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Cytologically based physical maps of the group-2 chromosomes of wheat

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Abstract We have constructed cytologically based physical maps (CBPMs), depicting the chromosomal distribution of RFLP markers, of the group-2 chromosomes of common wheat (*Triticum aestivum* L. em Thell). Twenty-one homozygous deletion lines for 2A, 2B, and 2D were used to allocate RFLP loci to 19 deletion-interval regions. A consensus CBPM was colinearily aligned with a consensus genetic map of group-2 chromosomes. The comparison revealed greater frequency of recombination in the distal regions. Several molecularly tagged chromosome regions were identified which may be within the resolving power of pulsed-field gel electrophoresis. The CBPMs show that the available probes completely mark the group-2 chromosomes, and landmark loci for sub-arm regions were identified for targeted-mapping.

Key words RFLPs · Deletion mapping · Recombination · Cereals

Introduction

Considerable progress has been made in recent years towards constructing genetic linkage maps of the 21 wheat (*Triticum aestivum*, 2n = 6x = 42, genomes AABBDD) chromosomes. Linkage maps compiled from RFLP, isozyme and seed protein data have been published for all the chromosome groups of wheat (Devos and Gale 1993), as well as for the diploid D-genome progenitor of wheat, *T. tauschii* (Gill et al. 1991; Lagudah et al. 1991). In the wheat maps, the markers are largely clustered around the centromere, indicating that there is more recombination in the distal portions of the chromosomes. This phenomenon has been noted in several studies among different cereal crops (Linde-Laursen 1979; Dvorak and Chen 1984; Curtis and Lukaszewski 1991; Lukaszewski 1992). In addition, physical mapping experiments using in situ hybridization, C-banding polymorphisms, and other cytogenetic techniques, have revealed that markers which gentically map near the centromere are physically located at a considerable distance from it (Dvorak et al. 1984; Lawrence and Appels 1986; Tsujimoto and Noda 1990). This lack of correlation between physical and genetic distance, points to the need for more detailed physical maps for use in chromosome walking and gene-cloning efforts.

Methods for constructing physical maps can be classified into two basic categories: molecularly based and cytogenetically based. Molecularly based methods include the construction of contigs using libraries of large genomic fragments, and long-range restriction mapping using rare cutting enzymes (Cheung et al. 1991). These methods are useful for fine-structure mapping of small areas of the genome, but are not feasible for physical mapping of the entire wheat genome. Cytogenetically based methods include in situ hybridization (reviewed in Jiang and Gill 1994), gentic mapping between polymorphic chromosome markers such as C-bands (Linde-Laursen 1979), and deletion mapping Werner et al. 1992). At the present time, only deletion mapping offers a practical method for constructing whole-genome physical maps.

We have undertaken the construction of cytologically based physical maps (CBPMs) of the 21 chromosomes of bread wheat. The general strategy was outlined previously (Werner et al. 1992; Gill et al. 1993). A CBPM consists of an idiogram of a chromosome depicting C-banding patterns, deletion breakpoints, and molecular markers allocated to each deletion interval. A CBPM can be aligned to a genetic linkage map for the same chromosome to compare marker distribution and variations in cM/bp along the chromosome.

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Apart from the possibilities of map-based cloning, wheat CBPMs have already provided important insights into cereal chromosome structure (reviewed in Gill and Gill 1994). Our recent work with the construction of CBPMs of wheat chromosomes group 4 (Mickelson-Young et al. 1995), 6 (Gill et al. 1993), 7 (Werner et al. 1992; Hohmann et al. 1994), and chromosome 1B (Kota et al. 1993), allow us to draw the following general conclusions. First, the distribution of recombination is non-random along the chromosome length, with distal regions being higher in recombination, as compared to proximal regions. Superimposed on this pattern is the occurrence of submicroscopic chromosomal regions that are hotspots of recombination and contain a high density of marker loci. There may be one or more of these regions per chromosome and they may lie in proximal or distal regions. We have calculated cM/bp values of less than 1 Mb for these regions, which may be accessible to long-range restriction mapping (Werner et al. 1992; Gill et al. 1993). It is imperative that we construct CBPMs for all the chromosomes of wheat because of the aforementioned reasons, and also because the specific patterns of recombination and marker distribution may vary. Wheat chromosome group-2 maps are reported here.

Materials and methods

Deletion lines

The deletion lines were generated from the backcross progeny of a monosomic addition line of a *T. cylindricum* (syn. *Aegilops cylindrica*) chromosome (Endo 1988). Plants carrying deleted chromosomes were identified by C-banding and were selfed to create homozygous lines (Endo and Gill 1995). A total of 21 homozygous deletion lines were generated for the group-2 chromosomes (one in 2AS, two in 2AL, five in 2BS, five in 2BL, three in 2DS and five in 2DL). The breakage point for each deleted chromosome was calculated as a fraction length (FL) of the distance from the centromere/total length of the arm, from a sample of at least five C-banded chromosomes (Mukai et al. 1990). Other aneuploid stocks used in this study were nulli-tetra (NT) stocks for each of the group-2 chromosomes (except chromosome 2A for which only a mono-2A-tetra-2D stock was available), and ditelosomic (DT) stocks for several of the group-2 chromosome arms (Sears 1966; Sears and Sears 1978).

Southern blotting

Genomic DNA of each of the deletion lines and aneuploid stocks was extracted using the CTAB method of Saghai-Maroof et al. (1984). For Southern analysis, 15 μg of each genomic DNA sample was electrophoresed through a 0.8% agarose gel and blotted onto a MSI nylon membrane. The probes used for physical mapping were generously provided from several sources. The KSU probes (D thru I) were isolated from a genomic library of *T. tauschii* and were provided by K. S. Gill. The WG, CDO, and BCD probes were isolated from genomic and cDNA libraries of wheat, oat and barley (Anderson et al. 1992), respectively, and were provided by Dr. M. E. Sorrels, Cornell University. The PSR probes are from genomic and cDNA libraries of wheat and were provided by Dr. M. D. Gale at the Cambridge Laboratory, Norwich, UK. The TAG clones were isolated from a genomic library of hexaploid wheat (Liu and Tsunewaki 1991) and were provided by Dr. K. Tsunewaki, Kyoto University. Twenty to fifty nanograms of each probe was labeled by the random primer method (Feinberg and Vogelstein 1983). After hybridization at 65°C for 1–2 days, the filters were washed in 0.12 × SSC and 0.1% SDS at 65°C. Filters were reused after stripping off the probe in a solution of 0.1 × SSC and 0.1% SDS at 95°C.

Physical mapping

The procedure for construction of the CBPMs was described by Werner et al. (1992). Data from the three physical maps were combined to create a consensus map, which was then compared to the genetic linkage map of the group-2 chromosomes of wheat (Devos et al. 1993).

Results and discussion

Deletion lines

The deletion lines with their fraction length (FL) values, which were used to construct the group-2 CBPMs, are listed in Table 1. Although far more deletions are available for 2A, the lines used were the only ones available in a homozygous condition (Endo and Gill 1995). Based on deletion breakpoints, the group-2 chromosomes can be sub-divided into 25 deletion-interval regions (Fig. 1). Taking together the deletion breakpoints and C-band markers, chromosome 2B has the best coverage, followed by 2D, whereas chromosome 2A has poor coverage.

Probes mapped

Information on the probes, the chromosomal location of the fragments to which they hybridize, the number of bands observed, the number of fragments allocated to A,

Table 1 Deletion lines and fraction-length (FL) values where breaks occurred, followed by the loss of the distal segment

Deletion lines	FL value
Chromosome 2A	
2AS-6	0.51
2AL-2	0.00
2AL-1	0.85
Chromosome 2B	
2BS-2	0.15
2BS-1	0.53
2BS-3	0.75
2BS-5	0.79
2BS-4	0.84
2BL-3	0.35
2BL-2	0.36
2BL-1	0.69
2BL-5	0.70
2BL-6	0.89
Chromosome 2D	
2DS-2	0.00
2DS-1	0.33
2DS-3	0.36
2DL-2	0.10
2DL-4	0.26
2DL-3	0.49
2DL-8	0.58
2DL-9	0.76

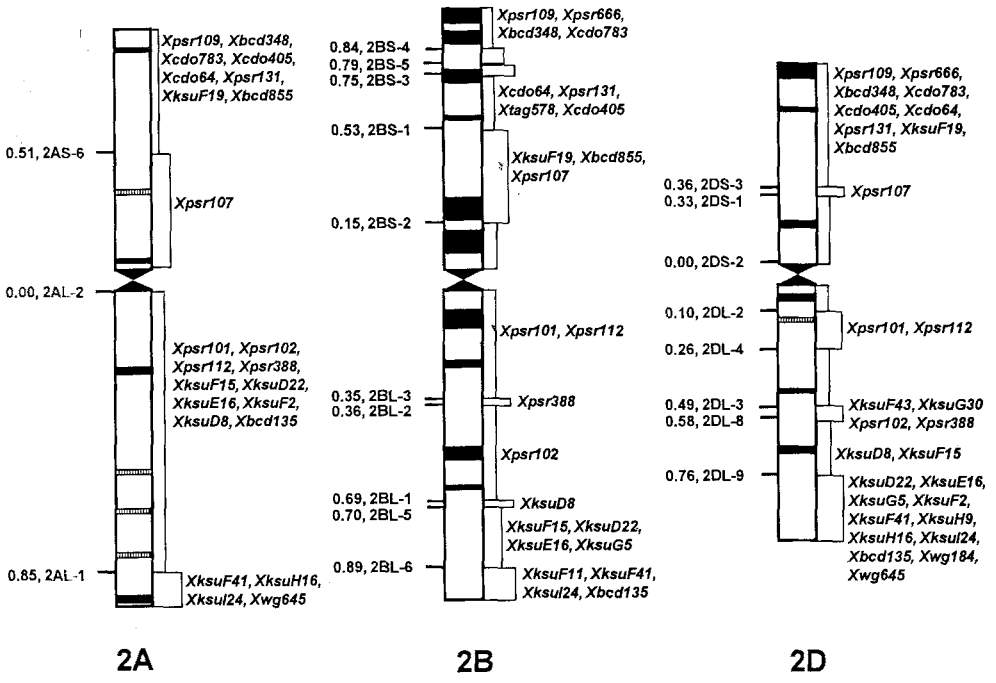

Fig. 1 Physical maps for C-banded group-2 chromosomes of wheat. Fraction-length measurements and deletion lines are shown on the left, and marker positions on the right

B or D genomes, and the informative restriction enzyme used, is given in Table 2. Overall 75 fragments were mapped with 30 probes; 23 on 2A, 24 on 2B and 28 on 2D. Thirteen probes hybridized to three fragments, one allocated to each of the 2A, 2B or 2D chromosomes (Table 2). The orthologous loci detected by these probes were mapped across A, B and D genomes (Fig. 1). These probes are single copy, appear to be highly conserved,

Table 2 Description of loci mapped with deletion stocks

Locus	Chromosome location	No. of fragments	No. of scorable fragments			Enzyme
			A	B	D	
Xbcd348	2S	6	3	1	1	*Hin*dIII
Xbcd855	2S	3	1	1	1	*Eco*RI
Xcdo064	2S	6	1	1	1	*Hin*dIII
Xcdo405	2S	3	1	1	1	*Eco*RI
Xcdo783	2S	9	2	1	2	*Hin*dIII
XksuF19	2S	4	1	1	1	*Hin*dIII
Xpsr107	2S	3	1	1	1	*Hin*dIII
Xpsr109	2S	15	2	4	3	*Hin*dIII
Xpsr131	2S	3	1	1	1	*Hin*dIII
Xpsr666	2S	2	0	1	0	*Hin*dIII
		3	0	0	1	*Eco*RI
Xtag578	2S	3	0	1	0	*Eco*RI
Xbcd135	2L	3	1	1	1	*Hin*dIII
XksuD8	2L	3	1	1	1	*Hin*dIII
XksuD22	2L	3	1	1	1	*Hin*dIII
XksuE16	2L	3	1	1	1	*Hin*dIII
XksuF2	2L	36	1	1	3	*Hin*dIII
XksuF11	2L	5	0	1	0	*Hin*dIII
XksuF15	2L	5	2	1	1	*Hin*dIII
XksuF41	2L	6	1	2	1	*Hin*dIII
XksuG5	2L	6	0	1	3	*Hin*dIII
XksuG30	2L	6	0	1	1	*Hin*dIII
XksuH9	2L	14	0	0	2	*Hin*dIII
XksuH16	2L	4	1	1	1	*Hin*dIII
Xksu124	2L	8	1	2	1	*Hin*dIII
Xpsr101	2L	3	1	1	1	*Hin*dIII
Xpsr102	2L	3	1	1	1	*Hin*dIII
Xpsr112	2L	3	1	1	1	*Hin*dIII
Xpsr388	2L	3	1	1	1	*Hin*dIII
Xwg184	2L	6	0	0	1	*Hin*dIII
Xwg645	2L	2	1	0	1	*Eco*RI

and identify excellent markers for comparative mapping across the Triticeae and probably beyond.

Another nine probes, although they detected anywhere from 4–36 fragments, were also used for mapping across the 2A, 2B and 2D chromosomes. However, not all fragments could be mapped on the group-2 chromosomes, and some may be located on other chromosomes. WG645 detected two fragments, one each on 2A and 2D and a null locus on 2B. The remaining eight probes did not detect loci across the three genomes.

Physical maps

The CBPMs of group-2 chromosomes are presented in Fig. 1. One or more fragments were allocated to 19 of the 25 deletion-interval regions. The relative position of the markers across homoeologous chromosomes, where such comparisons are feasible, appears to be conserved. However, the relative position of *Xpsr388* in chromosomes 2B and 2D differs by at least 14% (0.35–0.36 FL in 2BL vs 0.49–0.58 FL in 2DL) which is above the standard error of $\pm 6\%$. The reason for this discrepancy is not clear at present, but could be due to a small inversion in either 2B or 2D.

The MTCRs

The MTCRs are defined as molecularly tagged chromosome regions that are submicroscopic in size (0.1 FL or less) and are marked by one or more loci. Two MTCRs were identified in 2BL: one at FL 0.35–0.36 tagged by *Xpsr388*, and another one at FL 0.69–0.70 and tagged by *XksuD8*. Two potential MTCRs may exist in chromosome 2D: one in 2DS at FL 0.33–0.36, and another in 2DL at FL 0.49–0.58 (three markers allocated at an average distance of 0.03FL). When the relative positions of all the deletion breakpoints were indicated on a single consensus map, one additional MTCR became apparent at FL 0.51–0.53 marked by probes *XksuF19* and *Xbcd855*. Based on calculations presented elsewhere (Werner et al. 1992), MTCRs may be within the resolving power of pulsed-field electrophoresis.

Physical vs genetic map distance

A comparison of the consensus physical map for the group-2 chromosomes prepared in this study and the consensus genetic map of the group-2 wheat chromosomes presented by Devos et al. (1993) is shown in Fig. 2.

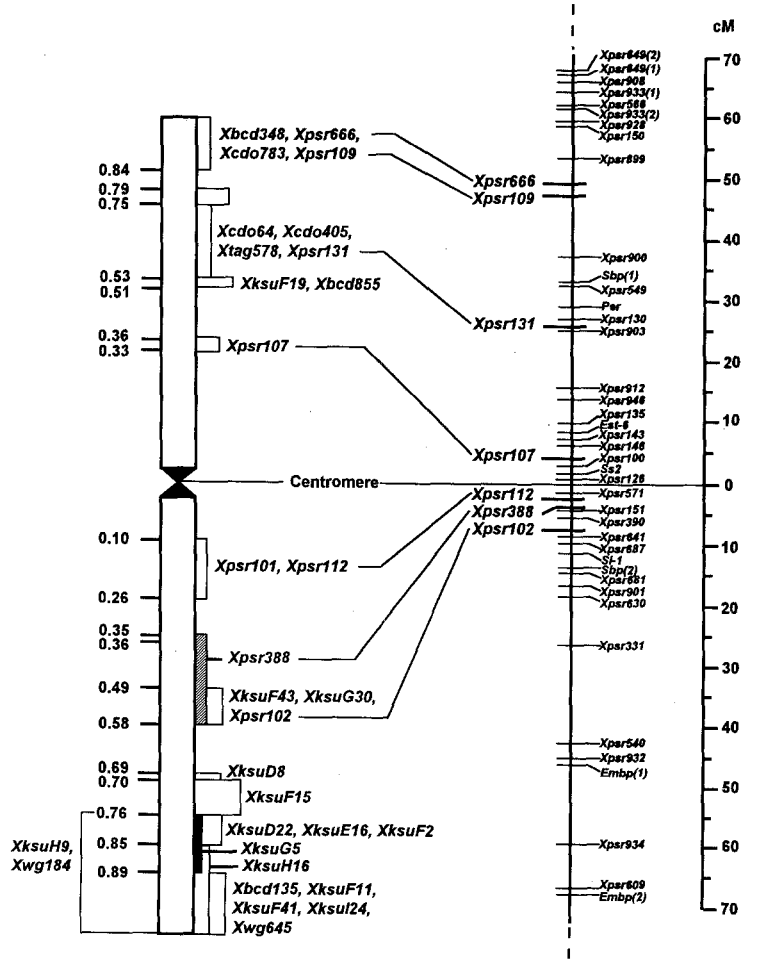

Fig. 2 Comparison of the consensus physcial map of the group-2 chromosomes of wheat (left) with the consensus genetic linkage map of wheat group-2 chromosomes. The scale in cM is indicated on the far right

Table 3 Comparison of subarm regions for recombination frequencies in group-2 chromosomes of wheat

Chrom. arm	FL interval	Length in FL	Approximate genetic distance (cM)	Genetic distance per 1% FL
2S	0–0.33	0.33	4	0.12
	0.33–0.84	0.51	42	0.82
	0.84–1.0	0.16	22	1.38
	0–1.0	1	68	0.68
2L	0–0.35	0.35	3	0.09
	0.35–0.54	0.19	4	0.21
	0.54–1.0	0.46	58	1.26
	0–1.0	1	65	0.65

The relative distances between commonly mapped markers provides information on the physical and genetic linkage lengths within that chromosomal region. The proximal 1/3rd of the 2S arm has a recombinational length of only 3 cM and the proximal 50% of the 2L arm has a genetic linkage length of only 7 cM. Based on the 2L genetic linkage data (Devos et al. 1993), it is most likely that markers *XksuF19* and *Xbcd855* in 2S (FL 0.51–0.53) are no more than 7 cM from the centromere. Considering that the length of each arm is 70 cM at a minimum, the recombination rate in the distal 50% of each arm is ten times greater than the proximal 50% of each arm. These data further confirm the inescapable conclusion that recombination is suppressed in the proximal regions surrounding the centromeres of wheat chromosomes (Werner et al. 1992; Gill et al. 1993). Dividing genetic distance (Devos et al. 1993) by the length of the region in FL, the average recombinational distances corresponding to 1% FL were calculated for each of the regions. The data in Table 3 show that recombination frequencies in the distal ends are at least ten-times higher than in the proximal regions of group-2 chromosomes.

Landmark loci

Considering the large genome size of wheat, it is our long-term objective to identify landmark loci for appropriate subregions of chromosome arms. The landmark loci must map across the three genomes and not have duplicate loci elsewhere. They should mark designated regions of chromosome arms. Considering the low frequency of recombination, a landmark locus located at FL 0.5 will be sufficient for many breeding and marker-based selection schemes to mark the proximal 75% of each arm. One landmark locus will be needed to mark each end of a chromosome arm. The number of landmark loci needed between FL 0.75 and 1.0 will depend on the genetic linkage length of those regions. Based on these considerations, some of the probes and deletion stocks that identify landmark loci for specific regions are listed (Table 4). Undoubtedly, there will be additions to the above list in the future, but the list in Table 4 will be useful for targeted-mapping of specific genes to specific regions of group-2 chromosomes.

Table 4 Loci and deletion stocks used to identify landmark loci for short and long arms of group-2 chromosomes of wheat

Locus	Arm location	FL position[a]	Diagnostic deletion stock[b]
Xpsr107	2S	0.35	2DS-3
Xbcd855	2S	0.52	2BS-1
Xbcd348	2S	>0.84	2BS-4
Xpsr112	2L	0.18	2DL-4
Xpsr102	2L	0.54	2DL-8
XksuD8	2L	0.70	2BL-5
Xbcd135	2L	0.87	2BL-6

[a] Average of proximal and distal FL values of the intervel in which the probe is mapped
[b] Marks the distal boundary of the deletion-interval region in which a probe in mapped

Conclusions

The data on physical mapping of group-2 chromosomes confirms almost complete homoeology in terms of gene synteny among chromosomes 2A, 2B, and 2D of wheat. A large number of orthologous loci were detected across the three chromosomes in the same linear order. Nineteen of the twenty-five deletion intervals have been tagged by one or more molecular markers. The marker coverage of the chromosomes in terms of their physical position is good, especially for chromosome 2B. Thus, targeted-mapping of specific chromosomal regions containing a gene of interest should be feasible. The patterns of marker distribution and recombination are similar to those described for the group 7, 6 and 1B chromosomes of wheat.

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