Theor Appl Genet (2000) 100:519–527

Y. Weng · N.A. Tuleen · G.E. Hart

Extended physical maps and a consensus physical map of the homoeologous group-6 chromosomes of wheat (*Triticum aestivum* L. em Thell.)

Received: 19 April 1999 / Accepted: 28 July 1999

Abstract Extended physical maps of chromosomes 6A, 6B and 6D of common wheat (*Triticum aestivum* L. em Thell., 2*n*=6*x*=42, AABBDD) were constructed with 107 DNA clones and 45 homoeologous group-6 deletion lines. Two-hundred and ten RFLP loci were mapped, including three orthologous loci with each of 34 clones, two orthologous loci with each of 31 clones, one locus with 40 clones, two paralogous loci with one clone, and four loci, including three orthologs and one paralog, with one clone. Fifty five, 74 and 81 loci were mapped in 6A, 6B and 6D, respectively. The linear orders of the mapped orthologous loci in 6A, 6B and 6D appear to be identical and 65 loci were placed on a group-6 consensus physical map. Comparison of the consensus physical map with eight linkage maps of homoeologous group-6 chromosomes from six Triticeae species disclosed that the linear orders of the loci on the maps are largely, if not entirely, conserved. The relative distributions of loci on the physical and linkage maps differ markedly, however. On most of the linkage maps, the loci are either distributed relatively evenly or clustered around the centromere. In contrast, approximately 90% of the loci on the three physical maps are located either in the distal one-half or the distal two-thirds of the six chromosome arms and most of the loci are clustered in two or three segments in each chromosome.

Key words Wheat · *Triticum aestivum* · Physical mapping · Deletion lines · RFLP

Communicated by B. Gill

Y. Weng · N.A. Tuleen · G.E. Hart (✉)
Department of Soil and Crop Sciences, Texas A & M University, College Station, TX 77843, USA

Present adress:
Y. Weng
Texas Agricultural Experimental Station,
Texas A & M University, 6500 Amarillo Blvd. West,
Amarillo, TX 79106, USA

Introduction

The method introduced by Endo (1988) for the systematic production of common wheat stocks containing terminal chromosomal deletions of various lengths has been used to develop 430 deletion lines involving all 21 wheat chromosomes (Endo and Gill 1996). Physical maps of RFLPs produced using these stocks have been reported for the chromosomes of each of the seven homoeologous chromosome groups of wheat (Werner et al. 1992a; Kota et al. 1993; Gill et al. 1993a, 1996a,b; Hohmann et al. 1994; Delaney et al. 1995a,b; Mickelson-Young et al. 1995), and genes controlling phenotypic traits (Endo and Mukai 1988; Endo et al. 1991; Mukai and Endo 1992; Gill et al. 1993b; Endo and Gill 1996), in situ hybridization sites (Mukai et al. 1990, 1991; Werner et al. 1992b) and biochemical markers (Yamamuri et al. 1994) have also been mapped using the stocks. Map-based cloning in wheat, made difficult by the large amount of DNA that it contains [16000 Mbp per haploid genome (Armuganathan and Earle 1991)], is likely to be facilitated by the results of these studies, which make it possible to convert linkage distances to physical distances (Werner et al. 1992a; Gill and Gill 1994). Except for the chromosomes of homoeologous groups 1, 5 and 7, however [in which, respectively, 50, 82 and 97 different DNA, protein, and morphological markers have been physically mapped using deletion lines (Hohmann et al. 1994; Gill et al. 1996a,b)], the number of physically mapped markers per chromosome is relatively small, with an average of less than one marker physically mapped per cM even in chromosome groups 5 and 7. In the case of homoeologous group 6, loci detected with 31 DNA clones have been physically mapped to-date (Gill et al. 1993a) while more than 250 DNA clones that hybridize to group-6 DNA fragments have been isolated (Hart 1997) and several group-6 deletion lines not previously studied are available for use (Endo and Gill 1996).

The present paper reports the construction of extended physical maps and a consensus physical map of the group-6 chromosomes of hexaploid wheat. Results of a

comparison of the consensus physical map with homoeologous group-6 linkage maps are also presented.

Materials and methods

Plant materials

Forty five homoeologous group-6 chromosome deletion lines of hexaploid wheat, including 11 6A deletion lines, 20 6B lines, and 14 6D lines, were analyzed. The lines were kindly provided by Dr. Bikram S. Gill, Kansas State University, Manhattan, Kansas. Thirty seven of the lines are homozygous for the deletion-containing chromosome, five are heterozygous, and three lines are hemizygous, i.e., the chromosome that contains the deletion is monosomic**.** The C-banding pattern of the deletion-containing chromosome arm of each deletion line and the fraction length (FL) (the length of the non-deleted arm-segment relative to the length of the whole arm) of most of the deletion-containing arms have been determined by microscopic analyses (Endo and Gill 1996). The chromosome and chromosome-arm locations of genetic markers were determined by analyses of hexaploid wheat cv Chinese Spring (CS) and homoeologous group-6 aneuploid stocks (Sears 1953, 1954, 1966), namely, the six CS group-6 compensating nullisomic-tetrasomic lines and five of the six possible CS group-6 ditelosomic stocks (ditelosomic 6AL is not available).

DNA manipulation

Genomic DNA isolation, restriction enzyme digestion, Southern blotting, probe labeling and hybridization were all performed as described by Devey and Hart (1993).

DNA clones

DNA clones known to hybridize to Triticeae homoeologous group-6 chromosomal fragments were used. These included genomic DNA (gDNA) clones, cDNA clones, and 'known-function' DNA clones isolated from wheat, barley, oat, *Triticum tauschii* (Coss.) Schmal., and rice. TAM clones (wheat gDNA and cDNA clones) were developed by Devey and Hart (1993). Other clones of unknown function that were used were as follows: ABC (barley cDNA) and ABG (barley gDNA), obtained from A. Kleinhofs, Pullman, Wash. (Kleinhofs et al. 1993); BCD (barley cDNA), CDO (oat cDNA), and WG (wheat gDNA) from M.E. Sorrells, Ithaca, N.Y. (Heun et al. 1991); cMWG (barley cDNA) and MWG (barley gDNA) from A. Graner, Grunbuch, Germany (Graner et al. 1991, 1994); FBA and FBB (wheat gDNA) from F. Quetier, Paris, France (Marino et al. 1996); Tag (wheat gDNA; loci are designated '*glk*') from R. Appels, Canberra, Australia,with permission of K. Tsunewaki, Kyoto, Japan (Liu and Tsunewaki 1991); ksu (*T. tauschii* gDNA) from B.S. Gill, Manhattan, KS (Gill et al. 1991b), PSR (wheat cDNA and gDNA) from M.D. Gale, Norwich, England (Chao et al. 1989); and RZ (rice cDNA) from S. McCouch, Ithaca, N.Y. (McCouch et al. 1988). The 'known-function' DNA clones that were used were as follows: 2119 [α-amylase-1; *Xpsr2*(*α-Amy-1*)] and pTag53 [wheat gliadin; *Xpsr10*(*Gli-2*)], obtained from M.D. Gale, Norwich, England (Bartels et al. 1983); 2437 [wheat carboxypeptidase; *Xpsr8*(*Cxp3*)] from C. Baulcombe, Norwich, England (Baulcombe et al. 1987); ES18 and ES35 [cDNAs for salt-stress-induced genes; *Xucd106*(*Esi18*) and *Xucd109*(*Esi35*), respectively] from J. Dvorak, Davis, Calif. (Patrick and Dvorak 1990); Hv5 [barley dehydrin; *Xcsb112*(*Dhn5*)] from T. Close, Riverside, Calif. (Close et al. 1989); pGC19 [wheat DNA-binding protein; *Xrsq805*(*Embp*)] from M. Guiltinan, Raleigh, N.C. (Guiltinan et al. 1990]; p26 and p28 [protein synthesis initiation factors; *Xuta1*(*Psif*) and *Xuta2*(*Psif*), respectively) from K. Browning, Austin, TX (Metz et al. 1992), and R6 (phosphoribulokinase cDNA; *Xpsr463*(*Prk*)] from T. Dyer, Norwich, England (Raines et al. 1989).

Map construction

The method of Werner et al. (1992a) was used to construct physical maps of 6A, 6B and 6D. Specifically, the locus or loci detected by a given probe was first assigned to a chromosome arm(s) based on the presence or absence of the locus (loci) in the lines by Southern-blot analysis of CS group-6 compensating nullisomic-tetrasomic and ditelosomic lines. Next, the locus (loci) was assigned to a chromosomal region by analysis of a panel of 6A, 6B and 6D deletion lines, with each locus being assigned to the region between the breakpoints of the largest deletion where the locus was present and the next largest deletion where the locus was absent.

A consensus physical map of the group-6 chromosomes of hexaploid wheat was constructed using clones that detected two or three orthologous loci. The order of loci on the consensus map was based on their relative order in individual chromosomes rather than on the FLs of the deletion-containing chromosomes. The order of loci on the consensus group-6 physical map of wheat was compared with consensus linkage maps of Triticeae group-6 chromosomes and hexaploid wheat group-6 chromosomes, with linkage maps of chromosomes 6A and 6B of *Triticum turgidum* L. (tetraploid wheat), 6D of *T. tauschii*, and 6A of *Triticum monococcum* L., with a consensus linkage map of 6H of *Hordeum vulgare* L., and with the group-6 component of a 6R linkage map of *Secale cereale* L, using either published maps or, if available at the GrainGenes website 'http://wheat.pw.usda.gov/', updated versions of them.

Results

Mapped loci

Altogether, 210 loci were mapped (Fig. 1) with 107 DNA clones, including 22 cDNA clones and 85 gDNA clones. Short-arm loci were mapped with 35 clones and long-arm loci with 73 clones. Three orthologous loci were mapped with 34 clones, two orthologous loci with 31 clones, one locus only with 40 clones, two paralogs with one clone (PSR301), and three orthologs and one paralog with one clone (PSR141). On average, 1.95 loci were mapped per clone. A few clones hybridized to as many as 5–10 fragments. Regardless of the number of fragments missing from the deletion lines for a chromosome arm, however, only one locus was assumed as long as all of the fragments were either present or absent in each deletion line.

Paralogous loci within a chromosome were mapped with PSR141 and PSR301. Three fragments, 0.2 kb, 0.7 kb and 1.1 kb in size, were released from the PSR141 plasmid when it was digested with *Hin*dIII and *Eco*RI,

Fig. 1 Physical maps of wheat chromosomes 6A, 6B and 6D. ▶ Short arms are at the top. The *black circles* indicate centromeres. Deletion-line breakpoints and fraction lengths (FLs) are indicated by the *horizontal line to the right* and the *number to the left* of each deletion-line symbol, respectively. The breakpoint positions are drawn approximately to scale. *nd*=not determined. *Darkened areas* within chromosome arms are C-bands (Endo and Gill, 1996). Symbols for orthologous loci mapped in 6A, 6B and 6D are in *boldface type*, those for orthologous loci mapped in two of the three chromosomes are *underlined*, and those for loci mapped in one chromosome only are in *plain text*. *Asterisks* designate loci physically mapped by Gill et al. (1993a). *Diamonds* identify loci that may be located either between the 6BS-2 and 6BS-6 breakpoints or between the 6BS-6 and 6BS-3 breakpoints (see text)

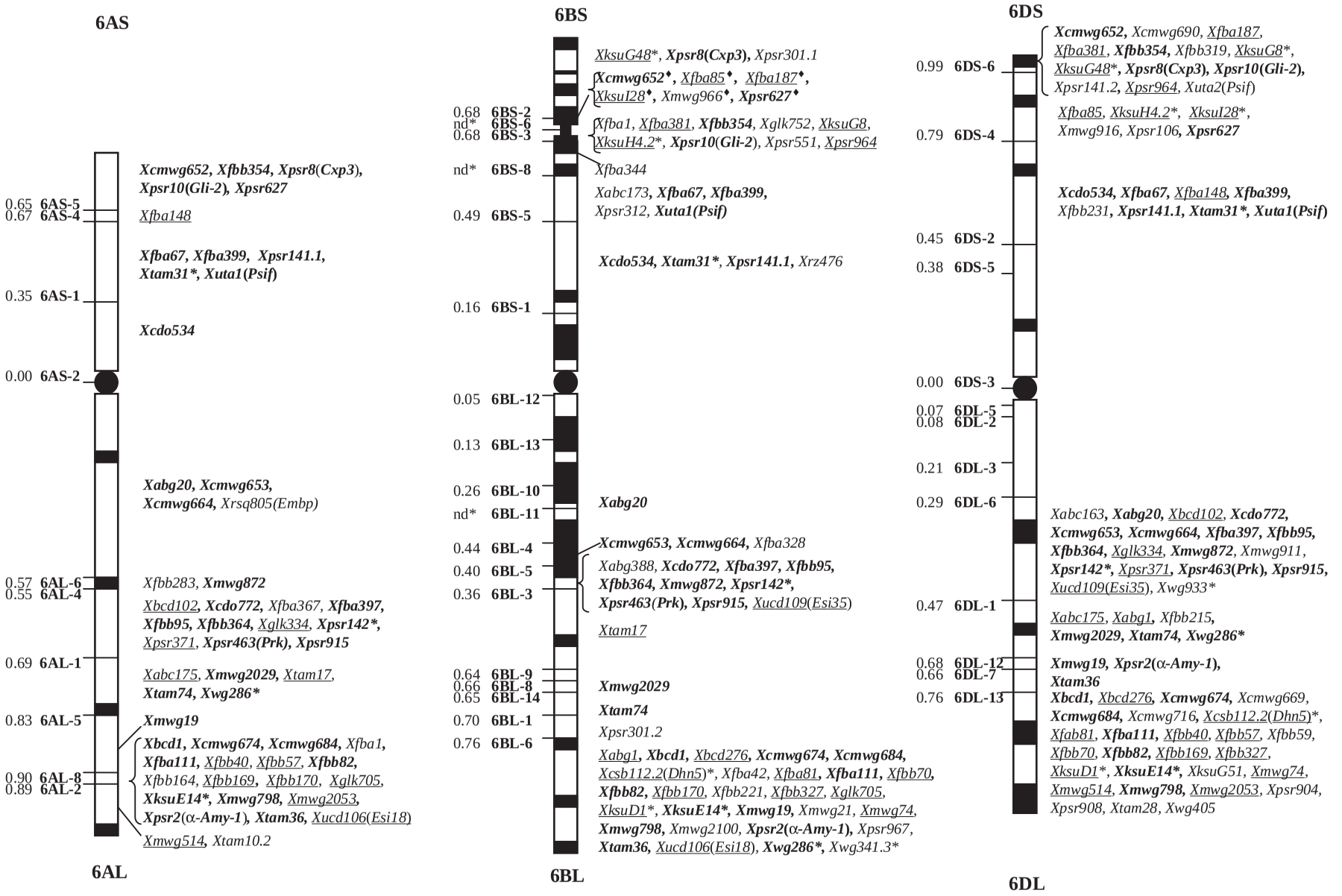
6AS
0.65 6AS-5
0.67 6AS-4
0.35 6AS-1
0.00 6AS-2
0.57 6AL-6
0.55 6AL-4
0.69 6AL-1
0.83 6AL-5
0.90 6AL-8
0.89 6AL-2
6AL
6BS
0.68 6BS-2
nd* 6BS-6
0.68 6BS-3
nd* 6BS-8
0.49 6BS-5
0.16 6BS-1
0.05 6BL-12
0.13 6BL-13
0.26 6BL-10
nd* 6BL-11
0.44 6BL-4
0.40 6BL-5
0.36 6BL-3
0.64 6BL-9
0.66 6BL-8
0.65 6BL-14
0.70 6BL-1
0.76 6BL-6
6BL
6DS
0.99 6DS-6
0.79 6DS-4
0.45 6DS-2
0.38 6DS-5
0.00 6DS-3
0.07 6DL-5
0.08 6DL-2
0.21 6DL-3
0.29 6DL-6
0.47 6DL-1
0.68 6DL-12
0.66 6DL-7
0.76 6DL-13
6DL

Table 1 Chromosome and chromosome-arm distribution of RFLP loci mapped in 6A, 6B and 6D

Locus type	Number of loci								
	Short arm			Long arm			Totals		
	6A	6B	6D	6A	6B	6D	6A	6B	6D
Member of orthologous set composed of three loci	11	11	11	24	24	24	35	35	35
Member of orthologous set composed of two loci	1	8	9	13	13	18	14	21	27
One locus	0	9	7	6	9	12	6	18	9
Totals	12	28	27	43	46	54	55	74	81

and four loci were mapped with the 0.7-kb and 1.1-kb fragments when they were used as probes, namely, *Xpsr141-6A.1, Xpsr141-6B.1,* and *Xpsr141-6D.1* in 6AS, 6BS, and 6DS, respectively, with the 1.1-kb probe and *Xpsr141-6D.2* in 6DS with the 0.7 kb probe. Loci in both 6BS and 6BL were mapped with PSR301.

Physical maps

Fifty five, 74, and 81 loci were physically mapped in 6A, 6B and 6D**,** respectively (see Fig. 1 and Table 1). The 6BS-6 deletion line was not analyzed with several of the short-arm clones and, as a consequence, a few 6BS loci were mapped only to the interval between the 6BS-2 and 6BS-3 breakpoints rather than to one or the other of the two segments between these breakpoints. Four of the analyzed deletion lines, 6AS-3, 6BS-9, 6BL-2 and 6DS-1, are not shown in Fig. 1. These lines are either heterozygous or hemizygous for the deletion-containing chromosome, and the presence or absence of a locus in the segment defined by each of these deletions, as opposed to the segment defined by an adjacent deletion, could not be determined. The other four heterozygous or hemizygous deletion lines that were examined, 6AS-2, 6DS-5, 6DS-3 and 6DL-2, are shown in Fig. 1, although no markers were mapped either in the segment defined by them or in the segment defined by the adjacent deletion in the same arm. Twenty four loci were mapped with clones used earlier for physical mapping of the group-6 chromosomes by Gill et al. (1993a). The map locations determined for common loci in the two studies are the same, except for two 6AL loci, *Xpsr142-6A* and *Xwg286-6A*. The former was mapped between FLs 0.41 and 0.55 and the latter between FLs 0.83 and 0.89 by Gill et al. (1993a), while our results place the former between FLs 0.55 and 0.83 and the latter between FLs 0.69 and 0.83.

Characteristics common to the maps of 6A, 6B and 6D (see Fig. 1 and Table 1) are the presence of approximately 90% of the mapped loci in the distal one-half or two-thirds of the two arms of each chromosome and the clustering of a large number of loci in two or three segments in each chromosome. For example, most of the mapped 6AS and 6AL loci are distal to the breakpoints in deletion lines 6AS-1 and 6AL-4, respectively. This establishes that most of the mapped loci are in the distal two-thirds of 6AS and the distal one-half of chromosome 6AL because the FLs of these deletions are 0.34 and 0.55, respectively. Only six loci [*Xcdo534-6A, Xtam31-6A, Xabg20-6A, Xcmwg653-6A, Xcmwg654-6A* and *Xrsq805*(*Embp*)*-6A*)] were mapped in the proximal one-third (6AS) and one-half (6AL) of the arms. Also, more than one-half of the mapped 6A loci (29 out of 55) are located in two segments that combined comprise approximately 15% of the FL of 6AL, namely, the segments between the 6AL-1 and 6AL-4 breakpoints and between the 6AL-8 and 6AL-2 breakpoints. Each of the 11 segments defined by the ten 6A deletion lines shown in Fig. 1 contains at least one marker, however.

Chromosome 6B is the longest of the group-6 chromosomes and has a satellite at the distal end of the short arm. As with 6A, most of the mapped 6B loci are located in the distal portions of the arms; only five loci (*Xcdo534-6A, Xtam31-6A, Xpsr141-6A.1, Xrz476-6A* and *Xabg20-6A*) were mapped in the proximal one-half of 6BS and the proximal one-third of 6BL. More than 80% of the mapped 6AL loci (38 of 46) are clustered in two regions, distal to the 6BL-6 breakpoint and between the 6BL-5 and 6BL-3 breakpoints. Also, more than one-half of the mapped short-arm loci are located in the satellite or immediately adjacent to it. Indeed, 86% of the mapped short-arm loci (24/28) are in the distal one-half of the arm (i.e., distal to the 6BS-5 breakpoint). Of the 19 segments defined by 18 deletion lines, 14 are tagged by 74 RFLP markers.

A 2BS-6BS reciprocal translocation was hypothesized by Devos et al. (1993), based on: (1) mapping of several RFLP loci near the distal ends of the linkage maps of 2AS and 2DS with clones that did not detect a 2BS locus and (2) mapping of a locus near the distal end of the 2BS linkage map with a clone that also detects loci located near the distal ends of the 6AS and 6DS linkage maps. Evidence supporting this translocation was not found in the present study. The five clones that detected loci in the distal 35% of 6AS also detected loci in both 6BS and 6DS and, although four clones that detected loci in the distal portion of 6DS (cMWG690, FBB319, MWG916, and p28) did not detect a 6BS locus, none of the clones has detected a 2BS locus (McIntosh et al. 1998) and only one has detected a 6A locus (MWG916, Du and Hart 1998). The 6BL-6 deletion line has an interstitial deletion that includes an awn-inhibitor gene (Endo and Gill

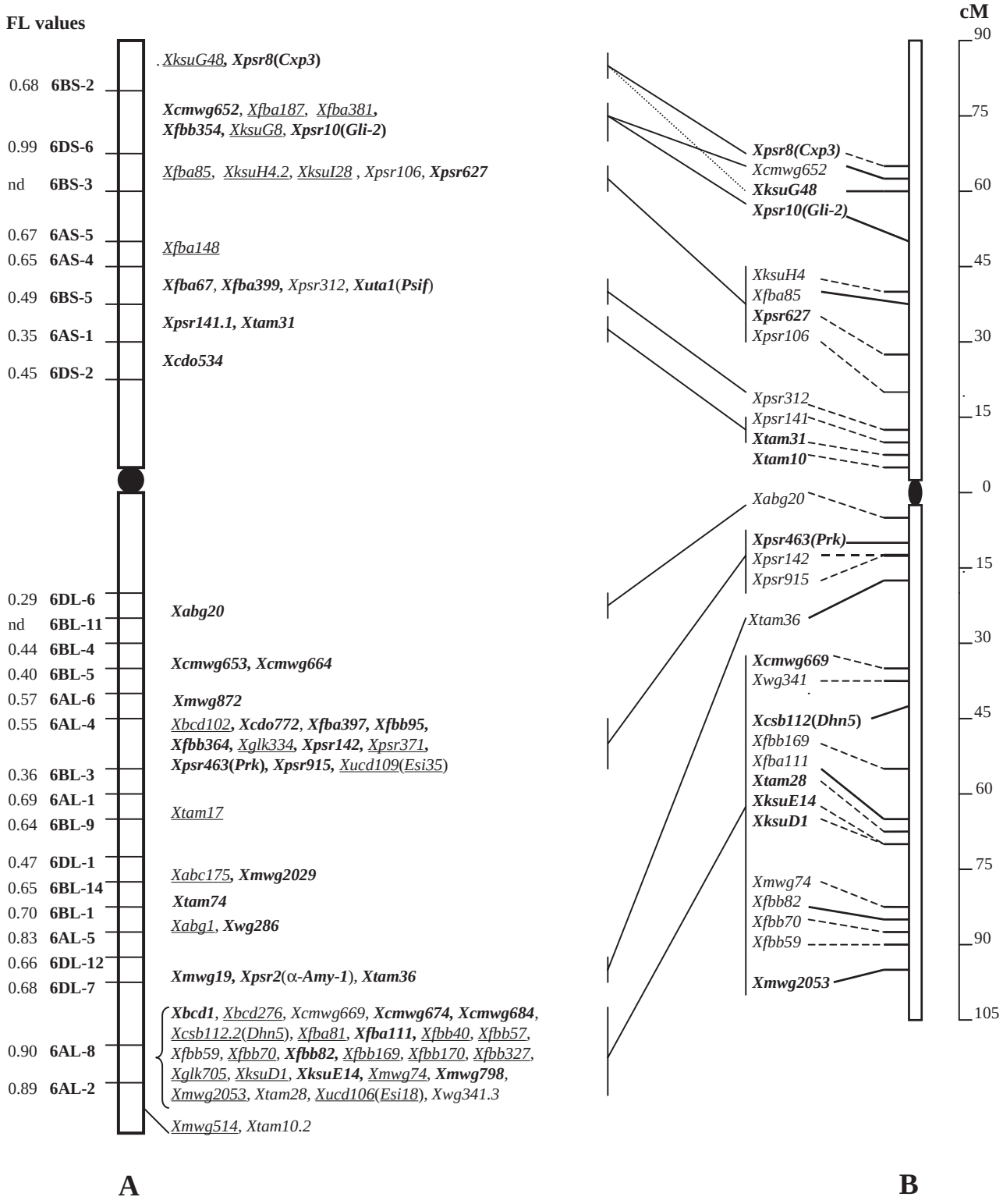

Fig. 2 Consensus physical map of the group-6 chromosomes of hexaploid wheat (**A**) and consensus Triticeae group-6 linkage map of Marino et al. (1996) (**B**). Short arms are at the top. *Solid lines* between the maps connect colinear loci and the *dashed line* between the maps connects loci mapped with the same clone that are not colinear. **A** The *black circle* designates the centromere. Deletion-line breakpoints and fraction lengths (FLs) are indicated by the horizontal line to the right and the *number* to the left of each deletion-line symbol, respectively. The breakpoint positions are not drawn to scale. *nd*=not determined. Symbols for loci mapped in 6A, 6B and 6D are in *boldface type*, those for loci mapped in two of the three chromosomes are *underlined*, and those for loci mapped in one chromsome only are in *plain text*. **B** The *black oval* designates the centromere. *Boldface type* designates loci whose orientation relative to each other was determinced accross two or more Triticeae genomes. Loci connected to the map with *solid lines* were placed on the hexaploid wheat 6A linkage map of Marino et al. (1996) at a LOD score ≥3.0 and the map locations shown for these markers are the same as they are on the 6A linkage map

1996). No 6BL marker located proximal to the 6BL-6 breakpoint was missing from 6BL-6, indicating that none of the 6BL markers analyzed in this study are located in the deleted segment.

All of the loci mapped in 6DS are located in the distal one-half of the arm but approximately 35% of the mapped 6DL loci (19 of 54) are located in the proximal one-half of the arm in one segment. Most of the loci mapped in the distal one-half of 6DL (27 of 36) are located in one segment, namely, the segment distal to the 6DL-13 breakpoint. Especially notable is the fact that 13 loci were mapped in 6DS in a segment with a FL of only 0.01, namely, the segment proximal to the 6DS-3 breakpoint. A similar concentration of loci is present in 6AL, where 18 loci were mapped between the breakpoints of 6AL-2 and 6AL-8. The shortest deletion of each of the other four arms is one-fourth or more of the arm in length, making it impossible to determine if similar concentrations of loci are present near the ends of these arms. Three or more loci were mapped in seven of the 14 segments defined by the 6D deletion lines.

The mapping data obtained in this study and the reported FLs of four pairs of deletion lines and of one group of three deletion lines are inconsistent. For example, the FL of 6AS-5 is 0.65 but the line contains a locus, *Xfba148-6A*, that is not present in 6AS-4, which has a FL of 0.67. The other deletion lines for which inconsistencies between FL values and marker data were observed are 6AL-8 (FL 0.90) and 6AL-2 (0.89); 6BL-4 (0.44), 6BL-5 (0.40) and 6BL-3 (0.36); 6BL-8 (0.66) and 6BL-14 (0.65); and 6DL-7 (0.66) and 6DL-12 (0.68)(see Fig. 1). Although an internal deficiency could cause a mapping data/FL-value discrepancy, all of these FL differences are within the standard error of ± 5% in the calculation of FL values that is regarded as an approximate estimation of the breakpoint positions (T.R. Endo and B.S. Gill, personal communication). The marker mapping data, by allowing accurate ordering of the breakpoints of deletion lines with similar FL values, thus provides a valuable characterization of deletion stocks.

Consensus physical map of the group-6 chromosomes

Because many clones detected two or three orthologous loci among the three group-6 chromosomes and the loci appear to be colinear, it was possible to construct a consensus physical map of the chromosomes. The order of loci on the map is based on the relative positions of loci in the three group-6 chromosomes as determined by deletion mapping, not on the FLs of the deletion lines. The consensus physical map is shown in Fig. 2 (A). Altogether, 35 sets of three orthologous loci and 30 sets of two orthologous loci were placed on the consensus map, 19 in the short arm and 46 in the long arm. Also shown on the map, for use in comparing it with linkage maps, are seven loci that were mapped in only one chromosome.

Two loci that are probably orthologous, *Xpsr964-6B* and *Xpsr964-6D*, were not used in constructing the consensus physical map. *Xpsr964-6D* was mapped 9-cM distal to *Xpsr8*(*Cxp3*)*-6D* and 13-cM distal to *XksuG48-6D* by Marino et al. (1996) and the three loci were mapped at a LOD score ≥ 3.0. In this study, however, *Xpsr964-6B* was mapped proximal to *Xpsr8*(*Cxp3*)*-6B* and *XksuG48-6B*. Whether this difference is due to a rearrangement, mapping of paralogous rather than orthologous loci with one or more of the clones, or experimental error is not known.

Discussion

Duplicated loci

Duplicated (i.e., paralogous) loci are present on almost all of the RFLP linkage and physical maps of Triticeae species constructed to date (e.g., Anderson et al. 1992; Kota et al. 1993; Hohmann et al. 1994; Nelson et al. 1995; Marino et al. 1996), and in large numbers on some maps. For example, more than 35% of the RFLP loci on the *T. tauschii* linkage map of Gill et al. (1991b) were mapped with clones that detected two or more loci. Also, more than 30% of the clones used by Dubcovsky et al. (1996) to construct a map of *T. monococcum* detected intra- and/or inter-chromosomal duplicated loci and nearly 30% of the clones used by Graner et al. (1994) in mapping the barley genome detected duplicated loci. In contrast, duplicated loci were mapped in this study with only 2 of 107 clones, namely, PSR141 and PSR301 (however, different loci than mapped previously were also detected with some clones; see below). Undoubtedly, the two principal reasons for the relatively low number of paralogous loci mapped in this as compared to other studies were: (1) the use, principally, of clones previously shown to detect single- or low-copy-number loci, and (2) mapping the chromosomes of one homoeologous group only, thereby eliminating the possibility of mapping paralogous loci located in different homoeologous groups.

Care must be taken to distinguish paralogous loci from orthologous loci when analyzing the relationships among chromosomes and chromosomal segments. Clearly, the mapping of paralogous loci rather than orthologous loci is the reason that some of the markers mapped with the same clones on linkage maps and the group-6 physical maps are not colinear (see below). In practice, the redundancy of duplicated loci can also create difficulties for physical mapping by means of marker-based chromosome landing (Zhang and Wing 1997). Therefore, DNA clones that detect both paralogous and orthologous loci should be used for this purpose with caution.

Ordering of deletion-line breakpoints on the consensus group-6 physical map

Because the order of loci and of deletion breakpoints on the maps of 6A, 6B, and 6D are based on physical mapping data rather than on the reported FLs of the deletion-

containing chromosome arms, the loci and deletion breakpoints on the consensus physical map of the group-6 chromosomes (Fig. 2) also are ordered on this basis. Apart from the difficulty in accurately measuring the FLs of deletion-containing chromosome arms of similar length, a disadvantage in using FLs to align deletion breakpoints on a consensus physical map is that deletion-containing chromosome arms in different lines may have the same FL and different absolute lengths, or they may have the same absolute length and different FLs. This is because neither the lengths of the chromosomes within a homoeologous group nor their arm ratios are necessarily the same (Morrison 1953; Gill et al. 1991a). The significance of this is emphasized by the finding that the orthologs to all of the 6A loci located in the interval between FLs 0.55 (6AL-4) and 0.69 (6AL-1) are located between the 6BL FLs 0.40 (6BL-5) and 0.36 (6BL-3) and between the 6DL FLs 0.29 (6DL-6) and 0.47 (6DL-1).

Comparison of the consensus physical map with other group-6 maps

To analyze the relationships between the physical maps constructed in this study and linkage maps constructed in other studies, the group-6 consensus physical map was compared with consensus linkage maps of the group-6 chromosomes of hexaploid wheat and the tribe Triticeae, with linkage maps of chromosomes 6A and 6B of tetraploid wheat, 6A of *T. monococcum* and 6D of *T. tauschii*, with a consensus linkage map of 6H of barley, and with the group-6 component of a 6R linkage map of rye. The consensus group-6 physical map produced in this study and the consensus Triticeae group-6 map of Marino et al. (1996) are shown and compared in Fig. 2 and a summary of the results of all of the comparisons is shown in Table 2.

Detection of paralogous loci rather than orthologous loci, chromosomal rearrangements, and experimental error, are the three most likely causes for the mapping of non-colinear loci with common clones. The consensus Triticeae linkage map of Marino et al. (1996) consists of 52 loci, 30 of which were detected with clones also used to produce the hexaploid wheat group-6 consensus physical map of this study. As shown in Fig. 2, 28 of the 30 loci detected with common clones are colinear on the two maps. The locus detected with TAM10 is near the centromere on the short-arm of the group-6 map of Marino et al. (1996) and in a distal position in the long arm of the 6AL physical map, making it clear that, in the absence of experimental error, they are paralogous loci. The detection of paralogous rather than orthologous loci with ksuG48 (which, like TAM10, is a low-copy number clone) may also explain the non-colinearity of the *XksuG48* loci on the two maps.

Table 2 shows that, altogether, seven clones that detected loci placed on the consensus physical map were earlier used to map loci that occupy non-colinear locations on one or more of the maps listed in the table. Five of the seven clones hybridize to low-copy number loci and three of these five clones detected loci located in different chromosome arms. It seems likely that paralogous rather than orthologous loci were mapped with these five clones. *Xcmwg653-6A* was mapped 1.5 cM from the centromere in 6AS of tetraploid wheat by Du and Hart (1998) while *Xcmwg653* loci were mapped in the proximal regions of 6AL, 6BL, and 6DL in this study. One or more data-point errors in the linkage study is the probable cause of this anomaly. Data point errors also may be the cause of the non-colinearity of the *Xmwg514* loci mapped in this study and by Qi et al. (1996).

In summary, comparison of the consensus physical map produced in this study with several linkage maps of Triticeae species does not provide evidence for chromo-

Table 2 Probes detecting loci located in non-colinear positions on the hexaploid wheat group-6 consensus physical map and Triticeae linkage maps

Linkage map	Probes detecting non-colinear loci	Number of loci mapped with common probes	Reference
Triticeae group-6 consensus	ksuG48[a], TAM10[a, b]	30	Marino et al. (1996)
T. aestivum group-6 consensus	ksuG8[a], PSR301[a]	16	Jia et al. (1996)
T. turgidum 6A	cMWG653[b], ksuG48[a], TAM10[a, b]	37	Du and Hart (1998)
T. turgidum 6B	ksuG8[a], PSR106[a, b] TAM10[a, b]	26	Du and Hart (1998)
T. monococcum $6A^m$	None	13	Dubcovsky et al. (1996)
T. tauschii $6D^t$	ksuG8[a]	12	Gill et al. (1991b)
H. vulgare 6H consensus	MWG514	20	Qi et al. (1996)
S. cereale 6R	PSR106[a, b]	5	Devos et al. (1993)
Totals	7	159	

[a] Low-copy number probe
[b] Loci mapped with the probe are located in different chromosome arms

somal rearrangements among the species. [As noted above, however (see Results), *Xpsr964–6D* was mapped distal to *Xpsr8*(*Cxp3*)*-6D* and *XksuG48–6D* by Marino et al. (1996) and *Xpsr964–6B* was mapped proximal to *Xpsr8*(*Cxp3*)*-6B* and *XksuG48–6B* in this study, and the cause of this difference is unknown.]

Distribution of recombination

As just noted, a high degree of colinearity among the physical maps produced in this study and linkage maps of Triticeae species is apparent. The distribution of the loci on the physical maps differs markedly from that found on linkage maps of Triticeae species, however. A clustering of loci in centromeric regions is common on Triticeae linkage maps, while 90% of the loci analyzed in this study were mapped in either the distal one-half or the distal two-thirds of the six chromosome arms. These differing distributions of loci are consistent with the relatively low frequency of recombination in the proximal regions of chromosome arms and the relatively high frequency of recombination in the distal regions that has been demonstrated in many studies of Triticeae species, including those of Dvorak and Chen (1984); Curtis and Lukaszewski (1991); Lukaszewski (1992); Werner et al. (1992a); Gill et al. (1993a); Alonso-Blanco et al. (1993); Mickelson-Young et al. (1995); and Gill et al. (1996a,b).

Usefulness of deletion lines and future studies

Deletion mapping, like aneuploid mapping, requires intergenomic polymorphism among orthologous loci while linkage mapping required polymorphism at individual loci among the parents of a segregating population. Consequently, some RFLP loci that could not be mapped in this study (due to the lack of intergenomic polymorphism) have been mapped in segregating populations and, conversely, some loci mapped in this study have not been mapped in a segregating population (due to the lack of segregating polymorphism). A combination of the two methods allows mapping a greater number of loci. Segregating populations are easier to produce than deletion lines but the latter have the advantage that the physical positions of loci can be unambiguously determined.

The results of this study demonstrate that the relative FLs of deletion-containing chromosome arms of closely similar length cannot always be determined accurately with the light microscope. Consequently, both light microscopic examination of C-banded chromosomes and physical mapping are desirable for this to be accomplished. To identify and order new deletion lines, it will be useful to select one or more molecular markers for each available deletion line and use them as landmarks for the line. These landmarks should have stable and strong hybridization signals, be easy to use, and detect one locus per homoeologue.

Werner et al. (1992), Gill and Gill (1993a), and Gill et al. (1996a,b) have discussed the potential of deletion lines for facilitating map-based cloning in wheat. Integration of linkage and physical maps allows estimating the frequency and distribution of recombination in defined chromosomal regions as well as the amount of DNA per cM. It is apparent that the ratios between cMs and megabases of DNA differ greatly in different regions in Triticeae chromosomes, with a higher ratio in distal as opposed to proximal regions of chromosome arms. This has important ramifications for gene cloning, making it apparent that cloning will be easier in the gene-rich distal regions of chromosomes than in proximal regions. Also, the information derived from the study of deletion maps can be used as a starting point in constructing high-resolution large-insert physical maps.

Acknowledgments We are grateful to Dr. B.S. Gill of Kansas State University for supplying us with seed stocks of the deletion lines used in this study. The research was supported by the Texas Agricultural Experiment Station and by a grant from the United States Department of Energy – National Science Foundation – USDA Joint Program. We are grateful to the Coordination Office of the International Triticeae Mapping Initiative at the University of California, Davis, for its role in procuring and managing the grant and for providing a forum for collaboration and discussion.

References

Alonso-Blanco C, Goicoechea RG, Roca A, Giraldez R (1993) A cytogenetic map of the entire length of rye chromosome 1R, including one translocation breakpoint, three enzyme loci and four C-bands. Theor Appl Genet 85:735–744

Anderson JA, Sorrells ME, Tanksley SD (1992) Development of a chromosome arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bartels D, Kim JD, Thompson RD (1983) The characterization of cDNA clones coding for wheat storage proteins. Nucleic Acids Res 11:2961–2977

Baulcombe DC, Barker RF, Jarvis MG (1987) A gibberellin-response wheat gene has homology to yeast carboxypeptidase Y. J Biol Chem 262:13726–13735

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

Close S, Kortt AA, Chandler PM (1989) A cDNA comparison of dehydration-induced protein (dehydrins) in barley and corn. Plant Mol Biol 13:95–108

Curtis CA, Lukaszewski AJ (1991) Genetic linkage between C-bands and storage protein genes of tetraploid wheat. Theor Appl Genet 81:245–252

Delaney D, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995a) Cytologically based physical maps of group-2 chromosomes of wheat. Theor Appl Genet 91:568–573

Delaney D, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995b) Cytologically based physical maps of group-3 chromosomes of wheat. Theor Appl Genet 91:780–782

Devey ME, Hart GE (1993) Chromosomal localization of intergenomic RFLP loci in hexaploid wheat. Genome 36:913–918

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

Du C, Hart GE (1998) *Triticum turgidum* L. 6A and 6B recombinant substitution lines: extended linkage maps and characterization of residual background alien genetic variation. Theor Appl Genet 96:645–653

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

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

Endo TR (1988) Induction of chromosome structural changes by a chromosome of *Aegilops cylindrica* L. in common wheat. J Hered 79:366–370

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Endo TR, Mukai Y (1988) Chromosome mapping of a *speltoid* suppression gene of *Triticum aestivum* L. based on a partial deletion in the long arm of chromosome 5A. Jpn J Genet 63:501–505

Endo TR, Mukai Y, Yamamoto M, Gill BS (1991) Physical mapping of a male-fertility gene of common wheat. Jpn J Genet 66:291–295

Gill KS, Gill BS (1994) Mapping in the realm of polyploidy: the wheat model. BioEssays 16:841–846

Gill BS, Friebe B, Endo TR (1991a) Standard karyotype, nomenclature system for the 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 (1993a) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374–381

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

Gill KS, Gill BS, Endo TR, Boyko EV (1996a) Identification and high-density mapping of gene-rich regions of group-5 chromosomes of wheat. Genetics 143:1001–1012

Gill KS, Gill BS, Endo JR, Taylor T (1996b) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

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

Graner A, Bauer E, Kellermann A, Kirchner S (1994) Progress in RFLP map construction in winter barley. Barley Genet Newslett 23:53–59

Guiltinan MJ, Marcotte WR, Quatrano RS (1990) A plant leucine zipper protein that recognizes an abscisic acid response element. Science 250:267–271

Hart GE (1997) Homoeologous group 6. In: McGuire PE, Qualset CO (eds) Progress in genome mapping of wheat and related species. Joint Proc of 5th and 6th Public Workshops of ITMI (September 1–3, 1995, Norwich, UK; August 30–31, 1996, Sydney, Australia), Genetic Resources Conservation Program, UC Davis, California, USA, pp 89–105

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of an RFLP map for barley (*Hordeum vulgare*). Genome 34:437–447

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

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD (1996) RFLP-based maps of the homoeologous group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. Theor Appl Genet 92:559–565

Kleinhofs A, Kilian A, Saghai Maroof M, Biyashev RM, Hayes PM, Chen F, Lapitan N, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman O, Skadsen R, Steffenson BJ (1993) A molecular, isozyme, and morphological map of the barley genome. Theor Appl Genet 86:705–762

Kota RS, Gill KS, Gill BS (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548–554

Liu YZ, Tsunewaki K (1991) RFLP analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Lukaszewaksi AJ (1992) Comparison of physical distribution of recombination in 1R in diploid rye and hexaploid triticale. Theor Appl Genet 83:1048–1053

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39:359–366

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

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, vol. 5. University Extension Press, University of Saskatchewan, Manitoba, Canada, pp 235

Metz AM, Timmer RT, Browning KS (1992) Isolation and sequence of a cDNA encoding the cap binding protein of wheat eukaryotic protein synthesis factor 4F. Nucleic Acids Res 20:4096

Mickelson-Young L, Endo TR, Gill BS (1995) A cytogenetic ladder map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007–1011

Morrison JW (1953) Chromosome behavior in wheat monosomics. Heredity 7:203–217

Mukai Y, Endo TR (1992) Physical mapping of a fertility-restoring gene against *Aegilops kotschyi* cytoplasm in wheat. Jpn J Genet 67:199–207

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. J Hered 81:290–295

Mukai Y, Endo TR, Gill BS (1991) Physical mapping of the 18S-26 S rRNA multigene family in common wheat: identification of a new locus. Chromosoma 100:71–78

Nelson JC, Sorrells ME, van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD (1995) Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, 7. Genetics 141:721–731

Patrick JG, Dvorak J (1990) Selective enrichment of cDNA from salt-stress-induced genes in the wheatgrass. Gene 95:173–177

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley maps. Genome 39:379–394

Raines CA, Longstaff M, Lloyd JC, Dyer TA (1989) Complete coding sequence of wheat phosphoribulokinase: development and light-dependent expression of the mRNA. Mol Gen Genet 220:43–48

Sears ER (1953) Nullisomic analysis in common wheat. Am Nat 87**:**245–252

Sears ER (1954) The aneuploids of common wheat. Missouri Agric Exp Stn Res Bull 572

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

Werner J, Endo TR, Gill BS (1992a) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311

Werner J, Kota RS, Gill BS, Endo TR (1992b) Distribution of telomeric repeats and their role in the healing process of broken chromosome ends in wheat. Genome 35:844–848

Yamamori M, Nakamura T, Endo TR (1994) Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor Appl Genet 89:179–184

Zhang HB, Wing RA (1997) Physical mapping of the rice genome with BACs. Plant Mol Biol 35:115–127