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High-Resolution Radiation Hybrid Map of Wheat Chromosome 1D

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ABSTRACT

Physical mapping methods that do not rely on meiotic recombination are necessary for complex polyploid genomes such as wheat (*Triticum aestivum* L.). This need is due to the uneven distribution of recombination and significant variation in genetic to physical distance ratios. One method that has proven valuable in a number of nonplant and plant systems is radiation hybrid (RH) mapping. This work presents, for the first time, a high-resolution radiation hybrid map of wheat chromosome 1D (D genome) in a tetraploid durum wheat (*T. turgidum* L., AB genomes) background. An RH panel of 87 lines was used to map 378 molecular markers, which detected 2312 chromosome breaks. The total map distance ranged from ~3,341 $cR_{35,000}$ for five major linkage groups to 11,773 $cR_{35,000}$ for a comprehensive map. The mapping resolution was estimated to be ~199 kb/break and provided the starting point for BAC contig alignment. To date, this is the highest resolution that has been obtained by plant RH mapping and serves as a first step for the development of RH resources in wheat.

BOTH bread wheat (*Triticum aestivum* L. $2n = 6x = 42$; A, B, and D genomes) and durum wheat (*T. turgidum* ssp. *durum* L. $2n = 4x = 28$; A and B genomes) have multiple genomes with chromosomes characterized by regions differing in gene density and distribution (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Sandhu and Gill 2002). Eighty percent of the wheat genome is composed of repetitive sequences (Smith and Flavell 1975). Colinearity in the order of loci in the wheat genomes is largely conserved except for a 4A-5A-7B translocation, a putative 2B-6B translocation, and two inversions on chromosome 4A (Hossain *et al.* 2004a; Peng *et al.* 2004). Gene density in wheat tends to increase with relative distance from the centromere, with some of the highest densities observed in several distal regions of the chromosomes (Akhunov *et al.* 2003; Erayman *et al.* 2004). However, the overall relationship between gene density and relative position on the chromosome is weak (Akhunov *et al.* 2003). As in many plant species, recombination along chromosome arms in wheat is not evenly distributed, being absent in proximal regions of all arms (Gill *et al.* 1993; Lukaszewski and Curtis 1993; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Endo and Gill 1996; Sandhu and Gill 2002). Recombination rate increases with approximately the square of the relative distance of a given segment from the centromere (Akhunov *et al.* 2003). Due to this uneven distribution of recombination, significant variation in genetic to physical distance ratios along the length of a given chromosome is evident ranging from ~17 Mb/cM in the most proximal region to ~1 Mb/cM in the most distal intervals (Akhunov *et al.* 2003; Erayman *et al.* 2004). A weak correlation was observed between relative gene density and recombination rate (Akhunov *et al.* 2003; Erayman *et al.* 2004). By some estimates one-fourth to one-third of the wheat genome, primarily around the centromeres, accounts for <1% of total recombination (Lukaszewski and Curtis 1993; Akhunov *et al.* 2003; Erayman *et al.* 2004). Thus, development of high-resolution genetic maps through the use of traditional recombination methods for at least one-fourth of the wheat genome will be extremely difficult. By some recent estimates nearly 30% of wheat genes are in recombination-poor regions that are thus inaccessible to map-based cloning (Erayman *et al.* 2004).

To effectively place genes and molecular markers by methods that do not rely on meiotic recombination,

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several tools have recently been developed that assign genes to chromosomal location on the basis of physical evidence. In wheat, polyploidy buffering allows segmental deletions and the substitution of whole chromosomes, chromosome arms, and sub-arm regions from one genome into another (Sears 1954, 1966; Gill *et al.* 1993; Joppa 1993; Endo and Gill 1996). These aneuploid and deletion stocks can effectively be used to place genes and markers to any given chromosome or chromosomal region. The first set of wheat lines missing entire chromosomes or chromosome arms was first described in 1954 (Sears 1954). Later a gametocidal chromosome of *Aegilops cylindrica* Host was used to generate chromosomal deletion lines in wheat (Endo 1988). This approach usually generates deletions due to a single break and loss of the remaining chromosomal region located distal to the initial breakpoint (Endo 1990). To date, a total of 430 deletion lines involving the 21 wheat chromosomes have been reported (Endo and Gill 1996) and a selected set of these lines representing a total of 159 chromosomal bins has been used to map 8241 expressed sequence tags (ESTs) (Qi *et al.* 2004; Hossain *et al.* 2004a; Peng *et al.* 2004). These bins cover large deletions with an average size of 35 Mb and have been useful in placing molecular markers to defined physical intervals. Although this method of chromosome bin assignment is effective in placing markers to chromosomal regions, the order of the markers within a given segment is largely unknown.

For detailed analysis and sequencing of the wheat genome, the construction of an accurate, sequence-ready, global physical (BAC-contig) map anchored to a high-resolution genetic map is needed (Gill *et al.* 2004). Construction of such a map in the wheat genome through genetic recombination methods would be difficult due to the uneven distribution of recombination across the genome. Conversely, mapping to chromosomal bins has proved to be extremely valuable for localizing molecular markers in wheat but cannot provide accurate region-specific ordering of these markers. Radiation hybrid (RH) mapping is another recombination-independent mapping approach that has been particularly successful in other organisms for high-resolution ordering of molecular markers. This method has been effectively applied to mapping human chromosomes as well as those of other animals (Cox *et al.* 1990). RH mapping was first described by Goss and Harris (1975, 1977) and subsequently by Benham *et al.* (1989) and Cox *et al.* (1990). This method relied on irradiating donor cells and fusing them with recipient cells. Thus, the irradiated chromosomes could be studied in the background of the recipient's cells (radiation hybrids). A panel of radiation hybrids with random breaks on the chromosomes of the donor can be used to precisely place and order molecular markers or genes along a chromosome or entire genome (Cox *et al.* 1990). An advantage of RH mapping is that different doses of radiation can be used to construct maps with varying levels of resolution (Gyapay *et al.* 1996; Lunetta *et al.* 1996; Schuler *et al.* 1996; Stewart *et al.* 1997). This work has been followed by RH mapping in a number of other animal species (McCarthy *et al.* 1997; Priat *et al.* 1998; Yerle *et al.* 1998; Hawken *et al.* 1999; Hukriede *et al.* 1999; Kwok *et al.* 1999; Murphy *et al.* 1999; Vignaux *et al.* 1999; Watanabe *et al.* 1999). In addition to whole-genome RH mapping, other researchers have focused on mapping specific chromosomes and chromosomal regions in different species as well (Hoover and Floros 1998; Barendse *et al.* 2000; Ozawa *et al.* 2000; Liu *et al.* 2002; Rink *et al.* 2002).

The first plant RH panel was constructed by irradiating maize chromosome 9 in an oat background (oat–maize addition lines) with 30-, 40-, and 50-krad γ-rays and subsequent characterization of these lines with maize-specific molecular markers (Riera-Lizarazu *et al.* 1996, 2000; Ananiev *et al.* 1997). Using a combination of maize-specific probes, the average maize marker retention frequency was estimated to be 85, 83, and 75% for the 30-, 40-, and 50-krad treatments, respectively. Subsequently, the maize chromosome 1 RH panel was developed and used to map 45 simple-sequence repeat (SSR) markers (Kynast *et al.* 2004). This work has been followed by generation of whole-genome RH panels in other species, which include barley (Wardrop *et al.* 2002, 2004) and cotton (Gao *et al.* 2004). All these reported RH maps in plants are of low to medium resolution. Recently we described the molecular cytogenetic characterization of an alloplasmic durum wheat (A and B genomes, *A. longissimum* S. & M. cytoplasm) line containing chromosome 1D (Hossain *et al.* 2004b) harboring scs^{ae}, a gene responsible for nuclear–cytoplasmic compatibility. To further analyze the area around scs^{ae}, seed from this line was irradiated with 35-krad γ-rays and used as the basis for generating a durum wheat radiation hybrid panel of 87 RH lines (DWRH-1D) containing various fragments of chromosome 1D (Hossain *et al.* 2004c). Initial analysis of the DWRH-1D panel with 39 molecular markers that included RFLP, SSR, and EST markers revealed 88 radiation-induced breaks on the 1D chromosome. In this study, we expand this initial analysis by assaying the DWRH-1D panel with a total of 378 markers and generating a map with a resolution of ~199 kb/break. This is the first published report of such a high-resolution genetic map of a plant chromosome using the RH mapping approach.

MATERIALS AND METHODS

Production of radiation hybrid lines: A detailed description of population development is provided in Hossain *et al.* (2004b,c). Briefly, a previously characterized aneuploid alloplasmic durum wheat line with the majority of chromosome 1D (where only a small portion of the long arm of chromosome 1D was replaced by homeologous counterpart from chromosome 1A) was used for generating the durum wheat radiation hybrid 1D (DWRH-1D) panel. This line is male

sterile and is maintained by crossing back as female parent to cultivated durum wheat. Selection for the *scs*^{ae} gene on chromosome 1D is used to guarantee the presence of at least a portion of 1D in recovered plants. Seeds from this line were irradiated with 35 krad of γ-rays. Plants that grew from irradiated seed were subsequently crossed to normal durum wheat to restore the A and B genomes. Subsequent generations of the DWRH-1D panel have been assayed with molecular markers to assess stability of the 1D fragment. Results reveal that the 1D fragment is stable over generations (Hossain *et al.* 2004b,c) Therefore, the absence of any marker in the DWRH-1D panel is the result of deletions in chromosome 1D due to irradiation.

DNA extraction and amplification: Genomic DNA extraction and marker assays were performed according to the methods of Hossain *et al.* (2004c). Analysis of EST and SSR amplifications was carried out on nondenaturing polyacrylamide gel electrophoresis using ethidium bromide staining.

Molecular markers: *AFLP markers:* Eleven AFLP primer combinations were used yielding a total of 209 informative markers. A Beckman CEQ 2000XL automated DNA sequencer (Beckman, Alameda, CA) was used for analysis of the fluorescent AFLP (fAFLP) markers according to the protocol of the manufacturer. Labeled *Eco*RI primers were obtained from Proligo (Boulder, CO). Components of the AFLP kit manufactured by Invitrogen (Carlsbad, CA) were used. A list of the AFLP marker loci used in this study is provided as a supplemental document (supplemental Table 1 at http://www.genetics.org/supplemental/). This list does not include AFLP loci that were retained in all samples. Fragments retained in all samples do not provide mapping information and are of no value in the current analysis.

RFLP, SSR, and EST markers: RH data from 39 RFLP and EST loci from our previous work (Hossain *et al.* 2004b,c) were included in the present study. This expanded analysis included a total of 378 molecular markers (209 fAFLP, 24 RFLP and SSR markers previously placed on the genetic map, and 134 ESTs previously localized onto group 1 homeologous chromosomes on the wheat deletion map).

Map construction: Analysis of the RH data was carried out by using Carthagene 0.999 (http://www.inra.fr/bia/T/Carthagene). Carthagene was selected for its ability to handle >350 markers, its user-friendly graphics interface, and its computing efficiency. Carthagene analyzes RH data by using an equal-retention model and computes all two-point distance information when the data set is initially loaded. Linkage groups were determined using the group command at a two-point LOD of 4.0 and a distance of 100 cR. The heap command was used to identify the map with the best likelihood.

The total number of obligate chromosome breaks was determined using the mapocb command for each data set, for each individual, and for each pair of consecutive markers in the map. An obligate chromosome break is declared if a 0 or 1 (or 1 and 0, where 0 is absent, 1 is present) is adjacent in one individual using a given map order. This is analogous to multipoint linkage map analysis based on genetic recombination in which there is no need for having 150 segregating loci to generate a 150-cM map of a chromosome or identify 150 recombination points.

Since the linkage groups as well as unlinked markers all belong to chromosome 1D, we generated a lower-confidence comprehensive map that included all markers in this study. This map included all the markers analyzed by using a two-point LOD of 2.0.

RESULTS

Radiation-induced breakages in chromosome 1D: Analysis of the DWRH-1D panel with the 378 markers indicated that 77% of the 87 individual lines had lost at least 1 marker. The number of markers missing per individual ranged from 18 to 134. Of a total of 378 markers, ~96% were lost at least once. The markers most frequently lost were BE590719 and BE518221. There were two regions of high marker retention reported in our previous study (Hossain *et al.* 2004c), one on the long arm and one near the telomere of the short arm. In this study we did detect the loss of BE444505, a marker previously placed at the proximal end of the retained region on the long arm. The markers—BCD1434, CDO388, BCD98, and MWG68 on the telomeric end of the short arm, and CDO98, BCD338, BCD921, BE490430, BE403322, BE442876, and BE443720 on the long arm of chromosome 1D—were retained in all lines. We did not analyze AFLP markers that were retained in all the lines as they did not provide useful information for this study. The high marker retention region on the long arm of chromosome 1D corresponds to the location of *scs*^{ae} that is linked to seed plumpness that was selected for during RH population development (Hossain *et al.* 2004c). The other region of high marker retention identified by the markers on the telomere of the short arm is curious because if we assume random distribution of markers, and therefore random retention frequencies for the rest of the markers, this region was not subjected to positive selection during panel development. This suggests that there exists the possibility of another gene that may contribute to seed plumpness, gametic selection, or an unknown factor in this region of chromosome 1D. Average marker retention frequency in the DWRH-1D panel assessed against the 378 markers was 74%. This value is lower than that previously reported by Hossain *et al.* (2004c) since AFLP marker loci (which represent the majority of the newly added information) that were always retained were excluded from analysis. However, this retention frequency is similar to that observed in other plant RH panels, such as maize and cotton. These frequencies may reflect the use of seed or pollen as the target of the radiation *vs.* the somatic cells frequently used in the mammalian and nonmammalian systems where retention frequencies ranging from 5 to 45% have been reported (Wardrop *et al.* 2002, 2004; Kynast *et al.* 2004).

Chromosome breakage analysis: The resolution of the RH map can be assayed by the distribution of the markers. Assuming random distribution, 368 of the 378 markers used in this study identified at least one break along chromosome 1D, and therefore the resolution of the RH map can be directly assayed by estimating the average distance between breaks. With 39 markers used in our previous study (Hossain *et al.* 2004c), 88 breaks were identified. In this study with 378 markers, we identified a total of 2312 obligate breaks, a 26-fold increase. The RH map resolution (average distance between breaks) was estimated in our previous study to be 5.4 Mb (size of chromosome/total breaks = 464 Mb/88

TABLE 1

Characteristics of five major linkage groups on chromosome 1D derived from DWRH-1D panel

Linkage groups	No. of markers	Marker type	Two-point LOD range			Total distance (cR)
			Lowest	Highest	Average	
A	25	EST	3.3	13.0	7.7	263.1
B	26	AFLP	2.6	12.5	7.1	704.6
C	34	AFLP	3.2	12.9	8.3	792.4
D	17	AFLP	4.8	12.0	8.3	451.8
E	56	AFLP	1.1	13.7	5.9	1129.0
Total	158					3340.9

The group command in Carthagene 0.999 was used to produce linkage groups at a distance of 100 cR and a two-point LOD score of 4.0. Four of the linkage groups (groups B–E) consisted only of AFLP markers, while one of the groups consisted only of EST markers (group A). The lowest two-point LOD, highest two-point LOD, and the average two-point LOD obtained per given marker pair in each linkage group are listed in the two-point LOD range column. Total distance indicates the distance covered by each linkage group.

breaks). In the present study, our average distance between breaks is ~199 kb/break, or a 27-fold increase in resolution. Therefore, increasing the total number of markers assayed on the panel has allowed detection of more breaks and greater map resolution.

Linkage analysis: The group command in Carthagene was used to generate linkage groups. Considering the size of this chromosome and map resolution determined by the number of obligate breaks, >2300 molecular markers would be needed to completely cover chromosome 1D. Since the total number of markers included in this study was only ~16% of this value, we expected that a number of linkage groups and unlinked markers would result from this RH mapping experiment. As expected with 378 markers a complete coverage of this chromosome by a single linkage group was not accomplished and we identified a total of 205 linkage groups and unlinked markers at a two-point LOD score of 4.0. Among these, there were 5 major linkage groups consisting of a total of 158 markers (~42% of all markers used) and 14 linkage groups with 2–4 markers totaling 33 additional markers (~9%). A total of 187 (~49%) markers were unlinked. Table 1 shows the distribution of the markers in the 5 major linkage groups. Four of these linkage groups, groups B, C, D, and E, consisted entirely of AFLP markers, whereas group A consisted entirely of ESTs (detailed information on these groups is presented in supplemental Tables 2–5 at http://www.genetics.org/supplemental/). These 5 linkage groups cover a total of 3341 $cR_{35,000}$ ($cR_{35,000}$ is defined as a unit of distance for a RH panel generated with 35 krad). This linkage analysis has allowed the ordering of previously binned EST markers (on the basis of the deletion mapping work, Peng *et al.*, 2004) onto our RH map without the need for polymorphism and/or large recombination-based populations (Figures 1 and 2).

Generation of a comprehensive map: Since the linkage groups as well as unlinked markers all belong to chromosome 1D, we generated a lower confidence (LOD 2.0) comprehensive map that included all markers in this study. This analysis produced a comprehensive map with a total length of 11,737 $cR_{35,000}$ (Figure 1). The five major linkage groups cover ~28.5% (3341/11,737) of this map or approximately one-fourth of the total chromosome length (~132 Mb).

Relationship between the RH map and genetic map: Selected RFLP and SSR markers, which were included in the development of a consensus wheat genetic map (Endo and Gill 1996), were chosen from our data set and the centiray distances between them were calculated using the comprehensive RH map of 11,737 $cR_{35,000}$ (Table 2). As expected, the comparison of the genetic map with RH map for chromosome 1D indicates an uneven distribution of recombination along the length of this chromosome (Table 2). On the basis of the RH analysis, on average, each centimorgan of the genetic linkage map corresponds to 3.2-Mb physical distance on this chromosome. The physical to genetic distance ratios along this chromosome ranged from 0.8 to 7.8 Mb/cM. These values are remarkably close to previous estimates based on EST mapping (Akhunov *et al.* 2003) or high-resolution recombination mapping and genome walking studies in wheat (Spielmeyer *et al.* 2000; Stein *et al.* 2000; Faris and Gill 2002; Faris *et al.* 2003; Feuillet *et al.* 2003; Huang *et al.* 2003; Olmos *et al.* 2003; Yan *et al.* 2003, 2004). The longest segment covered by markers *Xgwm232* and *Xgwm642* represents a region on the RH map containing both EST and AFLP marker classes. This large region likely covers both gene-rich and gene-poor areas due to the distribution of major marker types.

Ordering of ESTs using the RH map: To demonstrate the value of RH maps in ordering molecular markers within the large linkage groups, we used linkage group A as an example. Although all markers in this linkage group are from the chromosomal deletion bin 1DL2-0.50-0.80, the order of these markers with respect

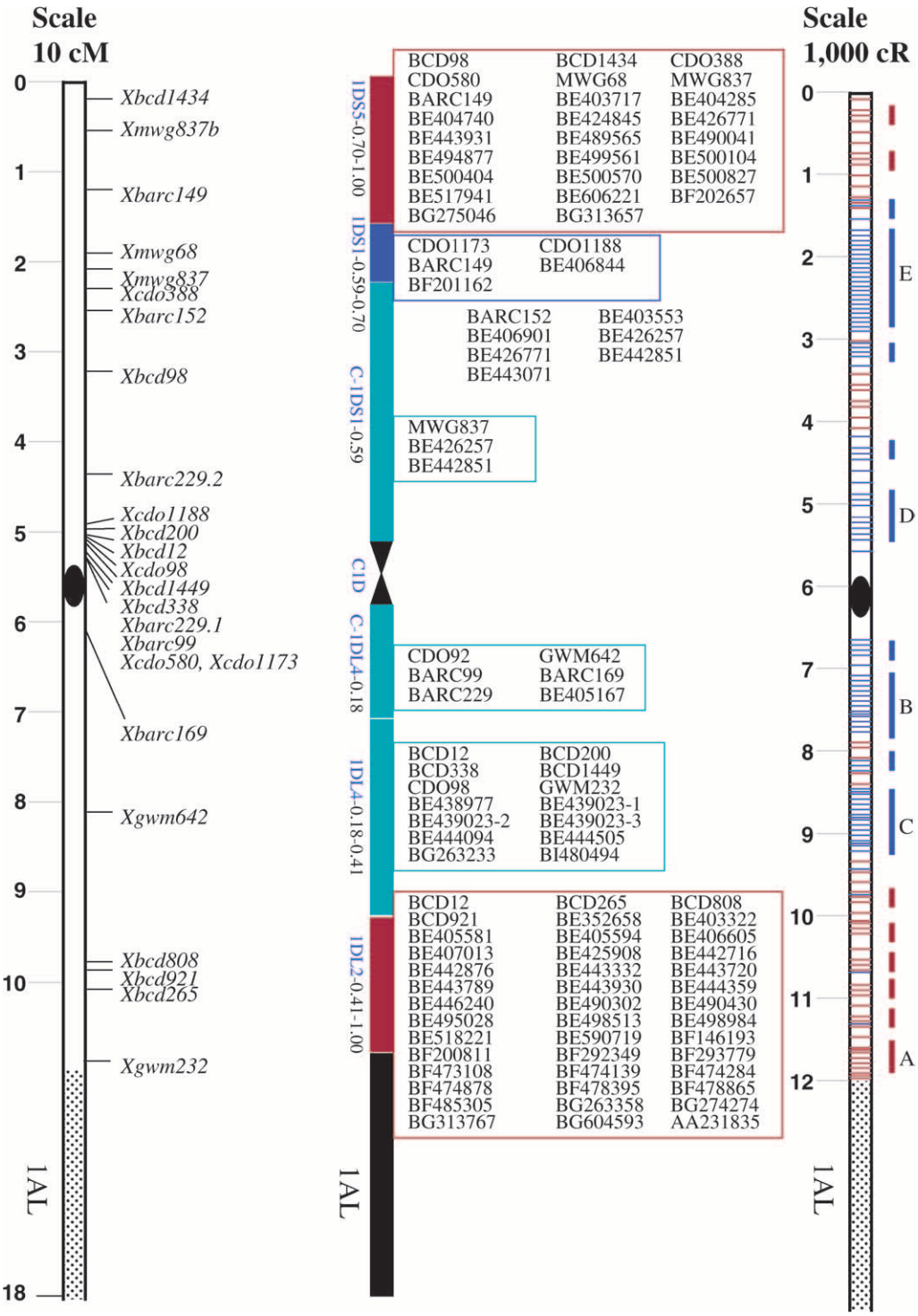
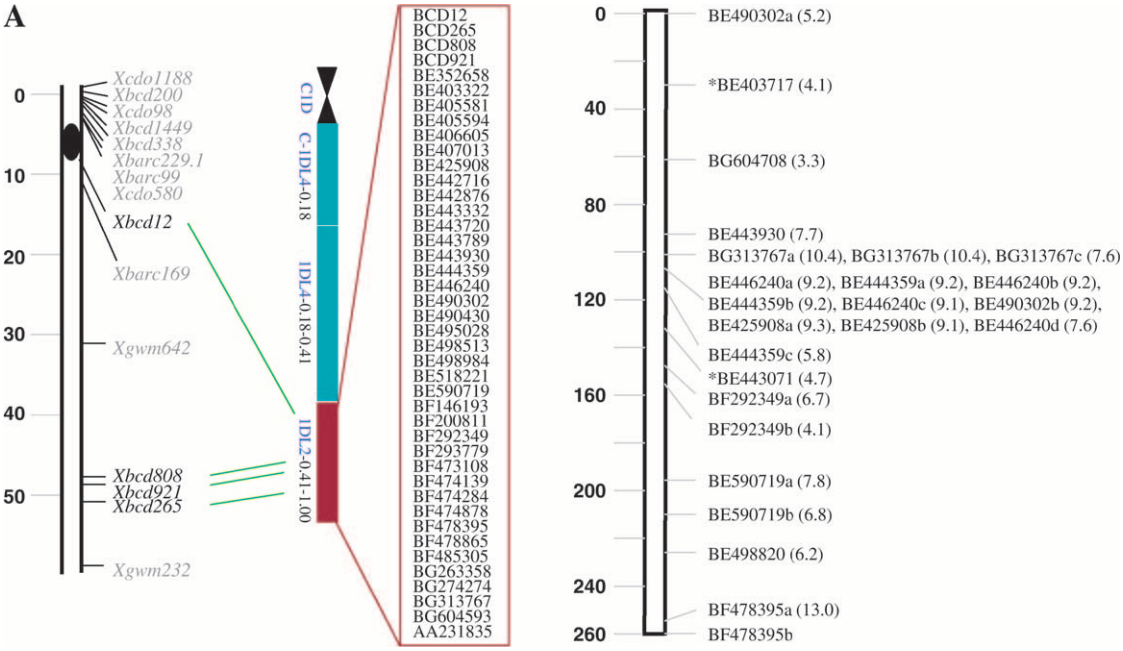


FIGURE 1.—Wheat chromosome 1D RH map (right) as compared with the genetic (left) and the deletion map (middle). The boxed markers on the deletion map refer to sequences assigned to a specific region on chromosome 1D. The nonboxed set of markers (seven total on 1DS) are assigned to a region covered by the two deletion breakpoints. Regions with high density (red), medium density (blue), and low density (teal) of mapped ESTs are identified on the deletion map (PENG *et al.* 2004). The RH map has an estimated total length of 11,737 $cR_{35,000}$ covered by 378 AFLP (marked in blue), EST, RFLP, and SSR (all marked in red) markers. The locations of major linkage groups (A–E) and minor linkage groups are indicated by lines on the right of the RH map with the major marker classes of that linkage group identified by blue (AFLP) and red (EST, RFLP, and SSR).

to each other is unknown. The RH mapping method allowed the ordering of these previously unordered ESTs (Figure 2a). This linkage group can also be used as an example of how an RH map is generated on the basis of marker retention frequencies (Figure 2b). A number of ESTs identified multiple PCR fragments, which were coretained (*e.g.*, BG313767, BE446240), possibly representing segments of the same gene or tandem duplications separated by radiation (*e.g.*, BE44359, BE292349, BE590719). For this linkage group, 2 of 84 individuals were missing the entire segment, 60 lines retained the entire segment, and the remaining 22 had different combinations of breaks within this region. Assuming breaks flanking a given marker in two different lines are not identical, a total of 36 unique breaks were evident for this region (Figure 2b). In terms of mapping, this pattern of chromosome breakage yielded a 263.1-$cR_{35,000}$ map of this linkage group.



B

Marker / Line #	BE490302a	BE403717	BG604708	BE443930	BG313767a	BG313767b	BG313767c	BE446240a	BE444359a	BE446240b	BE444359b	BE446240c	BE490302b	BE425908a	BE425908b	BE446240d	BE444359c	BE443071	BF292349a	BF292349b	BE590719a	BE590719b	BE498820	BF478395a	BF478395b	Number Obligate Breaks
186-29-3*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
186-10-2	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
186-16-3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
186-15-1	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
186-23-1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
185-8-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	1
185-23-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	1
185-1-1	+			+	+	+	+	+	+	+	+	+				+	+		+	+	+	+		-	-	1
185-43-1		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+					+	-	-	1
185-17-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	1
186-13-1	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	2
186-1-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	2
186-21-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	2
186-10-1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
186-7-1	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
185-9-4	+		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
186-11-4	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
185-26-3	+	+		+	+	+	+		+		+		+	+	+		+		+	+	-	+	+	+	+	2
186-2-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	2
186-22-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	2
186-7-5	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
186-21-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	2
186-33-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	3
185-31-2**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0

FIGURE 2.—The ability to order previously deletion-binned but unordered ESTs by RH mapping approach. (A) Comparison of maps generated by the recombination-based linkage, chromosome deletion bin, and radiation hybrid approach. EST loci placed in deletion bin 1DL2 (0.41–1.00 FL distance coverage of chromosome, middle) are part of linkage group A presented in centiray distances (right). Markers in this region cover the majority of the long arm recombination-based linkage map (left, centimorgan distances). The two-point LOD scores are shown in parentheses after the name of the EST in the RH map. Two ESTs indicated by asterisks were placed in bins other than 1DL2-0.41–1.00, but were placed within this linkage group in the RH analysis and represent possible multiple-copy ESTs or simply deletion mapping errors. (B) Actual marker retention data for this region identifying the critical lines used to determine the distance between various markers. The + sign indicates the presence of a marker in the line and the − sign (shaded boxes) indicates absence of that marker. Where marker information was not available the cell is left blank. Markers are organized according to their position on the RH map and critical breaks (shaded boxes) are clearly evident. Two individuals among the population were missing all of the markers within this linkage group (*e.g.*, line 186-29-3 genotype) and 60 individuals had all of the markers within this linkage group (*e.g.*, line 185-31-2 genotype). The total number of unique obligate breaks for each individual is indicated in the right column and totals to 36 unique breaks for this entire region.

TABLE 2

Alignment of wheat chromosome 1D consensus linkage map with the RH map

Molecular marker interval	cM	cR	Mb	Mb/cM	Major marker type
Barc229–mwg837	46.6	1326.6	51.8	1.1	ESTs
Bcd98–mwg837	12.8	1144.8	44.7	3.5	ESTs
Bcd200–cdo580	3.7	84.6	3.3	0.9	ESTs
Cdo580–barc229	9.3	259.2	10.1	1.1	ESTs
Barc 99–barc229	9.1	1272.9	49.7	5.5	ESTs
Barc99–bcd1449	1.3	161.1	6.3	4.8	ESTs
Gwm642–bcd1449	29.5	595.1	23.2	0.8	ESTs
Gwm232–gwm642	27.4	5489.9	214.2	7.8	AFLPs and ESTs
Bcd200–cdo98	1.2	94.7	3.7	3.1	ESTs
Average	15.6	1158.8	45.2	3.2	

Molecular markers used in developing wheat consensus genetic maps were used on the DWRH-1D panel and the distances between them (in centimorgans) were calculated on the basis of genetic maps. The total RH distance (in centirays) was calculated from the comprehensive RH map developed in this study. The physical distances between markers (in megabases) were calculated on the basis of estimated resolution of the comprehensive RH map.

Alignments of chromosome 1D contigs to the RH map: All group 1 contigs were extracted from the wheat physical mapping database and classified into three categories for analysis (http://wheat.pw.usda.gov/PhysicalMapping). High-priority contigs were those containing at least two or more markers that were unique and not found in any other contigs on chromosome 1D. Medium-priority contigs were those that contained markers that were also represented in other contigs. Low-priority contigs were those that contained markers present in many contigs. The last group of contigs was not included in this study due to the absence of controls for determining the markers assigned to each contig and being mapped in the present study. Given the chromosome 1D RH map resolution of ~199 kb, we were able to align six BAC contigs from the D-genome physical mapping project to the RH map (Table 3). These contigs were aligned on the basis of two to six marker loci each. Additional alignments will be readily possible as our RH map is populated with the same markers used to anchor chromosome 1D BACs. Since our comprehensive RH map is of low confidence (LOD 2.0) and the location of markers assigned to BAC contigs as well as the physical distance separating them is not precisely known, at this time exact comparison of centiray to megabase distance is not possible.

DISCUSSION

The level of radiation-induced breakage, recovery of subchromosome fragments, and ability to assay the breakage points using available marker systems without the need for marker polymorphism illustrate the power of RH mapping. We present here a high-resolution map of a single introgressed wheat D-genome chromosome in a tetraploid AABB background. The RH panel carries different subchromosome fragments of wheat chromosome 1D representing many different types of chromosome breaks in a single line (Hossain *et al.* 2004b). We initially placed 39 markers on this panel (Hossain *et al.* 2004c), and in this study we have placed 378 markers to determine their usefulness in wheat RH mapping. This targeted mapping effort was carried out by selectively placing different types of molecular markers including RFLPs, SSRs, AFLPs, and ESTs. The total RH map length ranges from 3341 $cR_{35,000}$ (for linkage groups) to 11,773 $cR_{35,000}$ (comprehensive map consisting of all markers)

TABLE 3

Alignment of chromosome 1D contigs to the RH map

Contig name	Markers used for alignment	Coretention frequency (%)
ctg605	cdo57-0.90–cdo92-0.30	97.7
ctg3467	BE500570-0.35–BE443071-0.80	86.2
ctg5162	BF293779-0.39–bcd808-0.50	89.7
ctg8420	BE423482-0.15–BE403717-0.80	83.9
ctg8420	BE494877-0.39	
ctg8420	BE403553-0.03	
ctg8420	BE406901-0.20–BE423482-0.15	83.9
ctg8420	BE406901-0.20–BE494877-0.39	86.2
ctg8420	BE403553-0.03–BE403717-0.80	82.8
ctg8420	BE403717-0.80–BE403553-1.00	82.8
ctg9212	BF475048-0.07–BE443931-0.62	97.7
ctg4625	BE489565-1.60–BE490041-0.50	93.1

Markers used in the wheat RH mapping work in this study were used to identify wheat D-genome BAC contigs derived from the wheat D-genome physical mapping project (http://wheat.pw.usda.gov/PhysicalMapping/index.html). In most cases, the left and right markers of a contig are given, as well as single markers that mapped to a particular contig. The ordering of these markers by using the comprehensive RH map (Figure 1) provides a method for the physical ordering of the contigs. The percentage of coretention frequency between two markers is calculated by subtracting the total number of breaks between those two markers from the total number of possible breaks (87) divided by the total number of possible breaks multiplied by 100.

and provides the resolution of ~199 kb, much higher than any previously reported maps of wheat.

The method of plant RH panel development is considerably different from that used in animal and other systems. In the nonplant systems, a donor cell line with functional markers such as thymidine kinase (TK+) or hypoxanthine phosphoribosyl transferase (HPRT+) is irradiated and then fused with recipient nonirradiated cell lines. Subsequently, fusion products with donor subchromosomal fragments are selected for the presence of TK+ or HPRT+ activity. The method used in plant radiation hybrid development (except for the barley panel where protoplasts carrying the gene for bialaphos resistance were fused to tobacco protoplasts; Wardrop *et al.* 2002, 2004) is significantly different and has implications for radiation dosages, screening of plants, selection, and retention frequency of the markers. The method used in plants is to irradiate seed, recover live fertile or infertile plants, possibly cross to recipient parent to generate stable segregants, and then assay lines for breakage using available marker systems of choice. Nuclei with highly fragmented chromosomes are not likely to survive subsequent cell divisions. In the plant RH work to date, including this study, high marker retention frequencies were detected as compared with those in other systems (Kynast *et al.* 2004; Wardrop *et al.* 2002, 2004). In comparison to the protoplast fusion method that has been demonstrated in barley (Wardrop *et al.* 2002, 2004), development of RH panels via seed irradiation, where the cytogenetic resources for panel development are available, has an added value because the RH lines can be propagated by seed with transmission of the subchromosome fragments retained in the RH_0 lines (Vales *et al.* 2004). Therefore, this serves as an unlimited resource for mapping different marker sources.

RH panel development via seed irradiation also has an advantage in species that have traditionally been identified as recalcitrant to the various tissue culture manipulations due to problems such as protoplast isolation and callus culture. Additional benefits include the relative ease of distribution of the entire or selected members of the RH panel and the ease of maintenance. The male-sterile RH plants in the DWRH-1D panel are induced to produce seed by simply pollinating the recipient with a durum line (Langdon-16). These lines are stable and can be used as an unlimited resource for follow-up studies. The development of a chromosome 1D panel with a lower radiation dose will complete the tool kit required for low- (nullisomic, ditelosomic, and deletion lines of wheat), medium- (low radiation dose), and high- (DWRH-1D) resolution mapping using the RH approach.

Pioneering work in plant RH analysis was carried out in maize using oat × maize panels. In that system, maize chromosomes and subchromosome fragments were isolated in the genome of oat and then assayed (Riera-Lizarazu 2000; Kynast *et al.* 2004). These efforts in maize with a haploid genome size of 2500 Mb has yielded maize RH panels for chromosomes 1 and 9 (Riera-Lizarazu 2000; Kynast *et al.* 2004). However, those studies have generated only low- to medium-resolution maps of specific maize chromosomes. These studies underscore the value of developing high-resolution physical maps for large and complex polyploid genomes such as wheat because of the limitations on the analysis of genome organization due to genome size, extensive gene or chromosome duplication and rearrangement, and absence of recombination in the majority of the genome. This effort will be complementary to other efforts to sequence segments of the wheat genome by overcoming the limitations of traditional recombination-based mapping and using methods that improve the features of bin mapping in wheat.

Over 16,000 EST loci were successfully placed onto various chromosomes by deletion bin mapping (Hossain *et al.* 2004a; Peng *et al.* 2004). Of these, 2212 EST loci have been placed on wheat homeologous group 1 chromosomes (Peng *et al.* 2004). Although these ESTs were placed onto seven bins for chromosome 1D (three long-arm and four short-arm bins), the relative order of the ESTs within the respective bins is not known. A portion of the ESTs that had been placed to chromosome 1D bins were ordered using the RH mapping approach. As seen in Table 1, linkage group A consists entirely of ESTs. Eighty-eight percent of the ESTs in linkage group A are from the bin 1DL2-0.50-0.80. The RH approach allowed ordering of these previously unordered ESTs (Figure 2), which included examples of EST sequence identifying multiple fragments within this region. The ability to order these multiple fragments is valuable for this may indicate the presence of gene families in a particular chromosomal area of interest where the physical distance between the various members can be calculated from this map. Additionally, these multiple fragments are valuable in providing an excellent substrate (markers that are physically close) for generating a high-resolution physical map (Figure 2b). As is evident from Figure 2b, the DWRH-1D panel represents an excellent array of breaks within this chromosome segment for high-resolution mapping. The only limitation to this work is the availability of a large number of evenly distributed markers.

To provide a more even coverage of chromosome 1D, AFLP markers were utilized demonstrating the value of this approach for ordering anonymous markers. The ability of the RH mapping approach to differentiate between gene-rich and gene-poor areas by ordering markers such as ESTs and SSRs and anonymous markers such as AFLPs (given by the best order of markers) can help in delineating a chromosomal region and allow detailed analysis of such a region for the purposes of gene identification as well as to provide direction to chromosome sequencing efforts that may be hampered

by the presence of large tracts of repetitive DNA. Linkage groups B through D are composed entirely of AFLP markers, which can potentially provide markers for BAC alignment and contig development in gene-poor regions.

RFLP, SSR, EST, and AFLP markers were used in this study to determine the distribution of the various classes of markers along this chromosome. Previous findings had shown that gene-rich areas are interspersed between gene-poor areas of the wheat genome (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Endo and Gill 1996; Sandhu and Gill 2002). Thus, we predicted that SSR, RFLP, and EST loci would cluster among themselves and interspersed among these clusters would be AFLP loci. Since the linkage groups as well as unlinked markers all belong to chromosome 1D, we generated a lower-confidence (LOD 2.0) comprehensive map that included all markers in this study (Figure 1). The distribution of various marker classes is an interesting feature of this comprehensive RH map. The EST, RFLP, and SSR markers tend to colocalize, possibly representing the gene-rich regions as they are mostly derived from gene sequences (*i.e.*, cDNA libraries), while AFLP markers are evenly distributed (Figure 2). The use of AFLP markers and their distribution pattern in this mapping work provided the glue needed to attain linkage between the AFLP marker clusters and the EST-based clusters. We estimate that >2300 markers would be required to cover the entire 1D chromosome and in subsequent studies plan to use all available EST and SSR markers in addition to more AFLP markers to saturate this map. This approach will subsequently allow the identification of either a large linkage group that encompasses all these markers or, alternatively, linkage groups that consist of both AFLP and EST/SSR markers.

An approach recommended for the systematic sequencing of complex genomes is a strategy in which both the clone-by-clone approach and the whole-genome shotgun sequencing (WGS) methods are combined (Green 2001). For this WGS approach to work efficiently, high-quality physical maps are essential, especially for the larger genomes, which includes wheat (Meyers *et al.* 2004). An RH approach to ordering molecular markers and developing a robust physical map is useful with the number of EST or full-length cDNA, the high C_0t, and methylation filtration sequencing projects that are underway in plants. The advantage of this strategy is that it requires only that a molecular marker be amplified to be placed on an RH map (Bentley and Dunham 1995). Since this method does not depend on meiotic recombination, high-resolution RH maps can be generated with fewer (<100) lines (Green 2001; Meyers *et al.* 2004) compared to the screening of thousands of individual lines to achieve a similar level of resolution by traditional mapping methods. Another advantage of RH mapping is that it does not require polymorphism in the target DNA sequence and therefore does not depend on enzyme-marker combinations that have traditionally been used to identify useful molecular markers. Additionally, primer sequences used in the RH map development can potentially be used to screen BAC libraries and assign contigs to chromosome regions. We demonstrate the value of the RH mapping approach in aligning BAC contigs (Table 3). Alignment and ordering of large-insert clones and markers within those contigs will allow the development of high-resolution maps linked to physical maps of a genome.

RH maps with the required resolution can be produced by altering the dosage of radiation, and in humans this method has been employed to develop RH panels with low, medium, and high resolution (Gyapay *et al.* 1996; Stewart *et al.* 1997). The extensive collection of cytogenetic stocks in wheat and its relatives provides the substrate for low- to medium-resolution physical mapping. The RH mapping approach reported here complements those stocks used in the development of high-resolution physical maps.

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