenclature used was described by Watson and Luig 1961) of stem rust which was multiplied on wheat genotype W195 which is highly susceptible to rust but resistant to powdery mildew. The freshly collected rust spores (kindly provided by Dr. Ian S. Dundas, Waite Agricultural Research Institute) were mixed with talcum powder (in a ratio of approximately 1:40) and sprayed on the plants already moistened with a fine spray of R.O. water. Seedlings were then transferred to a darkened humidity chamber at $25-30^0C$ for 12-14 hours. After this period, seedlings were placed under normal glasshouse conditions. Disease reactions were scored 10-14 days after inoculation. When plants gave a reaction which was difficult to score as resistant or susceptible due to light infection, a repeat inoculation was carried out after three weeks.

3.8. BYDV resistance screening:

For screening for BYDV resistance, 12-18 seedlings per genotype including the long arm recombinants and parental lines, were grown under glasshouse conditions. After 10-12 days following germination, at least 5-7 viruliferous aphids infected with BYDV-PAV$_{Adel}$ isolate and grown on susceptible oats variety 'Stout' (Kindly provided by Mr. Brendon King, Waite Agricultural Research Institute) were inoculated on each seedling (referred to as test entries). This BYDV isolate was originally collected from the field near Adelaide (for details see Collins 1996). Six plants from each of the parent line (also planted at the

same time) were kept away from the test entries and were used as uninoculated control populations. After 3 days, aphids were killed by spraying with Rogor insecticide and after 4-5 weeks the plants were observed for the symptoms (also see chapter 6). At this stage (4-5 weeks after inoculation) a sample of 6 randomly selected plants (4 plants from test entries and 2 from control populations) was subjected to Northern dot blot hybridisation analysis (Collins 1996) to determine the presence of BYDV in test entries.

3.9. Genomic *in situ* hybridisation:

The method used for genomic *in situ* hybridisation was adapted from Reader *et al.* (1994) with minor modifications. The total genomic DNA of *Agropyron intermedium* was mechanically sheared to 12-15 kbp by passing through a sterile syringe fitted with a 27G needle, 200 times (more if necessary) and then labelled with Fluorescein-11-dUTP (Fluorogreen, Amersham) by the nick translation method. The labelling reaction was stopped by adding 1/10 volume of 0.3M EDTA (pH 8.0). At this stage blocking DNA (total genomic DNA from wheat cv. CS, also sheared as described above) was added at 35-70X concentration of the probe DNA and the labelled probe DNA + the unlabelled blocking DNA was precipitated by ethanol precipitation.

Freshly-fixed root tips were treated with a solution of 10% pectinase + 1% Cellulase (Dr. Ralf G. Kynast, personal communication) in 10mM Citrate buffer (10 mM citric acid, 10 mM tri sodium citrate) at 37^0C for 1 hour and squashed in a drop of 45% acetic acid. After checking the quality of the slides under phase contrast, coverslips were flicked off after freezing with liquid nitrogen. The chromosome preparations were treated with 5μg/ml RNAase in 2XSSC at 37^0C for 45 minutes, fixed with 4% freshly depolymerised paraformaldehyde (pH 7.2) and dehydrated in an ice cold ethanol series (Reader *et al.* 1994). Forty μl of hybridisation mixture (per slide) containing 50% deionised formamide, 10% dextran sulphate, 0.1% SDS, 10 μg autoclaved salmon sperm DNA, 2XSSC and 400-500 ng of labelled *Agropyron* DNA plus 35-70X unlabelled blocking

DNA was denatured for 10 min at 90-95^{0}C, chilled on ice for 5 minutes and applied to the slide under a plastic coverslip. The slides were then incubated in a humidity chamber at 80-90^{0}C for 10 minutes and then at 37^{0}C for 18-24 hours. After hybridisation, slides were washed at 42^{0}C twice in 2XSSC for 5 minutes, once in 50% formamide in 2XSSC for 10 minutes, twice in 2XSSC for 5 minutes and then finally for 10 minutes in 2XSSC at room temperature. Chromosomes were counterstained with 0.25 μg/ml 4'-6-diamidino-2-phenylindole.2HCl (DAPI) in McIlvaine buffer (100 mM citric acid, 200 mM Na$_2$PO$_4$, pH 7.0, Dr. R. G. Kynast, personal communication) and 0.1 μg/ml Propidium Iodide (PI) in 1X phosphate buffered saline (PBS, Sigma) for 3 minutes each at room temperature. Preparations were mounted with Vectashield (Vector laboratories) and observed on an Olympus microscope fitted with reflected light fluorescence attachments and appropriate optical filters. The photographs were taken using "Kodak Ektachrome 160 T" film.

CHAPTER - 4

DETECTION OF POLYMORPHISM AMONG THE GROUP 7 HOMOEOLOGOUS CHROMOSOMES OF WHEAT AND *AGROPYRON INTERMEDIUM*

4.1. Introduction:

The family Gramineae includes many important cereal crops including wheat, barley, rice, maize, sorghum and oats. Detailed genetic maps based on molecular markers for various species within the family have been developed, including rice (McCouch *et al.* 1988), barley (Heun *et al.* 1991; Graner *et al.* 1991; Kleinhofs *et al.* 1993) and maize (Helentjaris *et al.* 1986). In comparison with these diploid species, the equivalent genetic map of hexaploid wheat is poorly developed mainly because of the relatively large number of chromosomes and the relative paucity of polymorphism at many loci. The large size of the wheat genome (estimated 16 billion bp per haploid nucleus and the higher proportion (75 %) of repetitive sequences of varying degree and length (for a review, see May and Appels 1987) have also added to the difficulty in constructing a linkage map of wheat chromosomes. Nevertheless, hexaploid wheat offers some unique advantages for genetic mapping as well, including (i) availability of nullisomic-tetrasomic (Sears 1966) and ditelosomic (Sears and Sears 1978) stocks for the 21 chromosomes, which allow easier location of the genes on a particular chromosome/chromosome arm, (ii) the triplication of genetic material, often allowing one probe to provide simultaneous classification of at least three independent loci in a single assay and (iii) a number of chromosomes / chromosome segments have been transferred and maintained in wheat from related species in the Triticeae (for a review, see Islam and Shepherd 1991).

RFLPs were first proposed by Botstein *et al.* (1980) to be used as genetic markers for construction of high resolution linkage maps. Since then they have been used to construct genetic maps in humans as well as in various plant species. Because the RFLPs

are almost unlimited in number and usually act as co-dominant genetic markers, thereby allowing individual alleles to be recognised in heterozygotes and also at homoeoloci, their use is generally not confounded by polyploidy. However, because of polyploidy and the narrow genetic base of wheat (Kihara 1944; McFadden and Sears 1946), many DNA probes show limited polymorphism among cultivars (Chao *et al.* 1989; Kam-Morgan *et al.* 1989). On the other hand, the wild relatives of wheat, including *T. tauschii* (Kam-Morgan *et al.* 1989; Gill *et al.* 1991b) and the primitive hexaploid *T. spelta* (Liu and Tsunewaki 1991) are rich in RFLPs and have been used to construct the genetic maps of cultivated wheats.

Before embarking on a study of homoeologous recombination between the group 7 chromosomes of wheat and *Agropyron*, it was necessary to find polymorphic marker loci for the chromosomes. In this chapter, detection of polymorphism for group 7 chromosomes of wheat and *Ag. intermedium* using different assay procedures including C-banding, isozyme analyses, PCR and RFLPs, is described.

4.2. Materials and methods:

4.2.1. Plant material:

Prior to their use in crossing programs or for detection of polymorphism, the chromosome constitution of each genotype used, was ascertained by root tip mitosis and meiotic studies of the pollen mother cells (PMCs). Some examples of these screenings are presented in Fig. 4.1. To ascertain the chromosome constitution (Table 4.1) of plants at least five counts per plant were made at metaphase of mitosis in root tips or at metaphase I in PMCs. Double monosomic plants (DM -5B,7A; -5B,7B; -5B,7D) were initially isolated from the selfed progeny of DM plants by root tip mitosis (2n = 40) and then confirmed at meiosis. The frequency of isolating plants with the correct chromosome configuration (19"+1'+1') was only about 10 % in these progeny. The presence or absence of critical wheat and/or *Ag. intermedium* chromosome / chromosome

arms in the different genetic stocks was ascertained using restriction fragment length polymorphism assays (section 4.3.4).

Table 4.1. Chromosome constitution of the genetic stocks used in this study (for further details refer to Chapter 3).

Genetic stock		Chromosome constitution	
Designation	Classification	Root tip mitosis	MI in PMC
CS	Euploid	2n=42	21"
L1	Alien addition	2n=44	22"
7Ai (7A/7B/7D)	Alien substitutions	2n=42	21"
TAF2d	Short arm addition	2n=42+2tS	---
F-17	Long arm addition	2n=42+2tL	---
W44	Alien substitution [(7Ai-2 (7D)]	2n=42	---
7H addition	Barley addition	2n=44	---
DM-5B,7A;-5B,7B;-5B,7D	Double monosomics of CS	2n=40	19"+1'+1'
NT 7A-7B, 7B-7A, 7D-7A	Nullisomic-tetrasomic for homoeologous group 7 chromosomes of CS	2n=42	---

--- = Not checked by meiosis

Fig. 4.1: Mitotic metaphase of (a) euploid wheat cv. CS (2n = 42), (b) Vilmorin 27 wheat - *Ag. intermedium* whole chromosome addition line L1 (2n = 44), (c) double monosomic 5B,7A (2n = 40) and (d) meiotic metaphase I of DM 5B,7A (19"+1'+1').
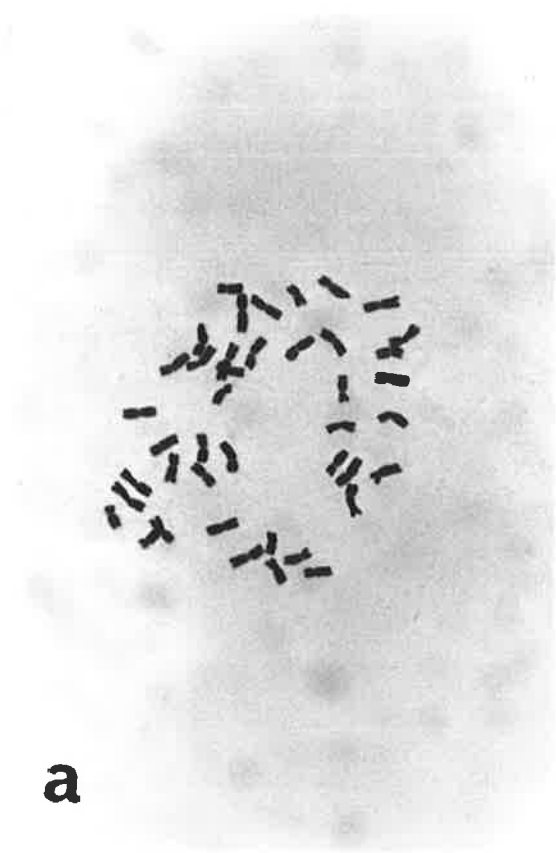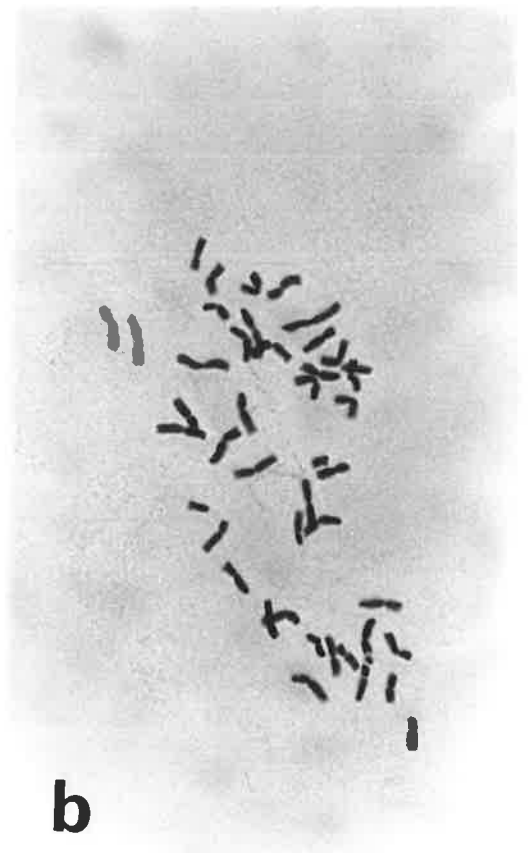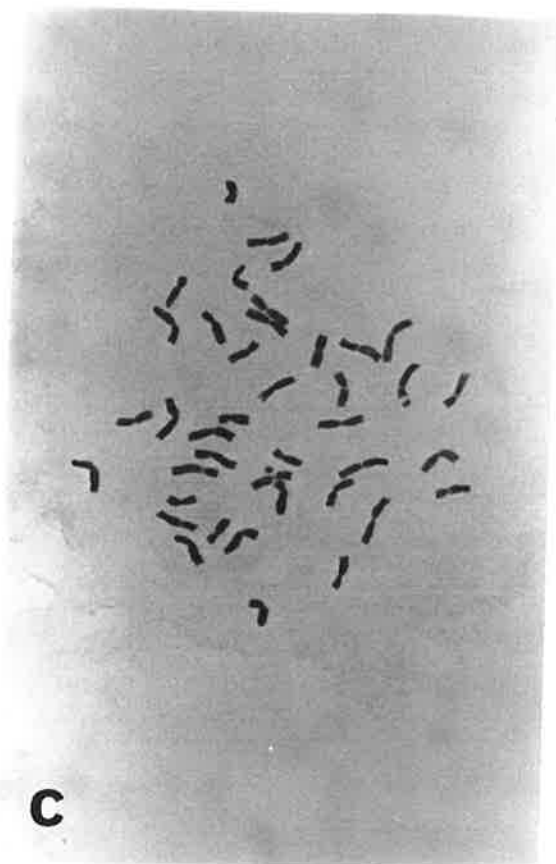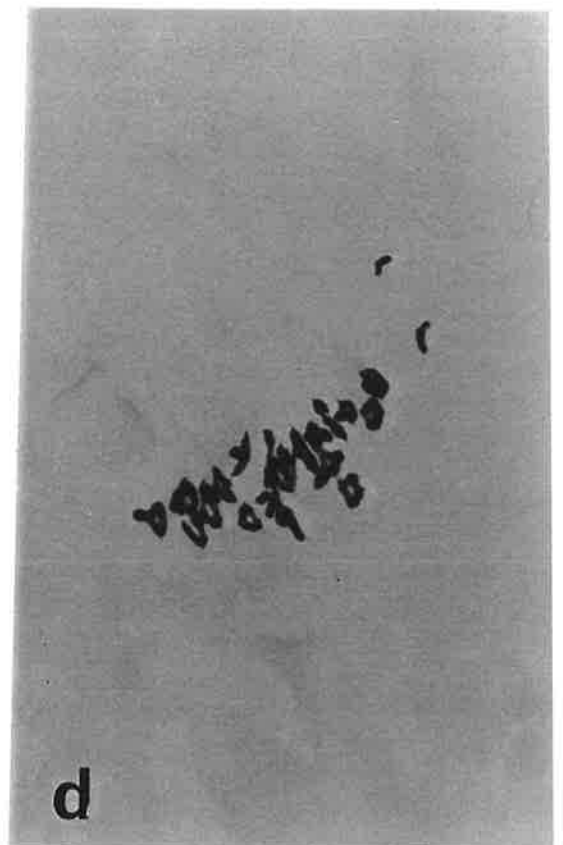
4.2.2. Assay procedures to detect polymorphism between different chromosomes:

Different assay procedures including C-banding, isozyme analyses (for α-amylase 2 and endopeptidase-1), analysis for WSP-1 (water soluble protein-1), polymerase chain reactions and restriction fragment length polymorphism, were employed to obtain useful polymorphism between group 7 homoeologous chromosomes of Triticeae. Detailed procedures for these assays have been given in chapter 3.

Fifty-seven different cDNA or genomic RFLP probes (Table 4.2) previously mapped on the different homoeologous group 7 chromosomes of the tribe Triticeae were tested on the experimental materials in Southern hybridisations to search for the required polymorphism.

Table 4.2. DNA probes used to detect polymorphism between group 7 homoeologous chromosomes in Triticeae .

Clone designation	Origin	Reference	Chromosome location reported	No. of probes tested
ABC / ABG	NABGMP[1]	Kleinhofs *et al.* 1993	Chr. 1 of barley (7H)	10
BCD	Cornell[2] NABGMP	Heun et at. 1991 Kleinhofs *et al.* 1993	Chr. 1 of barley (7H)	5
CDO	Cornell	Heun *et al.* 1991	Chr. 1 of barley (7H)	10
pTag	Japan[3]	Liu and Tsunewaki 1991	Homoeologous group 7 chromosomes of wheat and *T. spelta*	6
WG	Cornell	Heun *et al.* 1991	Chr. 1 of barley (7H)	7
ksu	Kansas[4]	Gill *et al.* 1991	7D (*T. tauschii*)	5
PSR	Cambridge[5]	Chao *et al.* 1989	Homoeologous group 7 chromosomes of wheat	14

1 = North American Barley Genome Mapping Project. 2 = Cornell Univ., NY, USA.
3 = Kyoto University, Japan. 4 = Kansas State University, USA.
5 = Institute of Plant Science Research, Cambridge laboratory, UK.

4.3. Results:

4.3.1. C-banding:

C-banding technique (as described in section 3.2) was employed on ditelosomic addition lines TAF2d and F-17 (having short and long arms, respectively, of chromosome 7Ai of *Ag. intermedium* added to the wheat genome). Both the long (Fig. 4.2 a) and short (C-banding pattern not presented) arms of chromosome 7Ai showed some faint C-bands at the telomeres, but neither arm exhibited any prominent diagnostic C-bands, indicating that this chromosome is largely composed of euchromatin. This result is consistent with the earlier findings of Friebe *et al.* (unpublished, cited in Friebe *et al.* 1992) and Hohmann *et al.* (1996) and further supported the hypothesis that chromosome 7Ai comes from the X genome of *Ag. intermedium* with an unknown origin (Dr. P. Banks, personal communication). In contrast to chromosome 7Ai, chromosome 7Ai-2 of *Ag. intermedium* [(added to wheat by Wienhues (1966)] present in the substitution line W44 [7Ai-2 (7D)] was easily distinguished from wheat chromosomes by the presence of a prominent C-band on the long arm, very close to the centromere (Fig. 4.2 b).

4.3.2. Analyses for isozymes and WSP-1:

Analyses of α-amylase 2 isozymes sometimes, showed an additional band of similar mobility as that described by Forster *et al.* (1987) for chromosome 7Ai in the addition line L1 as compared to euploid wheat cvs. CS and Vilmorin 27. However, this difference could not be reliably reproduced and it was not considered a suitable marker for the current work. Analysis of endopeptidase-1 and water soluble protein-1 (WSP-1) did not show polymorphism for the chromosome 7Ai.

Fig. 4.2: C-banding patterns of mitotic metaphase chromosomes in

(a) the ditelosomic addition line F-17. (Arrows point to the long arms of chromosome 7Ai of *Ag. intermedium*).

(b) the 7Ai-2 (7D) substitution line W44. (The arrow points to chromosome 7Ai-2 of *Ag. intermedium*).

a
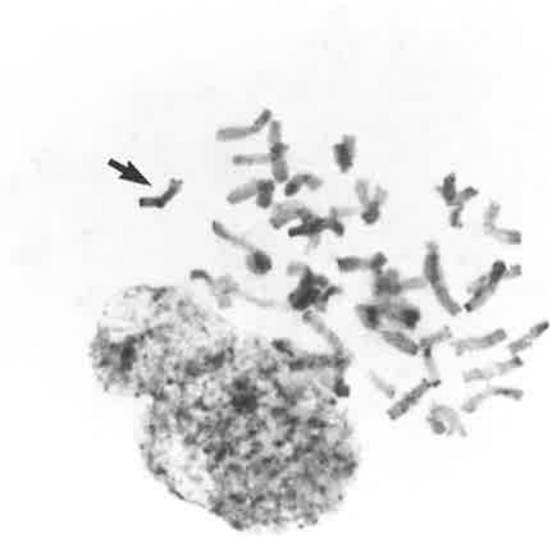
b

4.3.3. Polymerase chain reactions:

Most of the PCR primers used in the search for suitable polymorphisms had previously been reported to show PCR products specific for barley chromosome 1 (7H) (Weining and Langridge 1991; Talbert *et al.* 1994; Dr. T. Blake, personal communication). Therefore the disomic addition line of CS carrying chromosome 1 (7H) of barley (2n = 44, $21_W"+7H"$) was always included as a positive control in these assays. PCR products were assayed from DNA extracted from CS, Vilmorin 27, *Ag. intermedium*, L1 and 7Ai (7A) lines, to look for useful polymorphic differences among these genetic stocks. Polymorphic bands were detected for chromosome 1 of barley (7H) using A1, A2, and most of the intron splice junction (ISJ) and "sequence-tagged site" (STS) primers (details of the primers have been described in Chapter 3) but no reliably reproducible polymorphisms were obtained for chromosome 7Ai. An example of DNA amplification using primer set A1 and A2 is presented in Fig. 4.3.

4.3.4. Restriction fragment length polymorphisms:

The RFLP probes listed in Table 4.2 were tested on DNA extracted from reference stocks for *Ag. intermedium* chromosome 7Ai [*Ag. intermedium*, CS, Vilmorin 27, L1, 7Ai (7A), 7Ai (7B), 7Ai (7D)]) and nullisomic-tetrasomic (for group 7 homoeologous chromosomes of wheat cv. CS) lines, to search for restriction fragments characteristic for chromosome 7Ai of *Ag. intermedium* and/or group 7 homoeologous chromosomes of common wheat. Different restriction endonucleases were used to digest the DNA extracted from these lines to identify probe / restriction enzyme combination giving polymorphisms. Initially seven restriction enzymes, *BamH I*, *Bgl II*, *Dra I*, *Hind III*, *EcoR I*, *EcoR V* and *Xba I* (Promega) were used, but later, only four of them (excluding *Bgl II*, *EcoR I* and *Xba I*) were used routinely, mainly because it was found that increasing the number of restriction enzymes only slightly increased the level of polymorphism detected and also because polymorphism was frequently observed with more than one restriction enzyme for each probe.

Fig. 4.3: PCR products generated by primer set A1 and A2.

Template DNA from (1) barley cv. Betzes (2) wheat cv. CS (3) substitution line 7Ai (7A) (4) addition line L1 (21_W"+7Ai") (5) wheat cv. Vilmorin 27 (6) *Ag. intermedium* (7) barley chromosome 1 (7H) addition line (CS background). The arrow points to the diagnostic band for barley chromosome 1 (7H).

1   2   3   4   5   6   7
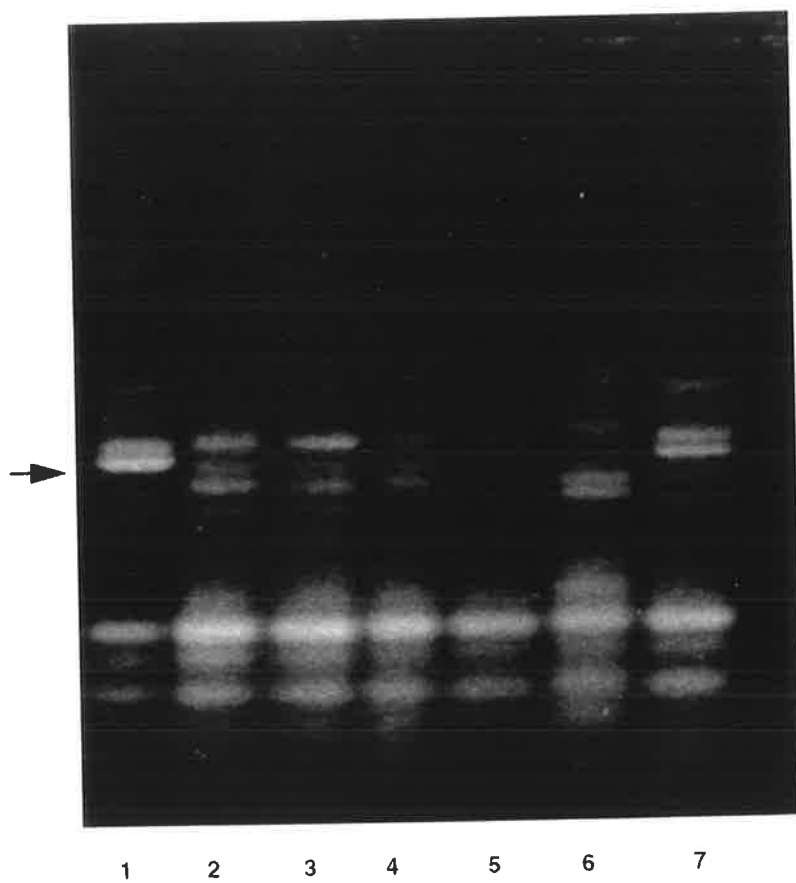
Fifteen probes revealed suitable polymorphisms by generating single or low copy number restriction fragment(s), specific for the group 7 homoeologous chromosomes under study (Table 4.3). In most cases, one restriction enzyme (*EcoR V*) was found to be sufficient to generate RFLPs between the chromosomes, but if none was observed with this enzyme then additional restriction enzymes were used. To determine the arm location for the locus associated with polymorphic probes, wheat - *Ag. intermedium* ditelosomic addition (TAF2d = 21$_W$"+tS", F-17 = 21$_W$"+tL") and wheat ditelosomic (dit. 7AS, dit. 7BS, dit. 7DS) lines were assayed. Because of the unavailability of homoeologous group 7 long arm ditelosomic lines of common wheat, absence of the chromosome specific band(s) in the short arm ditelosomic lines was taken to indicate the likely location of the probe loci on the long arm. Examples of these screenings are shown in Fig. 4.4.

In almost all the cases, the arm locations of the probes were in agreement with those previously describes in the published maps, except for the *Xwg719* locus (nomenclature used for RFLP loci adapted from McIntosh *et al.* 1994), which was previously mapped on the short arm of chromosome 1 (7H) of barley (Heun *et al.* 1991), while during present studies the locus was found to be on the long arms of chromosomes 7A, 7D and 7Ai. This result was in agreement with recently published maps for wheat homoeologous group 7 chromosomes (Hohmann *et al.* 1994, 1995) who also mapped the *Xwg719* locus on the long arms of homoeologous group 7 chromosomes of wheat.

Table 4.3. DNA probes and restriction enzymes used to detect restriction fragments specific for chromosome 7Ai and/or group 7 homoeologous chromosomes of wheat.

	Clone designation	Restriction enzyme used	Specific restriction fragment detected for chromosome(s)
Short arm markers			
1.	CDO475	*EcoR V*	7A, 7D
2.	CDO545	*EcoR V*	7Ai, 7A
3.	CDO595	*EcoR V*	7Ai, 7A, 7B, 7D
4.	PSR103	*Dra I*	7Ai, 7A, 7D
6.	PSR119	*Hind III*	7A, 7D
5.	PSR108	*EcoR V*	7A
7.	PSR152	*BamH I*	7Ai, 7B
Long arm markers			
8.	CDO347	*EcoR V*	7Ai, 7B
9.	CDO673	*EcoR V*	7Ai, 7A, 7B, 7D
10.	PSR117	*EcoR V*	7Ai, 7A, 7B, 7D
11.	PSR121	*EcoR V*	7Ai
12.	PSR129	*Hind III*	7Ai, 7A, 7B
13.	WG420	*BamH I*	7Ai, 7B
14.	WG686	*EcoR V*	7Ai, 7A, 7B, 7D
15.	WG719	*EcoR V*	7Ai, 7A, 7D

Note that all the possible probe - restriction enzyme combinations were not tested.

Fig. 4.4: Southern hybridisations of different probes to DNA from genetic stocks used during present study.

(a). Ethidium bromide stained gel and

(b). Southern hybridisations of the same filter using RFLP probe PSR103.

Total genomic DNA was digested with *Dra I*.

1 = Wheat cv. CS, 2 = *Ag. intermedium*, 3 = Addition line L1 (21$_W$"+7Ai"), 4 = Wheat cv. Vilmorin 27, 5 = Substitution line 7Ai (7A), 6 = NT 7A-7B, 7= Substitution line 7Ai (7B), 8 = NT 7B-7A, 9 = Substitution line 7Ai (7D), 10 = NT 7D-7A, 11= wheat-*Ag. intermedium* short arm addition line TAF2d (21$_W$"+tS"), 12 = wheat-*Ag. intermedium* long arm addition line F-17 (21$_W$"+tL").

(c). Southern hybridisation using RFLP probe WG 686. Total genomic DNA was digested with *EcoR V*.

(1) CS, (2) *Ag. intermedium*, (3) Addition line L1, (4) Vilmorin 27, (5) Substitution line 7Ai (7A) (6) NT 7A-7B, (7) Short arm addition line TAF2d, (8) Long arm addition line F-17 (21"$_W$+tL"), (9) NT 7B-7A, (10) NT 7D-7A

(d). Southern hybridisation using RFLP probe WG719. Total genomic DNA was digested with *EcoR V*.

(1) CS (2) *Ag. intermedium* (3) Addition line L1 (4) Vilmorin 27 (5) Substitution line 7Ai (7A) (6) NT 7A-7B (7) Short arm addition line TAF2d (8) Long arm addition line F-17 (9) Ditelosomic of wheat cv. CS (Dit.7AS) (10) Dit. 7BS (11) Dit. 7DS.

Note that probe WG686 produced two specific fragments for chromosomes 7A.

Arrows indicate the chromosome specific bands.

M = Molecular size markers (kbp) using *Hind III* digested λ DNA are shown on left.
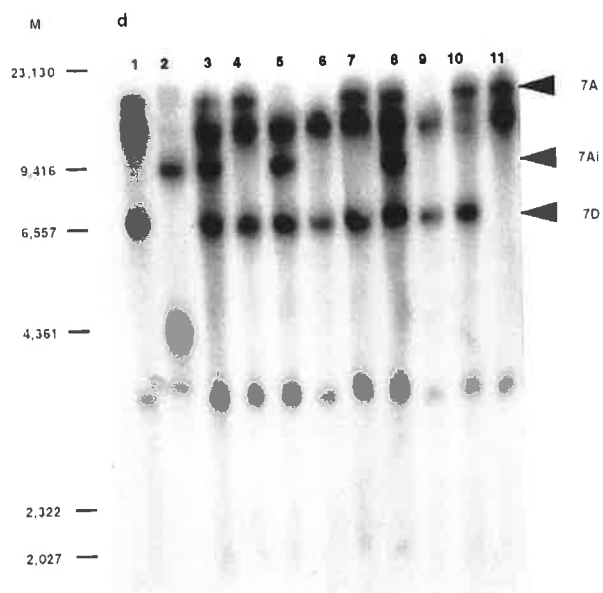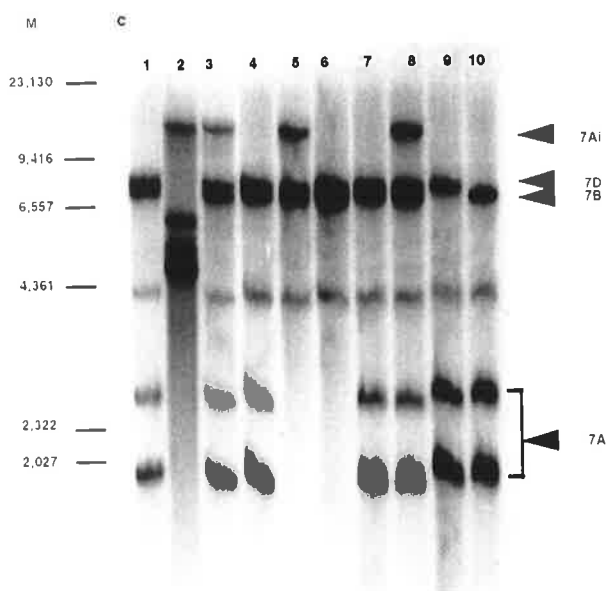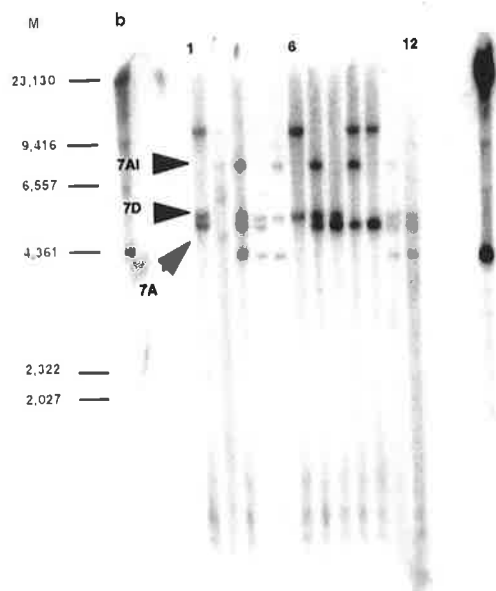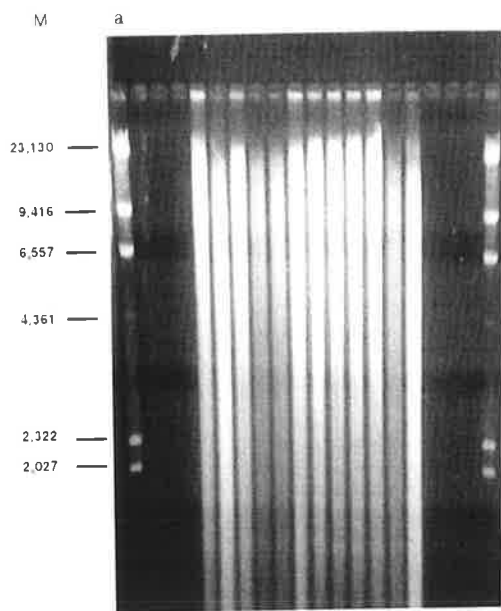
4.4. Discussion:

The failure to obtain useful polymorphisms for chromosome 7Ai using cytological, biochemical and PCR markers and generation of a substantial number of reliably reproducible polymorphisms for the chromosome using restriction fragment length assays indicated the greater value of RFLPs as molecular markers for the chromosome(s) across the genomes. The four restriction enzymes used during present studies (all having six base recognition sequences) had previously been reported to be useful in other mapping studies including those with wheat (Gill *et al.* 1991; Liu and Tsunewaki 1991; Chao *et al.* 1989), barley (Kleinhofs *et al.* 1993; Heun *et al.* 1991) and tomato (Miller and Tanksley 1990). Three of these enzymes (*Dra I, EcoR V, Hind III*) involved A or T for at least four of the six recognition sequences, which are known to generate more RFLPs in different plant species including wheat (Chao *et al.* 1989), maize and tomato (Helentjaris *et al.* 1986), rice (McCouch *et al.* 1988) and lettuce (Landry *et al.* 1987) as compared to C-G rich sequences. However, these findings were opposite to those obtained in human RFLPs where sites containing CpG were found to be more polymorphic (Barker *et al.* 1984).

In the present work, chromosome 7A was selected as the model wheat chromosome to study the process of wheat - alien homoeologous chromosome recombination, mainly to avoid possible complications from the known 7BS/4AL translocation (Naranjo *et al.* 1987) where a segment of the short arm of chromosome 7B has been translocated to the long arm of chromosome 4A. Such structural changes were expected to complicate interpretation of the homoeologous recombinant products.

Out of the fifteen polymorphic probes analysed, six probes, CDO -545, -595 (Short arm markers), CDO673, WG686, PSR -117, and -121 (long arm markers) were selected for detection and isolation of recombinants involving the targeted chromosomes 7Ai and 7A. These probes were selected because they appeared well spread along the length of the

chromosomes. Because a genetic map for chromosome 7Ai has not been published, the location and map distances between these loci on chromosome 7Ai could not be ascertained but their relative linear order was deduced from the published maps for group 7 homoeologous chromosomes of wheat and barley, assuming collinearity had been retained within these Triticeae species (for details, see review of literature).

The two short arm markers selected were known to be widely separated on the short arm of barley chromosome 1 (7H) (Heun *et al.* 1991) (Fig. 4.5). The long arm markers were also well spread along the length of the long arms of wheat and barley group 7 chromosomes and 4 markers were selected to allow any double cross-over products to be detected if they occurred in the long arms. In addition, all of these probes except one (PSR121) showed polymorphic bands with one restriction enzyme (*EcoR V*) for both the targeted chromosomes (7Ai and 7A), (PSR121 did not show any polymorphic band for chromosome 7A using *EcoR V*) enabling the same filters to be reused for all the Southern hybridisations. The cost of the enzymes and the ease of observing RFLPs were also taken into account before selection of the marker RFLP probes.

Fig. 4.5: Location and order of 15 polymorphic RFLP loci on genetic maps of group 7 homoeologous chromosomes of the Triticeae.

a = Chromosome 1 (7H) of barley (Heun *et al.* 1991).

b = Chromosome 1 (7H) of barley (Kleinhofs *et al.* 1993).

c = Homoeologous group 7 chromosomes of wheat (Chao *et al.* 1989).

C = Location of centromere.

Numbers above chromosomes are published genetic distances (cM).

† Genetic distance to the most distal locus reported on the published map.

* Indicates synteny based on the aneuploid stock, but where the contingency Chi-square test revealed no linkage (Chao *et al.* 1989).

** The breakpoint for 7BS / 4AL translocation (Naranjo *et al.* 1987) inferred to be proximal to *Xpsr119* (Chao *et al.* 1989).

Short arm   Long arm

| | 19.7 | | 56.2 | | 12.2 | | 2.9 | | 35.3 | | 31.6 | | 27.5 | | 3.7 † | | a |

Chromosome
1of barley (7H)

Xcdo *545*   Xcdo *475*   Xwg *719*   Xcdo *595*   Xcdo *673*   Xwg *686*   Xwg *420*   Xcdo *347*

C

| † 32.0 | | 67.1 | | 8.2 | | 46.5 | | 4.0 | | 22.3 † | | b |

Chromosome 1
of barley (7H)

Xcdo *475*   Xwg *719*   Xcdo *673*   Xpsr *129*   Xwg *420*

C

| | 3.0 | | 8.0 | | 3.0 | | 48.0 | | 12.0 | | 91.0 * | | c |

Homoeologous
group 7 chromosomes
of wheat

Xpsr ** *119*   Xpsr *108*   Xpsr *103*   Xpsr *152*   C   Xpsr *117*   Xpsr *129*   Xpsr *121*
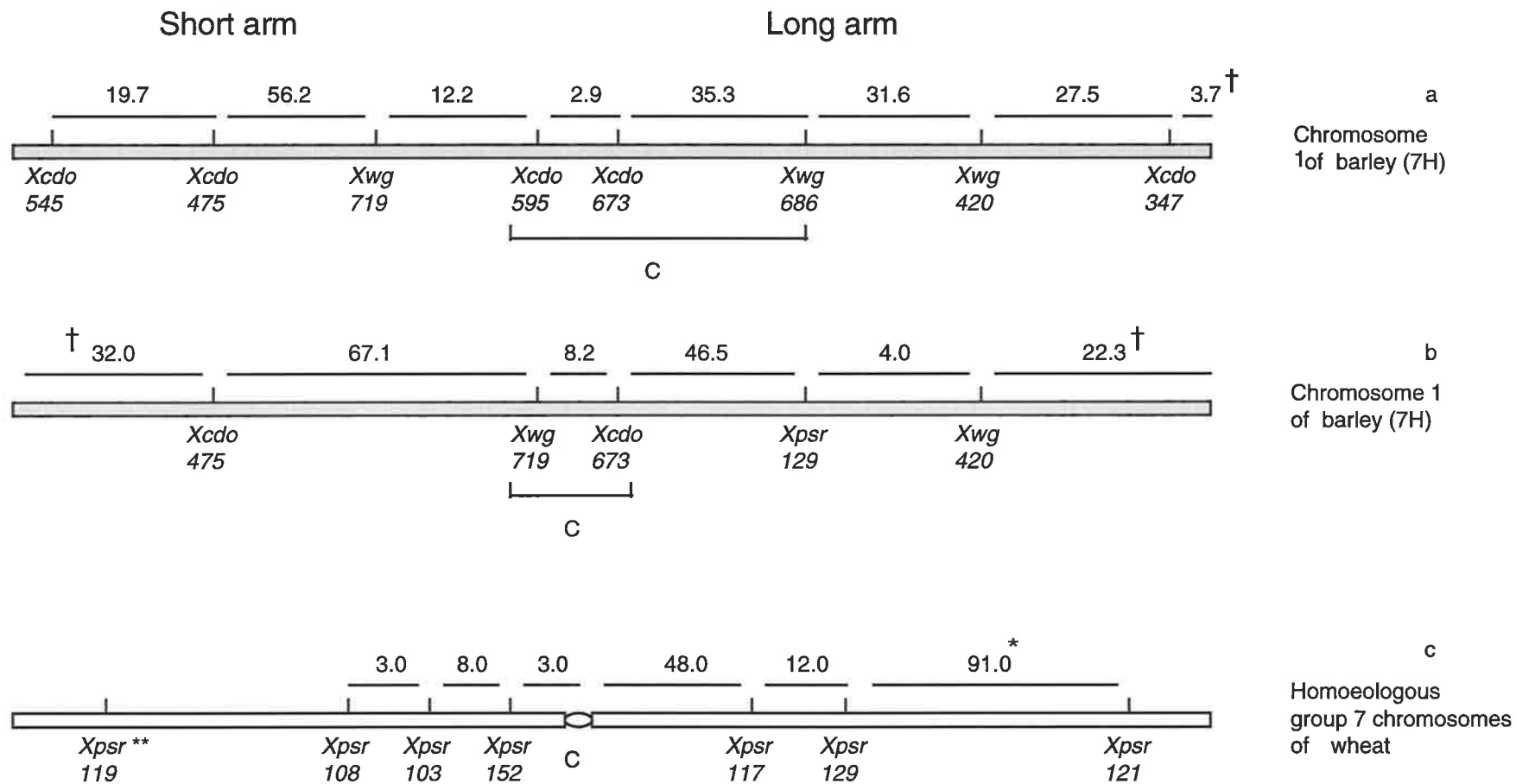
CHAPTER - 5

INDUCTION OF ALLOSYNDESIS AND ISOLATION OF WHEAT - *AGROPYRON* RECOMBINANT CHROMOSOMES

5.1. Introduction:

After E.R. Sears transferred the leaf rust resistance gene from *T. umbellulatum* (*Lr9*) into common wheat by irradiation-induced translocation (Sears 1956), much interest has been shown in introducing other useful genes (especially those conferring resistance to diseases and insects) into wheat from different alien species, including *Agropyron*. Several 56-chromosome amphiploid derivatives of wheat carrying a complete set of *Agropyron* chromosomes, and also some individual *Agropyron* chromosome addition, substitution and translocation lines have been produced. Most of these wheat derivatives carrying *Agropyron* chromatin have had limited use in practical breeding, because the alien chromatin usually carried genes which are detrimental to agronomic performance. However, some of these lines have proved to be excellent sources of resistance against various wheat pathogens and have been utilised in breeding programs and released as cultivars, including *Agropyron* chromosome segments containing genes *Lr -19, -24, -29, Sr -24, -25, -26* (Knott 1989).

Although various workers have claimed successful transformations with exogenous DNA in the Triticeae, the frequency and stability of the transformants obtained and reproducibility of the technique is still low (for reviews, see Vasil 1994; Maheshwari *et al.* 1995). Therefore, at present chromosome manipulation by sexual hybridisation followed by genetic recombination remains the most practicable method for utilising genes in wheat from other members of the tribe. Genetic recombination between wheat and the alien chromosomes is normally prevented by the stringent pairing control mechanism in wheat which restricts pairing to homologues. Following the demonstration

of the role of the *Ph1* gene in restricting meiotic pairing to homologous chromosomes (Okamoto 1957; Riley and Chapman 1958), there have been many efforts made to remove this restriction so that the alien chromosome segments carrying desirable genes can be induced to recombine with homoeologous wheat segments. Although a number of cytogenetic manipulations have been described to induce homoeologous chromosome pairing, during the present studies, Sears' (1977) *ph1b* mutant was selected because it offers some advantages over other available methods (details have been described in chapter 1).

To facilitate the detection of recombinants resulting from cross-overs between wheat and homoeologous alien chromosomes, it is necessary to have linked marker loci on the target chromosomes so that dissociation of these markers can be used to select putative recombinants as shown by Koebner and Shepherd (1985). For general applicability, such genetic markers should be widely spread over the genome, show a high degree of polymorphism, co-dominant expression and be assayed both easily and economically. Biochemical loci (seed storage proteins and isozymes) previously used as genetic markers, are co-dominant, easy to score and comparatively cheap to assay but are less polymorphic than DNA markers (Gale and Sharp 1988) and do not extend over a large part of the genome, especially in the proximal regions of the chromosomes close to the centromere (Islam and Shepherd 1992). Despite these limitations, biochemical markers have been used successfully to isolate a few wheat - alien recombinant chromosomes, over the past 10 years. For example, Koebner and Shepherd (1986), and Islam and Shepherd (1992) have used such markers to select rare wheat-rye and wheat-barley recombinant chromosomes, respectively.

Although co-dominant DNA markers (RFLPs) are more expensive to assay as compared to the biochemical markers, they are more generally applicable to such studies because they are more numerous and show more polymorphism than isozyme markers.

Furthermore unlike biochemical assays, RFLPs do not depend upon sample extraction from any particular tissue at a specific developmental stage; a sample of DNA from about 5-10 mg of any living plant tissue will suffice.

In this chapter, the production, identification and isolation of a large number of *ph1b*-induced wheat-*Agropyron* recombinant chromosomes, using RFLP markers for selection is reported. The recombinants were isolated in the F3 progeny of especially constructed genetic stocks monosomic for chromosomes 7A of common wheat and 7Ai of *Ag. intermedium* and homozygous or hemizygous *ph1b*. The main objectives of the study were to study the pattern of homoeologous chromosome recombination along the length of chromosomes 7A and 7Ai, but in doing so attempts were also made to transfer the genes conferring resistance to the stem rust fungus and barley yellow dwarf virus, present on the short (Cauderon *et al.* 1973) and long (Brettell *et al.* 1988) arms of chromosome 7Ai, respectively, into homoeologous chromosomes of bread wheat.

5.2. Materials and methods:

5.2.1. Selection of homozygous *ph1bph1b* plants:

Homozygous *ph1bph1b* plants to be used in the crossing program were selected from the selfed progeny of Sears' *ph1b* mutant and their chromosome pairing behaviour was checked at metaphase I. Reduction in overall chiasmata frequency (observed as a higher than normal number of univalents), along with the presence of multivalents in some of the PMCs (Fig. 5.1), was used initially as evidence for the correct genotype (Koebner and Shepherd 1985). Because the mutant line itself had undergone an unknown number of selfings, any plants showing a large number of quadrivalents and/or trivalents in the majority of the PMCs were not used in the crosses, to minimise the transmission of unbalanced gametes resulting from the accumulation of chromosome translocations within the wheat genomes.

Fig. 5.1: Configuration of chromosomes at MI in PMCs of a *ph1bph1b* plant. Note the occurrence of a substantial number of univalents, rod bivalents and multivalents. Diagnostic univalents (a) and multivalents (b) are arrowed.

a

b
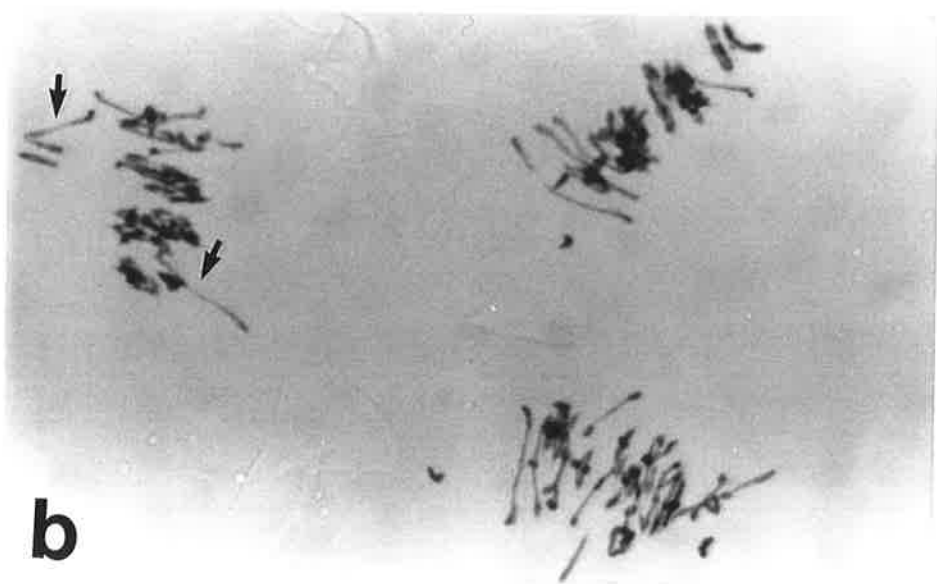
Later, a single copy cDNA probe PSR128 (described in chapter 3) was used to detect RFLPs between Chinese Spring (CS) and Sears' *ph1b* mutant, which are isogenic lines except for the deletion involving the *Ph1* locus. Total genomic DNA from CS and *ph1bph1b* plants (previously checked by meiotic pairing behaviour) was digested with six restriction enzymes (*BamH I, Bgl II, Dra I, EcoR I, EcoR V* and *Hind III*) and hybridised as described earlier (section 3.6, chapter 3). In all digestions, the homozygous *ph1bph1b* genotypes showed one less band than euploid CS (Fig. 5.2), indicating that the hybridisation site of the probe PSR128 lies within or very close to the deleted segment (on the long arm of chromosome 5B) in Sears' *ph1b* mutant. After the introduction of this assay with probe PSR128, meiotic data were no longer required to detect plants lacking the *Ph1* gene. Although all of the six restriction enzymes gave reliable and reproducible polymorphism for the locus, *EcoR V* was chosen for routine use to detect *Ph1* deficient genotypes, because the same filters could be reused to detect group 7 chromosome markers using the selected RFLP probes (described in section 4.4, Chapter 4).

Fig. 5.2: Hybridisation of the RFLP probe PSR128 to genomic DNA digested with

(A) *Dra I*, (B) *BamH I*, (C) *Bgl II*, (D) *EcoR I*, (E) *EcoR V* and (F) *Hind III*.

Each panel consists of six lanes.

Lanes 1&6 of each panel = DNA from euploid wheat cv CS.

Lanes 2-5 of each panel = DNA from four different Sears' *ph1b* mutant plants, previously isolated by studying metaphase I configuration of meiotic chromosomes.

The diagnostic bands for the *Ph1* locus are arrowed. (Note that using *BamH I*, two diagnostic bands for the *Ph1* locus were obtained.

M = Molecular size markers (kbp) using λ DNA digested with *Hind III* are given on left
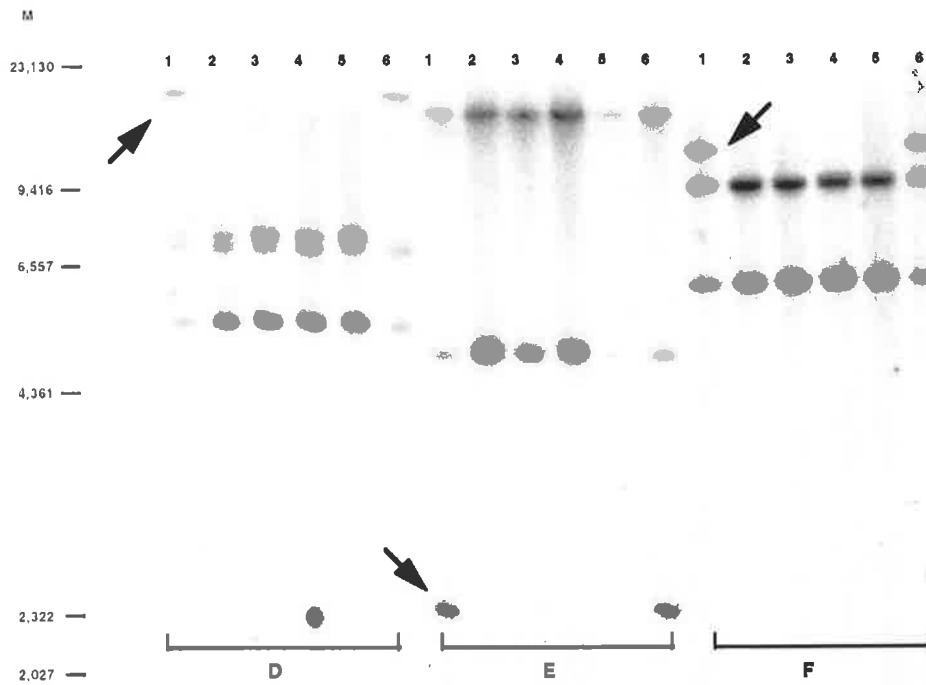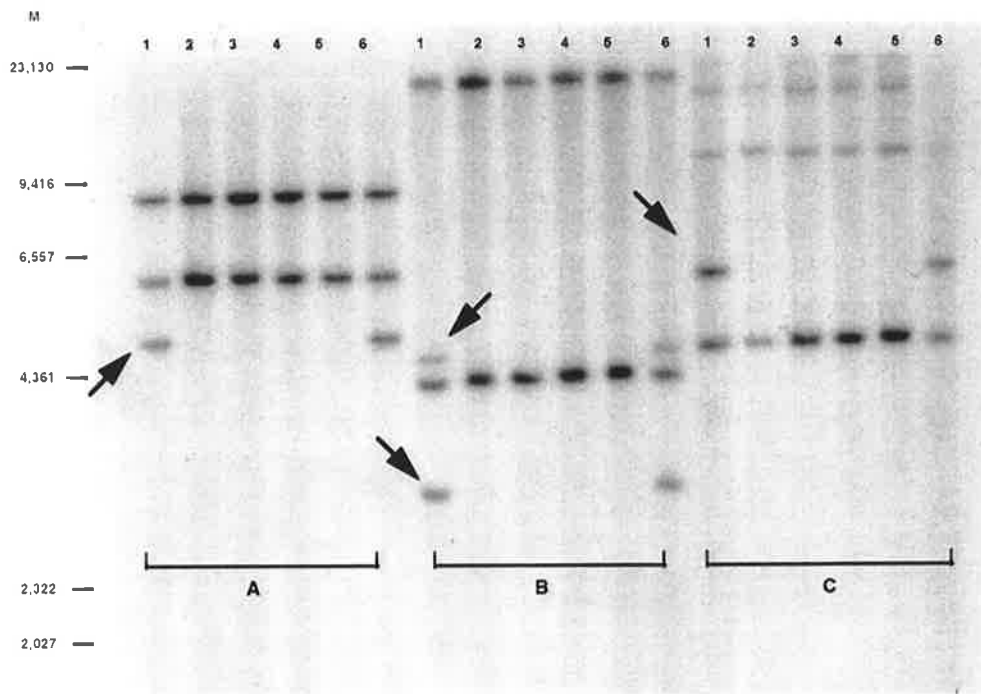
5.2.2. Crossing procedures:

The required progeny for screening for wheat-*Agropyron* recombinant chromosomes were produced using the following crossing procedures:

(i) Direct crossing of the 7Ai (7A) substitution line with Sears' *ph1b* mutant:

7Ai (7A) substitution line   X   Sears' *ph1b* mutant
(2n=42, 20$_W$"+7Ai")   (42$_W$, *ph1bph1b*)

$$\downarrow$$

F$_1$
(2n = 42, 20$_W$"+7A'+7Ai', *Ph1ph1b*)

$$\downarrow \oplus$$

F$_2$
Select 42 chromosome progeny
monosomic 7A, 7Ai, homozygous *ph1bph1b*
(2n=42, 40$_W$+7A'+7Ai', *ph1bph1b*)

$$\downarrow \oplus$$

Screen F$_3$ progeny for recombinants
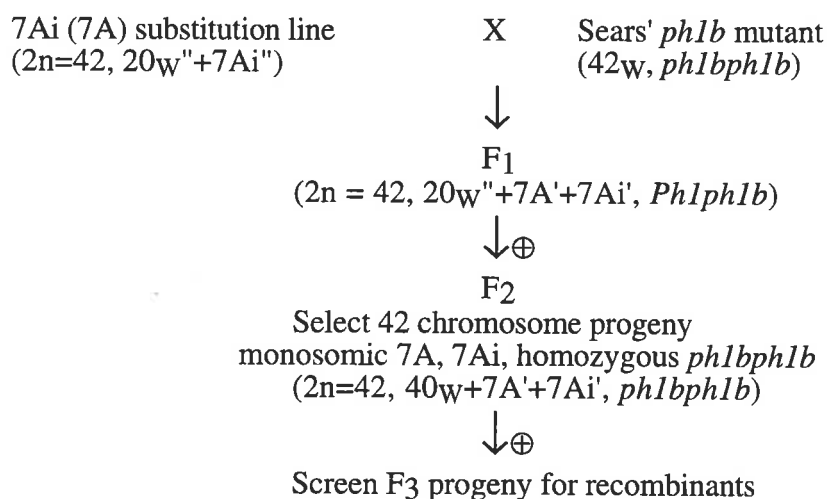
(ii) Production of triple monosomic plants (2n=41, 38$_W$ + 7A' + 7Ai' + 5B', *ph1b*):

Double monosomic 5B,7A   X   7Ai (7A) substitution line
(2n=40, 19$_W$"+5B'+7A')   (2n=42, 20$_W$"+7Ai")

$$\downarrow$$

F$_1$
Select 40 chromosome progeny   X   Sears' *ph1b* mutant
(2n = 40, 19$_W$"+5B'+7Ai', *Ph1*)   (42$_W$, *ph1bph1b*)

$$\downarrow$$

F$_2$
Select triple monosomic progeny
(2n=41, 38$_W$+5B'+7A'+7Ai', *ph1b*)

$$\downarrow \oplus$$

Screen F$_3$ progeny for recombinants
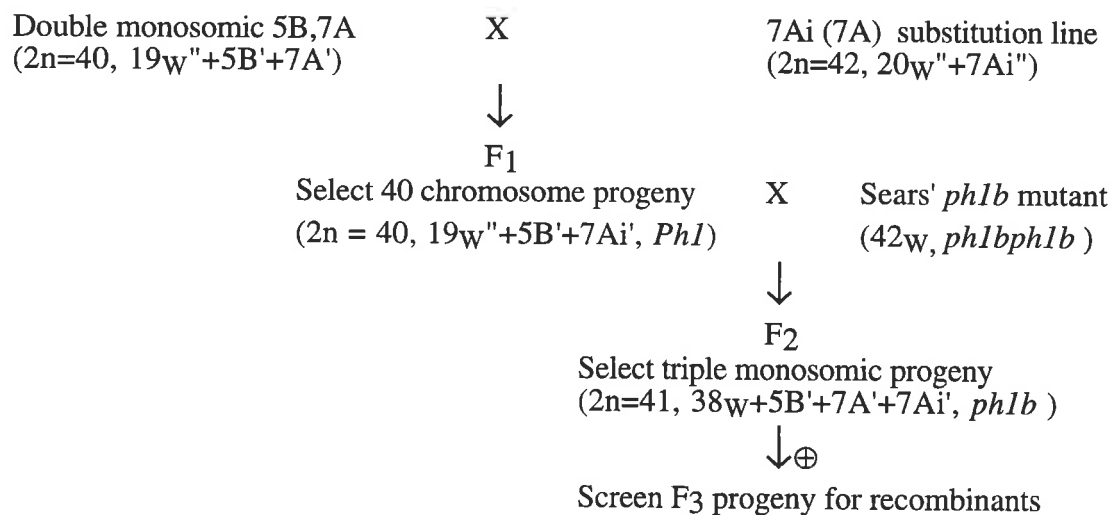
5.2.3. Detection of recombinants using co-dominant markers:

The efficiency with which recombinants can be detected, depends upon the type and spread of genetic markers on the chromosome segment(s) targeted for recombination. The co-dominant markers used in the present studies (RFLPs) were able to differentiate efficiently between parental and recombinant chromosomes and almost complete classification of the families was possible. As discussed by Mather (1951) and shown in Figs. 5.3, 5.4, F_2/F_3 progeny are very efficient for detecting recombinants when dealing with co-dominant markers closely linked in coupling phase. All recombinant gametes can be detected except the progeny resulting from union of reciprocal recombinant products (AB' X A'B), where the phenotype of the recombinant is the same as two parental phenotypes (Fig. 5.4). The expected frequency of the non-detectable recombinants (p^2 / 2) will be very small, when p is small, as expected in allosyndetic recombination. Another advantage of using the selfed F_3 progeny over the conventional testcross populations is the ease with which large numbers of progeny can be produced. Note that most recombinant progeny [2p(1-p)≈2p, if p is small) are expected to have a recombinant gamete combined with an unchanged parental gamete, whereas the detectable class having two recombinant gametes is expected to occur very infrequently (p^2 / 2).

Fig. 5.3: Expected gametic types from non-cross-overs (parental) and cross-over (recombinant) events involving short arms of targeted chromosomes 7A and 7Ai, carrying co-dominant marker alleles A, B and A', B', respectively.

A B   7Ai

A' B'   7A
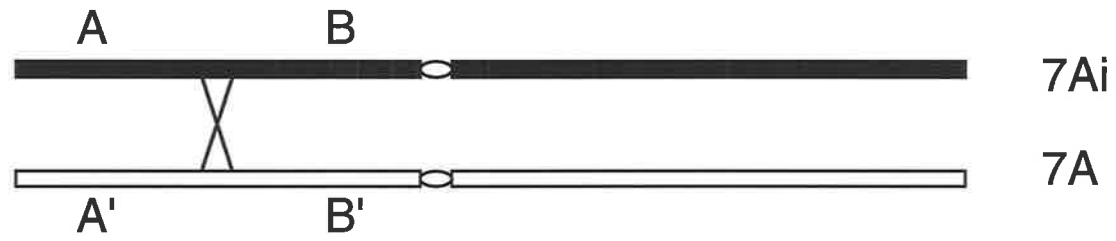
Gametes

parental   A B

               A' B'

Recombinant   A B'

               A' B

Fig. 5.4: Scheme for detecting recombinants in F_2 generation using co-dominant DNA markers.

P = Parental gametes, R = Recombinant gametes.

Genotypes are shown in smaller point letters in italics and the corresponding phenotypes are shown in bold large type in parentheses.

Note that all recombinants are detectable except those circled (see text for details) and the expected frequency of undetected recombinants is $p^2 / 2$, where p = frequency of recombination.

Dark grey - Parental gametes only [most frequent = $(1-p)^2$].

Light grey - One recombinant and one parental gamete (medium frequency, $2p(1-p) \approx 2p$ if p is small).

No shading - Two recombinant gametes (very low frequency, p^2).

$$F_1 \quad \frac{A\ B}{A'\ B'}$$

$\oplus \downarrow$

F_2

	P		R	
	A B	A' B'	A B'	A' B
P A B	$\frac{A\ B}{A\ B}$ (A, B)	$\frac{A\ B}{A'\ B'}$ (A,A',B,B')	$\frac{A\ B}{A\ B'}$ (A,B,B')	$\frac{A\ B}{A'\ B}$ (A,A',B)
A' B'	$\frac{A'\ B'}{A\ B}$ (A,A',B,B')	$\frac{A'\ B'}{A'\ B'}$ (A',B')	$\frac{A'\ B'}{A\ B'}$ (A,A',B')	$\frac{A'\ B'}{A'\ B}$ (A',B,B')
R A B'	$\frac{A\ B'}{A\ B}$ (A,B,B')	$\frac{A\ B'}{A'\ B'}$ (A,A',B')	$\frac{A\ B'}{A\ B'}$ (A,B')	$\frac{A\ B'}{A'\ B}$ (A,A',B,B')
A' B	$\frac{A'\ B}{A\ B}$ (A,A',B)	$\frac{A'B}{A'\ B'}$ (A',B,B')	$\frac{A'\ B}{A\ B'}$ (A,A',B,B')	$\frac{A'\ B}{A'\ B}$ (A',B)
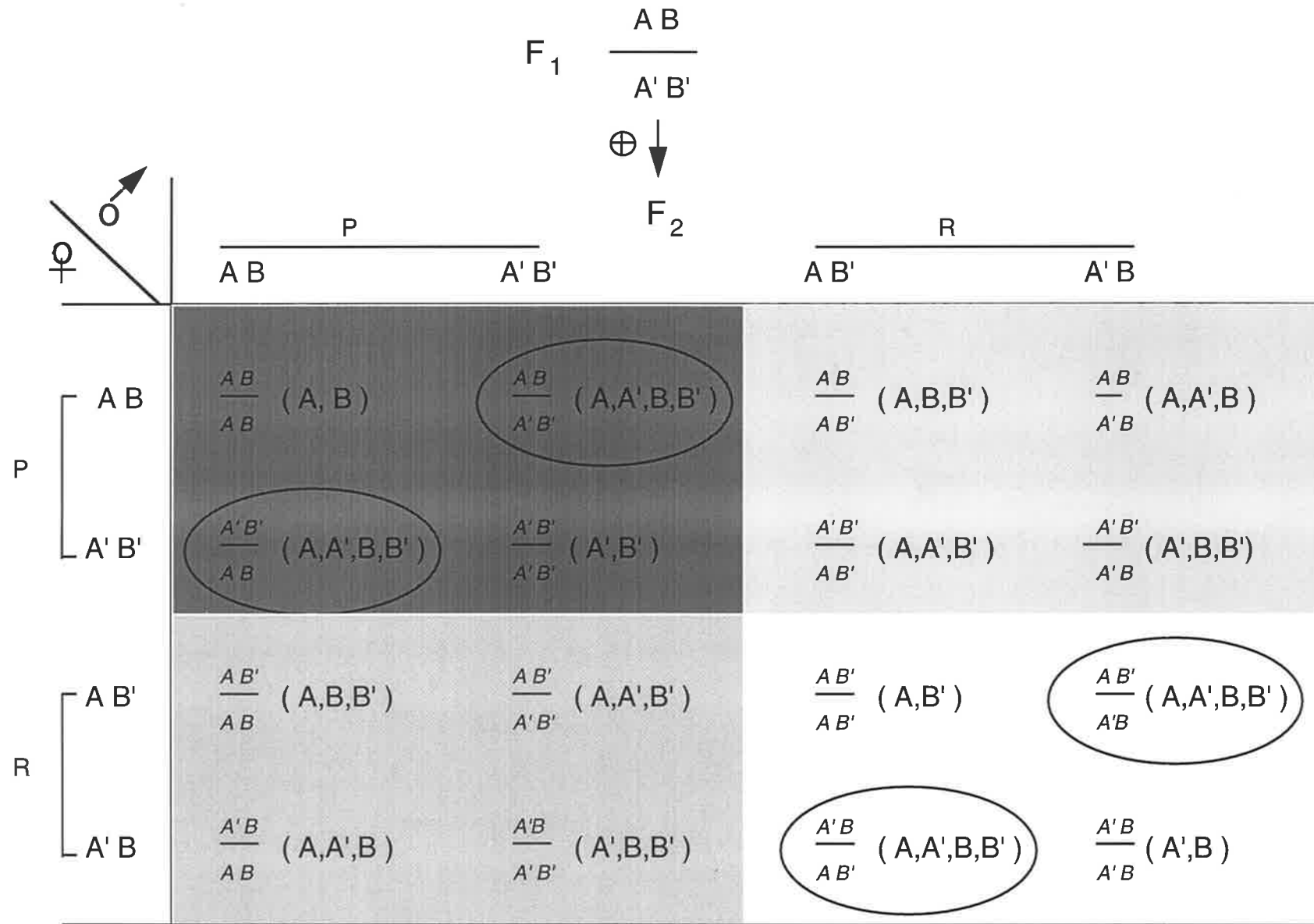
5.3. Results:

5.3.1. Isolation of critical F_2 families:

A total of ninety-six F_2 progeny plants were screened using cytology and RFLPs, to

obtain the required critical families having a single dose of chromosomes 7A and 7Ai and

being homozygous / hemizygous for the chromosome 5B carrying the *ph1b* allele (Table

5.1).

Table 5.1. Isolation of critical families in F_2 progeny from two different crossing procedures.

Crossing procedure	Chromosome constitution of		No. of F_2 progeny	
	parent F_1 plant	critical F_2 plant	screened	selected
Direct crossing	(2n=42, 20"+1'+1') (*Ph1ph1b*)	(2n=42, 40$_W$+7A'+7Ai') (*ph1bph1b*)	60	4
Triple monosomic	(2n=40, 19"+1'+1') (*Ph1*)	(2n=41, 38$_W$+5B'+7A'+7Ai') (*ph1b/-*)	36	2

In addition to the four selected families from direct crossing (family # -347, -351, -394,

-447, referred to as *ph1bph1b* families) and two selected families from triple monosomic

scheme (family # -430, -477, referred to as *ph1b/-* families) (Table 5.1), four plants (#

-332, -356, -376 and -392) having 2n=42 chromosomes and either *Ph1Ph1* or *Ph1ph1b*

(20"$_W$+7A'+7Ai', *Ph1-*, referred to as *Ph1-* families) were also selected as control

populations. All of the control (*Ph1-*) families were obtained through the direct crossing

procedure.

5.3.2. Screening of F_3 progeny:

All of the *Ph1* deficient families (*ph1bph1b* and *ph1b/-*), selected during the present

studies, showed reduced fertility, associated with the high level of non-homologous

pairing induced by deficiency of the *Ph1* locus (Sears 1981). Sears (1981) suggested that

using the less effective pairing mutants *ph1a* or *ph2*, might overcome this problem, but use of these mutants would be expected to produce lower frequencies of wheat-alien recombination. A total of 286 and 104 progeny plants from *ph1bph1b* and *ph1b/-* families, respectively, were screened using all six selected RFLP probes and 117 progeny from *Ph1-* families were screened for the two short arm markers only and all of the results are summarised in Table 5.2. The linear order of the loci for the long arm probes used in Table 5.2 (and subsequent tables), was based on the pattern of marker loci on recombinant chromosomes isolated during the present studies (described in later sections of this chapter) and the order chosen was the one which minimised the number of double cross-over events required to explain the observed patterns.

Particular chromosomes were inferred to be present or absent based on the corresponding presence or absence of the RFLP markers used. The parental chromosomes (7A and 7Ai) occurred in the F3 progeny in the approximate ratio of 2 (7Ai/7A) : 1 (7Ai) : 1 (7A), but in the last two cases (underlined) it was not established whether these chromosomes were present in one or two doses. Hypoploidy resulting from failure of transmission of both chromosomes (7A and 7Ai) was only observed in 5 of the 507 F3 progeny plants screened. When all markers of one arm only were absent it was assumed that a telocentric for the other arm was present due to misdivision of the centromere. The presence of these telocentric chromosomes was later confirmed by cytological analysis of root tip mitoses in their F4 progeny plants.

Any F3 progeny plants showing dissociated markers within the long or short arms of 7A or 7Ai were considered to be putative recombinants. The chances of confusing homoeologous recombinants with centromeric misdivision products were reduced by using at least two marker loci for each arm.

Table 5.2. Phenotypes of F3 progeny plants obtained from three types of families carrying chromosomes 7A and 7Ai in *Ph1* and non-*Ph1* backgrounds screened with RFLP probes specific for homoeologous group 7 chromosomes of Triticeae.

Phenotype of F3 progeny plants (RFLP loci)								No. of F3 progeny in families		
	Short arm markers		Long arm markers*							
Chromo-some	*Xcdo 545*	*Xcdo 595*	*Xcdo 673*	*Xpsr 117*	*Xwg 686*	*Xpsr 121*	Classifi-cation	*phlb phlb*	*phlb/ -*	*Ph1-* [†]
7Ai	+	+	+	+	+	+	7Ai/	130	35	64
7A	+	+	+	+	+		7A			
7Ai	+	+	+	+	+	+	7Ai	54	15	23
7A	-	-	-	-	-		alone			
7Ai	-	-	-	-	-	-	7A	59	23	27
7A	+	+	+	+	+		alone			
7Ai	-	-	-	-	-	-	Hypoploid	2	1	2
7A	-	-	-	-	-					
7Ai	+	+	-	-	-	-	Telo	2	1	
7Ai	-	-	+	+	+	+	Telo	1	1	
7A	+	+	-	-	-		Telo	1	0	
7A	-	-	+	+	+		Telo	1	2	
	Dissociation of markers within arm(s)						Recomb.	36	26	1
Total								286	104	117

* = The four long-arm-specific RFLP probes used were obtained from different maps (details described in chapter 4) and because of the unavailability of common markers on these maps, their consensus linear order could not be obtained. [The linear order of the long arm probes used here and in following tables was actually obtained later when recombinant chromosomes were isolated (see later sections of this chapter). The linear order was chosen on the basis of the minimum number of intra-arm double cross-over events required to explain the recombinant marker patterns observed.

+ = RFLP locus present,   - = RFLP locus absent].

† = Only short arm markers scored.

Telo = Telocentric chromosome present.

Recomb. = Putative recombinants (see Table 5.4 for details).

5.3.3. Frequency of allosyndetic recombination detected in different families:

A total of sixty-three F3 progeny plants with dissociated markers within the arm(s) (putative recombinant) were detected (also see section 5.3.7). Phenotypes of these putative recombinants are described in later sections of this chapter. Sixty-two of these putative recombinants were detected in *Ph1* deficient families (*ph1bph1b* and *ph1b/-*) and only one in the *Ph1-* families (Table 5.3). The data from the four *ph1bph1b* derived families were homogeneous for the frequency of recombinants detected (χ^2 = 1.73, 2 df, 0.30 < p < 0.50; because of small number of progeny screened, data from families # -347 and -351 were combined for analysis) and were therefore pooled as shown in Table 5.3; similar tests on the data from the two *ph1b/-* families (χ^2 = 0.35, 1 df, 0.50 < p < 0.70) also allowed pooling. The combined data of the F3 progeny of *ph1b/-* families showed an overall much higher percentage of recombination (25 %) as compared to the *ph1bph1b* families (12.6%). These two sets of data exhibited significant heterogeneity (χ^2 = 5.3, 1 df, 0.05 < p < 0.02) and thus can not be pooled.

Table 5.3. Frequency of putative recombinants observed in F3 progeny of different F2 families.

| Type of F2 family | F3 progeny | | % recomb. | χ^2 het. | df |
	Total screened	No. putative recombinants			
ph1bph1b					
# 347	19	4	21.1		
# 351	16	1	6.2		
# 394	143	14	9.8		
# 447	108	17	15.7		
pooled total	286	36	12.6	1.73	2
ph1b/-					
# 430	84	19	22.6		
# 477	20	7	35.0		
pooled total	104	26	25.0	0.29	1
Ph1-					
Total of 4 families	117	1	0.8		

5.3.4. Detection of wheat-*Agropyron* recombinant chromosomes:

A total of sixty-three F3 progeny plants with dissociated markers within the arm(s) (putative recombinant) were detected and they represented at least sixty-nine homoeologous cross-over events (since six F3 progeny plants showed dissociation of marker loci in both arms) (Table 5.4). The homoeologous recombination events involving the short arms of the two Triticeae chromosomes (7Ai and/or 7A) were detected with more than twice the frequency (47) as compared to those involving the long arms of the two chromosomes (22). The putative recombinants are listed in Table 5.4 and for convenience of description are described under three main headings; (a) progeny showing recombination in the short arms only, (b) progeny showing recombination in the long arms only and (c) progeny showing recombination in both the arms.

a. Progeny showing recombination in the short arm(s) only.

The probes CDO -545 and -595, specific for the short arms of group 7 homoeologous chromosomes of Triticeae, when hybridised to genomic DNA digested with *EcoR V*, distinguished RFLPs between chromosomes 7A and 7Ai easily and reliably. All of the F3 progeny plants showing dissociation between these two markers within the chromosome(s) were considered as putative short arm recombinants. An example of this screening is presented in Fig. 5.5. In most cases, the chromosomes with dissociated markers (putative recombinants) were present along with the other parental homoeologous chromosome targeted for allosyndetic recombination (Table 5.4). In particular, with recombinant types R1/7A and R2/7A, dissociation of 7AiS markers was observed in the presence of an intact 7A chromosome (where all the RFLP loci for 7A were present). Similarly with recombinant types R4/7Ai and R5/7Ai, dissociation of 7AS markers was detected in the presence of an intact 7Ai chromosome. In recombinant types R3/- and R6/- the chromosomes with dissociated markers were detected alone. Reciprocal dissociation of the short arm markers for both the chromosomes (7Ai and 7A)

Table 5.4. Phenotype of plants with dissociated markers (putative recombinants) detected in the F3 progeny of the critical F2 families, using RFLP markers.

| Phenotypes of F3 progeny plants (RFLP loci) | | | | | | | No. of F3 progeny plants in families* | | | Classification of recomb. and inferred |
| Short arm markers | | | Long arm markers | | | | | | | |
Chromo-some	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	phlb phlb	phlb /-	Phl-	chromosome constitution
a. Marker dissociation observed for short arms only										
Chromosome arm 7AiS										
7Ai	-	+	+	+	+	+	4^A	1^B	1^C	R1/7A
7A	+	+	+	+	+					
7Ai	+	-	-	-	-	-	6^D	0	0	R2/7A
7A	+	+	+	+	+					
7Ai	+	-	-	-	-	-	1^E	0	0	R3/-
7A	-	-	-	-	-					
Chromosome arm 7AS										
7Ai	+	+	+	+	+	+	7^F	8^G	0	R4/7Ai
7A	+	-	-	-	-					
7Ai	+	+	+	+	+	+	2^H	2^I	0	R5/7Ai
7A	-	+	+	+	+					
7Ai	-	-	-	-	-	-	2^J	0	0	R6/-
7A	-	+	+	+	+					
Chromosome arms 7AiS/7AS										
7Ai	-	+	+	+	+	+	3^K	1^L	0	R7/-
7A	+	-	-	-	-					
7Ai	+	-	-	-	-	-	2^M	1^N	0	R8/-
7A	-	+	+	+	+					
b. Marker dissociation observed for long arms only										
Chromosome arm 7AiL										
7Ai	+	+	+	-	-	-	1^O	3^P		R9/7A
7A	+	+	+	+	+					
7Ai	-	-	-	-	+	+	1^Q	0		R10/7A
7A	+	+	+	+	+					
7Ai	-	-	-	-	-	+	0	1^R		R11/7A
7A	+	+	+	+	+					
7Ai	+	+	-	-	-	+	1^S	0		R12/7A
7A	+	+	+	+	+					
Chromosome arm 7AL										
7Ai	+	+	+	+	+	+	1^T	0		R13/7Ai
7A	+	+	+	+	-					

Cont'd...

Phenotypes of F3 progeny plants (RFLP loci)

Chromo-some	Short arm markers			Long arm markers			No. of F3 progeny plants in families*			Classification of recomb. and inferred chromosome constitution
	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	ph1b ph1b	ph1b /-	Ph1-	
7Ai	+	+	+	+	+	+	1^U	0		R14/7Ai
7A	+	+	+	-	-					
7Ai	+	+	+	+	+	+	0	2^V		R15/7Ai
7A	-	-	-	+	-					
7Ai	+	+	-	-	-	-	1^W	0		R16/-
7A	-	-	-	+	+					
Chromosome arms 7AiL/7AL										
7Ai	+	+	+	-	-	-	0	2^X		R17/-
7A	-	-	-	+	+					
7Ai	-	-	-	+	+	+	1^Y	0		R18/-
7A	+	+	+	-	-					
7Ai	-	-	-	-	+	+	0	1^Z		R19/-
7A	+	+	+	+	-					
c. Marker dissociation observed for both arms										
7Ai	-	+	+	-	-	-	0	1^{Z1}		R20/7A
7A	+	+	+	+	+					
7Ai	+	-	-	+	+	+	0	1^{Z2}		R21/7A
7A	+	+	+	+	+					
7Ai	-	-	-	-	-	+	1^{Z3}	0		R22/-
7A	-	+	+	+	+					
7Ai	-	+	+	+	+	+	0	2^{Z4}		R23/-
7A	+	+	+	-	-					
7Ai	+	+	+	-	-	-	1^{Z5}	0		R24/-
7A	+	-	-	+	+					

* = Progeny from *Ph1*- families were screened with short arm markers only.

+ = RFLP locus present, - = RFLP locus absent.

A - Z5 = Plant # of F3 progeny plants. Identification number of the critical F2 family is given in parentheses. Sterile plants are indicated by asterisks.

A = # 876, 1045, 1118 (447); 1147 (394): **B** = # 1034* (430): **C** = # 1307 * (*Ph1*-): **D** = # 833 (351); 835, 1169, 1182 (394); 1050, 1089* (447): **E** = # 879 (447): **F**= # 803, 953 (347); 1047, 1048, 1094 (447); 1198, 1251 (394): **G**= # 898, 963 (477); 976, 986, 1011*, 1020, 1023*, 1025 (430): **H** = # 874 (447); 1218 (394): **I** = # 886, 896 (477): **J** = # 1078 (447); 1139* (394) **K** = # 1063, 1106 (447); 1180 (394): **L** = # 1009* (430): **M** = # 1052, 1053 (447): **N**= # 1019 (430): **O** = # 1170 (394): **P** = # 995, 1029* (430); 895 (477): **Q** = # 1146 (394): **R** = # 936 (430): **S** = # 1128* (447): **T** = # 817 (347): **U** = # 1175 (394): **V** = # 884, 891 (477): **W** = # 1199 (394): **X** = # 1003, 1038* (430): **Y** = # 1129 (447): **Z** = # 985 (430): **Z1** = # 942 (430): **Z2** = # 979* (430): **Z3** = # 1140 (394): **Z4** = # 1002, 1013 (430): **Z5** = # 805 (347).

Fig. 5.5: Dissociation of RFLP markers in the short arms of chromosomes 7A and 7Ai. DNA samples were digested with *EcoR V*. The same filter was hybridised sequentially with probes (a) CDO545 and (b) CDO595.

Note the dissociation phenotypes in lane 1 (plant # 1063), lane 2 (plant # 963) and lane 3 (plant # 1045) as follows:

		Xcdo545	*Xcdo595*
Plant # 1063 =	7Ai	-	+
	7A	+	-
Plant # 963 =	7Ai	+	+
	7A	+	-
Plant # 1045 =	7Ai	-	+
	7A	+	+

These three dissociations are described as putative recombinants in categories R7, R4 and R1, respectively, in Table 5.4.

M = Molecular size markers (kbp) using λ DNA digested with *Hind III* are shown on left.

a

M
23,130
9,416 — 7A
6,557 — 7A

4,361

2,322
2,027

b
23,130
9,416
6,557
7A

4,361

2,322
2,027 — 7A
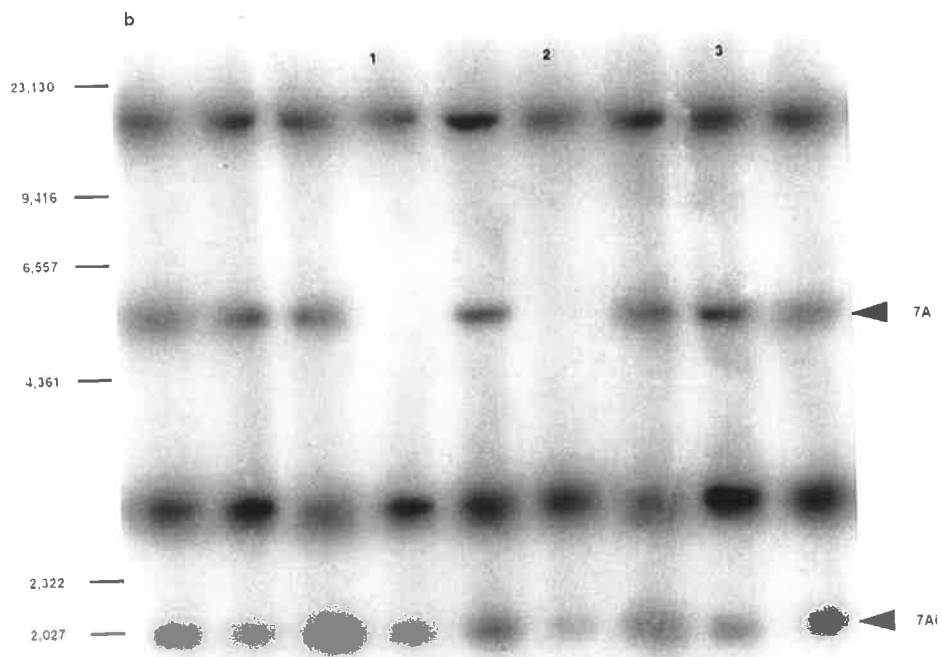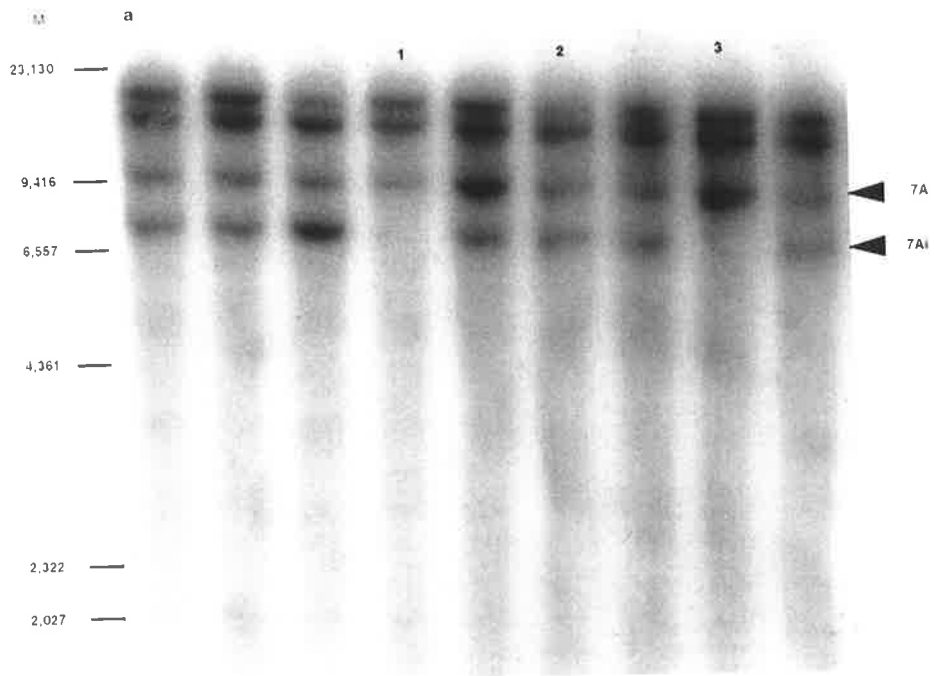
was also observed (R7/-, R8/-) which indicated the likely presence of a 7Ai-7A recombinant chromosome as a univalent.

b. Progeny showing recombination in the long arm(s) only.

Four RFLP probes (CDO673, WG686, PSR -117 and -121) specific for the long arms of group 7 homoeologous chromosomes of Triticeae were used to detect long arm recombinants. Three of these probes when hybridised to genomic DNA digested with *EcoR V*, detected RFLPs for both the chromosomes (7A and 7Ai), while the probe PSR121 under similar conditions only gave a specific RFLP for chromosome 7Ai. All F3 progeny plants showing dissociation between any of these markers within the chromosome(s) were kept as potential long arm recombinants. Altogether 16 progeny plants with dissociated long arm markers were observed (Table 5.4). Seven plants showed dissociation for 7AiL markers and were detected in the presence of an intact 7A chromosome (R9/7A - R12/7A). Four plants showed dissociation for 7AL markers and were detected in the presence of an intact 7Ai chromosome (R13/7Ai - R15/7Ai). One F3 progeny was detected having dissociated 7AL markers in the presence of just the short arm of chromosome 7Ai (R16/-). In four F3 progeny (R17/- to R19/-), reciprocal dissociation of the long arm markers was observed for both chromosomes 7Ai and 7A, indicating the possible presence of wheat-*Agropyron* recombinant chromosomes as univalents.

c. Progeny showing recombination in both the arms.

During the screening of F3 progeny plants, dissociation of the markers was also detected within both arms of either one or both the chromosomes. In total, six such putative recombinants were detected. In two plants (R20/7A and R21/7A) dissociation of markers was observed for both the arms of chromosome 7Ai only, while all the loci for chromosome 7A were present. In the remaining four plants (R22/- to R24/-), dissociation of the markers involved both the chromosomes (7Ai and 7A).

5.3.5. Progeny testing of the putative recombinants:

Progeny tests of the putative recombinants were carried out to confirm the dissociation phenotype but also to detect segregants carrying the recombinant chromosomes separated from both parental chromosomes so that their structure could be analysed further. That is, attempts were made to determine which homoeologous chromosomes were involved in the cross-over event and where the cross-over had occurred along the chromosome. Altogether 403 progeny from the 53 self-fertile non-parental F_3 progeny were studied using the same six RFLP markers. The progeny tested were mainly selfed F_4 progeny or backcrosses of the putative recombinants (used as female parent) to euploid or nullisomic 7A - tetrasomic 7B (NT 7A-7B) stock of CS and these are referred to as the primary derivatives. However, in a few cases, F_5 progeny from selected F_4 plants carrying the recombinant chromosome and progeny plants from backcrosses of the selected F_4 plants (used as female parent) to euploid or NT 7A-7B stock of CS, were also analysed and these are referred to as secondary derivatives. In many cases, the reduced fertility of the putative recombinant plants after one or more meiotic cycles in the *Ph1* deficient background restricted the number of progeny seed available for analysis.

The detailed data for all these progeny tests are presented in Appendix (Note: The order of the putative recombinants in the Appendix corresponds strictly to that presented in Table 5.4). The data relating to recombinant chromosomes which apparently resulted from a single cross-over event are summarised in Table 5.5 and are described in section 5.3.5.1. The inferred cross-overs and structure of these recombinant chromosomes are shown diagrammatically in Fig. 5.6a. The data where evidence was obtained for the occurrence of more than one homoeologous cross-over event in the original recombinant plant (F_3 progeny) are summarised in Table 5.6 and discussed separately in section 5.3.5.2. The inferred origin and structure of these recombinant chromosomes are shown in Fig. 5.6b.

These progeny tests confirmed the original classification of almost all of the non-parental self-fertile F3 progeny plants. New combinations of the marker loci were detected in the progeny tests with a comparatively low frequency and it was suspected that these may have arisen from a new cross-over event, during the subsequent meiosis after their isolation, since these F3 plants were still deficient for the *Ph1* locus.

5.3.5.1. Recombinants carrying single cross-over products:

The detailed results of these progeny tests are given in Appendix and summarised in Table 5.5 and for convenience of description the results are described under two main headings; (a) putative short arm recombinants and (b) putative long arm recombinants .

(a). Putative short arm recombinants.

The progeny test of the four self-fertile putative recombinants in category R1 (where dissociation of the markers was observed for 7AiS, Table 5.4), revealed recombinant chromosomes in two of them (# -1045, -1118, Table 5.5) involving the short arms of chromosomes 7A and 7Ai [phenotype: 7Ai (- + + + + +), 7A (+ - - - -) with inferred chromosome structure 7AS-7AiS.7AiL, using the nomenclature of Koebner and Miller 1986]. The inferred origin and structure of these recombinant chromosomes are shown in Fig. 5.6a (chromotype a). The progeny of the other two putative recombinants in R1 (# -876, -1147) did not gave a recombinant chromosome on its own and hence they are listed as ?-7AiS in Table 5.5. Instead, in their progeny the dissociated 7AiS markers were always present with a normal copy of chromosome 7A or the progeny had an intact 7A chromosome alone. One plant with neither 7A or 7Ai markers (designated as nullisomic in Table 5.5) and another plant with a possible telocentric chromosome (listed under 'other' in Table 5.5) were observed among the progeny of the plants in this category (the detailed phenotypes of these plants are given in Appendix). All of these four plants in category R1 originated from the *ph1b* mutant families.

Table 5.5. Summary of the progeny tests of plants carrying recombinant chromosomes resulting from putative single homoeologous cross-overs (for detailed phenotypes see Appendix-A).

| Classification of the recomb-inant and plant # of F3 progeny | Phenotype of the recombinant chromosome isolated alone | Number of progeny plants having | | | | | | Interpretation of the recombinant (the structure reads from distal short arm to distal long arm) |
		Recombinant chromosome present alone	present with a parental chromosome (7A or 7Ai)	Parental chromosome only (7A or 7Ai)	Nulli-somic	Other[1]	Total	
a. Putative short arm recombinants.								
R1								
876			3 (1)^	1 (1)		(1)	7	?-7AiS
1045	[7Ai= - +++++]† [7A = + - - - -]	3	3	6			12	7AS-7AiS.7AiL
1118	[7Ai= - +++++] [7A = + - - - -]	2	1		1		4	7AS-7AiS.7AiL
1147			2	1			3	?-7AiS
R2								
833			2 (3)	6			11	7AiS-?
835	[7Ai= + - - - - -] [7A = - + + + +]	2 (10)	2		(4)		18	7AiS-7AS.7AL
1169	[7Ai= + - - - - -] [7A = - + + + +]	1	2	1			4	7AiS-7AS.7AL
1182			3	1			4	7AiS-?
1050	[7Ai= + - - - - -] [7A = - + + + +]	5	2	5			12	7AiS-7AS.7AL
R3								
879	[7Ai= + - - - - -] [7A = - - - - -]	4 (6)			(5)		15	7AiS-7X*
R4								
803			4 (2)	(1)			7	7AS-?
953			3 (3)	1			7	7AS-?
1047			1	2			3	7AS-?
1048			3	1			4	7AS-?
1094	[7Ai= - +++++] [7A = + - - - -]	1		3			4	7AS-7AiS.7AiL
1198			2	2			4	7AS-?
1251			3				3	7AS-?
898	[7Ai= - +++++] [7A = + - - - -]	(4)	3 (1)	1 (2)			11	7AS-7AiS.7AiL

Table 5.5 Continued

Classification of the recomb-inant and plant # of F3 progeny	Phenotype of the recombinant chromosome isolated alone	Number of progeny plants having		Parental chromosome only (7A or 7Ai)	Nulli-somic	Other[1]	Total	Interpretation of the recombinant (the structure reads from distal short arm to distal long arm)
		Recombinant chromosome						
		present alone	present with a parental chromosome (7A or 7Ai)					
963	[7Ai= - +++++] [7A = + - - - -]	(6)	2 (3)	2 (4)			17	7AS-7AiS.7AiL
976	[7Ai= - +++++] [7A = + - - - -]	1	3				4	7AS-7AiS.7AiL
986			2	1			3	7AS-?
1020			4				4	7AS-?
1025			3	1			4	7AS-?
R 5 874			4 (3)				7	?-7AS
1218	[7Ai= + - - - - -] [7A = - ++++]	2	2				4	7AiS-7AS.7AL
886	[7Ai= + - - - - -] [7A = - ++++]	2 (3)	1			1	7	7AiS-7AS.7AL
896	[7Ai= + - - - - -] [7A = - ++++]	1 (3)	1	2			7	7AiS-7AS.7AL
R 6 1078	[7Ai= - - - - - -] [7A = - ++++]	11					11	7X-7AS.7AL
R 7 1063	[7Ai= - +++++] [7A = + - - - -]	3					3	7AS-7AiS.7AiL
1106	[7Ai= - +++++] [7A = + - - - -]	5					5	7AS-7AiS.7AiL
1180	[7Ai= - +++++] [7A = + - - - -]	5					5	7AS-7AiS.7AiL
R 8 1052	[7Ai= + - - - - -] [7A = - ++++]	5 (3)			1		9	7AiS-7AS.7AL
1053	[7Ai= + - - - - -] [7A = - ++++]	3					3	7AiS-7AS.7AL
1019	[7Ai= + - - - - -] [7A = - ++++]	7			4		11	7AiS-7AS.7AL

Table 5.5 Continued

Classification of the recomb-inant and plant # of F3 progeny	Phenotype of the recombinant chromosome isolated alone	Number of progeny plants having		Parental chromosome only (7A or 7Ai)	Nulli-somic	Other[!]	Total	Interpretation of the recombinant (the structure reads from distal short arm to distal long arm)
		Recombinant chromosome						
		present alone	present with a parental chromosome (7A or 7Ai)					
b. Putative long arm recombinants.								
R9								
1170	[7Ai= +++ - - -] [7A = - - - ++]	5	4	4			13	7AiS.7AiL-7AL
995	[7Ai= +++ - - -] [7A = - - - ++]	1	4	6	1		12	7AiS.7AiL-7AL
R10								
1146			2	1		1	4	?-7AiL
R11								
936	[7Ai= - - - - - +] [7A = +++++]	6		4			10	7AS.7AL-7AiL
R13								
817	[7Ai= - - - - ++] [7A = ++++ -]	1	1	5		1	8	7AS.7AL-7AiL
R14								
1175	[7Ai= - - - +++] [7A = +++ - -]	2	2	2			6	7AS.7AL-7AiL
R16								
1199	[7Ai= - - - - - - -] [7A = - - - ++]	5	2			1	8	7X-7AL
R17								
1003	[7Ai= +++ - - -] [7A = - - - ++]	9			3		12	7AiS.7AiL-7AL
R18								
1129	[7Ai= - - - +++] [7A = +++ - -]	6					6	7AS.7AL-7AiL
R19								
985	[7Ai= - - - - ++] [7A = ++++ -]	3			3		6	7AS.7AL-7AiL

! = Detection of new dissociation of markers and/or detection of telocentric chromosome.
^ = Number of primary derivatives are given first and number of secondary derivatives are given in parentheses.
? = Recombinant chromosome could not be isolated alone.
† = The order of the probe loci is same as used in Table 5.4 and Appendix-A.
+ = RFLP locus present, - = RFLP locus absent.
* = The non-7A wheat chromosome involved in recombination could not be identified.

Fig. 5.6 a: Diagrams showing inferred types of cross-over resulting in recombinant chromosomes and the inferred structure of the recombinant chromosomes (chromotypes) isolated during present studies (described in Table 5.5, section 5.3.5.1).

S= short arm, L= long arm, C = position of centromere.

1=*Xcdo545*, 2=*Xcdo595*, 3=*Xcdo673*, 4=*Xpsr117*, 5=*Xwg686*, 6=*Xpsr121*.

a-i = Chromotypes inferred.

a = 7AS-7AiS.7AiL, recombinants # -1045, -1118 (R1), -1094, -898, -963, -976 (R4), -1063, -1106, -1180 (R7).

b = 7AiS-7AS.7AL, recombinants # -835, -1169, -1050 (R2), -1218, -886, -896 (R5), -1052, -1053, -1019 (R8).

c = 7AiS-7X, recombinant # 879 (R3).

d = 7X-7AS.7AL, recombinant # 1078 (R6).

e = 7AiS.7AiL-7AL, recombinants # -1170, -995 (R9), -1003 (R17).

f = 7AS.7AL-7AiL, recombinants # -1175 (R14), -1129 (R18).

g = 7AS.7AL-7AiL, recombinant # -936 (R11).

h = 7AS.7AL-7AiL, recombinants # -817 (R13), -985 (R19).

i = 7X-7AL, recombinant # 1199 (R16).

Note: (1) only the inferred structure of those recombinant chromosomes isolated away from parental chromosomes has been presented.

(2) the positions of the probe loci reflect linear order but not actual genetic or physical distances.

Inferred cross-over types

type 1

type 2

type 3

S   L

1 2 C 3 4 5 6   Chromotypes

a

b

c

d

7Ai chromatin

7A chromatin

Non-7A (7X) wheat chromatin

Inferred cross-over types

type 4

type 5

type 6

type 7

S   L

1 2 C 3 4 5 6   Chromotypes

e

f

g

h

i
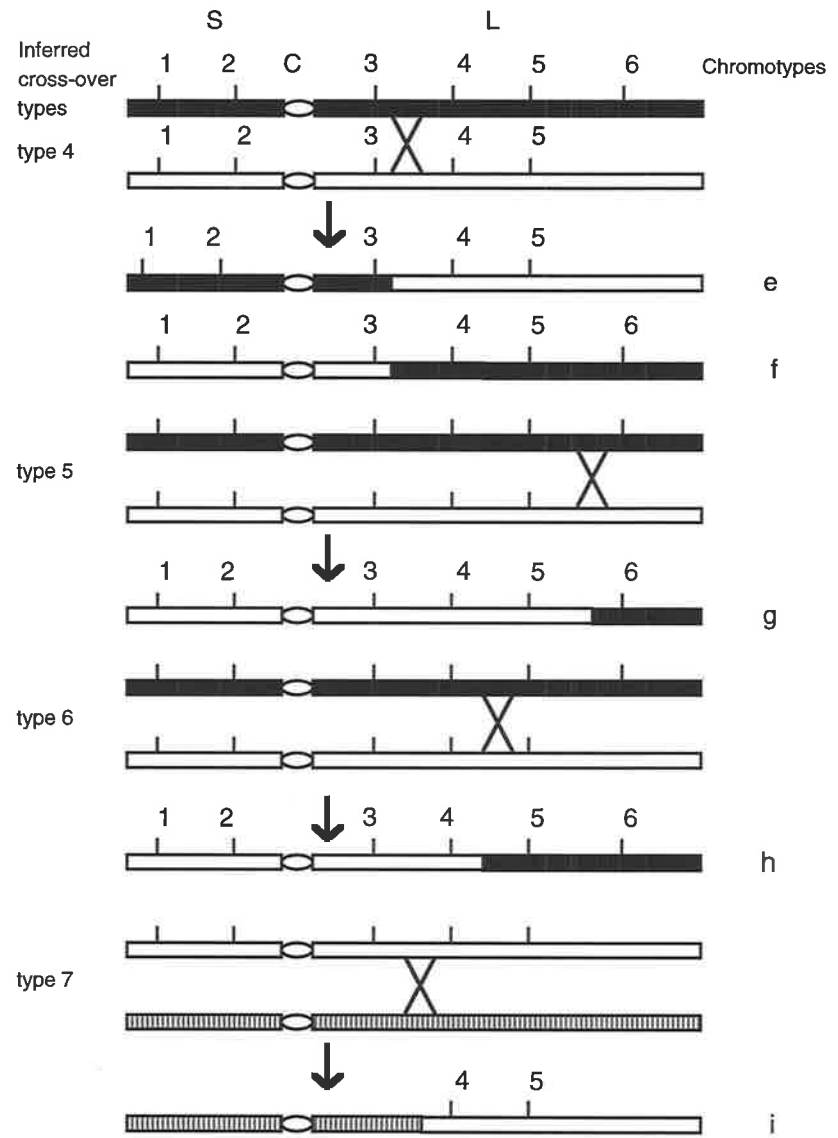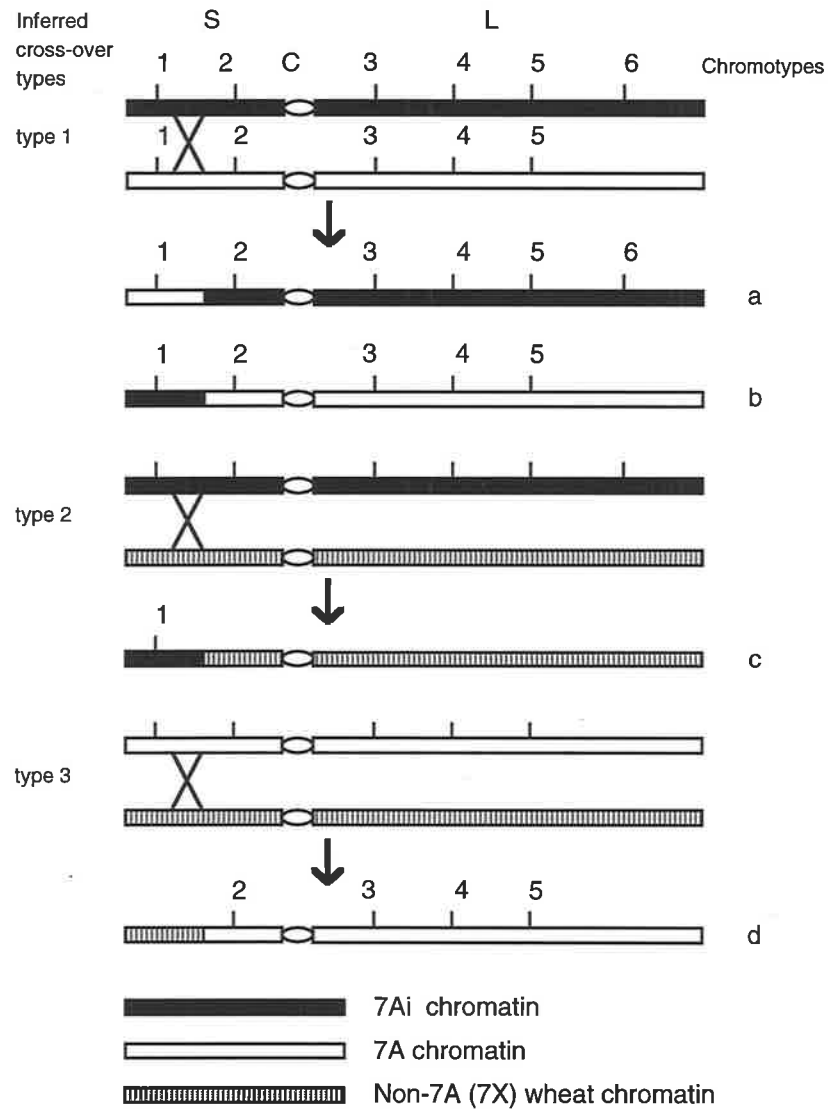
The only plant in the *Ph1-* control families showing dissociated short arm markers (# 1307, category R1, Table 5.4) could not be progeny tested because it was sterile.

Progeny tests of five putative recombinants in category R2, revealed recombinant chromosomes with inferred structure 7AiS-7AS.7AL in three plants (# - 835, -1169, -1050) and the likely origin and structure of the recombinant chromosomes is shown in Fig.5.6a (chromotype b). The other two self-fertile plants in this category (# -833, -1182) did not give an isolated recombinant chromosome in their progeny and therefore the nature of the recombination event could not be determined.

The absence of 7A markers and presence of only one 7AiS marker (*Xcdo545-7Ai*) in the F3 progeny plant # 879 (R3) could have arisen from recombination between 7AiS and another wheat group 7 chromosome (other than 7A) or from deletion of the major portion of chromosome 7Ai. The presence of this altered phenotype in most of the progeny favoured the idea of a 7Ai - non-7A recombinant chromosome (listed as 7AiS-7X, Table 5.5 and shown as chromotype c in Fig. 5.6a) rather than a deletion. The unidentified wheat chromosome is expected to be a group 7 homoeologous chromosome of wheat (7B or 7D), because in the absence of the *Ph1* gene, homoeologous chromosome pairing is favoured over non-homoeologous pairing. Hence this unidentified wheat chromosome has been designated as 7X at this stage. The five nullisomic plants among the secondary derivatives of this putative recombinant must have resulted from failure of transmission of the 7AiS-7X recombinant chromosome, to some of the female gametes, when plant # 1422 (a progeny of # 879) was crossed as female parent with NT 7A-7B (Appendix).

Thirteen recombinants in category R4 were progeny tested and two of these (# -1094, -976) gave isolated recombinant chromosomes in primary derivatives and another two (# -898, -963) gave similar chromosomes in secondary derivatives and the inferred structure of all four recombinant chromosomes was again 7AS-7AiS.7AiL (Table 5.5, chromotype

a, Fig. 5.6a). The remaining nine recombinants of category R4 failed to give a recombinant chromosome on its own in their progeny and therefore the nature of the recombination event could not be deduced.

Four recombinants in category R5 were progeny tested and three of them (# -1218, -886, -896) gave isolated recombinant chromosome of the 7AiS-7AS.7AL type (chromotype b, Fig. 5.6a). Only one of the recombinants in this category (# 874) failed to give a recombinant chromosome alone.

All progeny from recombinant # 1078 (R6) had the phenotype [7Ai (- - - - - -), 7A (- + + + +)] and these may have arisen from wheat - wheat recombination or a terminal deletion of chromosome 7A. With the information presently available it is difficult to distinguish between these two possibilities but the presence of this altered phenotype in all 11 progeny plants tested favoured the idea of a wheat - wheat recombination event (described as 7X-7AS.7AL, Table 5.5 and shown as chromotype d, Fig. 5.6a) rather than a terminal deletion.

The putative recombinants having dissociated short arm markers for both chromosomes 7A and 7Ai [recombinants # -1063, -1106, -1180 (R7), and -1052, -1053, -1019 (R8)] appeared to have recombinant chromosomes present as a univalent with structures 7AS-7AiS.7AiL (chromotype a, Fig.5.6a) and 7AiS-7AS.7AL (chromotype b, Fig. 5.6a), respectively. Their high rate of transmission to the progeny is most likely due to pollen carrying the translocated chromosome having a competitive advantage over 20-chromosome nullihaploid wheat pollen in fertilisation. The five progeny with a nullisomic phenotype were all derived from crosses between a primary derivative as female parent to NT 7A-7B (Appendix) and no selection favouring the recombinant chromosomes is expected in female gametes.

b. Putative long arm recombinants.

The progeny test of the two long arm recombinants in category R9 (# -1170, -995) revealed recombinant chromosomes with inferred structure 7AiS.7AiL-7AL (chromotype e, Fig. 5.6a). The third self-fertile plant in this category (# 895) gave progeny which could not be explained by a single cross-over event and these results are included in Table 5.6 and discussed in section 5.3.5.2.

The four progeny of plant # 1146 (R10) did not give a recombinant chromosome separated from an intact 7A chromosome, hence its structure could not be deduced. However, one of the progeny plants showed a new dissociation phenotype, consistent with a new recombination event in the previous meiosis (phenotype described in Appendix).

Ten progeny of F_3 plant # 936 (R11) were studied and four of them expressed only 7A markers while the remaining 6 progeny had the phenotype: 7Ai (- - - - - +), 7A (+ + + + +). The absence of a diagnostic band for chromosome 7A with the PSR121 - *EcoR V* probe - restriction enzyme combination, prevented a decision being made on whether a supposed 7AS.7AL-7AiL (chromotype g, Fig. 5.6a) chromosome was present alone or with an intact 7A.

The single plant in R12 (# 1128) could not be progeny tested because it was sterile. Long arm recombinant chromosomes with phenotype 7Ai (- - - - + +), 7A (+ + + + -), (chromotype h, Fig. 5.6a) and 7Ai (- - - + + +), 7A (+ + + - -) (chromotype f, Fig. 5.6a) were isolated in the F_4 progeny of plants # -817 (R13) and -1175 (R14), respectively. The progeny types obtained with putative recombinants # -884, -891 (R15) could not be accounted for by single cross-over events, so these are included in Table 5.6 and discussed in section 5.3.5.2.

Plant # 1199 (R16) when progeny tested showed the likely presence of a telocentric for the short arm of chromosome 7Ai , produced as a result of misdivision of the centromere [Such misdivisions are known to occur more frequently when an alien chromosome is present as a univalent in wheat background, Sears (1981)]. The presence of two 7AL markers only (*Xpsr117* and *Xwg686* loci) in 5 of the progeny indicated the possible occurrence of wheat-wheat recombination (listed as 7X-7AL in Table 5.5 and shown as chromotype i, Fig. 5.6a).

The 7AiS.7AiL-7AL (# 1003, R17) and 7AS.7AL-7AiL [# -1129 (R18), -985, (R19)] types of long arm recombinant chromosomes when present alone, presumably as univalents, transmitted with high frequency to their progeny, as observed previously with the equivalent type of short arm recombinant chromosomes. The inferred structure of the chromosomes in these three types of recombinants are shown as chromotypes e, f and h, respectively, in Fig. 5.6a.

In summary, from among 34 self-fertile short arm recombinants, nine 7AS-7AiS.7AiL and nine 7AiS-7AS.7AL recombinant chromosomes could be isolated. Two of the short arm recombinants seem to have involved non-targeted homoeologous cross-over products (7AiS-7X and 7X-7AS.7AL) involving a non-7A wheat chromosome (7X). In ten and four putative short arm recombinants, where dissociation of marker loci were observed for 7AS and 7AiS, respectively, the recombinant chromosomes could not be isolated alone (also see section 5.3.6). For the ten long arm recombinants arising from a single cross-over event, 5 and 3 recombinants having 7AS.7AL-7AiL and 7AiS.7AiL-7AL recombinant chromosomes, respectively, were isolated alone. One non-targeted long arm recombinant was isolated having a wheat-wheat translocation (7X-7AL). The recombinant chromosome present in plant # 1146 (R10) was the only long arm recombinant chromosome in this group which could not be isolated alone.

5.3.5.2. Recombinants resulting from two or more homoeologous cross-over events:

When the progeny test of the putative recombinants gave results which could not be explained by a single cross-over event, they have been grouped together and the results are summarised in Table 5.6 and Fig. 5.6b and discussed in this section.

The phenotypes of the progeny derived from putative recombinant # 895 (R9) indicated that more than one recombinant chromosome must be present in the original plant. One of these recombinant chromosomes (described as Rec 1, Table 5.6) was isolated in two of the primary derivatives (# -1470, 1515, Appendix) and all of the secondary derivatives from these two primary derivatives (Table 5.6, Appendix). Its phenotype [7Ai (- + + - - -), 7A (+ - - - -)] suggested that it had structure 7AS-7AiS.7AiL-7X (chromotype j, Fig. 5.6b) and besides occurring alone, it occurred with an intact 7A chromosome in four other progeny (# -1471, -1514, -1516, -1581, Appendix). Four other progeny of plant # 895 (# -1472, -1473, -1517, -1582, Appendix) apparently expressed an additional 7AiS marker (*Xcdo545-7Ai*) than present in 'Rec. 1', and the presence of this additional marker was assumed to come from an additional recombinant chromosome of type 7AiS-7X. However, since this chromosome was not isolated alone it has been listed as 7AiS-? (Rec.2, Table 5.6). This recombinant chromosome could not be detected in the original recombinant plant # 895 because of the masking effects of the other loci present.

The phenotypes of the putative recombinants # -884, -891 (R15) suggested that they had come from a double cross-over (one cross-over proximal and one distal to the *Xpsr117* locus, respectively) involving 7A and another unidentified wheat chromosome. This putative wheat-wheat recombinant chromosome (?-7AL-?) was not transmitted to any of the seven progeny derived from plant # 884, but, 6 of the 11 progeny derived from plant # 891, carried this recombinant chromosome along with a normal chromosome 7Ai (Table 5.6, Appendix).

Table 5.6. Summary of the progeny tests of plants carrying putative recombinant chromosomes resulting from two or more homoeologous cross-over events (for detailed phenotypes see Appendix-A).

| Classification of rec. and plant # of F$_3$ progeny | Number of progeny plants having | | | | | | | Structure (in bold) and phenotype of the recombinant chromosomes. The structure reads from distal short to distal long arm. (Note: The order of probe loci is same as in previous tables |
| | Recombinant chromosome 1 | | Recombinant chromosome 2 | | Parental chromo-alone (7A or 7Ai) | Other | Total | |
	alone	present with a parental chromosome (7A or 7Ai)	alone	present with recomb. chromosome 1				
R9								
895*	2 (10)^	4		2+2†	1		21	**7AS-7AiS.7AiL-7X**(Rec.1) (7Ai = - + + -- -) (7A = + ----) **7AiS-?** (Rec. 2) (7Ai = + -----) (7A = -----)
R15								
884**					7		7	
891**		5 (1)			1 (2)	2!	11	**?-7AL-?** (7Ai = ------) (7A = --- + -)
R20								
942*	(2)	4 (1)			(1)	1!	9	**7AS-7AiS.7AiL-7AL** (7Ai = - ++ ---) (7A = + -- + +)
R22								
1140	2		3	3		1$	9	**7X-7AS.7AL** (Rec. 1) (7Ai = ------) (7A = - + + + +) **7X-7AiL** (Rec. 2) (7Ai = ----- +) (7A = -----)
R23								
1002*	3			7			10	**7AS.7AL-7AiL** (Rec. 1) (7Ai = --- +++) (7A = +++- -) **?-7AiS.7AiL-?** (Rec. 2) (7Ai = - + + --) (7A = - - - --)
1013*	2			3			5	**7AS-7AiS.7AiL** (Rec. 1) (7Ai = - +++++) (7A = + ----) **?-7AS.7AL-?** (Rec. 2) (7Ai = ------) (7A = - + + --)
R24								
805*	3			6			9	**7AiS.7AiL-7AL** (Rec. 1) (7Ai = + + + --) (7A = --- + +) **7AS-?** (Rec. 2) (7Ai = -----) (7A = + ----)

^ = # of primary derivatives are given first and number of secondary derivatives are given in parentheses.
! = Detection of new dissociation of marker loci. $ = Nullisomic.
† = In two derivatives (# -1472, -1473) both the recombinant chromosomes were present along with an intact 7A, while in two derivatives (# -1517, 1582) only the two recombinant chromosomes were present.
* = The inferred recombinant chromosome Rec.2 was not isolated alone.
** = Only one recombinant chromosome resulting from a double cross-over event was inferred.

Fig. 5.6 b: Diagrams showing inferred types of cross-over resulting in recombinant chromosomes and the inferred structure of the recombinant chromosomes (chromotypes) isolated during present studies (described in Table 5.6, section 5.3.5.2).

S= short arm, L= long arm, C=position of centromere.

1=*Xcdo545*, 2=*Xcdo595*, 3=*Xcdo673*, 4=*Xpsr117*, 5=*Xwg686*, 6=*Xpsr121*.

+ Rec.2 = The other recombinant chromosome inferred to be present (as discussed in section 5.3.5.2) was not isolated alone.

j-p = Chromotypes inferred.

j = 7AS-7AiS.7AiL-7X, Rec.1, recombinant # 895 (R9).

k = 7AS-7AiS.7AiL-7AL, recombinant # 942 (R20).

l = 7X-7AS.7AL, Rec.1, recombinant # 1140 (R22).

m = 7X-7AiL, Rec.2, recombinant # 1140 (R22).

n = 7AS.7AL-7AiL, Rec. 1, recombinant # 1002 (R23).

o = 7AS-7AiS.7AiL, Rec. 1, recombinant # 1013 (R23).

p = 7AiS.7AiL-7AL, Rec. 1, recombinant # 805 (R24).

Note: (1) only the inferred structure of those recombinant chromosomes isolated away from parental chromosomes has been presented.

(2) the positions of the probe loci reflect linear order but not actual genetic or physical distances.

Inferred cross-over types

type 8
type 9
type 10
type 11

type 12
type 13
type 14

Chromotypes

j + Rec. 2
k
l
m

n + Rec. 2
o + Rec. 2
p + Rec. 2

■ 7Ai chromatin

□ 7A chromatin

▥ Non-7A (7X) wheat chromatin

▨ Non-7A (7X) wheat chromatin
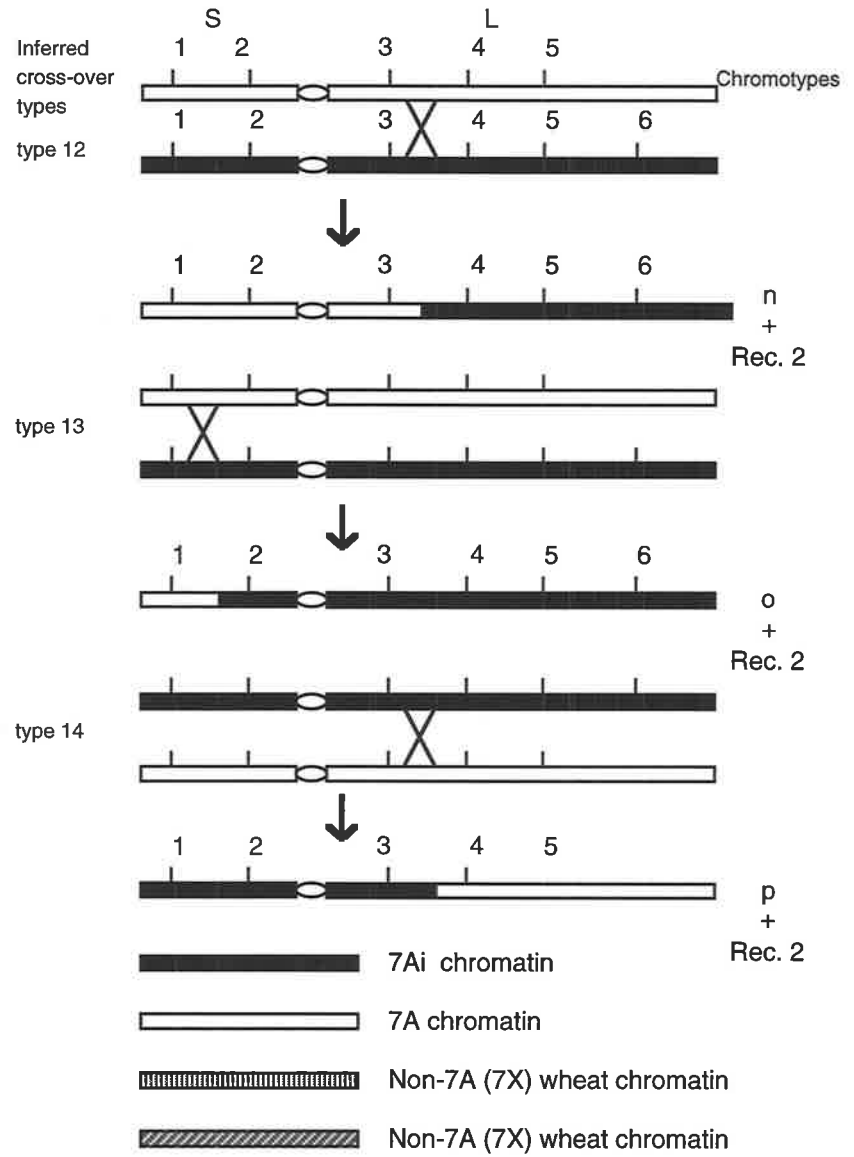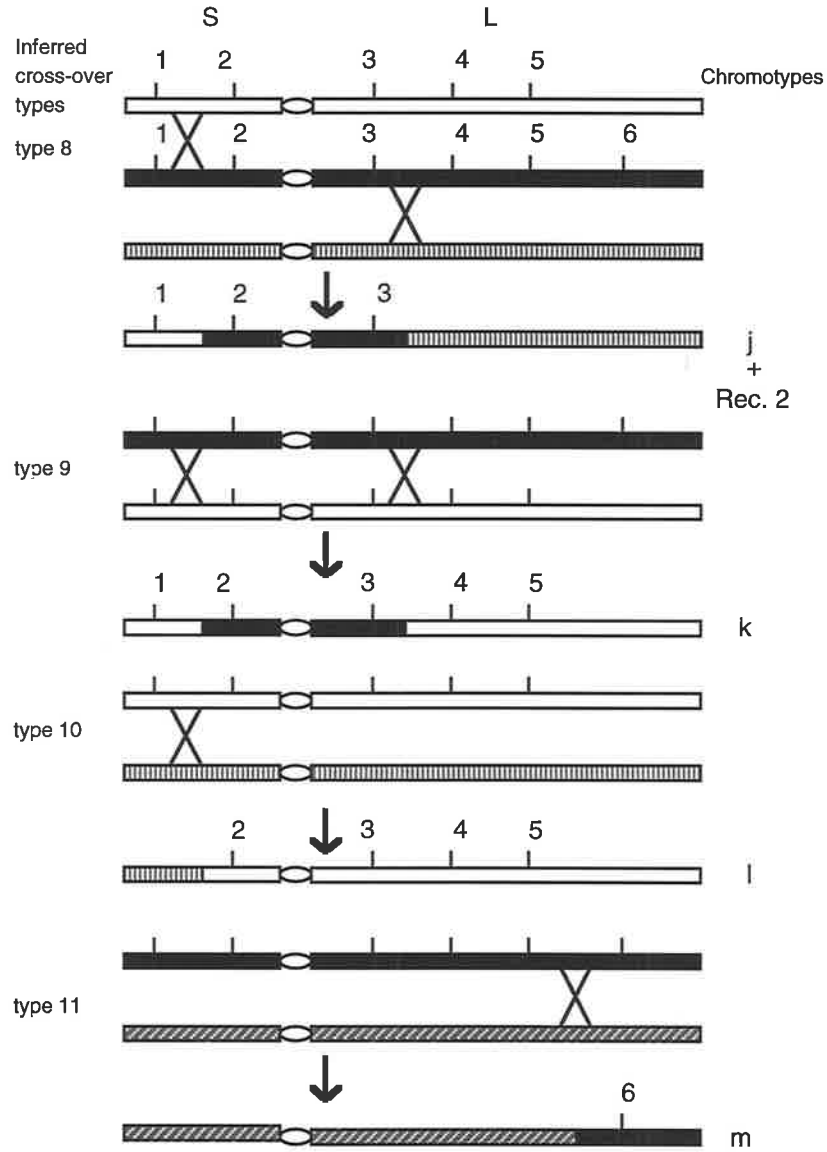
Among the progeny derived from plant # 942 (R20), a recombinant chromosome was isolated in two secondary derivatives (# -1748, -1750, Table 5.6, Appendix) and its phenotype [7Ai (- + + - - -), 7A (+ - - + +)] suggested the structure 7AS-7AiS.7AiL-7AL. Such a recombinant chromosome with intercalary *Agropyron* markers could have arisen from a double cross-over between chromosomes 7A and 7Ai, with one cross-over in each arm (chromotype k, Fig. 5.6b).

The only recombinant in category R21 (# 979) could not be progeny tested because it was sterile.

The phenotypes of the progeny from the four recombinants # 1140 (R22), -1002, -1013 (R23) and # 805 (R24) indicated that each of these plants possessed two recombinant chromosomes. In the progeny of plant # 1140, both the recombinant chromosomes [phenotypes: 7Ai (- - - - - -), 7A (- + + + +) and 7Ai (- - - - - +), 7A (- - - - -)] having structures 7X-7AS.7AL and 7X-7AiL, respectively, were isolated (listed as Rec.1, and Rec.2, respectively, Table 5.6) in two (# -1660, -1664, Appendix) and three (# -1663, -1665, -1955, Appendix) progeny, respectively. These recombinant chromosomes have been shown as chromotypes l and m, respectively, in Fig. 5.6b.

In progeny of the remaining three plants [# -1002, -1013 (R23), -805 (R24)], only one of the recombinant chromosomes could be isolated. With plant # -1002 (R23), one recombinant chromosome (described as Rec.1 in Table 5.6) with the inferred structure 7AS.7AL-7AiL (chromotype n, Fig. 5.6b) was isolated in 3 progeny (# -1867, -1868, -1869, Appendix) . The other supposed recombinant chromosome (with inferred structure ?-7AiS.7AiL-?, listed as Rec.2 in Table 5.6) appears to be present always with Rec. 1 in seven progeny (Table 5.6, Appendix).

With plant # 1013 (R23), a recombinant chromosome with inferred structure 7AS-7AiS.7AiL (chromotype o, Fig. 5.6b) was isolated alone in two primary derivatives (# -1882, -1884, Appendix). The other supposed recombinant chromosome with inferred structure ?-7AS-7AL.? (Rec.2, Table 5.6) appears to be present along with Rec.1 in 3 progeny (# -1881, -1883, -1885, Appendix) and could not be isolated alone.

With plant # 895 (R24), one recombinant chromosome (listed as Rec. 1, Table 5.6) with the inferred structure 7AiS.7AiL-7AL (chromotype p, Fig. 5.6b) was isolated alone in three progeny (# -1404, -1847 -1848, Appendix), while in remaining 6 progeny, this recombinant chromosome (listed as Rec. 2, Table 5.6, inferred structure 7AS-?) appears to be present along with Rec. 1.

In summary, a total of eight recombinant plants, apparently resulting from more than one homoeologous cross-over events were progeny tested. The results indicated that five of the original recombinant plants [(# -895 (R9), -1140 (R22), -1002, -1013 (R23), and -805 (R24)] contained two recombinant chromosomes. However, both of the supposed recombinant chromosomes (listed as Rec. 1 and Rec. 2, Table 5.6) could be isolated in only one of the plants (#1140, R22). In the other four plants [# -895 (R9), -1002, -1013 (R23), -805 (R24)], only one of the recombinant chromosomes (listed as Rec. 1, Table 5.6) was isolated alone. The other inferred recombinant chromosome (listed as Rec. 2, Table 5.6) either was not transmitted in the progeny or was always present along with the other recombinant chromosome (Rec. 1, Table 5.6).

The other three plants in this group [# -884, -891 (R15), -942 (R20)] appeared to have resulted from double cross-over events. With plants # -884, -891 (R15) the putative recombinant chromosome (?-7AL-?, Table 5.6) is thought to have arisen from an intra-arm double cross-over event involving chromosome 7A and another wheat chromosome,

but since this recombinant chromosome was not isolated alone, its structure could not be deduced.

In the case of recombination between wheat and *Agropyron* chromosomes, there was no evidence for the occurrence of any double cross-overs between the long arms of chromosomes 7A and 7Ai. However, one double cross-over product was isolated (recombinant # 942, R20), apparently involving a cross-over in the short arms and another cross-over involving the long arms of these chromosomes (Table 5.6, chromotype k, Fig. 5.6b).

5.3.6. Follow-up progeny tests of putative short arm recombinants carrying single cross-over products:

As described in section 5.3.5.1, among the 34 self-fertile putative short arm recombinants, only 20 gave recombinant chromosomes which could be isolated away from parental 7A and 7Ai chromosomes in the progeny tests. In the remaining 14 cases, the recombinant chromosome was either present along with a parental chromosome or only the parental chromosome was present in the progeny. In these cases additional progeny seeds (where available) from 12 of the 14 putative short arm recombinants were screened in follow-up tests using just the two short arm markers (CDO -545, -595). In these follow-up progeny tests, two more recombinants gave isolated recombinant chromosomes with structures 7AS-7AiS (# 1147, R1) and 7AiS-7AS (# 953, R4) (Table 5.7). These progeny tests indicated that other recombinant chromosomes of similar structures might be isolated from the other putative recombinants if more progeny could be screened. Alternatively, if the recombinant chromosome involved recombination between 7A or 7Ai and another group 7 wheat chromosome (7B or 7D) and the original recombinant was homozygous for an intact 7A or 7Ai chromosome, it would not be possible to isolate the recombinant chromosome alone in selfed progeny no matter how many progeny were screened. One of the short arm markers (CDO545) gave specific RFLPs for chromosomes 7A and 7Ai with *EcoR V* but no specific band for chromosome 7B and/or 7D. Hence, additional markers showing specific fragments for chromosomes 7Ai, 7B and 7D will be required to screen the progeny of these putative recombinants in order to try and isolate a recombinant chromosome involving 7A (or 7Ai) and 7B or 7D, and to deduce its structure.

Table 5.7. Follow-up progeny tests of putative short arm recombinants carrying single cross-overs products. (Note: Only short arm markers were used for screening).

Classification of the recomb-inant and plant # of F3 progeny	Phenotype of the recombinant chromosome isolated alone	Number of progeny plants having		Parental chromosome only (7A or 7Ai)	Nulli-somic	Total	Interpretation of the recombinant (distal segment listed first)
		Recombinant chromosome					
		present alone	present with a parental chromosome (7A or 7Ai)				
R 1 876			3	4		7	?-7AiS
1147	[7Ai = - +] [7A = + -]	1	3			4	7AS-7AiS
R 2 833			2	2		4	7AiS-?
1182			1			1	7AiS-?
R 4 803			2	2		4	7AS-?
953	[7Ai = + -] [7A = - +]	2	1	2		5	7AiS-7AS
1048			2			2	7AS-?
1198			1	2	1	4	7AS-?
1251			3			3	7AS-?
1020			3			3	7AS-?
1025			1			1	7AS-?
R 5 874			3			3	?-7AS

5.3.7. Detection of an F3 progeny plant with a possible interstitial deletion

Six RFLP probes were used for to screen F3 progeny for dissociation of markers within the short and long arms of chromosome 7A and/or 7Ai and a total of 63 putative recombinants were detected. However, one plant (# 1079) gave an ambiguous result when tested with long arm probe CDO673. The overall signal was weak and it was originally classified as + (?) and not recorded as a dissociation for 7Ai marker. When some of these membranes were hybridised with additional RFLP probes to find other polymorphisms for chromosomes 7A and/or 7Ai, this plant (# 1079) was found to lack the *Xwg719-7Ai* locus whereas all the other loci for chromosome 7Ai and 7A were present. Thus it now classified as a putative recombinant. Progeny of this plant were analysed using seven RFLP probes (including WG719) and the results are presented in Table 5.8. These results indicate that plant # 1079 had a complicated structure and appeared to contain a short arm recombinant chromosome of 7AiS-7AS.7AL type, which was likely present in the F3 plant # 1079 (not detected initially because of the masking effect of the parental 7A and altered 7Ai loci present). This short arm recombinant chromosome [phenotype 7Ai (+ - - - - - -), 7A (- + + + + +)] was isolated alone in two of the progeny, while it was present along with an intact 7A chromosome in two other progeny (Table 5.8).

Because of the absence of the *Xcdo673 -7Ai* locus in all the progeny tested, it must have also been absent in the original F3 plant # 1079, even though it was scored as + (?) due to what now appears to have been a background smear. It can be inferred that plant # 1079 had at least three chromosomes contributing to the observed phenotype; (i) an intact 7A chromosome, (ii) a recombinant 7AiS-7AS.7AL chromosome and (iii) a 7Ai chromosome with phenotype (+ + - - + + +). Such a phenotype could has arisen from (i) interstitial deletion on the long arm of chromosome 7Ai or (ii) a double cross-over event between chromosome 7Ai and a wheat chromosome, with one cross-over

proximal to *Xcdo673* and one cross-over distal to *Xwg719* loci. However, at present, insufficient data are available to differentiate between these two possibilities (also see Chapter 7 for discussion). This plant (# 1079) has not been included in the Tables and other calculations because the short arm recombinant inferred to be present in this plant was actually not isolated during the F3 progeny tests and the 7Ai chromosome with missing *Xcdo673* and *Xwg719* loci is more likely to be a result of deletion rather than recombination (see Chapter 7 for detailed discussion). Because these progeny tests were conducted late in this study, these progeny plants were not included in further analyses.

Table 5.8. RFLP phenotypes of F3 plant # 1079 and its F4 progeny.

Plant #	Chromosome	Phenotype (RFLP loci)						
		Xcdo 545	*Xcdo 595*	*Xcdo 673*	*Xwg 719*	*Xpsr 117*	*Xwg 686*	*Xpsr 121*
1079	7Ai	+	+	+?	-	+	+	+
	7A	+	+	+	+	+	+	

Progeny test of plant # 1079

No. of F4 progeny

10	7Ai	+	+	-	-	+	+	+
	7A	+	+	+	+	+	+	
2	7Ai	+	-	-	-	-	-	-
	7A	+	+	+	+	+	+	
2	7Ai	+	-	-	-	-	-	-
	7A	-	+	+	+	+	+	

+ = RFLP locus present, - = RFLP locus absent.

Note: Frequency of isolating 7AiS-7AS.7AL recombinant chromosome (isolated alone in two progeny and with an intact 7A chromosome in two other progeny) indicate that it is not a new recombination event because of second round in *Ph1* deficient conditions but it must have been present in the original plant (# 1079).

5.4. Discussion:

The use of Sears' *ph1b* mutant to induce homoeologous recombination in the present study was found to be successful in incorporating the alien chromatin into homoeologous wheat chromosomes. The approach of using RFLP loci as genetic markers proved highly successful not only for detection and isolation of higher frequencies of wheat-alien chromosome recombination (16 %) but for the types and diversity of the recombinants as well.

The overall high frequency (63 %) of non-recombinant (parental) alien chromosome (7Ai) detected in F3 progeny of *Ph1* deficient families, was in agreement with Banks *et al.* (1995) who observed that 67 % of the BYDV resistant progeny from crosses between addition line L1 (21$_W$"+7Ai") X euploid wheat (21$_W$"), carried an intact 7Ai chromosome. This might be due to chromosome 7Ai having large homoeologous regions and good compensating capabilities for its homoeologous wheat chromosomes (Hohmann *et al.* 1996) or alternatively it might be due to the presence of a segregation distortion factor (*Sd-1*), which is known to be present on several *Agropyron* chromosomes of homoeologous group 7 and results in the preferential transmission of those chromosomes through pollen (Hart and Tuleen 1983; Zhang and Dvorak 1990).

The very low rate of putative homoeologous recombination (less than 1%) detected in *Ph1-* families as compared to that detected in *Ph1* deficient families (average 16 %) was consistent with the known effect of the *Ph1* gene in restricting meiotic pairing to homologues. These results supported the previous findings that in the presence of *Ph1* gene, wheat chromosomes do not pair or only rarely pair with the homoeologous *Agropyron* chromosomes (Dvorak 1972; Dvorak and Knott 1974).

In the present studies a more than two fold increase in the frequency of allosyndetic recombination was observed when the *ph1b* deletion was present in hemizygous

condition (*ph1b/-* families) as compared to homozygous *ph1bph1b* families. Varying rates of success in inducing homoeologous chromosome pairing, have been claimed in the literature using different methods. Riley and Chapman (1967) suggested that maximum homoeologous pairing can be achieved when *Ph1* is removed or suppressed and the 5BS pairing promoter genes are retained. Wang *et al.* (1977) found that the frequency of pairing between chromosome 4B of common wheat and a homoeologous chromosome of *Ag. intermedium,* carrying resistance to wheat streak mosaic virus, was higher in the presence of two doses of *ph1b* (16 %) compared to one dose (7 %). Yasumuro *et al.* (1981) reported a two fold increase in pairing between chromosomes 6D of common wheat and 6Ag of *Ag. elongatum* in a nullisomic 5B background (4.9 %) compared to a hemizygous *ph1b* condition (2.5 %). However, the results of these two wheat-*Agropyron* studies were based on chromosome pairing frequencies only and no genetic evidence was provided to show that this increased pairing results in increased number of recombinant chromosomes. In a later study, a three fold increase in the rate of allosyndetic recombination between the short arms of homoeologous group 1 chromosomes of common wheat and cereal rye, was reported using nullisomy of chromosome 5B compared to Sears' *ph1b* mutant (Koebner and Shepherd 1986). More recently Islam and Shepherd (1992) reported an approximate three fold increase in the rate of allosyndetic recombination between chromosome arm 3HL of barley and chromosome 3A of common wheat in the presence of two doses of *ph1b* (1.48 %) compared to one dose of *ph1b* (0.45 %). However, none of these experiments (including the present one) were conducted under controlled environmental conditions and different populations within an experiment were grown during different seasons, which might have affected the rate of allosyndetic recombination, since it is known that chromosome pairing may be inhibited by heat in various plant species, including wheat (for a review, see Loidl 1990). Hence all of these findings should be considered with caution and a larger experiment with more controlled environmental conditions should be conducted before drawing any

firm conclusions regarding the relative efficiency of different methods in inducing allosyndetic recombination between chromosomes of wheat and related alien species.

In the present study, an approximate 2.5 fold increase in the frequency of isolation of short arm recombinants over the long arm recombinants indicated the possible greater homoeology between the short arms of the two Triticeae chromosomes. It is not likely that the observed differences in rate of allosyndetic recombination between the short and long arms of these chromosomes were due to the positions of the selected markers used for screening F3 progeny, because the RFLP probes chosen appeared to be well separated along the lengths of each arm (see Chapter 4 for details). Two alternative explanations for these observed results are discussed below.

(i) It was reported recently that the short arms of group 7 homoeologous chromosomes of wheat have a genetic length of 140 cM, while the long arms have genetic length of only 70 cM (Hohmann *et al.* 1995). Based on the increasing evidence for the presence of highly conserved blocks of genetic material within Triticeae chromosomes, it is speculated that this similarity might extend to genetic lengths also in the case of chromosome 7Ai of *Ag. intermedium*. One possibility is that the increased genetic length of the short arms of the two chromosomes is due to more pairing recognition sites (recombination hot spots) allowing them to recombine more frequently in a *Ph1* deficient background as compared to the long arms.

(ii) Alternatively the lower frequency of homoeologous recombination between the long arms of the two chromosomes might be due to structural changes which have occurred during the evolution of these Triticeae species. Translocations involving chromosomes in homoeologous groups 4, 5 and 7 have been found in wheat and rye. It has been reported that a part of the short arm of the "original" 7B chromosome of wheat has been translocated to the long arm of the present 4A and a part of the original 5AL has been translocated to the present 7BS (Naranjo *et al.* 1987; Chao *et al.* 1989; Liu *et al.* 1992). King *et al.* (1994) using RFLP analyses reported that the wheat-*Agropyron* addition line

L1 (having homoeologous chromosomes of group 7 of *Ag. intermedium* added to wheat genome by Cauderon 1966) does not possess the 4/7 translocations, found in wheat. However, Figueiras *et al.* (1986) reported that the addition line L1 carries an isozyme loci for acid phosphatase, which is normally associated with homoeologous chromosomes of group 4 of common wheat (for a review, see Hart 1987), suggesting the presence of a 4/7 translocation (presumably different from that found in wheat) in chromosome 7Ai.

At present, the available data are not sufficient to decide between these two possibilities and hence it is not known why the short arm recombinants were obtained at a much higher rate than the long arm recombinants.

In the present study, if double cross-overs had occurred within the short arms of the two Triticeae chromosomes, they could not have been detected because only two short arm markers were used for screening. However, four widely separated RFLP markers were used to detect homoeologous recombination along the long arms of these two chromosomes and although recombinant chromosomes with varying lengths of *Agropyron* chromatin introgressed onto wheat chromosomes were detected, there was no convincing evidence of any double cross-over within the long arms of these two chromosomes On the other hand, Jena *et al.* (1992) detected a large number of intercalated double cross-over products in hybrids between *Oryza sativa* L. and *O. officinalis* Wall, and suggested that an unconventional recombination system is operating in rice. Within Triticeae species, Lukaszewski (1995) studied distribution of translocation breakpoints in homoeologous recombinants involving group 1 chromosomes of wheat, 1R of rye and 7S of *T. speltoides* and found only one double cross-over product among 313 homoeologous recombinant chromosomes. Dubcovsky *et al.* (1996$^{\alpha}$) also reported that no double cross-over products occurred in 4B-4D recombinant chromosomes of wheat. It may therefore be concluded that

in attempting to reduce the length of alien segments in wheat-alien chromosome translocation lines, rather than searching for interstitial segments resulting from double cross-over products, Sears' approach of intercrossing two overlapping types of distal and proximal homoeologous recombinants (Sears 1981) should be adopted. The detection of overlapping types of recombinant chromosomes is therefore very important for this type of chromosome engineering in wheat and can be used to obtain agronomically desirable interstitial recombinants through homologous cross-overs (also see Chapter 6).

The occurrence of cross-over products involving non targeted chromosomes including 7Ai-non-7A and/or wheat-wheat translocations (e.g. 7AiS-7X, 7X-7AS.7AL, 7X-7AL) inferred during the present studies, was in agreement with earlier findings. During his attempts to transfer the leaf rust resistance gene *Lr24* from chromosome 3Ag of *Ag. elongatum* onto chromosome 3D of common wheat, Sears (1972, 1981) found that four out of twenty-seven recombinants involved 3Ag - non-3D cross-over products. Also Dvorak and Gorham (1992) found that one out of forty recombinants induced using the *phlc* mutant to transfer K^+/Na^+ discrimination locus *Kna1* from chromosome 4D of *T. aestivum* into chromosome 4B of durum wheat, one recombinant involved crossing over between 4D and a chromosome other than 4B.

Further characterisation of the recombinant chromosomes described in this chapter, was required to determine their agronomic properties and to define the cross-over points more precisely and hence to more accurately map the distribution of chiasmata along the length of chromosomes 7A and 7Ai. These detailed analyses using molecular, cytological and agronomic methods are described in the following chapter.

CHAPTER - 6

CHARACTERISATION OF THE RECOMBINANTS

6.1. Introduction:

In most of the wheat-alien translocation lines reported in the literature, the recombinant chromosomes have larger alien segment than necessary to incorporate the desirable genes and the surplus alien chromatin often results in agronomically undesirable effects (see review of literature). Therefore, to be useful in wheat breeding, it is often necessary to reduce the size of the transferred alien segment, and strategies to reduce the amount of alien genetic material in the translocated lines require prior knowledge of the amount and location of the alien chromatin present in the introgressed chromosome.

In order to fully characterise the wheat-alien recombinant chromosomes and establish how much alien chromatin has been introgressed into wheat chromosome(s), genetic markers are required to monitor the genetic make-up of the alien chromosome segment. In particular, markers specific for the alien chromatin will be more informative than extrapolating from the presence or absence of specific wheat markers in the translocated chromosome. During the past few years, several assay procedures have been used to estimate the amount of alien chromatin in wheat-alien translocation lines. Initially the length of the alien segment and the approximate positions of the homoeologous cross-over points in the wheat-alien recombinant chromosomes were inferred by determining the amount of pairing between the recombinant chromosomes carrying the alien resistance gene and the relevant wheat telocentric chromosomes (e.g. Sears 1981).

More recently, several species-specific repeated nucleotide sequences have been isolated and cloned (e.g. McIntyre *et al.* 1988; Zhang and Dvorak 1990) and these repeated

sequences have been shown to hybridise in Southern blots extensively with the DNA of the alien chromosomes. Direct visualisation of alien chromatin introgressed into the wheat genome, using these repeated sequences as labelled probes onto the metaphase spreads of mitotic chromosomes has also been carried out (Kim *et al.* 1993; Bournival *et al.* 1994). Genomic *in situ* hybridisation has also been used extensively for the characterisation of wheat-alien recombinant chromosomes (e.g. Schwarzacher *et al.* 1992). These techniques have been useful in the characterisation of alien introgressions but are not yet suitable for the efficient screening of large progeny populations for the detection of the recombinants.

The studies reported in this chapter were aimed at characterising the wheat-*Agropyron* recombinant chromosomes (isolated during present studies) in several ways:

(i) To determine the location and length of the alien chromosome segment introgressed into wheat chromosomes using:

(a) Genetic makers - RFLPs specific for group 7 homoeologous chromosomes, previously found polymorphic for chromosomes 7A and/or 7Ai.

(b) Genomic *in situ* hybridisations using labelled total genomic DNA from *Agropyron intermedium* as the probe.

(ii) To determine the agronomic characteristics of the recombinants, particularly their reaction to stem rust and BYDV, and thereby to locate the approximate position of the gene controlling these useful character(s) on the alien chromosome.

6.2. Materials and methods:

From the total of 63 recombinants detected during present studies, 52 produced progeny seed and it was planned to characterise all of these recombinants using additional RFLP probes. However, when selecting progeny from these 52 putative recombinants for further analysis, plants carrying the recombinant chromosome could not be obtained for six of them. Hence only 46 recombinants have been analysed with additional RFLP probes to obtain more information on the structure of the recombinant chromosomes. Preference was given to the progeny having the recombinant chromosomes in a *Ph1* background, i.e. progeny from crossing the putative recombinants with euploid CS or the nullisomic 7A-tetrasomic 7B stock, to avoid the complications which could occur from new episodes of homoeologous recombination. When such plants were not available, progeny plants possessing the recombinant chromosomes free of parental 7A or 7Ai chromosomes were preferentially chosen over plants carrying the recombinant chromosomes along with the intact parental chromosome(s). Where more than one recombinant chromosome was inferred to be present in a given plant [recombinants # -895 (R9); -1140 (R22); -1002, -1013 (R23) and -805 (R24), described in section 5.3.5.2], those progeny plants possessing only one of the recombinant chromosomes were selected for further testing. However, because of the unavailability of progeny plants having the above described criteria when this study was initiated, in a few cases sib progeny were used.

In addition to the six RFLP probes already used for the isolation of the recombinant chromosomes, nine more DNA probes [CDO475, PSR -119, -103, -108, -152 (short arm markers) and CDO347, PSR129 and WG -719, -420 (long arm markers)] were used to further define the cross-over points giving rise to the recombinants. These probes and the restriction enzymes used with them have been described in Chapter 4.

The *Agropyron* segment in two of the short arm recombinants was physically characterised by genomic *in situ* hybridisation using fluoro-labelled *Agropyron intermedium* DNA as probe (see Chapter 3 for detailed methods).

The progeny carrying the short arm recombinant chromosomes with 7AiS dissociated markers were screened with stem rust (pathotype: '21-2,3,7') and the progeny carrying long arm recombinant chromosomes with 7AiL dissociated markers were screened with barley yellow dwarf virus (serotype: BYDV-PAV$_{Adel.}$) (Details of the methods used are given in Chapter 3).

6.3. Results:

6.3.1. RFLP analyses:

6.3.1.1. Location of cross-over points:

Seven of the fifteen DNA probes used showed polymorphic band(s) for the short arm of 7A and/or 7Ai and eight for the long arms of these chromosomes. For the short arm, three probes (CDO -545, -595, PSR103) distinguished RFLPs with both the 7A and 7Ai chromosomes targeted for recombination, while three probes (CDO475, PSR -119, -108) produced restriction fragments specific for 7A only, and one (PSR152) for 7Ai only (using the restriction enzymes described in Chapter 4). For the long arm, five probes (CDO673, PSR -117, -129 and WG -719, -686) distinguished RFLPs with both the 7A and 7Ai chromosomes, while the three remaining probes (CDO347, PSR121, and WG420) produced restriction fragments specific for only chromosome 7Ai. The linear order of these 15 probe loci along chromosomes 7A and 7Ai was deduced by inspection of the phenotypes of the recombinant chromosomes. The linear order which required the minimum number of double cross-overs to explain the pattern of allosyndetic products

obtained was chosen as the most likely order along these chromosomes and this order has been used in Table 6.1 (also see section 6.3.1.2). Where probes showed polymorphism for only 7A or 7Ai, it has been assumed that the order is the same on the other chromosome.

Out of the total of 41 short arm recombinants detected in this work, 34 were analysed with the 15 RFLP probes. In 18 of the recombinant chromosomes, breakpoints were detected in both the 7A and 7Ai chromosomes (Table 6.1), but because of the lack of polymorphism for chromosome 7Ai with probes CDO475, PSR -119, -108 the exact location of the breakpoints on chromosome 7Ai could not be detected in the majority of the cases. In these cases the cross-overs could have occurred anywhere between *Xcdo545* and *Xpsr103* loci. In contrast, breakpoints were detected more precisely on chromosome 7A because 6 out of 7 short arm specific probes (all except PSR152) produced 7A specific bands. Among these 18 recombinants, the recombinant chromosome present in progeny # 1882 (F3 progeny # 1013, R23) showed a likely duplication for the *Xpsr103* locus.

In 16 of the short arm recombinant chromosomes analysed, breakpoints could be detected in only one of the chromosomes 7A or 7Ai because of the presence of a parental chromosome (7A or 7Ai) present along with the recombinant chromosome.

For chromosome 7A, a total of 30 breakpoints were detected (Table 6.1). Fourteen breakpoints occurred between the loci *Xcdo475* and *Xpsr108* , ten between *Xpsr119* and *Xcdo475* , three between *Xpsr108* and *Xpsr103* and only one between loci *Xcdo545* and *Xpsr119*. Because of the unavailability of a diagnostic band for 7A using the probe PSR152, in progeny # -2136 and -1882 the breakpoints could not be detected between two consecutive loci and were inferred to occur somewhere between three consecutive loci (*Xpsr103 - Xcdo595*).

Table 6.1. Characterisation of the recombinant chromosomes using 15 RFLP probes specific for group 7 homoeologous chromosomes of Triticeae. The order of the probe loci used, was chosen which needed minimum double cross-over products to explain the phenotypes observed. The loci between which cross-overs were detected are underlined.

of the progeny tested (# of parent F3 plant and category of recomb is given in parenthesis)

RFLP loci

	Short arm							Long arm							
	Xcdo 545	Xpsr 119	Xcdo 475	Xpsr 108	Xpsr 103	Xpsr 152	Xcdo 595	Xwg 719	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 129	Xcdo 347	Xpsr 121	Xwg 420

Cross-overs detected on the short arm(s)

1991 (1045, R1), 2147 (1118, R1), 1768 (963, R4), 1833 (1106, R7), 2108 (976, R4) Total = 5

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	−			±	+	+	+	+	+	+	+	+	+	+	+
7A	+	+	±	−	−		−	−	−	−	−	−			

2152 (1147, R1) Total = 1

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	−			±	+	+	+	+	+	+	+	+	+	+	
7A	+	+	+	+	+		+	+	+	+	+				

2208 (833, R2), 2160 (1182, R2) Total = 2

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+					−	−	−	−	−	−	−	−	−	−
7A	+	+	+	+	+		+	+	+	+	+	+			

1730 (835, R2), 2156 (1169, R2), 2167 (1218, R5), 2187 (1052, R8) Total = 4

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+					−	−	−	−	−	−	−	−	−	−
7A	−	±	±	+	+		+	+	+	+	+	+			

1973 (1050, R2), 2199 (896, R5), 2131 (1053, R8), 2183 (1019, R8) Total = 4

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+					−	−	−	−	−	−	−	−	−	−
7A	−	−	−	±	+		+	+	+	+	+	+			

1738 (879, R3) Total = 1

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+					−	−	−	−	−	−	−	−	−	−
7A	−	−	−	−	−		−	−	−	−	−	−			

2202 (803, R4), 2205 (953, R4), 2127 (1048, R4), 2111 (986, R4), 2119 (1025, R4) Total = 5

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+			+	+	+	+	+	+	+	+	+	+	+	+
7A	+	±	−	−	−		−	−	−	−	−	−			

2122 (1047, R4), 2164 (1198, R4), 2173 (1251, R4) Total = 3

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+			+	+	+	+	+	+	+	+	+	+	+	+
7A	+	+	±	−	−		−	−	−	−	−	−			

2142 (1094, R4) Total = 1

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	−			±	+	+	+	+	+	+	+	+	+	+	+
7A	+	+	+	±	−		−	−	−	−	−	−			

1739 (898, R4) Total = 1

	545	119	475	108	103	152	595	719	673	117	686	129	347	121	420
7Ai	+				+	+	+	+	+	+	+	+	+	+	+
7A	+	+	+	±	−		−	−	−	−	−	−			

Table 6.1 Continued

| | RFLP loci | | | | | | | | | | | | | | |
| | Short arm | | | | | | | Long arm | | | | | | | |
Plant #	Xcdo 545	Xpsr 119	Xcdo 475	Xpsr 108	Xpsr 103	Xpsr 152	Xcdo 595	Xwg 719	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 129	Xcdo 347	Xpsr 121	Xwg 420
2116 (1020, R4)) Total = 1															
7Ai	+				+	+	+	+	+	+	+	+	+	+	
7A	±	-	-	-	-		-	-	-	-	-	-			
2177 (874, R5) Total = 1															
7Ai	+				+	+	+	+	+	+	+	+	+	+	+
7A	-	-	-	±	+		+	+	+	+	+	+			
2190 (886, R5) Total = 1															
7Ai	±				-	-	-	-	-	-	-	-	-	-	-
7A	-	-	-	-	±		+	+	+	+	+	+			
1841 (1078, R6) Total = 1															
7Ai	-				-	-	-	-	-	-	-	-	-	-	-
7A	-	-	-	±	+		+	+	+	+	+	+			
2136 (1063, R7) Total = 1															
7Ai	-				-	±	+	+	+	+	+	+	+	+	+
7A	+	+	+	+	±		-	-	-	-	-	-			
1836 (1180, R7) Total = 1															
7Ai	-				+	+	+	+	+	+	+	+	+	+	+
7A	+	±	-	-	-		-	-	-	-	-				
1882 (1013, R23) Total = 1															
7Ai	-				+	+	+	+	+	+	+	+	+	+	+
7A	+	+	+	+	±		-	-	-	-	-	-			

Cross-overs detected on the long arm(s)

	Xcdo 545	Xpsr 119	Xcdo 475	Xpsr 108	Xpsr 103	Xpsr 152	Xcdo 595	Xwg 719	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 129	Xcdo 347	Xpsr 121	Xwg 420
1813 (1170, R9) Total = 1															
7Ai	+				+	+	+	±	-	-	-	-	-	-	-
7A	+	+	+	+	+		+	+	+	+	+	+			
1805 (1146, R10) Total = 1															
7Ai	-				-	-	-	-	-	-	±	+	+	+	+
7A	+	+	+	+	+		+	+	+	+	+	+			
1780 (936, R11) Total = 1															
7Ai	-				-	-	-	-	-	-	-	-	±	+	+
7A	+	+	+	+	+		+	+	+	+	+	+			
1873 (1175, R14), 1867 (1002, R23) Total = 2															
7Ai	-				-	-	-	-	-	±	+	+	+	+	+
7A	+	+	+	+	+		+	+	±	-	-	-			

Table 6.1 Continued

| | RFLP loci | | | | | | | | | | | | | | |
| | Short arm | | | | | | | Long arm | | | | | | | |
Plant #	Xcdo 545	Xpsr 119	Xcdo 475	Xpsr 108	Xpsr 103	Xpsr 152	Xcdo 595	Xwg 719	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 129	Xcdo 347	Xpsr 121	Xwg 420
1893 (1199, R16) Total = 1															
7Ai	-				-	-	-	-	-	-	-	-	-	-	-
7A	-	-	-	-	-		-	-	-	+	+	+			
1889 (1003, R17) Total = 1															
7Ai	+				+	+	+	+	+	-	-	-	-	-	-
7A	-	-	-	-	-		-	-	-	+	+	+			
1800 (1129, R18), 1786 (985, R19) Total = 2															
7Ai	-				-	-	-	-	-	-	+	+	+	+	+
7A	+	+	+	+	+		+	+	+	+	-	-	-		
Cross-overs detected on both the arms of chromosomes 7A and/or 7Ai															
1750 (942, R20) Total = 1															
7Ai	-				+	+	+	+	+	-	-	-	-	-	-
7A	+	+	+	-	-		-	-	-	+	+	+			
2196 (895, R9) Total = 1															
7Ai	-				+	+	+	+	-	-	-	-	-	-	-
7A	+	+	+	+	-		-	-	-	-	-	-			
1846 (805, R24) Total = 1															
7Ai	+				+	+	+	+	+	-	-	-	-	-	-
7A	+	+	+	-	-		-	-	-	+	+	+			

+ = RFLP locus present, - = RFLP locus absent

Nine progeny carrying long arm recombinant chromosomes only were analysed using the same 15 RFLP probes. In three progeny (# -1867, -1873, -1889) breakpoints were detected with equal precision on both chromosomes 7A and 7Ai with crossing-over occurring between *Xcdo673* and *Xpsr117* loci. In two progeny (# -1800, -1786) breakpoints were detected between *Xpsr117* and *Xwg686* loci also on both the chromosomes. For three progeny (# -1813, -1805, -1780) breakpoints were detected on chromosome 7Ai only, where they occurred between loci *Xwg719* - *Xcdo673*, *Xpsr117* - *Xwg686* and *Xpsr129* - *Xcdo347*, respectively. In one progeny (# 1893) the breakpoint was detected on chromosome 7A only and it was observed between loci *Xcdo673* and *Xpsr117*.

Three progeny showing dissociation of selective markers in both arms were also analysed. In the recombinant chromosome present in progeny # 1750 (F3 plant # 942, R20), two breakpoints were detected, one on the short arm and one on the long arm, and each breakpoint was detected in both chromosomes 7A and 7Ai. The short arm breakpoints were inferred to be located between loci *Xcdo475* and *Xpsr108*, while the long arm breakpoints occurred between loci *Xcdo673* and *Xpsr117*. For the recombinant chromosome present in progeny # 2196 (F3 plant # 895, R9) breakpoints were detected on short arms of both the chromosomes, where these were inferred to be located between loci *Xpsr108* - *Xpsr103* and long arm of chromosome 7Ai only, where it occurred between loci *Xwg719* -*Xcdo673*. For the recombinant chromosome present in plant # 1846 (F3 plant # 805, R24) breakpoints were detected on long arms of both the chromosomes where these occurred between loci *Xcdo673-Xpsr117* and short arm of chromosome 7A only, where it occurred between loci *Xcdo475* and *Xpsr108*.

6.3.1.2. Relative linear order of the probe loci:

The RFLP probes used during present studies were selected from different genetic maps (for details, see Chapter 4) and because of the unavailability of common markers on these maps, the exact position and relative linear order of these loci were not known at the start of these studies. The relative order of the probe loci was deduced after analysing the present data and it was based on the order which required only single cross-overs or the minimum number of double cross-overs, to explain the patterns of allosyndetic recombination products observed. This relative order (used in Table 6.1) is shown diagrammatically in Fig. 6.1, where the position of the markers do not reflect genetic or physical distances apart. However, the order of the loci *Xpsr152 - Xcdo595* (on the short arm) and *Xwg686 - Xpsr129, Xcdo347 - Xpsr121* and *XpsrÍ21 - Xwg420* (on the long arm) remains arbitrary because no recombination was detected between these loci in the present study and the linear order chosen reflects their position in earlier published maps of group 7 chromosomes of wheat (Chao *et al.* 1989; Hohmann *et al.* 1994, 1995) and barley (Heun *et al.* 1991; Kleinhofs *et al.* 1991). In all cases, except one (*Xwg719*) the relative linear orders of the probe loci were in agreement with those previously described in the published maps. Probe locus *Xwg719*, was previously mapped on the short arm of chromosome 1 (7H) of barley (Heun *et al.* 1991), but in the present study the locus was found to be on the long arms of chromosomes 7A and 7Ai. This result is in agreement with recently published physical maps of wheat homoeologous group 7 chromosomes (Hohmann *et al.* 1994, 1995) who also mapped the *Xwg719* locus on the long arms of homoeologous group 7 chromosomes of wheat.

Fig. 6.1: Chromosomal / chromosome arm location and relative linear order of the probe loci used for characterisation of the recombinant chromosomes

C=Location of the centromere.

The loci marked with a W and A indicate that the probe produced polymorphic bands only for wheat and *Agropyron*, respectively.

Note that the position of the probe loci do not reflect genetic or physical distances.
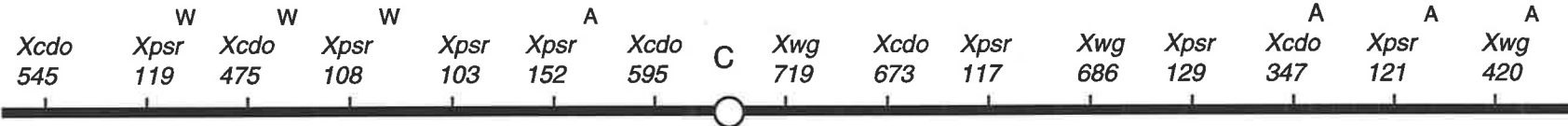
6.3.1.3. Structure of the recombinant chromosomes:

On the basis of the recombination breakpoints deduced from testing with DNA probes, the structure of the wheat-*Agropyron* recombinant chromosomes which had been isolated alone, were inferred and these are presented in Fig. 6.2. In inferring these structures, where the RFLP probes used were polymorphic for only one of the chromosomes (either 7A or 7Ai), when a locus was found to be absent on the marked chromosome it was assumed that the corresponding locus was present on the unmarked chromosome. It should also be noted that the breakpoints indicate their relative linear order only and do not reflect actual genetic or physical locations.

Among the progeny carrying the short arm recombinant chromosomes, nine progeny [# -1730, -2156, -2167, -2187 (chromotype A, Fig. 6.2a), -1973 , -2199, -2131, 2183 (chromotype B, Fig. 6.2a), -2190 (chromotype C, Fig. 6.2a)] were detected having varying lengths of distal *Agropyron* segments introgressed onto chromosome 7A of common wheat. Eight progeny [# -1836 (chromotype D, Fig. 6.2a), -1991, -2147, -1768, -2108, -1833 (chromotype E, Fig. 6.2a), -2142 (chromotype F, Fig. 6.2a), -2136 (chromotype G, Fig. 6.2a)] were detected having short arm recombinant chromosomes with varying lengths of distal wheat and proximal *Agropyron* segments.

Among the progeny carrying long arm recombinant chromosomes, five [# -1780 (chromotype H, Fig. 6.2b), -1800 , -1786 (chromotype I, Fig. 6.2b), -1873 , -1867 (chromotype J, Fig. 6.2b)] were detected carrying the recombinant chromosomes having varying lengths of distal *Agropyron* segment(s) introgressed onto chromosome 7A of wheat, while only one progeny [# 1889, (chromotype K, Fig. 6.2b)] was detected carrying a recombinant chromosome with proximal *Agropyron* and distal wheat chromatin.

For the progeny showing dissociation of markers on both the arms, the structure of two types of recombinant chromosomes was deduced and these are shown as chromotypes L and M (Fig. 6.2b). The recombinant chromosomes in progeny # 2142 (chromotype L, Fig. 6.2b) appeared to have an intercalary *Agropyron* segment recombined into chromosome 7A, while the recombinant chromosome in progeny # 2196 (chromotype M, Fig. 6.2b) appeared to be made up of a distal short arm segment from chromosome 7A, an intercalary (proximal short and long arm) segment from chromosome 7Ai and a distal long arm segment from an unidentified wheat chromosome.

The data presented in Table 6.1 and the structure of the recombinant chromosomes (Fig. 6.2a and b) showed that most of the short arm breakpoints detected were distal to *Xpsr108* and proximal to *Xcdo545* loci, while most of the long arm breakpoints detected were proximal to *Xcdo347* and distal to *Xcdo673*. In contrast, very few or no breakpoints were detected between the most distal and the most proximal loci on both the (short and long) arms of the chromosomes 7A and 7Ai. This indicated the possible presence of recombination hot spots on the two chromosomes between the loci described.

6.3.1.4. Distribution of chiasmata frequencies along chromosome arm 7AS:

A total of 63 putative recombinants were detected among 390 F3 progeny having *Ph1* deficient conditions (see Chapter 5 for details), giving a crude rate of 16 % homoeologous recombination which represents a gametic recombination frequency of approximately 8.0 % (for details of recombination frequencies, see section 5.2.3, Chapter 5). Out of these 63 putative recombinants, 46 were characterised using 15 RFLP probes (for details see materials and methods in this Chapter) and in 33 of the putative recombinants, breakpoints were detected on the short arm of chromosome 7A. Among these, 30 breakpoints were detected in putative short arm recombinants and 3 in the putative recombinants involving

Fig. 6.2 a : Inferred structure (chromotypes) and frequencies of the short arm recombinant chromosomes isolated away from parental chromosomes.

The likely linear order of the probe loci is shown at the top of the figure. The loci marked with a W and A indicate that the probe produced polymorphic bands only for wheat and *Agropyron*, respectively. C = position of centromere.

A - G = Chromotypes of the recombinant chromosomes present alone in the progeny plant tested.

Plant # of the progeny tested is given first, # of F_3 plant and the category of recombination is given in parentheses.

A = # -1730 (835, R2), -2156 (1169, R2), -2167 (1218, R5), -2187 (1052, R8)

B = # -1973 (1050, R2), -2199 (896, R5), -2131 (1053, R8), 2183 (1019, R8)

C = # 2190 (886, R5)

D = # 1836 (1180, R7)

E = # -1991 (1045, R1), -2147 (1118, R1), -1768 (963, R4), -2108 (976, R4), -1833 (1106, R7)

F = # 2142 (1094, R4)

G = # 2136 (1063, R7)

Short arm   Long arm

| W | W | W | | A | | | | | | | | A | A | A |

Xcdo 545, Xpsr 119, Xcdo 475, Xpsr 108, Xpsr 103, Xpsr 152, Xcdo 595, C, Xwg 719, Xcdo 673, Xpsr 117, Xwg 686, Xpsr 129, Xcdo 347, Xpsr 121, Xwg 420

Chromotype and frequency

A (4)
B (4)
C (1)
D (1)
E (5)
F (1)
G (1)
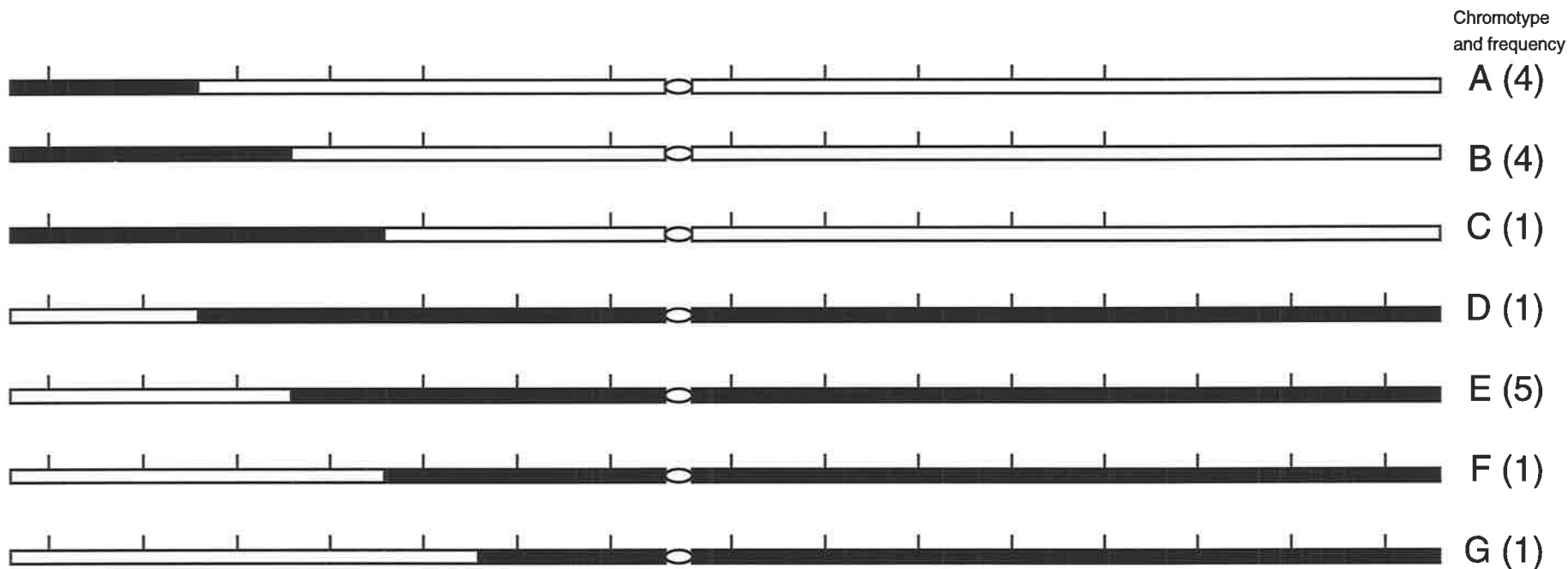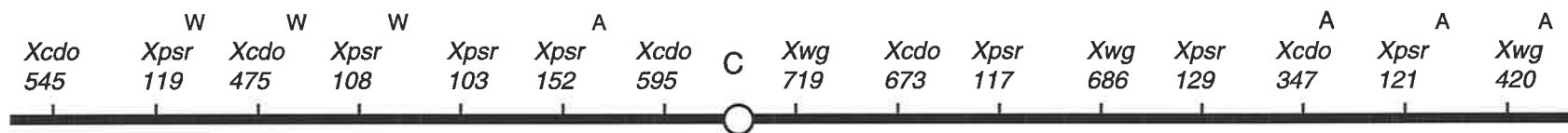
■■■ = 7Ai chromatin   ☐ = 7A chromatin

Fig. 6.2. b : Inferred structure (chromotypes) and frequencies of the long (H, I, J, K) and both (long and short) arms (L, M) recombinant chromosomes isolated away from parental chromosomes.

The likely linear order of the probe loci is shown at the top of the figure. The loci marked with a W and A indicate that the probe produced polymorphic bands only for wheat and *Agropyron*, respectively. C = position of centromere.

H - M = Chromotypes of the recombinant chromosomes present alone in the progeny plant tested.

Plant # of the progeny tested is given first, # of F$_3$ plant and category of recombination is given in parentheses.

H = # -1780 (936, R11)

I = # -1800 (1129, R18), -1786 (985, R19)

J = # -1873 (1175, R14), -1867 (1002, R23)

K = # 1889 (1003, R17)

L = # 1750 (942, R20)

M = # 2196 (895, R9)

Short arm | Long arm

W W W A A A A

Xcdo 545 | Xpsr 119 | Xcdo 475 | Xpsr 108 | Xpsr 103 | Xpsr 152 | Xcdo 595 | C | Xwg 719 | Xcdo 673 | Xpsr 117 | Xwg 686 | Xpsr 129 | Xcdo 347 | Xpsr 121 | Xwg 420

Chromotype and frequency

H (1)

I (2)

J (2)

K (1)

L (1)

M (1)
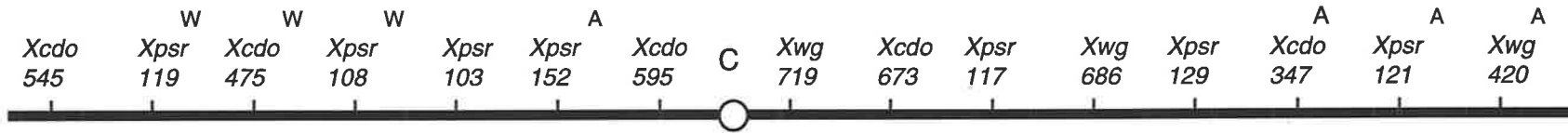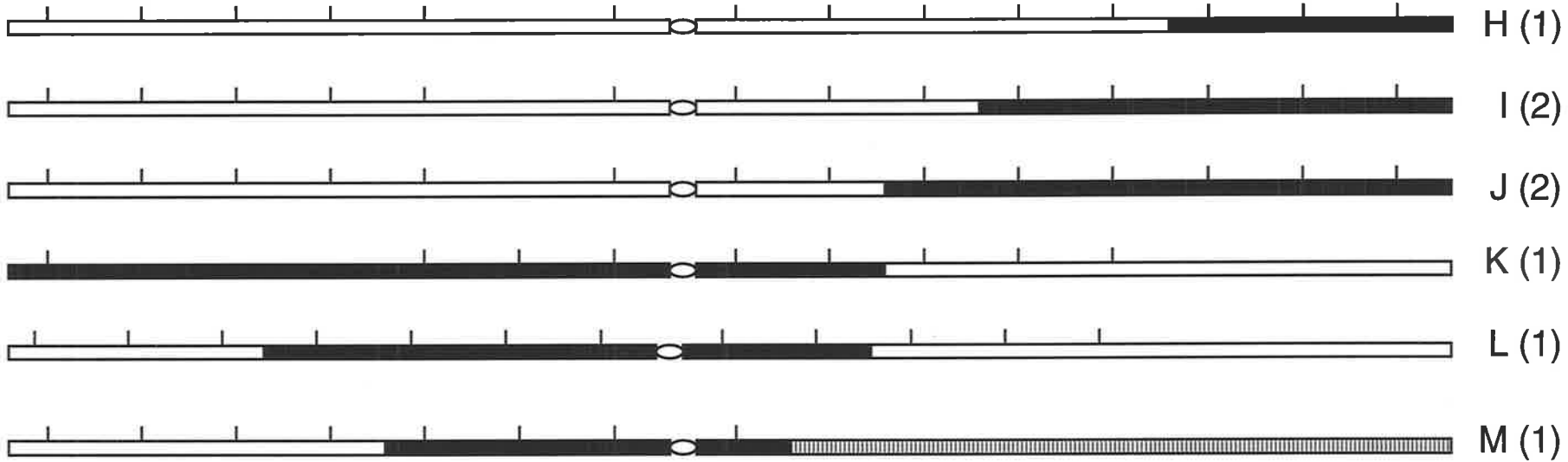
▬▬▬ = 7Ai chromatin   ▭ = 7A chromatin   ▥ = Non-7A wheat chromatin

both the arms (Table 6.1). One, ten, sixteen, four and 2 breakpoints were detected between the loci *Xcdo545-Xpsr119*, *Xpsr119-Xcdo475*, *Xcdo475-Xpsr108*, *Xpsr108-Xpsr103*, *Xpsr103-Xcdo595*, respectively (Table 6.1). (Note: Because of the unavailability of a 7AS specific band using the probe PSR152, breakpoints could not be detected between *Xpsr103-Xpsr152*, rather these were detected between *Xpsr103-Xcdo595*). Frequencies and distribution of chromosome breakpoints along the length of chromosome arm 7AS in these 33 homoeologous cross-over products were analysed and compared with the published corresponding map distances calculated from homologous cross-overs (where available). All of these results are summarised in Fig. 6.3. Similar analyses for 7AL are not presented here because of the low numbers of long arm recombinants detected during the present studies. Similar analyses for chromosome 7Ai are also not presented because in majority of the short arm recombinants, due to the absence of polymorphic bands for chromosome 7Ai using probes PSR119, CDO475 and PSR108, precise breakpoints on chromosome 7Ai could not be determined and these had to be inferred from the corresponding breakpoints on chromosome 7A.

To construct the genetic map based upon homoeologous recombination (presented in Fig. 6.3), crude gametic recombination frequencies (p values) were estimated from the observed number of recombinants, based on the expected frequency of recombinants being 2p when p is small (see section 5.2.3 for details). The maximum recombination frequency (p) was observed between loci *Xcdo475-Xpsr108* (2.1%) followed by 1.3 % observed between loci *Xpsr119-Xcdo475*. The recombination frequencies among other short arm loci (*Xcdo545-Xpsr119*, *Xpsr108-Xpsr103* and *Xpsr103-Xpsr152/Xcdo595*) were 1% or less. The lower estimates (maximum 'p' value being less than 3%) of gametic recombination frequencies obtained during present work are in agreement with the rare

occurrence of homoeologous recombination as compared to homologous recombination in wheat.

A precise comparisons of these observed homoeologous recombination frequencies with those of homologous recombination frequencies is not possible because of the unavailability of common markers among different maps published earlier. Nevertheless, in general the patterns of chiasmata distribution observed during present work are in agreement with the previously published results for homologous recombination in wheat and related chromosomes. Higher homologous recombination frequencies were reported between the loci *Xpsr119-Xpsr108* (Gale *et al.* 1993, map 'E', Fig. 6.3), *Xcdo545-Xcdo475* (Nelson *et al.* 1995, map 'F', Fig. 6.3), *Xpsr119-Xcdo475* (Dubcovsky *et al.* 1996, map 'G', Fig. 6.3). More or less similar patterns of recombination were observed for the homoeologous recombinants detected in present work, where highest recombination frequencies were detected between loci *Xpsr119-Xcdo475* and *Xcdo475-Xpsr108*. In both present work (based upon homoeologous recombination) and previously published reports (for homologous recombination), relatively lower recombination frequencies were observed among the loci located proximally (see maps C, D, E and G, Fig. 6.3) except with barley chromosome 1 (7H) (Heun et al. 1991), where higher recombination frequencies were reported between the loci *Xcdo475-Xpsr595* (map H, Fig. 6.3), which indicate the possible different structure of chromosome 1 (7H) of barley compared to those of homoeologous group 7 chromosomes of wheat and *Ag. intermedium*. This is further strengthened by the observation that a probe WG719, which was mapped on short arm of chromosome 1 of barley (7H) (Heun et al. 1991), was mapped on long arm of group 7 homoeologous chromosomes of wheat and chromosome 7Ai of *Ag. intermedium* during the present study (see Chapter 4 and section 6.3.1.2. of this Chapter for details).

Fig. 6.3 : Frequencies and distribution of homoeologous cross-overs along chromosome arm 7AS detected during present work and their comparison with the published genetic maps based upon homologous cross-overs.

Order of the probe loci (shown on the top of the Figure) is same as used in Table 6.1 and Figs. 6.2a & b.

c = position of centromere.

A. Homoeologous recombination (present work):

A1 = Number of homoeologous cross-overs detected in regions specified.

A2 = Conversion to % (N/390 * 100) which approximately equals 2p (see text for details)

A3 = Estimated p values from A2

B. Published genetic maps for common RFLP markers (based upon homologous recombination):

B1 = Group 7 homoeologous chromosomes of wheat (Chao *et al.* 1989).

B2 = Group 7 homoeologous chromosomes of wheat (Gale *et al.* 1993).

B3 = Group 7 homoeologous chromosomes of wheat (Nelson *et al.* 1995).

B4 = G = Chromosome 7A^m of *T. monococcum* (Dubcovsky *et al.* 1996).

B5 = Chromosome 1 (7H) of barley (Heun *et al.* 1991).

* = No recombination detected between loci *Xpsr152 - Xcdo595*

** = Probe PSR152 gave polymorphic band for chromosome 7Ai of *Ag. intermedium* only.

Note: The positions of loci shown at the top of the figure are based upon the linear order which was obtained in present work and these positions do not reflect genetic or physical distances.

Chromosome arm 7AS

Markers left to right: Xcdo 545, Xpsr 119, Xcdo 475, Xpsr 108, Xpsr 103, Xpsr 152**, Xcdo 595*, c
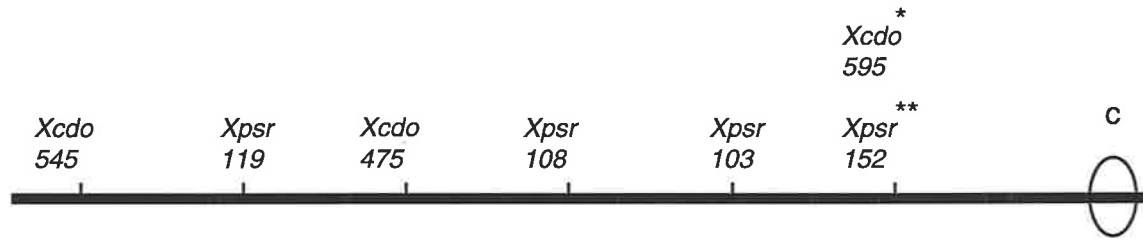
Homoeologous recombination

1	10	16	4	2	A1
0.3 %	2.6 %	4.1 %	1.0 %	0.5 %	A2
0.15 %	1.3 %	2.1 %	0.5 %	0.25 %	A3

Homologous recombination

			3 cM	8 cM	B1
	64.5 cM		3 cM	1.5 cM	B2
47 cM					B3
	36.7cM	5.2 cM			B4
19.7 cM		68.4 cM			B5

Most of the probes described above have recently been mapped physically along the length of short arms of homoeologous group 7 chromosomes of wheat (Werner et al. 1992; Chen and Gustafson 1995; Hohmann *et al.* 1994, 1995) where they are clustered in the distal halves of the chromosome arm. Hence all the chiasmata positions inferred during the present studies are physically situated in the distal halves of the chromosome arms.

The pattern of distribution of chiasmata frequency observed during present work is in agreement with observations of Lukaszweski (1995) who studied physical distribution of breakpoints in homoeologous recombinants involving chromosomes 1A, 1B, 1D of wheat, 1R or rye and the long arms of chromosome 7S of *T. speltoides* and reported that translocation breakpoints were concentrated in the distal halves of the chromosome arms and were absent in the proximal halves of the chromosome arms (also see Chapter 7 for further discussion).

6.3.2 . Genomic *in situ* hybridisation:

The C-banding technique could not be used to distinguish chromosome 7Ai (see Chapter 4), hence the alternative technique of genomic *in situ* hybridisation was applied to visualise the transferred alien segment in wheat-*Agropyron* recombinant chromosomes. Total genomic DNA from *Ag. intermedium* was used as the labelled probe along with 60-100X concentration of unlabelled DNA from wheat cv. CS (Detailed procedures are given in Chapter 3). Identification of the whole alien chromosome (7Ai) or the telocentrics of chromosome 7Ai (7AiS and/or 7AiL) in a wheat background was much easier than the identification of the *Agropyron* segment present in recombinant chromosomes. The relative concentration of labelled *Agropyron* DNA and unlabelled blocking DNA from wheat cv. CS was found to be critical for obtaining a clear signal for *Agropyron* chromatin present in recombinant chromosomes. Clear signals were obtained for whole chromosome

7Ai (photograph not shown) and/or telocentric of 7Ai (shown in Fig. 6.4a) in a wheat background using 60-70X concentration of unlabelled blocking DNA but a higher concentration of the unlabelled blocking DNA (100 X) was found necessary to obtain clear signals for the alien segment present in the recombinant chromosomes (shown in Fig. 6.4b and 6.4c). In many preparations wheat chromosomes showed cross-hybridisation with the *Agropyron* labelled DNA and to minimise this it was found necessary to employ a high stringency final wash (60 % formamide in 0.5 X SSC for 10 minutes at $60^{0}C$).

Only two of the short arm recombinant chromosomes have been characterised using the GISH technique (Fig. 6.4b and c). Both of these recombinant chromosomes, present in progeny # -2187 (F3 plant # 1052, R8) and -1608 (F3 plant # 1019, R8) had distal segments of *Agropyron* chromosome 7Ai introgressed into chromosome 7A of wheat (RFLP loci *Xcdo545*, *Xpsr119* and *Xcdo545, Xpsr119, Xcdo475* from *Agropyron* chromosome (7Ai), respectively were present or inferred to be present). The GISH results also indicated that the recombinant chromosomes were present in homozygous condition in both of these progeny (Note two fluorescing dots in interphase nuclei, Fig. 6.4b, c). This homozygosity may has arisen because both of the F3 progeny isolated (# 1052, -1019) carried the 7AiS-7AS.7AL recombinant chromosome alone (presumably as a univalent) and after selfing for two generations there are two opportunities to obtain the homozygote.

Fig. 6.4: Detection of *Agropyron* chromatin using genomic *in situ* hybridisation on mitotic metaphase chromosomes of

(a) Wheat - *Ag. intermedium* short arm addition line (TAF2d, $2n = 21_W"+tS"$).

(b) Recombinant plant # 2187 (F3 plant # 1052, R8).

(c) Recombinant plant # 1608 (F3 plant # 1019, R8).

Arrows point to *Agropyron* chromatin.
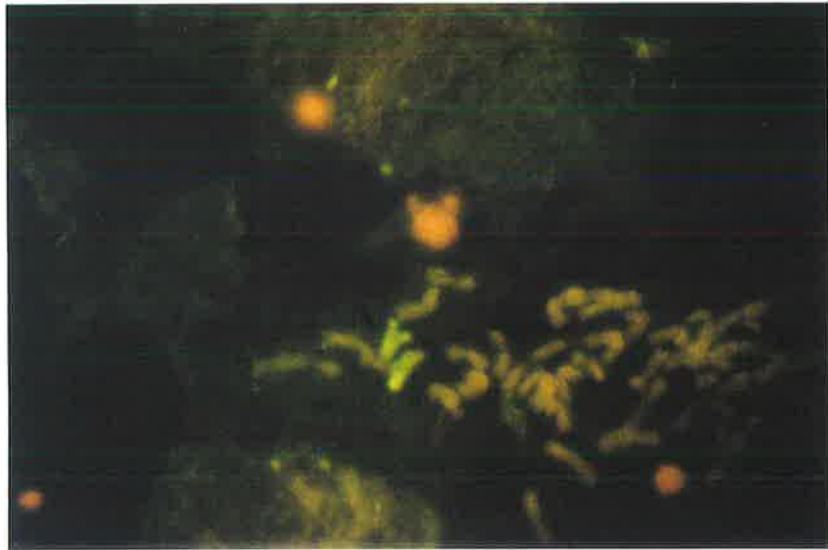
a
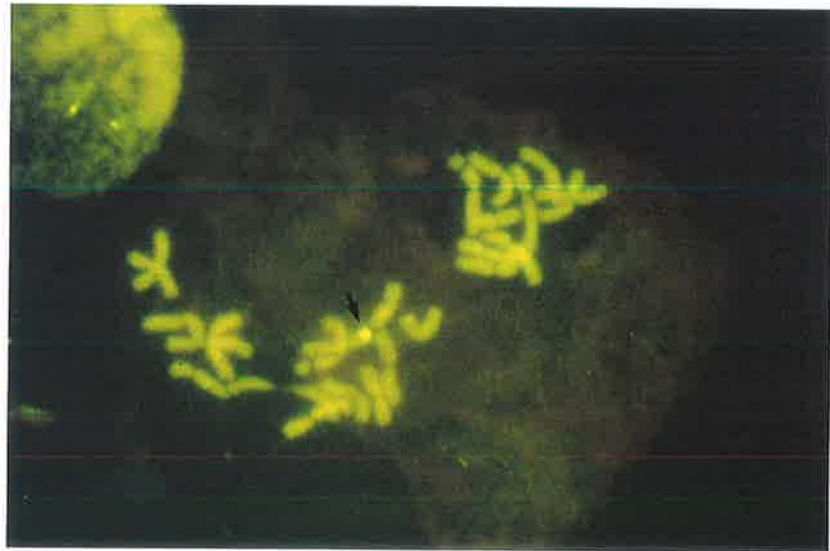
b
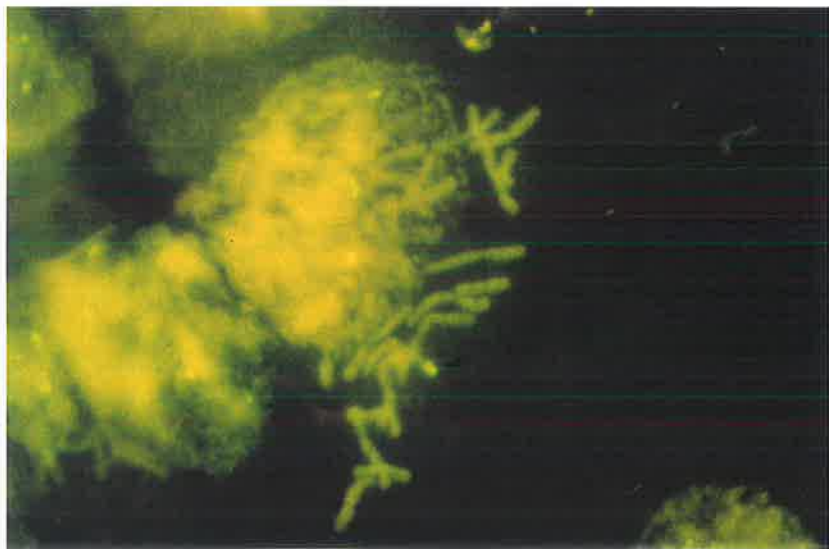
c

6.3.3. Disease reaction of the progeny carrying the putative recombinant chromosomes:

6.3.3.1. Screening of short arm recombinants for stem rust reaction:

The telocentric of chromosome 7Ai present in ditelosomic addition line TAF2d (21$_W$"+7AiS") produced by Cauderon (1966) and Cauderon *et al.* (1973) carries the stem rust resistance gene *SrAgi* (McIntosh *et al.* 1995). This line (designated as α arm addition line by Cauderon *et al.* 1973 and designated as long arm addition line by McIntosh *et al.* 1995) has been designated the short arm addition line in the present study. The controversy in designation of the long and short arms of the 7Ai chromosome has been explained previously, (see section 2.6, Chapter 2). Progeny carrying the short arm recombinant chromosomes (present alone), except derivatives of F3 progeny # 1052 because of shortage of seed, were screened with stem rust using the procedures described in Chapter 3. Where possible the progeny derived from the same plants used for RFLP characterisation were tested for rust reaction, but in some cases, because of the partial sterility of those plants, progeny from sib plants were used instead. Six to eighteen progeny from each of the recombinant and twelve plants from each of the parent lines [including euploid CS, Vilmorin 27, L1 addition line (21$_W$"+7Ai"), 7Ai (7A) substitution line and short arm addition line (TAF2d=21$_W$"+7AiS")] were inoculated with wheat stem rust inoculum (pathotype "21-2,3,7") and infection types were recorded 14 days after inoculation. The plants carrying the *SrAgi* resistance gene from *Ag. intermedium* [addition (L1), substitution (7Ai (7A) and ditelosomic addition (TAF2d) lines] developed only small restricted pustules surrounded by chlorotic tissues compared to the susceptible wheat cvs. CS and Vilmorin 27, which developed large pustules (Fig. 6.4) and the infected leaves of susceptible plants died earlier as compared to the leaves from the resistant plants. The plants carrying the recombinant chromosomes, which gave a reaction equivalent to that of wheat cvs. CS and Vilmorin 27 were classified as susceptible (S) and those with a reaction

similar to L1, 7Ai (7A) substitution and TAF2d lines were classified as resistant (R). In a few cases where clear resistant or susceptible reactions were not observed after the first inoculation, the plants were re-inoculated and infection types were recorded 14 days later. The results of all of these screening are shown in Table 6.2.

Table 6.2. Stem rust reaction and frequency of progeny carrying short arm recombinant chromosomes.

# of parent plant used to obtain the progeny for rust testing	Plant # of parent F$_3$ progeny and category of recombination	Number of progeny with rust reaction		Total number of progeny tested	Phenotype inferred for the parent plant	Chromotype of the parent recombinant as shown in Fig. 6.2 a
		R	S			
1992[*]	1045, R1	0	12	12	S	E
2147	1118, R1	0	11	11	S	E
1730	835, R2	0	6	6	S	A
2156	1169, R2	0	10	10	S	A
1973	1050, R2	10	2	12	R	B
2143[*]	1094, R4	0	18	18	S	F
1739	898, R4	11	1	12	R	-
1768	963, R4	0	6	6	S	E
2108	976, R4	0	11	11	S	E
2167	1218, R5	0	6	6	S	A
2190	886, R5	9	3	12	R	C
2199	896, R5	10	1	11	R	B
2136	1063, R7	0	7	7	S	G
1833	1106, R7	0	6	6	S	E
1836	1180, R7	13	4	17	R	D
2131	1053, R8	8	3	11	R	B
2183	1019, R8	6	0	6	R	B

S = Susceptible, R = Resistant
* = For plant # -1992 and -2143, chromotypes of sib plants # -1991 and -2142, respectively, were used.

Fig. 6.5. Stem rust reaction of reference parental genotypes.

a = Resistant reaction of 7Ai (7A) substitution line.

b = Susceptible reaction of wheat cv. CS.
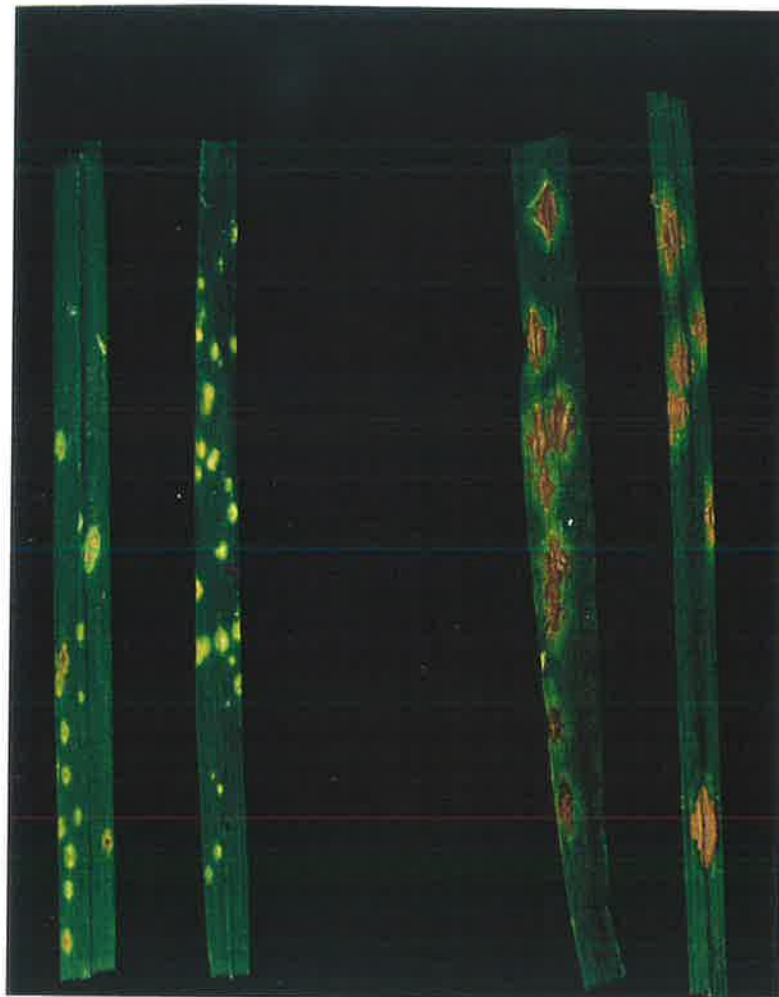
a   b

It was assumed that the few susceptible plants observed in the progeny of the resistant recombinants arose because of absence of the recombinant chromosome due to segregation. To confirm that the resistant and susceptible segregants were related to the presence or absence of the recombinant chromosome in the progeny, respectively, a sample of eight progeny (four resistant and four susceptible) was analysed using RFLPs. Extracted DNA from these 8 test entries was digested with *EcoR V* and hybridised with the probes CDO -545 and -475. The results of these screening are given in Table 6.3. Comparison of the probes CDO -545 and -475 readings for these entries with those obtained earlier for the parent plants, indicate clearly that S plants are lacking the critical *Xcdo475-7Ai* segment. The recombinant chromosome is either absent (test plant # 3, parent plant # 1992) or if present (test plant # 4, 1, 6, parent plant # 1992, 2143, 2143, respectively) it is positive (+) for *Xcdo475-7A* locus i.e. inferred minus (-) for *Xcdo475-7Ai* locus. Alternatively R plants are all carrying the recombinant chromosome and -ve for *Xcdo475-7A* locus i.e. inferred positive for *Xcdo475-7Ai* locus. As expected, a susceptible plant in the progeny of resistant recombinant # 1973 did not carry a recombinant chromosome, while a resistant plant from the same family had the recombinant chromosome.

Table 6.3. RFLP phenotypes of selected rust resistant and susceptible progeny plants

Plant # and rust reaction of the R and S progeny used for RFLP analysis	# and chromotype of parent plant carrying the recombinant chromosome	Phenotype (RFLP loci)									
		of the R & S progeny		of the parent plant (reproduced from Table 6.1)*							
		Xcdo 545	Xcdo 475	Xcdo 545	Xpsr 119	Xcdo 475	Xpsr 108	Xpsr 103	Xpsr 152	Xcdo 595	
Plant # 4, S	1992 (E)**	-		-				+	+	+	7Ai
		+	+	+	+	+	-	-		-	7A
Plant # 3, S	1973 (B)	-		+				-	-	-	7Ai
		-	-	-	-	-	+	+		+	7A
Plant # 8, R	1973 (B)	+		+				-	-	-	7Ai
		-	-	-	-	-	+	+		+	7A
Plant # 1, S	2143 (F)**	-		-				+	+	+	7Ai
		+	+	+	+	+	+	-		-	7A
Plant # 6, S	2143 (F)**	-		-				+	+	+	7Ai
		+	+	+	+	+	+	-		-	7A
Plant #2, R	2199 (B)	+		+				-	-	-	7Ai
		-	-	-	-	-	+	+		+	7A
Plant # 2, R	1836 (D)	-		-				+	+	+	7Ai
		+	-	+	+	-	-	-		-	
Plant # 4, R	1836 (D)	-		-				+	+	+	7Ai
		+	-	+	+	-	-	-		-	

S = Susceptible, R = resistant, + = RFLP locus present, - = RFLP locus absent
* = For the convenience of comparison, data of the parent recombinant (for the short arms only) have been reproduced from Table 6.1.
** = For plant # -1992 and -2143, chromotypes of sib plants # -1991 and -2142, respectively, were used.

The results of rust testing and structure of the recombinant chromosomes indicated that resistant types (chromotypes B, C and D, Fig. 6.2a) all had *Agropyron* chromatin in the region *Xcdo475* and susceptible types (chromotypes A, E, F, G, Fig. 6.2a) lacked this region. The critical comparisons are between chromotypes A and B, which both carry distal *Agropyron* chromatin but the plants with chromotype A are susceptible and plants with chromotype B are resistant. Unfortunately probes in these regions were not polymorphic for chromosome 7Ai so the boundary of the segment carrying the *SrAgi* gene could not be clearly defined. However, we may infer from the 7A markers (assuming collinearity of markers between 7A and 7Ai in this region) that the gene is located on an *Agropyron* segment bounded distally by *Xpsr119* and proximally by *Xpsr108*. Because of the importance of this segment of *Agropyron* chromosome for locating the *SrAgi* gene, several other restriction enzymes (*EcoR I*, *Hind III*, *Dra I*) were used in an attempt to find polymorphism for the *Xcdo545-7Ai* locus but no reliably reproducible polymorphism was obtained.

The occurrence of two overlapping types of resistant recombinants (present in chromotypes B and D, Fig. 6.2 a) could be used to obtain a secondary recombinant with an intercalary segment of chromosome 7Ai (containing the rust resistance gene) translocated onto chromosome 7A of common wheat by homologous recombination, as originally proposed by Sears (1981). These two recombinants have been inter-crossed and the progeny will be screened to obtain homologous cross-over products.

6.3.3..2. Screening of long arm recombinants for BYDV reaction:

All of the progeny having 7AiL dissociated markers were screened with barley yellow dwarf virus (serotype: BYDV-PAV$_{Adel}$). Eighteen progeny plants from each of the putative long arm recombinants and twelve plants from each of the parent (including wheat cvs. CS, Vilmorin 27, L1 addition, 7Ai(7A) substitution and F-17 (long arm addition, =21$_W$"+7AiL") lines were inoculated with infected aphids and these plants are referred to as test entries (Detailed procedures have been described in Chapter 3). In addition, six plants from each of the parent lines were planted under the same conditions and were used as uninoculated controls. The symptoms of infection (stunted growth, and yellowing of leaves) started to appear 4-6 weeks after inoculation in the test entries, while uninoculated control plants showed normal growth. Six and two randomly selected plants from the test entries and uninoculated control populations, respectively, were subjected to Northern blot hybridisations (Collins 1996) to determine if the test plants were infected with BYDV. This test indicated the presence of virus in the test entries, while the uninoculated control plants did not show the presence of virus. Unfortunately no clear differences in symptom expression could be observed between the supposedly resistant and susceptible parental lines tested. The L1 addition, 7Ai(7A) substitution and the long arm addition (F-17) lines showed more or less similar symptoms to those of euploid wheat cultivars CS and Vilmorin 27, indicating that the BYDV resistant gene reported to be present on the long arm of chromosome 7Ai (Brettell *et al.* 1988; Banks *et al.* 1995) is not effective under glass house conditions at least against the BYDV isolate used during the present studies (BYDV-PAV$_{Adel}$.). All the progeny carrying the long arm recombinant chromosomes (irrespective of the marker constitution of the recombinant chromosomes) showed more or less similar symptoms as those showed by the parental lines. This experiment was repeated under similar glass house conditions to confirm the results. In the repeat experiment, 6 plants from each of the parent and 3 plants from each of the recombinant lines were tested using

the same procedures. Similar results were obtained for the repeat experiment and no clear differences in the symptom expression were observed between euploid wheat cultivars (CS and Vilmorin 27) and wheat-*Agropyron* addition / substitution / recombinant lines. Because of the absence of clear cut difference between suspected resistant and susceptible parental plants these tests were not continued.

6.4. Discussion:

The conventional pairing studies of chromosomes at metaphase I used earlier for the characterisation of wheat-alien recombinant chromosomes by Sears (1972, 1981) are not only time consuming and require a lot of crossing with tester stocks but also cannot accurately define the breakpoints mainly because it is not possible to measure the frequency of pairing precisely because of desynapsis of chromosomes at metaphase I (Fu and Sears 1973).

The alternative approach of using chromosome banding techniques (Friebe *et al.* 1992) to locate the breakpoints and to identify the wheat chromosome involved in the translocations are also unsuccessful when the alien segment involved in the translocation is devoid of heterochromatic bands, like chromosome 7Ai of *Ag. intermedium*, which is mainly composed of euchromatin.

The recent developments of DNA technology (including RFLPs and *in situ* hybridisation) have made valuable contributions towards the characterisation of wheat-alien recombinant chromosomes. During the present studies, more accurate definition of cross-over points for the recombinant chromosomes resulting from homoeologous pairing and recombination were identified using DNA probes. The results of these RFLP analyses combined with those of rust testing also allowed the stem rust resistance gene (*SrAgi*) to be located on the

distal segment of short arm of chromosome 7Ai of *Ag. intermedium* . The distal location of the rust resistance locus was confirmed by the results of *in situ* hybridisation (shown in Fig. 6.4) where with progeny # 1608, the fluoro labelled *Agropyron* DNA hybridised just to the distal part of the recombinant chromosome and progeny from a sib plant of the same family (progeny from # 2183) were found to be resistant to the stem rust pathotype '21-2,3,7'.

The cross hybridisation of the labelled *Agropyron* DNA with wheat chromosomes observed during the present *in situ* hybridisation studies was in agreement with a recently published report of Hohmann *et al.* (1996) who reported that the labelled *Ag. intermedium* DNA weakly hybridised to a set of small wheat chromosomes which were most likely D genome chromosomes (Hohmann *et al.* 1996). The use of *Agropyron* amplified sequences (Hohmann and Appels, unpublished, cited in Hohmann *et al.* loc cit) has also revealed that the *Agropyron* genome shares more homology with the D genome than the A and B genomes. In addition, it has been shown that the transfer of *Agropyron* chromatin conferring resistance to WSMV (Friebe *et al.* 1991), BYDV (Banks *et al.* 1995) and stem rust (Kim *et al.* 1993) present in compensating substitution / translocation lines, often involve chromosomes of *Agropyron* and the D genome of wheat.

Recombinant plants resistant to stem rust and with overlapping segments of alien chromatin in the recombinant chromosome, were detected in the present study (chromotypes B, C and D, Fig. 6.2. a). These overlapping recombinant chromosomes carrying stem rust resistant gene *SrAgi* on *Agropyron* segments of varying length could be used to develop a secondary recombinant type having an interstitial alien segment as proposed by Sears (1981) and shown in Fig. 6.6. The recombinant chromosomes present in chromotype B (Fig. 6.2. a) are expected to be more useful than those in chromotype C for this type of chromosome engineering because they have a shorter distal alien segment.

Fig. 6.6 Scheme for producing a secondary recombinant with an interstitial segment of *Agropyron* carrying stem rust resistance, from intercrossing two primary recombinant chromosomes.

The linear order of the probe loci is given on the top. C=position of centromere

1 = Resistant homoeologous cross-over product (chromotype B, reproduced from Fig. 6.2. a)

2 = Resistant homoeologous cross-over product (chromotype D, reproduced from Fig. 6.2. a)

3 = Wheat chromosome having interstitial segment of *Agropyron* carrying the stem rust resistant gene *SrAgi* from chromosome 7Ai.

Short arm   Long arm

| | | W | W | W | | | A | | C | | | | | | A | | A | A |

Xcdo 545   *Xpsr* 119   *Xcdo* 475   *Xpsr* 108   *Xpsr* 103   *Xpsr* 152   *Xcdo* 595   C   *Xwg* 719   *Xcdo* 673   *Xpsr* 117   *Xwg* 686   *Xpsr* 129   *Xcdo* 347   *Xpsr* 121   *Xwg* 420

SrAgi
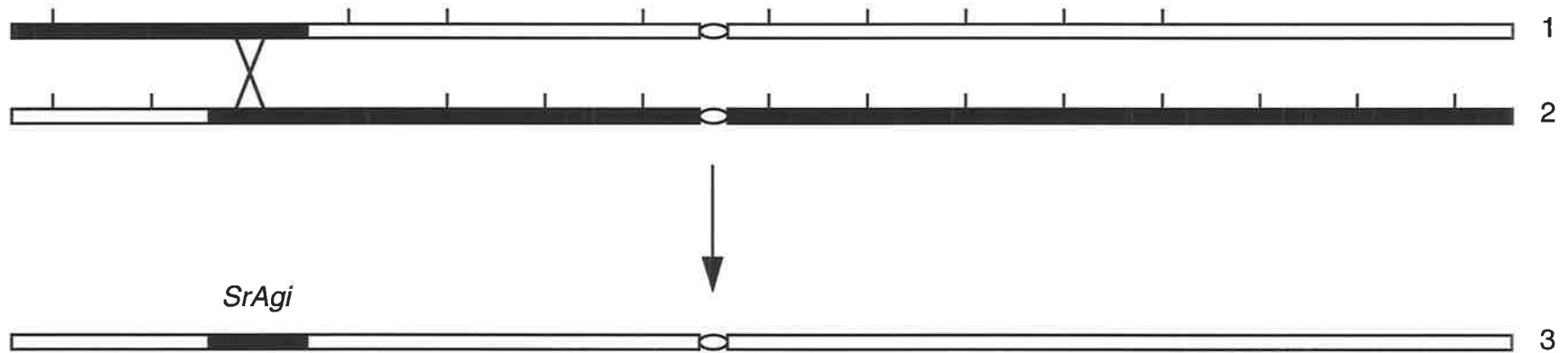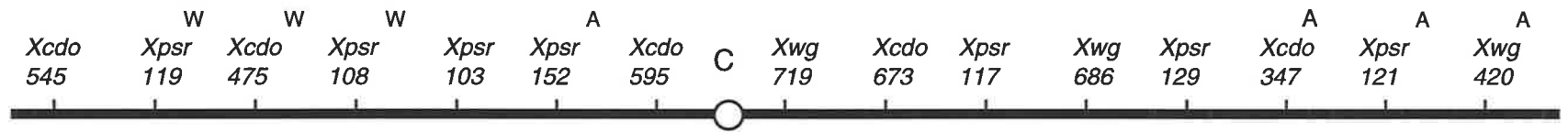
■ = 7Ai chromatin   □ = 7A chromatin

Introgression of this and other rust resistance genes from wild relatives will not only provide additional sources of resistance for gene pyramiding in wheat but will also offer better chances of tagging the alien segment with a molecular marker because of the increased level of polymorphism introduced over that present in wheat (Chao *et al.* 1989). In addition, molecular tagging of these alien genes might also help in locating other genes on homoeologous chromosomes over a wide range of wheat relatives. The presence of restriction fragments specific for the alien segment will allow DNA markers to be used to identify the presence of the new resistance genes in the background of wheat breeding populations without having to carry out an actual rust test.

BYDV is probably the most destructive viral disease of wheat and other small grains cereals. All wheat cultivars are vulnerable to BYDV infection and crop loss and only one gene conferring tolerance (reduced infection) has been reported in wheat so far (Singh *et al.* 1993). Unfortunately the supposed BYDV resistance gene on chromosome 7Ai (Brettell *et al.* 1988; Banks *et al.* 1995) does not provide good resistance to the strain of the virus used in the present study.

CHAPTER - 7
GENERAL DISCUSSION

The results obtained in this research project demonstrate the value of manipulating the *Ph1* gene of wheat [using Sears' (1977) *ph1b* mutant] for the induction of genetic recombination between wheat and related alien chromosomes and also the value of co-dominant DNA markers in detection, isolation and characterisation of such recombinant chromosomes.

During the present studies, which were undertaken to gain a better understanding of patterns of homoeologous recombination between wheat and alien chromosomes, chromosome 7Ai of *Ag. intermedium* was chosen as the model alien chromosome because of the reported presence of agronomically important genes [conferring resistances to wheat stem rust (Cauderon *et al.* 1973) and barley yellow dwarf virus (Brettell *et al.* 1988)] on this chromosome.

It is known that in the presence of the diploidisation system controlling chromosome pairing [mainly because of presence of the *Ph1* gene (Okamoto 1957; Riley and Chapman 1958)], wheat chromosomes do not, or very rarely, recombine with homoeologous alien chromosomes. Although the use of ionising radiations has resulted in a few wheat-alien recombinant chromosomes carrying agronomically useful alien genes (e.g. Sears 1956), such translocations are generally not considered suitable for practical breeding because of (i) their rare occurrence and (ii) the non-compensating nature of the translocations induced. For example, Sharma and Knott (1966) using the 7Ag chromosome from *Ag. elongatum*, analysed 357 radiation treated rust resistant offspring and found only one 7Ag-7D recombinant chromosome which had normal transmission through the male gamete. It is unlikely that radiation-induced breakage would occur at precisely equivalent points in the two homoeologous chromosomes, hence it is unlikely that the transferred

alien segment will be fully equivalent to the wheat segment replaced, which will lead to deletion or duplication of genetic material.

More recently cell-culture methods have been used to induce recombination between wheat and alien chromosomes. For example, Banks *et al.* (1995) utilised the disomic addition line L1 carrying chromosome 7Ai of *Ag. intermedium* (produced by Cauderon 1966) in cell culture and were able to isolate fourteen putative wheat-*Agropyron* recombinant lines carrying resistance to barley yellow dwarf virus. By studying chromosome pairing and Southern hybridisations using the dispersed repetitive probe pEleAcc2, isolated from *Ag. elongatum* (U. Hohmann and R. Appels unpublished, cited in Banks *et al.* 1995), they concluded that the recombinant lines carried less than the complete long arm of chromosome 7Ai. Later, in more comprehensive studies using C-banding, RFLP, deletion mapping and GISH techniques, Hohmann *et al.* (1996) showed that the amount of alien chromatin introgressed into these BYDV-resistant recombinant lines had been underestimated previously and many of these lines carried more than a complete arm of chromosome 7Ai. In addition, some non-reciprocal translocations for the most proximal region of the chromosome arm 7DL were also identified, which resulted in duplication. Some non-homoeologous exchanges (1BS-7AiS.7AiL) were also identified in these lines, indicating that the cell-culture derived translocations might not have much use in practical breeding.

In contrast to the problems posed by ionising radiations and cell-culture derived translocations, the recombinant chromosomes obtained during the present work utilising Sears *ph1b* mutant are compensating type of homoeologous cross-over products and therefore are likely to be free of deleterious chromosomal abnormalities. Only 1 of the 63 recombinants detected during the present study, showed a possible duplication for the *Xpsr103* locus on the short arm (details described in Chapter 6). One possible interstitial deletion on the long arm (described in section 5.3.7, Chapter 5) was also detected during

This work which has been discussed in a later section of this Chapter. Also the recombinant chromosomes reported in this thesis generally have smaller alien segments introgressed into wheat chromosomes than obtained with the cell-culture derived recombinants. Therefore, it is conclude that manipulation of the *Ph1* system is a more efficient and practicable method for the introgression of useful alien genes from related species into wheat chromosomes than either ionising radiations or tissue culture methods.

Following the induction of allosyndesis between wheat and homoeologous alien chromosomes, the most critical step is to detect and isolate the recombinant chromosomes. The efficiency with which wheat-alien recombinant chromosomes can be detected, relies upon the type, number and spread of genetic markers available for the chromosome segments to be recombined. These markers should ideally be co-dominant in nature so that heterozygotes can be recognised and should have no pleiotropic effect on traits of agronomic importance. In previous studies for the isolation of wheat-alien recombinant chromosomes, cytological, morphogenetic and/or biochemical markers were used (details have been described earlier). Chromosome pairing behaviour although used successfully by Riley *et al.* (1968a, b) and Sears (1972, 1973, 1981) to detect the wheat-alien recombinant chromosomes, has a serious limitation. Chromosome pairing observed at metaphase I is only a fraction of synapsis which takes place at prophase I (Fu and Sears 1973). If desynapsis of the homoeologous bivalents occur, then these paired chromosomes will appear as univalents at metaphase I. So, at least some univalents observed at metaphase would have been synapsed at pachytene / zygotene stages. Hence, an accurate estimate of the frequency and position of wheat-alien chromosome cross-over breakpoints cannot be obtained using these chromosome pairing analyses.

When using biochemical markers, the wheat genomes remain rather poorly characterised for such markers (Koebner and Shepherd 1985). The paucity of these biochemical markers has restricted their use in the isolation of wheat-alien recombinant chromosomes.

For example, Koebner and Shepherd (1986) and Islam and Shepherd (1992) could only detect less than 2% recombination between wheat-rye and wheat-barley chromosomes, using seed storage proteins and isozyme loci as genetic markers, respectively. The high frequency of wheat-alien chromosome recombination detected in the present work (16%) may be due mainly to the close genetic similarity of wheat and *Agropyron* chromosomes compared to those of wheat and rye or wheat and barley. However, recombination studies using *T. umbellulatum* (which is genetically more closely related to wheat than is *Ag. intermedium*) as the alien source, yielded less than 8 % allosyndetic recombinants involving both arms when seed storage protein loci were used as the genetic markers (Koebner and Shepherd 1987). It is likely that the rate of allosyndetic recombinations reported in these previous studies reflects the narrow spread of the selective biochemical markers than achievable using the more numerous and widely dispersed DNA markers.

The present utilisation of variation in the DNA sequences detected as restriction fragment length polymorphisms used for screening of F3 progeny to detect wheat-alien chromosome cross-over products not only gave a high frequency of allosyndetic recombinants (16%), but also could identify reciprocal recombinant products directly in some F3 progeny (recombinants described in categories R7, R8, R17, R18, R19, Table 5.4, Chapter 5). In addition, because of their dispersed nature, these co-dominant DNA marker loci also allow the alien segment introgressed to be more closely monitored. As both the group 7 wheat and *Ag. intermedium* chromosomes were almost equally characterised using RFLPs, only those recombinants would escape detection where two reciprocal recombinant gametes have united to produce a phenotype similar to that of parental phenotypes (as discussed in section 5.2.3). The higher cost per assay involved with RFLP markers compared to biochemical markers is a limiting factor at present, but with the recent progress in conversion of RFLP probes into PCR primers (e.g. Talbert *et al.* 1994) this problem may be overcome in the near future. In the present study the cost of the assay was reduced by selecting probes that showed polymorphism with the same

restriction enzyme (*EcoR V*) and then the same membrane could be used for all six probes used for screening the F3 progeny. This procedure also reduced the chances of sampling / loading errors which can occur when new sampling has to done for every assay.

A practical problem common in all schemes aimed at introgression of alien genetic material into wheat is the isolation of the recombinant chromosome alone i.e. isolation of a progeny plant where recombinant chromosome is present without one of the targeted parental chromosome(s) involved in the transfer. As discussed in section 5.2.3 and shown in the results described in Table 5.4, in most cases (49 out of 63 total recombinants isolated during present work), recombinant chromosomes were present along with an intact parental chromosome [expected frequency of obtaining such progeny is 2p(1-p), (which approximates 2p if p is small). In such cases the recombinant chromosomes have to be isolated through progeny tests (as described in Chapter 5) and after their isolation the recombinant chromosomes can be characterised. But self-fertilisation of the plants carrying the recombinant chromosome, while remaining *Ph1* deficient, results in unbalanced genotypes because of high level of non-homoeologous pairing of wheat chromosomes. Furthermore the occurrence of new episodes of homoeologous cross-overs would add to the complexity of characterising the recombinant chromosomes. It is therefore desirable to cross the plants having recombinant products with euploid wheat or genetic stocks of wheat which are nullisomic for the critical wheat chromosome involved in allosyndesis, in the generation they are first isolated.

An alternative practice to improve the protocol would be to pollinate the critical F2 plants having single doses of the alien chromosome and a wheat homoeologous chromosome (targeted chromosomes) and being *Ph1* deficient ($40_W+1_A'+1_W'$, *ph1bph1b* or $38_W+1_W'+1_A'+5B'$, *ph1b*) with either euploid wheat or preferably the wheat stock

nullisomic for the targeted wheat chromosome and tetrasomic for a homoeologous wheat chromosome. The resulting progeny could then be screened to select recombinant chromosomes. This strategy would be most useful where a high level of allosyndetic recombination is expected. For example, it could be efficient with chromosome 7Ai of *Ag. intermedium* used during present work, which yielded an overall rate of 16 % allosyndetic recombination. If 20-30 spikes of the critical F2 plants had been pollinated with NT 7A-7B or NT 7A-7D stocks of CS (to give approximate 400-500 seeds), recombinant 7A-7Ai chromosomes could have been isolated directly during the first screening without having to do progeny tests. Thus a few days extra work involving these crosses could save at least 2 growing seasons and also the large number of progeny tests carried out (a total of 403 F4/F5 or backcross progeny were analysed in the presnt study, Chapter 5, Appendix) to isolate progeny plants having the same recombinant chromosome isolated away from the parental chromosome(s) involved in allosyndesis. Furthermore this procedure would re-introduce the *Ph1* gene immediately and prevent any further homoeologous recombination in the next generation.

A further question arising from the study of wheat-alien homoeologous chromosome recombination is the distribution and number of chiasmata which ultimately give rise to the recombinant chromosomes. The comparison of frequencies and distribution of the homoeologous cross-overs along chromosome arm 7AS (detected during present work) with those of homologous cross-overs between the same RFLP loci (published earlier) indicated similar patterns of chiasmata distribution in both homologous and homoeologous recombination (see section 6.3.1.4., Chapter 6 for details). Based on the linear order of the probe loci obtained for chromosomes 7A and 7Ai (see Chapters 5 and 6 for details) and the consensus physical maps published earlier for homoeologous group 7 chromosomes of Triticeae (for details see Chapters 4 and 6), the cross-over breakpoints inferred for the recombinant chromosomes isolated during present work were mostly concentrated around the RFLP loci located in the intercalary regions of the chromosome

arms and not or very few recombination were detected among the most proximal and most distal loci (details have been described in Chapter 6). These results are in agreement with the previous reports where recombination in wheat chromosomes was found to be concentrated in the distal regions of the chromosomes (Dvorak and Chen 1984; Kleinhofs et al. 1988; Flavell et al. 1987; Curtis et al. 1991; Lukaszweski (1995). Lukaszweski and Curtis (1993) speculated that the distal concentration of recombination in wheat and possibly in other Triticeae species was a consequence of telomeric initiation of chromosome pairing which created favourable conditions for the establishment of distal chiasmata. Strong positive chiasma interference would then reduce the probability of crossing over in the proximal region. Kim et al. (1993) suggested that either recombination in the proximal half of the chromosome is suppressed or that suitable markers for this region are rare and, therefore, have not been found.

Like the regions close to the centromere, the telomeric regions of Triticeae chromosomes also contain few RFLP detection sites (e.g. see Chen and Gustafson 1995). This lack of RFLP detection sites in the telomeric region of Triticeae chromosomes could result from some degree of similarity in structure with the centromeric regions. Similar structures were reported for *Drosophila melanogaster* , and some vertebrates including mouse (Lefever 1971; Meyne et al. 1990; Kipling et al. 1991), where similar nucleotide sequences [e.g. $(TTAGGG)_n$] were found in common in the telomeric and centromeric regions of the chromosomes. Irrespective of the reason, it is clear that areas of high and low levels of recombination exist in wheat as in other animal and plant species. It is clear that more-recombination studies are necessary to more accurately define these regions in wheat chromosomes.

Based on the increasing evidence in favour of distal localisation of chiasmata and suppression of recombination in the proximal regions of Triticeae chromosomes (as discussed in the preceding paragraphs), it is more likely that the phenotype observed for

F3 progeny plant # 1079 (described in section 5.3.7, Chapter 5) arose from a deletion in the proximal region of the long arm of chromosome 7Ai rather than a double cross-over event involving chromosome 7Ai and another wheat chromosome (for details refer to section 5.3.7, Chapter 5). This supposed deletion involving 2 long arm markers (*Xwg719* and *Xcdo673* loci) might possibly have arisen from a paracentric inversion or unequal crossing over. It could not be a pericentric inversion because all evidence from telosomic analyses indicated that all RFLP loci were on corresponding arms of the group 7 homoeologous chromosomes of wheat and *Ag. intermedium*. However, more detailed data is required to explain the observed phenotype in plant # 1079

The wheat-*Agropyron* recombinant lines produced during the present study have provided a large number of genotypes with potentially different breakpoints along the wheat homoeologous group 7 chromosomes. These lines are genetically equivalent to deletion lines and therefore could be used to establish the linear order of markers along the length of homoeologous group 7 wheat chromosomes, which is considered to be more important than knowledge of accurate linkage distances between the markers (Chen *et al.* 1994b). An advantage of using these wheat-alien recombinant lines over the analysis of wheat X wheat mapping populations is that a greater level of polymorphism for RFLPs is expected between wheat and alien species compared to that within wheat line(s). Also studying the frequency and distribution of chiasmata resulting from wheat-alien chromosome pairing could contribute fundamental knowledge regarding the genome structure and evolution of the two species and as a whole for Triticeae. Also in practical terms, such recombinant lines will lead to the incorporation of useful genes from wild relatives into common wheat and thereby increase the gene pool available to wheat breeders.

The value of marker assisted selection in breeding programs has ben established. Testing for disease resistance is often laborious and time consuming. Additionally, once a line is

selected with one gene conferring resistance, it is difficult to incorporate other resistant genes without some method to independently test for each gene. If a molecular marker locus completely linked to the gene of interest can be found, it could be used in breeding programs to select for the resistance gene without conducting an actual disease screening test. Obviously more RFLP markers are required giving specific fragments with the chromosome 7Ai segment and close to the *SrAgi* gene which has been introgressed in chromosome 7A of wheat present in plants carrying the recombinant chromosomes with chromotypes B, C, D (Fig. 6.2^α, Chapter 6).

The isolation of rust resistant lines in the present work with distal and proximal *Agropyron* segments including an overlapping segment carrying the stem rust resistant gene, makes it possible to reduce the amount of alien chromatin by a homologous cross-over in the overlapping regions as suggested by Sears (1981) and already discussed in Chapter 6. These resistant recombinants with an overlapping alien segment have been inter-crossed and following homologous crossing over between the common *Agropyron* segment it is hoped to obtain a derived translocation line that carries the resistance gene on an interstitial segment in the F_2 generation.

Additional markers are required to isolate and characterise the recombinant chromosomes which have so far not been isolated alone (details described in section 5.3.6) and which might have involved a non-targeted wheat chromosome (likely to be chromosome 7B and/or 7D). Also characterisation of all the recombinant chromosomes using genomic *in-situ* hybridisations will be useful for a better understanding the physical nature of the allosyndetic recombination products. The failure to obtain a clear difference in expression between supposedly BYDV resistant and susceptible parent lines when tested with BYDV indicated the need to search for genes with better resistance against BYDV in the wild relatives of wheat, since only one gene (*Bydv1*) conferring tolerance against BYDV has been reported in wheat (Singh *et al.* 1993).

The wild relatives of wheat offer a large number of useful genes of agronomic importance including those offering resistances to diseases / insects, high yield potential and better quality. Utilisation of these alien genes can also compensate for the lost genetic variability of wheat, resulting from the prolonged deliberate selection for homozygous high yielding pure lines as opposed to the low yielding but genetically more variable landraces. The procedures described in this thesis can be used more efficiently to incorporate useful alien genes from related species into wheat. But the immediate use of the approaches described herein might be in reducing the amount of alien chromatin in order to remove the linked deleterious genes present in many wheat-alien recombinant chromosomes already isolated.

Appendix

Phenotypes of the progeny of self-fertile non-parental (putative recombinant) plants.

Category of recombinant and plant # of F$_3$ progeny. Identification # of F$_2$ family is given in parentheses	RFLP loci						Chromosome	Plant # of progeny
	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121		
R1	-	+	+	+	+	+	**7Ai**	
876 (447)	+	+	+	+	+		**7A**	
Progeny of 876								
	-	+	+	+	+	+	7Ai	1418, 1419
	+	+	+	+	+		7A	1421
	-	-	-	-	-	-	7Ai	1420
	+	+	+	+	+		7A	
Progeny of 1418								
	-	-	-	-	-	-	7Ai	2174
	+	+	+	+	+		7A	
	-	+	+	+	+	+	7Ai	2175
	+	+	+	+	+		7A	
	-	-	+	+	+	+	7Ai	2176*
	+	+	+	+	+		7A	
R1	-	+	+	+	+	+	**7Ai**	
1045 (447)	+	+	+	+	+		**7A**	
Progeny of 1045								
	-	+	+	+	+	+	7Ai	1627, 1628
	+	+	+	+	+		7A	1630
	-	-	-	-	-	-	7Ai	1629
	+	+	+	+	+		7A	
1045 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1631, 1632
	+	+	+	+	+		7A	1634, 1990 1993
	-	+	+	+	+	+	7Ai	1633, 1991
	+	-	-	-	-		7A	1992
R1	-	+	+	+	+	+	**7Ai**	
1118 (447)	+	+	+	+	+		**7A**	
Progeny of 1118								
	-	-	-	-	-	-	7Ai	2146
	-	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	2147, 2149
	+	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	2148
	+	+	+	+	+		7A	

Note: The putative recombinants appear in the same sequence as in Tables 5.4 (That is from **R1** to **R24**).

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R1	-	+	+	+	+	+	7Ai	
1147 (394)	+	+	+	+	+		7A	
Progeny of 1147								
	-	-	-	-	-	-	7Ai	2150
	+	+	+	+	+		7A	
	-	+	+	+	+	+	7Ai	2152, 2153
	+	+	+	+	+		7A	
R1	-	+	+	+	+	+	7Ai	Sterile
1034 (430)	+	+	+	+	+		7A	
R1	-	+	+	+	+	+	7Ai	Sterile
1307 (*Ph1/-*)	+	+	+	+	+		7A	
R2	+	-	-	-	-	-	7Ai	
833 (351)	+	+	+	+	+		7A	
Progeny of 833								
	-	-	-	-	-	-	7Ai	1494, 1496
	+	+	+	+	+		7A	1497, 2210
								2212, 2213
	+	-	-	-	-	-	7Ai	1495, 2211
	+	+	+	+	+		7A	
Progeny of 1495								
	+	-	-	-	-	-	7Ai	2207, 2208
	+	+	+	+	+		7A	2209
R2	+	-	-	-	-	-	7Ai	
835 (394)	+	+	+	+	+		7A	
Progeny of 835								
	+	-	-	-	-	-	7Ai	1410, 1411
	-	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	1412, 1413
	+	+	+	+	+		7A	
Progeny of 1411								
	+	-	-	-	-	-	7Ai	1723, 1724
	-	+	+	+	+		7A	1725, 1726
1411 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1727, 1728
	-	-	-	-	-		7A	1948, 1949
	+	-	-	-	-	-	7Ai	1729, 1730
	-	+	+	+	+		7A	1946, 1947
								1951, 1952

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R2	+	-	-	-	-	-	**7Ai**	
1169 (394)	+	+	+	+	+		**7A**	
Progeny of 1169								
	-	-	-	-	-	-	7Ai	2154
	+	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	2155, 2157
	+	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	2156
	-	+	+	+	+		7A	
R2	+	-	-	-	-	-	**7Ai**	
1182 (394)	+	+	+	+	+		**7A**	
progeny of 1182								
	+	-	-	-	-	-	7Ai	2158, 2160
	+	+	+	+	+		7A	2161
	-	-	-	-	-	-	7Ai	2159
	+	+	+	+	+		7A	
R2	+	-	-	-	-	-	**7Ai**	
1050 (447)	+	+	+	+	+		**7A**	
Progeny of 1050								
	-	-	-	-	-	-	7Ai	1635, 1638
	+	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	1636, 1637
	+	+	+	+	+		7A	
1050 X NT 7A-7B								
	+	-	-	-	-	-	7Ai	1639, 1640
	-	+	+	+	+		7A	1641, 1973 1974
	-	-	-	-	-	-	7Ai	1642, 1971
	+	+	+	+	+		7A	1972
R2	+	-	-	-	-	-	**7Ai**	**Sterile**
1089 (447)	+	+	+	+	+		**7A**	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R3	+	-	-	-	-	-	**7Ai**	
879 (447)	-	-	-	-	-		**7A**	
Progeny of 879								
	+	-	-	-	-	-	7Ai	1422, 1423
	-	-	-	-	-		7A	1424, 1425
Progeny of 1422								
	+	-	-	-	-	-	7Ai	1731, 1732
	-	-	-	-	-		7A	1733
1422 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1734, 1735
	-	-	-	-	-		7A	1737, 1941 1943
	+	-	-	-	-	-	7Ai	1736, 1738
	-	-	-	-	-		7A	1942
R4	+	+	+	+	+	+	**7Ai**	
803 (347)	+	-	-	-	-		**7A**	
Progeny of 803								
	+	+	+	+	+	+	7Ai	1482, 1483
	+	-	-	-	-		7A	1484, 1485
Progeny of 1482								
	+	+	+	+	+	+	7Ai	2201, 2202
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2203
	-	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
953 (347)	+	-	-	-	-		**7A**	
Progeny of 953								
	+	+	+	+	+	+	7Ai	1486, 1487
	+	-	-	-	-		7A	1488,
	+	+	+	+	+	+	7Ai	1489
	-	-	-	-	-		7A	
Progeny of 1486								
	+	+	+	+	+	+	7Ai	2204, 2205
	+	-	-	-	-		7A	2206
R4	+	+	+	+	+	+	**7Ai**	
1047 (447)	+	-	-	-	-		**7A**	
Progeny of 1047								
	+	+	+	+	+	+	7Ai	2122
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2123, 2125
	-	-	-	-	-		7A	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R4	+	+	+	+	+	+	**7Ai**	
1048 (447)	+	-	-	-	-		**7A**	
Progeny of 1048								
	+	+	+	+	+	+	7Ai	2126, 2127
	+	-	-	-	-		7A	2129
	+	+	+	+	+	+	7Ai	2128
	-	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
1094 (447)	+	-	-	-	-		**7A**	
Progeny of 1094								
	-	+	+	+	+	+	7Ai	2142
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2143, 2144
	-	-	-	-	-		7A	2145
R4	+	+	+	+	+	+	**7Ai**	
1198 (394)	+	-	-	-	-		**7A**	
Progeny of 1198								
	+	+	+	+	+	+	7Ai	2162, 2165
	-	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2163, 2164
	+	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
1251 (394)	+	-	-	-	-		**7A**	
Progeny of 1251								
	+	+	+	+	+	+	7Ai	2170, 2171
	+	-	-	-	-		7A	2173
R4	+	+	+	+	+	+	**7Ai**	
898 (477)	+	-	-	-	-		**7A**	
Progeny of 898								
	+	+	+	+	+	+	7Ai	1438, 1440
	+	-	-	-	-		7A	1441
	+	+	+	+	+	+	7Ai	1439
	-	-	-	-	-		7A	
Progeny of 1440								
	+	+	+	+	+	+	7Ai	1739
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	1740
	-	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	1741, 1742
	+	-	-	-	-		7A	
1440 X NT 7A-7B								
	-	+	+	+	+	+	7Ai	1743, 1744
	+	-	-	-	-		7A	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
1440XNT7A-7B (Cont'd)	+	+	+	+	+	+	7Ai	1745
	-	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
963 (477)	+	-	-	-	-		**7A**	
Progeny of 963	+	+	+	+	+	+	7Ai	1478, 1479
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	1480, 1481
	-	-	-	-	-		7A	
Progeny of 1479	+	+	+	+	+	+	7Ai	1763, 1765 1766
	+	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	1764
	+	-	-	-	-		7A	
1479 X NT 7A-7B	+	+	+	+	+	+	7Ai	1767, 1770 1978, 1980
	-	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	1768, 1769 1979, 1981 1982
	+	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
976 (430)	+	-	-	-	-		**7A**	
Progeny of 976	+	+	+	+	+	+	7Ai	2106, 2107 2109
	+	-	-	-	-		7A	
	-	+	+	+	+	+	7Ai	2108
	+	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	
986 (430)	+	-	-	-	-		**7A**	
Progeny of 986	+	+	+	+	+	+	7Ai	2110, 2111
	+	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2112
	-	-	-	-	-		7A	
R4	+	+	+	+	+	+	**7Ai**	**Sterile**
1011 (430)	+	-	-	-	-		**7A**	
R4	+	+	+	+	+	+	**7Ai**	
1020 (430)	+	-	-	-	-		**7A**	
Progeny of 1020	+	+	+	+	+	+	7Ai	2114, 2115
	+	-	-	-	-		7A	2116, 2117

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R4	+	+	+	+	+	+	7Ai	Sterile
1023 (430)	+	-	-	-	-		7A	
R4	+	+	+	+	+	+	7Ai	
1025 (430)	+	-	-	-	-		7A	
Progeny of 1025								
	+	+	+	+	+	+	7Ai	2118, 2119
	+	-	-	-	-		7A	2121
	+	+	+	+	+	+	7Ai	2120
	-	-	-	-	-		7A	
R5	+	+	+	+	+	+	7Ai	
874 (447)	-	+	+	+	+		7A	
Progeny of 874								
	+	+	+	+	+	+	7Ai	1462, 1463
	-	+	+	+	+		7A	1464, 1465
Progeny of 1463								
	+	+	+	+	+	+	7Ai	2177, 2178
	-	+	+	+	+		7A	2179
R5	+	+	+	+	+	+	7Ai	
1218 (394)	-	+	+	+	+		7A	
Progeny of 1218								
	+	+	+	+	+	+	7Ai	2166, 2169
	-	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	2167, 2168
	-	+	+	+	+		7A	
R5	+	+	+	+	+	+	7Ai	
886 (477)	-	+	+	+	+		7A	
Progeny of 886								
	+	-	-	+	+	+	7Ai	1430*
	-	+	+	+	+		7A	
	+	-	-	-	-	-	7Ai	1431, 1432
	-	+	+	+	+		7A	
	+	+	+	+	+	+	7Ai	1433
	-	+	+	+	+		7A	
Progeny of 1432								
	+	-	-	-	-	-	7Ai	2189, 2190
	-	+	+	+	+		7A	2191

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R5	+	+	+	+	+	+	7Ai	
896 (477)	-	+	+	+	+		7A	
Progeny of 896								
	+	-	-	-	-	-	7Ai	1474
	-	+	+	+	+		7A	
	+	+	+	+	+	+	7Ai	1475, 1477
	-	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	1476
	-	+	+	+	+		7A	
Progeny of 1474								
	+	-	-	-	-	-	7Ai	2198, 2199
	-	+	+	+	+		7A	2200
R6	-	-	-	-	-	-	7Ai	
1078 (447)	-	+	+	+	+		7A	
Progeny of 1078								
	-	-	-	-	-	-	7Ai	1643, 1644
	-	+	+	+	+		7A	1645, 1646
								1839, 1840
								1841, 1842
								1843, 1844
1078 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1647
	-	+	+	+	+		7A	
R6	-	-	-	-	-	-	7Ai	**Sterile**
1139 (394)	-	+	+	+	+		7A	
R7	-	+	+	+	+	+	7Ai	
1063 (447)	+	-	-	-	-		7A	
Progeny of 1063								
	-	+	+	+	+	+	7Ai	2134, 2136
	+	-	-	-	-		7A	2137
R7	-	+		+	+	+	7Ai	
1106 (447)	+	-		-	-		7A	
Progeny of 1106								
	-	+	+	+	+	+	7Ai	1829, 1830
	+	-	-	-	-		7A	1831, 1832
								1833
R7	-	+	+	+	+	+	7Ai	
1180 (394)	+	-	-	-	-		7A	
Progeny of 1180								
	-	+	+	+	+	+	7Ai	1834, 1835
	+	-	-	-	-		7A	1836, 1837
								1838

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R7	-	+	+	+	+	+	7Ai	Sterile
1009 (430)	+	-	-	-	-		7A	
R8	+	-	-	-	-	-	7Ai	
1052 (447)	-	+	+	+	+		7A	
Progeny of 1052	+	-	-	-	-	-	7Ai	1667, 1668
	-	+	+	+	+		7A	1669, 1670
1052 X NT 7A-7B	+	-	-	-	-	-	7Ai	1671
	-	+	+	+	+		7A	
	-	-	-	-	-	-	7Ai	1672
	-	-	-	-	-		7A	
Progeny of 1667	+	-	-	-	-	-	7Ai	2186, 2187
	-	+	+	+	+		7A	2188
R8	+	-	-	-	-	-	7Ai	
1053 (447)	-	+	+	+	+		7A	
Progeny of 1053	+	-	-	-	-	-	7Ai	2130, 2131
	-	+	+	+	+		7A	2133
R8	+	-	-	-	-	-	7Ai	
1019 (430)	-	+	+	+	+		7A	
Progeny of 1019	+	-	-	-	-	-	7Ai	1603, 1604
	-	+	+	+	+		7A	1605, 1606
1019 X NT 7A-7B	-	-	-	-	-	-	7Ai	1607, 1609
	-	-	-	-	-		7A	1610, 1944
	+	-	-	-	-	-	7Ai	1608, 1945
	-	+	+	+	+		7A	2183
R9	+	+	+	-	-	-	7Ai	
1170 (394)	+	+	+	+	+		7A	
Progeny of 1170	-	-	-	-	-	-	7Ai	1518, 1520
	+	+	+	+	+		7A	1809, 1811
	+	+	+	-	-	-	7Ai	1519, 1521
	-	-	-	+	+		7A	1523, 1524 1525
	+	+	+	-	-	-	7Ai	1522, 1812
	+	+	+	+	+		7A	1813, 1814

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R9	+	+	+	-	-	-	**7Ai**	
995 (430)	+	+	+	+	+		**7A**	
Progeny of 995								
	+	+	+	-	-	-	7Ai	1587, 1588
	+	+	+	+	+		7A	
	+	+	+	-	-	-	7Ai	1590
	-	-	-	+	+		7A	
995 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1593
	+	+	+	+	+		7A	
	-	-	-	-	-	-	7Ai	1594
	-	-	-	-	-		7A	
995 X CS								
	+	+	+	-	-	-	7Ai	1957, 1963
	+	+	+	+	+		7A	
	-	-	-	-	-	-	7Ai	1958, 1959
	+	+	+	+	+		7A	1960, 1961 1964
R9	+	+	+	-	-	-	**7Ai**	**Sterile**
1029 (430)	+	+	+	+	+		**7A**	
R9	+	+	+	-	-	-	**7Ai**	
895 (477)	+	+	+	+	+		**7A**	
Progeny of 895								
	-	+	+	-	-	-	7Ai	1470, 1515
	+	-	-	-	-		7A	
	-	+	+	-	-	-	7Ai	1471, 1514
	+	+	+	+	+		7A	1516, 1581
	+	+	+	-	-	-	7Ai	1472, 1473
	+	+	+	+	+		7A	
	+	+	+	-	-	-	7Ai	1517, 1582
	+	-	-	-	-		7A	
	-	-	-	-	-	-	7Ai	1579
	+	+	+	+	+		7A	
Progeny of 1515								
	-	+	+	-	-	-	7Ai	2195, 2196
	+	-	-	-	-		7A	2197
1470 X NT 7A-7B								
	-	+	+	-	-	-	7Ai	1759, 1760
	+	-	-	-	-		7A	1761, 1966 1967, 1968 1969

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R10	-	-	-	-	+	+	7Ai	
1146 (394)	+	+	+	+	+		7A	
Progeny of 1146								
	-	-	-	-	-	-	7Ai	1803
	+	+	+	+	+		7A	
	-	-	-	-	+	+	7Ai	1804, 1805
	+	+	+	+	+		7A	
	-	-	-	-	-	+	7Ai	1807*
	+	+	+	+	+		7A	
R11	-	-	-	-	-	+	7Ai	
936 (430)	+	+	+	+	+		7A	
Progeny of 936								
	-	-	-	-	-	-	7Ai	1450, 1779
	+	+	+	+	+		7A	1781, 1782
	-	-	-	-	-	+	7Ai	1451, 1452
	+	+	+	+	+		7A	1453, 1780
								1783, 1784
R12	+	+	-	-	-	+	7Ai	**Sterile**
1128 (447)	+	+	+	+	+		7A	
R13	+	+	+	+	+	+	7Ai	
817 (347)	+	+	+	+	-		7A	
Progeny of 817								
	+	+	+	+	+	+	7Ai	1406, 1408
	-	-	-	-	-		7A	1858, 1859
								1862
	+	+	+	-	+	+	7Ai	1407*
	+	+	+	+	-		7A	
	-	-	-	-	+	+	7Ai	1857
	+	+	+	+	-		7A	
	+	+	+	+	+	+	7Ai	1861
	+	+	+	+	-		7A	
R14	+	+	+	+	+	+	7Ai	
1175 (394)	+	+	+	-	-		7A	
Progeny of 1175								
	-	-	-	+	+	+	7Ai	1873, 1876
	+	+	+	-	-		7A	
	+	+	+	+	+	+	7Ai	1874, 1875
	+	+	+	-	-		7A	
	+	+	+	+	+	+	7Ai	1877, 1878
	-	-	-	-	-		7A	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R15	+	+	+	+	+	+	**7Ai**	
884 (477)	-	-	-	+	-		**7A**	
Progeny of 884								
	+	+	+	+	+	+	7Ai	1426, 1427
	-	-	-	-	-		7A	1428, 1429
								1507, 1508
								1509
R15	+	+	+	+	+	+	**7Ai**	
891 (477)	-	-	-	+	-		**7A**	
Progeny of 891								
	+	+	+	+	+	+	7Ai	1434, 1435
	-	-	-	+	-		7A	1436, 1437
								1511
	+	+	+	-	-	-	7Ai	1510*
	-	-	-	+	-		7A	
	+	+	+	+	+	+	7Ai	1512
	-	-	-	-	-		7A	
	+	+	+	+	+	-	7Ai	1513*
	-	-	-	+	-		7A	
Progeny of 1435								
	+	+	+	+	+	+	7Ai	2192, 2194
	-	-	-	-	-		7A	
	+	+	+	+	+	+	7Ai	2193
	-	-	-	+	-		7A	
R16	+	+	-	-	-	-	**7Ai**	
1199 (394)	-	-	-	+	+		**7A**	
Progeny of 1199								
	+	-	-	-	-	-	7Ai	1891*
	-	-	-	+	+		7A	
	-	-	-	-	-	-	7Ai	1892, 1893
	-	-	-	+	+		7A	1895, 1896
								1897
	+	+	-	-	-	-	7Ai	1894, 1898
	-	-	-	+	+		7A	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R17	+	+	+	-	-	-	**7Ai**	
1003 (430)	-	-	-	+	+		**7A**	
Progeny of 1003								
	+	+	+	-	-	-	7Ai	1595, 1596
	-	-	-	+	+		7A	1597, 1598
								1887, 1888
								1889, 1890
1003 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1599, 1600
	-	-	-	-	-		7A	1602
	+	+	+	-	-	-	7Ai	1601
	-	-	-	+	+		7A	
R17	+	+	+	-	-	-	**7Ai**	**Sterile**
1038 (430)	-	-	-	+	+		**7A**	
R18	-	-	-	+	+	+	**7Ai**	
1129 (447)	+	+	+	-	-		**7A**	
Progeny of 1129								
	-	-	-	+	+	+	7Ai	1797, 1798
	+	+	+	-	-		7A	1799, 1800
								1801, 1802
R19	-	-	-	-	+	+	**7Ai**	
985 (430)	+	+	+	+	-		**7A**	
Progeny of 985								
	-	-	-	-	-	-	7Ai	1785, 1788
	-	-	-	-	-		7A	1790
	-	-	-	-	+	+	7Ai	1786, 1787
	+	+	+	+	-		7A	1789
R20	-	+	+	-	-	-	**7Ai**	
942 (430)	+	+	+	+	+		**7A**	
Progeny of 942								
	-	+	+	-	-	-	7Ai	1454, 1455
	+	+	+	+	+		7A	1456, 1879
Progeny of 1456								
	-	+	+	-	-	-	7Ai	1747*
	+	-	+	+	+		7A	
	-	+	+	-	-	-	7Ai	1748, 1750
	+	-	-	+	+		7A	
	-	+	+	-	-	-	7Ai	1749
	+	+	+	+	+		7A	
1456 X NT 7A-7B								
	-	-	-	-	-	-	7Ai	1751
	+	+	+	+	+		7A	

Plant #	Xcdo 545	Xcdo 595	Xcdo 673	Xpsr 117	Xwg 686	Xpsr 121	Chromosome	Plant # of progeny
R21	+	-	-	+	+	+	**7Ai**	**Sterile**
979 (430)	+	+	+	+	+		**7A**	
R22	-	-	-	-	-	+	**7Ai**	
1140 (394)	-	+	+	+	+		**7A**	
Progeny of 1140								
	-	-	-	-	-	+	7Ai	1659, 1661
	-	+	+	+	+		7A	1662
	-	-	-	-	-	-	7Ai	1660
	-	+	+	+	+			
1140 X NT 7A-7B								
	-	-	-	-	-	+	7Ai	1663, 1665
	-	-	-	-	-		7A	1955
	-	-	-	-	-	-	7Ai	1664
	-	+	+	+	+		7A	
	-	-	-	-	-	-	7Ai	1666
	-	-	-	-	-		7A	
R23	-	+	+	+	+	+	**7Ai**	
1002 (430)	+	+	+	-	-		**7A**	
Progeny of 1002								
	-	+	+	+	+	+	7Ai	1863, 1864
	+	+	+	-	-		7A	1865, 1866 1870, 1871 1872
	-	-	-	+	+	+	7Ai	1867, 1868
	+	+	+	-	-		7A	1869
R23	-	+	+	+	+	+	**7Ai**	
1013 (430)	+	+	+	-	-		**7A**	
Progeny of 1013								
	-	+	+	+	+	+	7Ai	1881, 1883
	+	+	+	-	-		7A	1885
	-	+	+	+	+	+	7Ai	1882, 1884
	+	-	-	-	-		7A	
R24	+	+	+	-	-	-	**7Ai**	
805 (347)	+	-	-	+	+		**7A**	
Progeny of 805								
	+	+	+	-	-	-	7Ai	1402, 1405
	+	-	-	+	+		7A	1845, 1846 1849, 1850
	+	+	+	-	-	-	7Ai	1404, 1847
	-	-	-	+	+		7A	1848

* = Detection of new dissociation of markers including telocentric chromosome.

REFERENCES

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Nat Acad Sci USA 90: 7980-7984

Ahn S, Anderson, JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241: 483-490

Ainsworth CC, Johnson HM, Jackson EA, Miller TE, Gale MD (1984) The chromosomal locations of leaf peroxidase genes in hexaploid wheat, rye and barley. Theor Appl Genet 69: 205-210

Alani E, Padmore R, Kleckner N (1990) Analysis of wild type and *Rad 50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell 61: 419-436

Anamthawat-Jonsson K, Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1990) Discrimination between closely related Triticeae species using genomic DNA as probe. Theor Appl Genet 79: 721-728

Autrique E, Singh RP, Tanksley SD, Sorrells ME (1995) Molecular markers for four leaf rust resistance genes introgressed into wheat from wild relatives. Genome 38: 75-83

Banks PM, Larkin PJ, Bariana HS, Lagudah ES, Appels R, Waterhouse PM, Brettell RIS, Chen X, Xu HJ, Xin ZY, Qian YT, Zhou XM, Cheng ZM, Zhou GH (1995) The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. Genome 38: 395-405

Barker D, Schafer M, White R (1984) Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell 36: 131-138

Bennett MD (1982) Nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and the implications of the order for genome evolution and phenotypic variation. In: Dover GA, Flavell RB (eds) Genome evolution. Academic press, London, pp 239-261

Bennett MD (1983) The spatial distribution of chromosomes. In: Brandham PE, Bennett MD (eds) 2nd Kew Chromosome Conf, Royal Bot Garden, Kew, Surrey, UK, pp 71-79

Bhambani RN, Kuspira J, Kenny J, Sadasivaiah RS (1984) A study on somatic association and its relevance to meiotic pairing using haploids of barley (*Hordeum vulgare*, n=x=7). Can J Genet Cytol 26: 302-207

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Gent 32: 314-331

Bournival B, Obanni M, Abad A, Ohm H, Mackenzie S (1994) Isolation of a new species specific repetitive sequence from *Thinopyrum elongatum* and its use in the studies of alien translocations. Genome 37: 97-104

Bowden WM (1959) The taxonomy and nomenclature of the wheat, barley and rye and their wild relatives. Can J Bot 37: 647-684

Brettell RIS, Banks PM, Cauderon Y, Chen X, Cheng ZM, Larkin PJ, Waterhouse PM (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann Appl Biol 113: 599-603

Cauderon Y (1966) Etude cytogenetique de l'evolution du materiel issu de croisement entre *Triticum aestivum* et *Agropyron intermedium*. 1. Creation de types d'addition stables. Ann Amelior Plant 16:43-70

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 401-407

Chao S, Sharp PJ, Worland AJ, Warham, EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78: 495-504

Chen JM, Gustafson JP (1995) Physical mapping of restriction fragment length polymorphisms (RFLPs) in homoeologous group 7 chromosomes of wheat by *in situ* hybridisation. Heredity 75: 225-233

Chen PD, Tsujimoto H, Gill BS (1994a) Transfer of ph^1 genes promoting homoeologous pairing from *Triticum speltoides* to common wheat. Theor Appl Genet 88: 97-101

Chen Z, Devey M, Tullen NA, Hart GE (1994b) Use of recombinant substitution lines in the construction of RFLP based genetic maps of chromosome 6A and 6B of tetraploid wheat (*Triticum turgidum* L.). Theor Appl Genet 89: 703-712

Chojecki AJS, Gale MD (1982) Genetic control of glucose phosphate isomerase in wheat and related species. Heredity 49: 337-347

Clarke B, Stancombe P, Money T, Foote T, Moore G (1992) Targeting deletion (homoeologous chromosome pairing locus) or addition line single copy sequences from cereal genomes. Nucleic Acid Res 20: 1289-1292

Collins NC (1996) Genetics of barley yellow dwarf virus resistance in barley and rice. Ph.D thesis, University of Adelaide, Adelaide, Australia

Curtis CA, Lukaszewski AJ, Chrzastek M (1991) Metaphase-I pairing of deficient chromosomes and genetic mapping of deficiency breakpoints in common wheat. Genome 34: 553-560

Darlington CD (1931) Meiosis. Biol Rev VI(3): 221-264

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye and barley. Theor Appl Genet 85: 649-652

Devos KM, Millan T, Gale MD (1993a) Comparative RFLP maps of the homoeologous group 2 chromosomes of wheat, rye and barley. Theor Appl Genet 85: 784-792

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993b) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85: 673-680

Devos KM, Chao S, Li QY, Simonetti MC, Gale MD (1994) Relationship between chromosome 9 of maize and wheat homoeologous group 7 chromosomes. Genetics 138: 1287-1292

Dewey DR (1962) The genome structure of intermediate wheatgrass. J Hered 53: 282-290

Dover GA, Riley R (1972) The prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. Nature (London) 240: 159-161

Driscoll CJ (1973) Minor genes affecting homoeologous pairing in hybrid between wheat and relate genera. Genetics 74: [Suppl] S66 (Abstr)

Driscoll CJ, Bielig LM, Dervey NL (1979) An analysis of frequencies of chromosome configuration in wheat and wheat hybrids. Genetics 91: 755-767

Dubcovsky J, Santa-Maria G, Epstein E, Luo M-C, Dvorak J (1996a) Mapping of the K^+/Na^+ discrimination locus *Kna1* in wheat. Theor Appl Genet 92: 448-454

Dubcovsky J, Luo M-C, Zhong G-Y, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996b) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143: 983-999

Dundas IS, Shepherd KW, Fisher JM (1988) Transfer of cereal cyst nematode resistance from rye chromosome 6R into wheat. In: McWhirter KS, Downes RW, Reid BJ (eds) Proc 9th Australian Plant Breeding Conference, Wagga Wagga, Australia, pp 101-102

Dvorak J (1972) Genetic variability in *Aegilops speltoides* affecting homoeologous pairing in wheat. Can J Genet Cytol 14: 371-380

Dvorak J (1981) Chromosome differentiation in polyploid species of *Elytrigia*, with especial reference to the evolution of diploid-like chromosome pairing in polyploid species. Can J Genet Cytol 23: 287-303

Dvorak J (1987) Chromosomal distribution of genes in diploid *Elytrigia elongata* that promote or suppress pairing of wheat homoeologous chromosomes. Genome 29: 34-40

Dvorak J, Chen K-C (1984) Distribution of nonstructural variation between wheat cultivars along chromosome arm 6Bp: evidence from linkage map and physical map of the arm. Genetics 106:325-333

Dvorak J, Gorham J (1992) Methodology of gene transfer by homoeologous recombination into *Triticum turgidum*: transfer of K^+/Na^+ discrimination from *Triticum aestivum*. Genome 35: 639-646

Dvorak J, Knott DR (1974) Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can J Genet Cytol 16: 399-417

Dvorak J, Chen K-C, Giorgi B (1984) The C-band pattern of a *Ph⁻* mutant of durum wheat. Can J Genet Cytol 26: 360-363

Dvorak J, Terlizzi PD, Zhang H-B, Resta P (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21-31

Eizenga GC (1987) Locating the *Agropyron* segment in wheat-*Agropyron* transfer no. 12. Genome 29: 365-366

Faberge AC (1942) Homologous chromosome pairing: the physical problem. J Genet 43: 121-144

Fedak G (1985) Alien species as sources of physiological traits for wheat improvement. Euphytica 34: 673-680

Fedotova YS, Bogdanov YF, Gadzhiyeva SA, Sosnikhina SA, Smirnov VG, Mikhailova EI (1994) Meiotic mutants of rye *Secale cereale* L. 2. The nonhomologous synapsis in desynaptic mutants *Sy7* and *Sy10*. Theor Appl Genet 88: 1029-1036

Feldman M (1966) The effect of chromosome 5B, 5D and 5A on chromosome pairing in *Triticum aestivum*. Proc Nat Acad Sci USA 55: 1447-1453

Feldman M (1968) Regulation of somatic association and meiotic pairing in common wheat. In: Finlay KW, Shepherd KW (eds) Proc IIIrd Int Wheat Genet Symp, Canberra, pp 169-178

Feldman M (1993) Cytogenetic activity and mode of action of the pairing homoeologous (*Ph1*) gene of wheat. Crop Sci 33: 894-897

Feldman M, Avivi L (1973) The pattern of chromosomal arrangement in nuclei of common wheat and its genetic control. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 675-684

Feldman M, Avivi L (1984) Ordered arrangement of chromosomes in wheat. Chromosomes Today 8: 181-189

Feldman M, Mello-Sampayo T (1967) Suppression of homoeologous pairing in hybrids polyploid wheats X *T. speltoides*. Can J Genet Cytol 9: 307-313

Fernandez-Calvin B, Orellana J (1994) Metaphase I-bound arms frequency and genome analysis in wheat-*Aegilops* hybrids. 3. Similar relationships between the B genome of wheat and S or S' genomes of *Ae. speltoides*, *Ae. longissima* and *Ae. sharonensis*. Theor Appl Genet 88: 1043-1049

Figueiras AM, Gonzalez-Jaen MT, Benito C (1986) Biochemical evidence of homoeology between *Triticum aestivum* and *Agropyron intermedium* chromosomes. Theor Appl Genet 72: 826-832

Flavell RB, Bennett MD, Seal AG, Hutchinson J (1987) Chromosome structure and organization. In: Lupton FGH (ed) Wheat breeding: its scientific basis. Chapman and Hall, London, pp 211-268

Forster BP, Reader SM, Forsyth SA, Koebner RMD, Miller TE, Gale MD, Cauderon Y (1987) An assessment of the homoeology of six *Agropyron intermedium* chromosomes added to wheat. Genet Res 50: 91-97

Frankel OH (1970) Genetic conservation in perspective. In: Frankel OH, Bennett E (eds) Genetic resources in plants - their exploitation and conservation. Blackwell Scientific Publ, Oxford, pp 469-489

Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991) Identification of alien chromatin specifying resistance to wheat streak mosaic virus and greenbug in wheat germplasm by C-banding and *in-situ* hybridizations. Theor Appl Genet 81: 381-389

Friebe B, Zeller FJ, Mukai Y, Forster BP, Bartos P, McIntosh RA (1992) Characterization of rust resistant wheat-*Agropyron intermedium* derivatives by C-banding, *in situ* hybridization and isozyme analysis. Theor Appl Genet 83: 775-782

Fu TK, Sears ER (1973) The relationship between chiasmata and crossing over in *Triticum aestivum*. Genetics 75: 231-246

Gale MD, Miller TE (1987) The introduction of alien genetic variation in wheat. In: Lupton FGH (ed) Wheat Breeding: its scientific basis. Chapman and Hall, London, New York, pp 173-210

Gale MD, Sharp PJ (1988) Genetic markers in wheat - development and prospects. In: Miller TE, Koebner RMD (eds) Proc 7th Int wheat Genet Symp, Cambridge, pp 469-475

Gale MD, Law CN, Chojecki AJ, Kempton RA (1983) Genetic control of α-amylase production in wheat. Theor Appl Genet 64: 309-316

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1993) Genetic maps of hexaploid wheat. In: Li SZ, Xin ZY (eds) Proc 8th Int Wheat Genet Symp, Beijing pp:29-31

Gerechter-Amitai ZK, Wahl I, Vardi A, Zohary D (1971) Transfer of stem rust seedling resistance from wild diploid einkorn to tetraploid durum wheat by means of a triploid hybrid bridge. Euphytica 20: 281-285

Gill BS (1993) Molecular cytogenetics analysis in wheat. Crop Sci 33: 902-908

Gill BS, Chen PD (1987) Role of cytoplasm specific introgression in the evolution of the polyploid wheats. Proc Nat Acad Sci USA 84: 6800-6804

Gill KS, Gill BS (1991) A DNA fragment mapped within the submicroscopic deletion of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. Genetics 129: 257-260

Gill BS, Friebe B, Endo TR (1991a) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34: 830-839

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991b) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34: 362-374

Gill KS, Gill BS, Endo TR, Mukai Y (1993) Fine physical mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. Genetics 134: 1231-1236

Gillies CB (1975) Synaptonemal complex and chromosome structure. Annu Rev Genet 9: 91-109

Gillies CB (1984) The synaptonemal complex in plants. CRC Crit Rev Plant Sci. 2(2): 81-116

Giorgi B, Cuozzo L (1980) Homoeologous pairing in a *Ph* mutant of tetraploid wheat crossed with rye. Cereal Res Commun 8: 485-490

Giroux CN (1988) Chromosome synapsis and meiotic recombination. In: Kucherlapati R, Smith GR (eds) Genetic Recombination. Am Soc Microbiology, Washington, pp 465-496

Graner A, Jahoor A, Schondelmeier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83: 250-256

Gualandi G, Ceoloni C, Feldman M, Avivi L (1984) Spindle sensitivity to Isopropyl N-phenyl-carbamate and griseofulvin of common wheat plants carrying different doses of the *Ph1* gene. Can J Genet Cytol 26: 119-127

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistance in 'Hope' and related cultivars to wheat rusts. Z Pflanzenzucht 83: 350-367

Hart GE (1973) Homoeologous gene evolution in hexaploid wheat. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 805-810

Hart GE (1979) Genetical and chromosomal relationships among the wheats and their relatives. In: Redei GP (ed) 11th Stadler Genet Symp, Missouri, Columbia, Mo., pp 9-29

Hart GE (1987) Genetic and biochemical studies of enzymes. In: Heyne EG (ed) Wheat and wheat improvement. 2nd ed. Am Soc Agron, Madison, pp 199-214

Hart GE, Langston PJ (1977) Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity 39: 263-277

Hart GE, Tuleen NH (1983) Chromosomal location of eleven *Elytrigia elongata* (=*Agropyron elongatum*) isozyme structural genes. Genet Res 41: 181-202

Helbaek H (1959) Domestication of food plants in the old world. Science (Washington, D. C.) 130: 365-372

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72: 761-769

Henderson SA (1988) Four effects of elevated temperature on chiasma formation in the locust *Schistocerca gregaria*. Heredity 60: 387-401

Heslop-Harrison JS (1992) Molecular cytogenetics, cytology and genomic comparisons in the Triticeae. Hereditas 116: 93-99

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34: 437-447

Hobolth P (1981) Chromosome pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. Carlsberg Res Commun 46: 129-173

Hohmann U, Endo TR, Gill KS, Gill BS (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. Mol Gen Genet 245: 644-653

Hohmann U, Graner A, Endo TR, Gill BS, Herrmann RG (1995) Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. Theor Appl Genet 91: 618-626

Hohmann U, Badaeva K, Busch W, Friebe B, Gill BS (1996) Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome 39: 336-347

Holm PB (1986) Chromosome pairing and chiasma formation in allohexaploid wheat, *Triticum aestivum* analyzed by spreading of meiotic nuclei. Carlsberg Res Commun 51: 239-294

Holm PB, Wang X, Wischmann B (1988) An ultrastructural analysis of the effect of chromosome 5B on chromosome pairing in allohexaploid wheat. In: Brandham PE (ed) 3rd Kew chromosome Conf, Roy Bot Garden, Kew, Surrey, UK, pp 281-291

Hotta Y, Stern H (1971) Analysis of DNA synthesis during meiotic prophase in *Lilium*. J Mol Biol 55: 337-355

Huskins CL (1932) A cytological study of Vilmorin's unfixable dwarf wheat. J Genet 25:113

Irick H (1994) A new function for heterochromatin. Chromosoma 103: 1-3

Islam AKMR, Shepherd KW (1991) Alien genetic variation in wheat improvement. In: Gupta PK, Tsuchiya T (eds) Chromosome engineering in plants: Genetics, Breeding and Evolution. Vol. A. Elsevier Science Publishers, Amsterdam, pp 291-312

Islam AKMR, Shepherd KW (1992) Production of wheat-barley recombinant chromosomes through induced homoeologous pairing. 1. Isolation of recombinants involving barley arms 3HL and 6HL. Theor Appl Genet 83: 489-494

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161-174

Jampates R, Dvorak J (1986) Location of the *ph1* locus in metaphase chromosome map and the linkage map of the 5Bq arm of wheat. Can J Genet Cytol 28: 511-519

Jena KK, Khush GS, Kochert G (1992) RFLP analysis of rice (*Oryza sativa* L.) introgression lines. Theor Appl Genet 84: 608-616

Joshi BD, Singh D (1978) Introduction of alien variation into bread wheat. In Ramanujam S (ed) Proc 5th Int Wheat Genet Symp, New Delhi, pp 342-348

Jouve N, Diez N, Rodrigues M (1980) C-banding in 6X triticale X *Secale cereale* L. hybrid cytogenetics. Theor Appl Genet 57: 75-79

Kam-Morgan LNW, Gill BS, Mutthukrishnan S (1989) DNA restriction fragment length polymorphism: a strategy for genetic mapping of D genome of wheat. Genome 32: 724-732

Kato T, Yamagata H (1980) Reduction of meiotic homologous chromosome pairing due to high temperature in common wheat. Jpn J Genet 55: 337-348

Kerby K, Kuspira J (1987) The phylogeny of the polyploid wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). Genome 29: 722-737

Khan IA, Shepherd KW (1994) Isolation of wheat-*Agropyron* recombinant chromosomes through induced homoeologous pairing using Sears' *ph1b* mutant and DNA markers. In: Paul J, Dundas IS, Shepherd KW, Hollamby GH (eds) Proc 7th Wheat Breed Assemb, Adelaide, Australia, pp 309-312

Khan IA, Shepherd KW (1995) Patterns of homoeologous recombination between chromosomes 7Ai and 7A in Triticeae. Unifying plant genomes: comparisons, conservations and collinearity. 50th SEB Symp, Cambridge, pp 30

Kihara H (1944) Discovery of the DD-analyser, one of the ancestors of *vulgare* wheats. Agric Hortic 19: 889-890

Kim N-S, Armstrong K, Knott DR (1993) Molecular detection of *Lophopyrum* chromatin in wheat-*Lophopyrum* recombinants and their use in the physical mapping of chromosome 7D. Theor Appl Genet 85:561-567

Kim N-S, Whelan EDP, Fedak G, Armstrong K (1992) Identification of a *Triticum-Agropyron* non-compensating translocation line and detection of *Agropyron* chromatin using an *Agropyron* species specific probe. Genome 35: 541-544

King IP, Purdie KA, Liu CJ, Reader SM, Pittaway TS, Orford SE, Miller TE (1994) Detection of interchromosomal translocation within the Triticeae by RFLP analysis. Genome 37: 882-887

Kipling D, Ackford HE, Taylor BA, Cooke HJ (1991) Mouse minor satellite DNA genetically maps to the centromere and is physically linked to the proximal telomere. Genomics 11: 235-241

Kleinhofs A, Chao S, Sharp PJ (1988) Mapping of nitrate reductase gene in barley and wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int wheat Genet Symp, Cambridge, pp 541-546

Kleinhofs A, Kilian A, Saghai Maroof, MA, Biyashev RM, Hayes, P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dhaleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86: 705-712

Knott DR (1962) Inheritance of rust resistance. VIII. Additional studies on Kenya varieties of wheat. Crop Sci 2: 130-132

Knott DR (1971) The transfer of genes for disease resistance from alien species to wheat by induced translocations. In: IAEA (ed) Mutation breeding for disease resistance. International Atomic Energy Agency, Vienna, pp 67-77

Knott DR (1987) Transferring alien genes to wheat. In: Heyne EG (ed) Wheat and wheat improvement. 2nd ed. Am Soc Agron, Madison, pp 462-471

Knott DR (1989) The wheat rusts-Breeding for resistance. Monographs on Theoretical and Applied Genetics 12. Springer-Verlag, Berlin, Heidelberg, New York

Koebner RMD, Miller TE (1986) A note on the nomenclature for translocated chromosomes in the Triticeae. Cereal Res Commun 14: 315-316

Koebner RMD, Shepherd KW (1985) Induction of recombination between rye chromosome 1RL and wheat chromosomes. Theor Appl Genet 71:208-215

Koebner RMD, Shepherd KW (1986) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinants. Theor Appl Genet 73:197-208

Koebner RMD, Shepherd KW (1987) Allosyndetic recombination between a chromosome of *Aegilops umbellulata* and wheat chromosomes. Heredity 59: 33-45

Koebner RMD, Shepherd KW, Appels R (1986) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 2. Characterisation of recombinants. Theor Appl Genet 73: 209-217

Koebner RMD, Miller TE, Snape JW, Law CN (1988) Wheat endopeptidase: genetic control, polymorphism, intrachromosomal gene location and alien variation. Genome 30: 186-192

Konarev VG (1983) The nature and origin of wheat genomes on the data of grain proteins, immunochemistry and electrophoresis. In Sakamoto S (ed) Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 65-75

Landry BS, Kessel R, Leung H, Michelmore RW (1987) Comparison of restriction endonucleases and sources of probes for their efficiency in detecting fragment length polymorphisms in lettuce (*Lactuca sativa* L.). Theor Appl Genet 74: 646-653

Lapitan NLV, Sears RG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat X rye hybrids regenerated from tissue culture. Theor Appl Genet 68: 547-554

Larkin PJ, Ryan SA, Brettell RIS, Scowcroft WR (1984) Heritable somaclonal variation in wheat. Theor Appl Genet 78: 443-456

Larkin PJ, Spindler LH, Banks PM (1990) The use of cell culture to restructure plant genomes for introgressive breeding. In: Kimber G (ed) Proc 2nd Int Symp on chromosome engineering in plants, Missouri, Columbia, Mo., pp 80-89

Larkin PJ, Banks PM, Lagudah ES, Appels R, Xio C, Zhiyong X, Ohm HW, McIntosh RA (1995) Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. Genome 38: 385-394

Larsen J (1973) The role of chromosomal interchanges in the evolution of hexaploid wheat, *Triticum aestivum.* In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 87-93

Larson RI, Atkinson TG (1973) Wheat-*Agropyron* chromosome substitution lines as sources of resistance to wheat streak mosaic virus and its vector, *Aceria tulipae.* In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 173-177

Lay CL, Wells DG, Gardner WS (1971) Immunity from wheat streak mosaic virus in irradiated *Agrotricum* progenies. Crop Sci 11: 431-432

Le HT, Armstrong KC (1991) *In-situ* hybridisation as a rapid means to assess meiotic pairing and detection of alien DNA transfers in interphase cells of wide crosses involving wheat and rye. Mol Gen Genet 225: 33-37

Lefever G Jr.(1971) Salivary chromosome bands and the frequency of crossing over in *Drosophila melanogaster.* Genetics 67: 497-513

Lelley T, Kazman E, Devos KM, Gale MD (1995) Use of RFLPs to determine the chromosome composition of tetraploid triticale (A/B) (A/B) RR. Genome 38: 250-254

Liang GH, Wang RR-C, Nibbett CL, Heyne EG (1979) Registration of B-6-37-1 wheat germplasm. Crop Sci 19: 421

Liu CJ, Chao S, Gale MD (1989) *Wsp-1*, a set of genes controlling water-soluble proteins in wheat and related species. Gen Res 54: 173-181

Liu Y-G, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. 2. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66: 617-633

Liu, CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Non-homoeologous translocation between group 4, 5, and 7 chromosomes with in wheat and rye. Theor Appl Genet 83: 305-312

Loidl J (1989) Effects of elevated temperature on meiotic chromosome synapsis in *Allium ursinum*. Chromosoma 97: 449-458

Loidl J (1990) The initiation of meiotic chromosome pairing: the cytological view. Genome 33: 759-778

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90: 714-719

Lukaszewski AJ, Curtis CA. (1993) Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor Appl Genet 86: 121-127

Maguire MP (1974) A new model for homologous chromosome pairing. Caryologia 27: 349-357

Maguire MP (1988) Interactive meiotic systems. In: Gustafson JP, Appels R (eds) Chromosome structure and function. Impact of new concepts. Plenum Press, New York, pp 117-144

Maguire MP, Riess RW, Paredes AM (1993) Evidence from a maize desynaptic mutant points to a probable role of synaptonemal complex central region components in provision for subsequent chiasma maintenance. Genome 36 : 797-807

Maheshwari N, Rajalakshmi K, Baweja K, Dhir SK, Chowdhry CN, Maheshwari SC (1995) In vitro culture of wheat and genetic transformation - retrospect and prospect. Crit Rev Plant Sci 14: 149-178

Marais GF (1992) The modification of a common wheat-*Thinopyrum distichum* translocated chromosome with a locus homoeoallelic to *Lr19*. Theor Appl Genet 85: 73-78

Mather K (1951) The measurement of linkage in heredity. 2nd ed. Methuen's monographs on biological subjects. Methuen and Co. Ltd, London pp 149

May CE, Appels R (1987) The molecular genetics of wheat: towards an understanding of 16 billion base pairs of DNA. In: Heyne EG (ed) Wheat and wheat improvement, 2nd ed. Am Soc of Agron, Madison, pp 165-198

McCouch SR, Kockert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76: 815-829

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37: 81-89

McIntosh RA (1988) Catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int wheat Genet Symp, Cambridge, pp 1225-1324

McIntosh RA, Hart GE, Gale MD (1994) Guidelines for nomenclature of biochemical / molecular loci in wheat and related species; revision of section 5 and new section 6. In: "Graingenes", URL gopher://greengenes.cit.cornell. edu: 70/00/brouse/Nomenclature%20of%20Biochemical%20and%20Molecular %20Loci%20in%20wheat. Accessed on 29 Aug. 1996.

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. Common wealth Scientific and Industrial Research Organisation (CSIRO), Australia

McIntyre CL, Clarke C, Appels R (1988) Amplification and dispersion of repeated DNA sequences in the Triticeae. Plant Syst Evol 160: 39-59

McKee BD, Karpen G (1990) *Drosophila* ribosomal RNA genes function as an X-Y pairing site during male meiosis. Cell 61: 61-72

McKee BD, Habera L, Vrana A (1992) Evidence that the intergenic spacer of *Drosophila melanogaster* rRNA gene function as X-Y pairing sites in male meiosis, and a general model for achiasmata pairing. Genetics 132: 529-544

Mello-Sampayo T (1971) Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. Nature New Biol 230: 22-23

Mettin D, Bluthner WD, Schlegel G (1973) Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 179-184

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK (1990) Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequences in vertebrate chromosomes. Chromosoma 99: 3-10

Miller JC, Tanksley SD (1990) Effect of different restriction enzymes, probe source and probe length on detecting restriction fragment length polymorphism in tomato. Theor Appl Genet 80: 385-389

Miller TE, Reader SM, Gale MD (1983) The effect of homoeologous group 3 chromosomes on chromosome pairing and crossability in *Triticum aestivum*. Can J Genet Cytol 25: 634-641

Moens PB (1973) Mechanism of chromosome synapsis at meiotic prophase. Int Rev Cytol 35: 117-134

Morris R, Sears ER (1967) The cytogenetics of wheat and its relatives. In: Quisenberry KS, Reitz LP (eds) Wheat and wheat improvement. Ist ed. Am Soc Agron pp 19-87

Moses MJ (1968) Synaptonemal complex. Annu Rev Genet 2: 363-412

Mukai Y, Gill BS (1991) Detection of barley chromatin added to wheat by genomic *in situ* hybridisation. Genome 34: 448-452

Naranjo T, Roca A, Goicoechea PG, Giraldez R (1987) Arm homology of wheat and rye chromosomes. Genome 29: 873-882

Nelson OE, Clary GB (1952) Genetic control of semisterility in maize. J Hered 43: 205-210

Nelson JC, Sorrells ME, VanDeynze AE, Lu YH, Atkinson MD, Bernard M, Leroy P, Faris JD, Anderson JA (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5 and 7. Genetics 141: 721-731

Nishikawa K, Nobuhara M (1971) Genetic studies of α-amylase isozymes in wheat. 1. Location of genes and variation in tetra- and hexaploid wheat. Jpn J Genet 46: 345-358

Okamoto M (1957) Asynaptic effect of chromosome V. Wheat Inf Serv 5: 6

O'Mara JG (1940) Cytogenetic studies on Triticale. 1. A method for determining the effect of individual *Secale* chromosomes on *Triticum*. Genetics 25: 401-408

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46: 39-90

Rayburn AL, Gill BS (1985) Use of biotin-labelled probes to map specific DNA sequences on wheat chromosomes. J Hered 76: 78-81

Reader SM, Abbo S, Purdie KA, King IP, Miller TE (1994) Direct labelling of plant chromosomes by rapid *in situ* hybridisation. Trends in Genetics 10: 265-266

Renwick JH (1971) The mapping of human chromosomes. Annu Rev Genet 5: 81-120

Riley R (1960) The diploidization of polyploid wheat. Heredity 15: 407-429

Riley R (1966) The genetic regulation of meiotic behaviour in wheat and its relatives. In: Mackay J (ed) Proc IInd Int Wheat Genet Symp, Lund, 1963. Hereditas [Suppl] Vol. 2. pp: 395-408

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature (London) 182: 713-715

Riley R, Chapman V (1967) Effect of 5BS in suppressing the expression of altered dosage of 5BL on meiotic chromosome pairing in *Triticum aestivum*. Nature (London) 216: 60-62

Riley R, Flavell RB (1977) A first view of the meiotic process. Philos Trans R Soc London, Ser, B, 277: 191-199

Riley R, Kimber G (1966) The transfer of alien genetic variation to wheat. Annual report of the Plant Breeding Institute, Cambridge, 6-36

Riley R, Chapman V, Belfield AM (1966) Induced mutation affecting the control of meiotic pairing in *Triticum aestivum*. Nature (London) 211: 368-369

Riley R, Chapman V, Johnson R (1968a) The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. Genet Res 12: 199-219

Riley R, Chapman V, Johnson R (1968b) Introduction of yellow rust resistance of *Aegilops comosa* by genetically induced homoeologous recombination. Nature (London) 217: 383-384

Riley R, Chapman V, Miller TE (1973) The determination of meiotic chromosome pairing. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 731-738

Riley R, Unrau J, Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49: 90-98

Roberts PA (1972) Differences in synaptic affinity of chromosome arms of *D. melanogaster* revealed by differential sensitivity to translocation heterozygosity. Genetics 71: 401-415

Rogowsky PM, Guidet FLY, Langridge P, Shepherd KW, Koebner RMD (1991) Isolation and characterisation of wheat-rye recombinants involving chromosome arm 1DS of wheat. Theor Appl Genet 82: 537-544

Rowland GG, Kerber ER (1974) Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can J Genet Cytol 16: 137-144

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York

Schlegel R, Boerner A, Thiele V, Melz G (1991) The effect of the *ph1* gene in diploid rye, *Secale cereale* L. Genome 34: 913-917

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) *In-situ* localisation of parental genomes in a wide hybrid. Ann Bot 64: 315-324

Schwarzacher T, Anamthawat-Jonsson K, Harrison GE, Islam AKMR, Jia JZ, King IP, Leitch AR, Miller TE, Reader SM, Rogers WJ, Shi M, Heslop-Harrison JS (1992). Genomic *in situ* hybridisation to identify alien chromosomes and chromosome segments in wheat. Theor Appl Genet 84: 778-786

Sears ER (1952) Homoeologous chromosomes in *Triticum aestivum*. Genetics 37: 624

Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp Biol 9: 1-21

Sears ER (1966) Nullisomic -tetrasomic combinations in hexaploid wheat In:. Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver and Boyd, London, pp 29-45

Sear ER (1972) Chromosome engineering in wheat. Staddler Genet Symp, Vol. 4. Missouri, Columbia, pp 23-38

Sears ER (1973) *Agropyron*-Wheat transfers induced by homoeologous pairing. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 191-199

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet 10: 31-51

Sears ER (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19: 585-593

Sears ER (1981) Transfer of alien genetic material to wheat. In: Evans LT, Peacock WJ (eds) Wheat Science: today and tomorrow. Cambridge University Press, Cambridge, pp 75-89

Sears ER (1993) Use of radiation transfer alien chromosome segments to wheat. Crop Sci 33: 897-901

Sears ER, Sears LMS (1978) The telocentric chromosomes of common wheats. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp, New Delhi, pp 389-407

Sharma D, Knott DR (1966) The transfer of leaf-rust resistance from *Agropyron* to *Triticum aestivum* by irradiation. Can J Genet Cytol 8: 137-143

Sharma HC, Gill BS (1983a) Current status of wide hybridization in wheat. Euphytica 32: 17-31

Sharma HC, Gill BS (1983b) New hybrids between *Agropyron* and wheat. Theor Appl Genet 66: 111-121

Sharma HC, Gill BS, Uymoto JK (1984) High levels of resistance in *Agropyron* species to barley yellow dwarf and wheat streak mosaic viruses. Phytopath Z 110: 143-147

Sharma HC, Ohm HW, Lister RM, Foster JE, Shuklas RH (1989) Response of wheatgrasses and wheat X wheatgrass hybrid to barley yellow dwarf virus. Theor Appl Genet 77: 369-374

Sharma HC, Ohm HW, Goulart L, Lister R, Appels R, Benlhabib O (1995) Introgression and characterisation of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome 38: 406-413

Sharp PJ, Desai S, Chao S, Gale MD (1988) Isolation, characterisation and application of a set of 14 RFLP probes identifying each homoeologous chromosome arm on the Triticeae. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge, pp 639-646

Shepherd KW (1973) Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 745-760

Shepherd KW, Islam AKMR (1988) Forth compendium of wheat-alien chromosome lines. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge, pp: 1373-1398

Singh RP, Burnett PA, Albaran M, Rajaram S (1993) *Bydv1*: a gene for tolerance to barley yellow dwarf virus in bread wheats. Crop Sci 33: 231-234

Sinigovets ME (1974) The inheritance of resistance to common bunt in wheat-*Agropyron* hybrids. Genetics (USSR) 10: 25-31

Smith EL, Schlehuber AM, Young HC Jr, Edwards LH (1968) Registration of agent wheat. Crop Sci 8: 511-512

Stern H, Hotta Y (1987) The biochemistry of meiosis. In: Moens PB (ed) Meiosis. Acad Press, Orlando, USA, pp 303-331

Stoddard SL, Gill BS, Lommel SA (1987) Genetic expression of wheat streak mosaic virus resistance in two wheat-wheatgrass hybrids. Crop Sci 27: 514-519

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of "Sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87: 789-794

The TT (1973) Chromosome location of genes conditioning stem rust resistance transferred from diploid to hexaploid wheat. Nature New Biol 241: 256

The TT, Baker EP (1970) Homoeologous relationships between two *Agropyron intermedium* chromosomes and wheat. Wheat Inf Serv 31: 29-31

Vasil IK (1994) Molecular improvement in cereals. Plant Mol Biol 25: 925-937

Wall AM, Riley R, Gale MD (1971) The position of a locus on chromosome 5B of *Triticum aestivum* affecting homoeologous meiotic pairing. Genet Res 18: 329-339

Wang ML, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Comparative RFLP based genetic maps of barley chromosome 5 (1H) and rye chromosome 1R. Theor Appl Genet 84: 339-344

Wang RC, Liang GH, Heyne EG (1977) Effectiveness of *ph* gene in inducing homoeologous chromosome pairing in *Agrotricum*. Theor Appl Genet 51: 139-142

Watson IA, Luig NH (1961) Leaf rust on wheat in Australia: a systematic scheme for the classification of strains. Proc Linn Soc NSW, Australia, 86: 241-250

Werner JE, Endo TR, Gill BS (1992) Towards cytogenetically based physical map of the wheat genome. Proc Nat Acad Sci USA 89: 11307-11311

Weining S, Langridge P (1991) Identification and mapping of polymorphism in cereals based on polymerase chain reaction. Theor Appl Genet 82: 209-216

Whelan EPD, Hart GE (1988) A spontaneous translocation that transfers wheat curl mite resistance from decaploid *Agropyron elongatum* to common wheat. Genome 30: 289-292

Wienhues A (1966) Transfer of rust resistance of *Agropyron* to wheat by addition, substitution and translocation. In: Mackay J (ed) Proc 2nd Int Wheat Genet Symp, Lund, 1963. Hereditas [Suppl] Vol. 2. pp 328-341

Wienhues A (1973) Translocations between wheat chromosomes and *Agropyron* chromosomes conditioning rust resistance. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 201-207

Xie DX, Devos KM, Moore G, Gale MD (1993) RFLP based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 87: 70-74

Xin ZY, Brettlell RIS, Cheng ZM, Waterhouse PM, Appels R, Banks PM, Zhou GH, Chen X, Larkin PJ (1988) Characterization of a potential source of barley yellow dwarf virus resistance for wheat. Genome 30: 250-257

Yamamori M (1994) An N-band marker for gene *Lr18* for resistance to leaf rust in wheat. Theor Appl Genet 89: 643-646

Yasumuro Y, Morris R, Sharma DC, Schmidt JW (1981) Induced pairing between a wheat (*Triticum aestivum*) and an *Agropyron elongatum* chromosome. Can J Genet Cytol 23: 49-56

Zeller FJ (1973) 1B/1R Wheat-rye chromosome substitutions and translocations. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Missouri, Columbia, Mo., pp 209-221

Zhang, H-B, Dvorak J (1990) Characterisation and distribution of an interspersed repeated nucleotide sequence from *Lophopyrum elongatum* and mapping of a segregation-distortion factor with it. Genome 33: 927-936