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Genetics

Toward a cytogenetically based physical map of the wheat genome

(gametocidal chromosome/restriction fragment length polymorphism/polyploidy/deletion mapping)

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ABSTRACT **Bread wheat (*Triticum aestivum* L. em Thell) is well suited for cytogenetic analysis because the genome, buffered by polyploidy, can tolerate structurally and numerically engineered chromosomes for analysis over infinite generations. This feature of polyploidy can be used in developing a high-resolution, cytogenetically based physical map of the wheat genome. We show that numerous deletions, observed in the progeny of a monosomic addition of a chromosome from *Triticum cylindricum* in wheat, result from single breakpoints and a concomitant loss of distal fragments. Breakages occurred in euchromatic and heterochromatic regions. Forty-one deletions for chromosomes 7A, 7B, and 7D, and a set of genetically mapped DNA probes, were used to construct physical maps. Recombination was low in proximal chromosomal regions and very high toward the distal ends. Deletion mapping was more efficient than genetic mapping in resolving the order of proximal loci. Despite variation in size and arm ratio, relative gene position was largely conserved among chromosomes 7A, 7B, and 7D and a consensus group 7 physical map was constructed. Several molecularly tagged chromosome regions (MTCRs) of approximately one to a few million base pairs were identified that may be resolved by long-range mapping of DNA fragments. Thus, a cytogenetically based physical map may be used to integrate chromosome and DNA-based maps. The MTCRs may simplify strategies for cloning of agronomically useful genes despite the genetic complexity and the large genome size of wheat.**

The integration of data from linkage analysis of phenotypic and restriction fragment length polymorphism (RFLP) loci may lead to detection of a RFLP tightly linked to a gene controlling a phenotypic trait, potentially leading to cloning of the gene with an unknown product, exhibiting the phenotype by reverse genetics (1). However, genetic linkage distances may not correspond to the actual physical distances between loci. The unit of recombination, which measures the genetic distance between loci, might represent anywhere from 10 to 100,000 kilobases of DNA in a specific region of the genome due to nonrandom distribution of recombination along the chromosome length (2). Distortion of the linkage map in comparison to the estimated physical size of the region may result from suppression of recombination, especially near the centromere or in regions containing heterochromatin (3–7). Therefore, a necessary step to move from a linked marker toward a gene is to first create a physical map of the chromosome region (8).

Cytogenetically based physical maps are based on ordering loci (from a genetic linkage map) with respect to the cytological landmarks of a chromosome. The first requirement for constructing cytogenetically based physical maps is the availability of cytological techniques to identify individual chromosomes. The second requirement is the ability to generate cytogenetically altered stocks. Both of the above requirements are met in bread wheat (*Triticum aestivum* L.; $2n = 6x = 42$; AABBDD).

First, idiograms of C-banded chromosomes of common wheat have been constructed (9). Second, an efficient system for generating deletion stocks has been reported in wheat. A gametocidal chromosome from *Triticum cylindricum* Host. (syn. *Aegilops cylindrica*) causes frequent chromosome breakages when introduced into wheat (10). Most chromosome breakages occur in gametes lacking the alien chromosome and result in plants with pure wheat background. Stable homozygous deletion lines can be recovered because of the polyploid nature of wheat.

We are using an array of deletion lines involving 21 chromosomes of wheat to integrate newly developing genetic and physical maps. The objectives of this research were to construct cytogenetically based physical maps of molecular markers for the group 7 chromosomes of common wheat. The physical map was then compared to the genetic map (11) to analyze recombination in defined chromosome regions.

MATERIALS AND METHODS

Deletion Stocks. Subarm aneuploids containing partial arm deletions in individual chromosomes in wheat cultivar Chinese Spring (CS) were generated by backcrossing a monosomic addition of a *T. cylindricum* chromosome to CS (10). Plants with chromosomal aberrations (mainly deletions) were identified by the C-banding technique (12). The breakpoint (BP) for each deleted chromosome was calculated as a fraction length (FL) of the distance from the centromere from a sample of at least five C-banded chromosomes (13).

Probes and Southern Analysis. Sixteen cDNA clones (11, 14) were used for physical mapping. Genomic DNA was isolated from the deletion and aneuploid stocks of CS nullisomic tetrasomics, nullisomic for one chromosome pair and tetrasomic for a corresponding homologous pair; and ditelosomics, nullisomic for a chromosome arm. Fifteen-microgram samples of total genomic DNA, digested with *Eco*RI or *Hin*dIII restriction endonucleases (except *Dra* I digestion for *Nra, Sac* I for 121), were electrophoresed in 0.8% agarose gel for 18–20 hr at 50 V and blotted onto a Magnagraph membrane (MSI) following the manufacturer's recommendations.

Hybridization was performed with 50 ng of insert DNA of the appropriate clone labeled with ^{32}P by random-primer extension (15), and the membranes were treated as described (16). Filters were reused after washing in 0.1× standard saline citrate/0.2% SDS at 95°C.

Map Construction. First, a locus was assigned to a chromosome arm by Southern analysis of nullisomic tetrasomics

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Abbreviations: RFLP, restriction fragment length polymorphism; cM, centimorgan(s); BP, breakpoint; FL, fraction length; MTCR, molecularly tagged chromosome region.

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and ditelosomics. Next, a panel of deletions of 7A, 7B, 7D, and 4AL (long arm of chromosome 4A) were scored for the presence or absence of the specific fragment(s) representing RFLP loci. Chromosome 4A deletions were analyzed because its long arm contains a segment derived from chromosome 7B (17). Each locus was assigned to the chromosome region between the BP of the largest deletion where the band was present and the next larger deletion where the band was absent. Chromosome 7D is inverted in Figs. 3 and 5 such that the cytologically long arm is in the position of the short arm. This is because the genetically identified 7DS arm, which is homoeologous to the 7AS and 7BS arms, is cytologically the longer arm (7DL) of chromosome 7D and vice versa (9). In spite of the size discrepancy, we will use the generally accepted genetic nomenclature to describe group 7 arms.

The physical maps constructed for chromosomes 7B and 7D were compared to corresponding genetic maps (due to the lack of polymorphism in the A genome, the genetic map of 7A is not well developed). Placement of the loci on the physical map was in accordance with observed BPs. The placement on the linkage map was in relation to other genes based on recombination values. The two maps were positioned relative to each other by equating to the centromere.

RESULTS

Deletion Stocks. Forty-one homozygous deletion lines were isolated for group 7 chromosomes (23, 10, and 8 for 7A, 7B, and 7D, respectively) and nine for 4AL (Fig. 1). Cytological analysis indicated that each selected deletion resulted from a single chromosome breakage with a concomitant loss of the distal fragment. One chromosome 7A had deletions at both ends. A greater number of deletions were obtained for chromosome 7A, particularly the long arm, as compared to 7B and 7D. Chromosome breakages were distributed throughout the chromosome arms regardless of the location of heterochromatin and euchromatin. In chromosome 7B, five BPs were in the heterochromatic region and five BPs were in the euchromatic region.

Physical Mapping. An example of the chromosome assignment of locus 129 is shown in Fig. 2. The abbreviation 129 is for the locus *Xpsr129* and the clone used to study the locus is anonymous DNA (X) clone Psr129. The nullisomic tetrasomic and ditelosomic analysis identified specific arms in which a fragment detected by clone 129 is located. Based on deletion analysis, locus 129 is physically localized into the regions between BPs 0.77 and 0.80 on 7AL, 0.69 and 0.83 on 7BL, and 0.76 and 0.82 on 7DL (Fig. 3). In this way, 16 RFLP loci were physically mapped on 7A, 13 on 7B, 14 on 7D, and 3 on 4AL (Figs. 3 and 4).

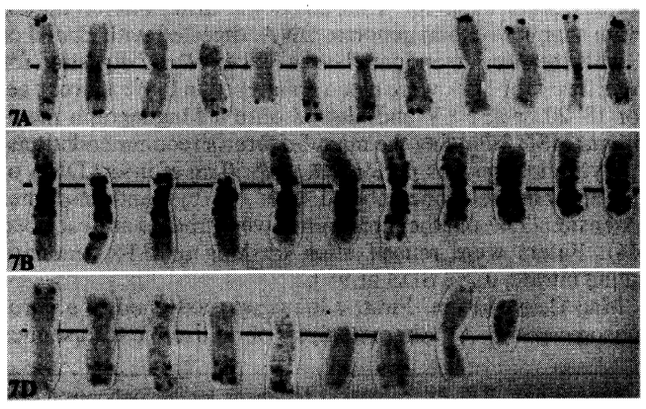

FIG. 1. C-banded chromosomes of wheat. A normal wheat chromosome is shown on the left followed by an array of short arm and long arm deletions. Note chromosome 7A with deletions at both ends (no. 5 from the left).

Allocation of Molecular Markers: Size of the Region. The physical maps of 7A, 7B, 7D, and 4AL showing regional location of RFLP loci were constructed (Fig. 3). The 16 loci on 7A were allocated to 13 specific regions. Clone 129 detects a duplicate locus on 7A and a single locus each on 7B and 7D. The 13 loci on 7B were allocated into 8 regions. Two clones, 103 and 152, were physically mapped to a tiny segment. The 14 loci on 7D were allocated into 7 regions.

Order of DNA Markers. The relative physical order of the loci on 7A, 7B, and 7D was similar with one exception. The order of loci 72 and 129 on 7AL and 7BL arms is reversed. Their physical order on 7DS could not be determined because both mapped into the same region.

Size of the 7B Segment Translocated to the 4AL Arm. Loci 119, 160, and *Nra* map to the distal segment of 7AS, 7DL, and 4AL and mark the segment of 7BS that was translocated to the 4AL arm. The *Nra* locus lies proximal to loci 119 and 160 (see 7A map). The same order of loci was observed on 4AL. The *Nra* locus on 4AL was mapped to the 0.75–0.80 segment. Thus, at least 20% of the distal region of 4AL is derived from a translocation from the 7BS terminus.

FL Position of Loci in Physical Maps. If rearrangements have not occurred, the FL values should be the same among homologous chromosomes. As an example, locus 103 maps at a similar FL value on 7AS (0.26–0.29) and 7BS (0.27). All loci mapped within overlapping regions (SEM, ±6%). However, a greater sample of deletions is needed for chromosomes 7B and 7D before FL gene position can be compared across genomes for many other loci.

Consensus Physical Map. A working consensus physical map of group 7 chromosomes was constructed based on the assumption that FL is conserved for most loci (Fig. 3). This provides a greater sample of deletions (41 in this case) for a more precise physical mapping of individual loci on a single map. As an example, locus 65 maps between 0 and 0.20 on 7AS, 0.16 and 0.27 on 7BS, and 0 and 0.15 on 7DL. Therefore, it is localized at position 0.15–0.16 on the consensus physical map. The consensus map also permits ordering of two or more loci that map to a common region on an individual chromosome map. This is illustrated by ordering of loci 103, 152, 150, and 108 on the consensus map, which otherwise mapped in groups of 2 or 3 in the individual chromosome maps (Fig. 3).

Comparison of Physical and Genetic Maps. The linear order of the loci mapped on the physical and genetic maps is identical in most cases (Fig. 5). There was a reversal of order for loci 72 and 129 on 7B (Fig. 5) and loci 103 and 152 on 7D (see genetic map in Fig. 5 and consensus group 7 map in Fig. 3). Large discrepancies between physical and genetic map distances are apparent from comparison of the physical and genetic maps of chromosomes 7B and 7D. Loci that were close to the centromere from the genetic analysis are physically localized to more distal chromosomal regions. The loci 65 and 165 on each side of the centromere on 7B are 7 centimorgans (cM) apart and yet physically span the proximal 25% of total chromosome length. This area of 7B consists of a large block of heterochromatin. However, a similar distortion is observed for chromosome 7D, which lacks a centromeric heterochromatin block.

For the 7BL arm, the proximal region from centromere to 0.3 FL has a genetic length of <7 cM. The region from FL 0.3–0.7 has a genetic length of 15 cM and that from FL 0.7–0.85 has a genetic length of 38 cM. The distal region from FL 0.7 to telomere has a genetic length of >100 cM, indicating a high rate of recombination. A similar distribution of recombination along the chromosome length was observed for 7D. Because of this feature of recombination (suppression in the proximal 70% of the chromosome and high recombination in the distal ends), the genetic maps of 7B and 7D are compressed in the middle and greatly expanded at both ends.

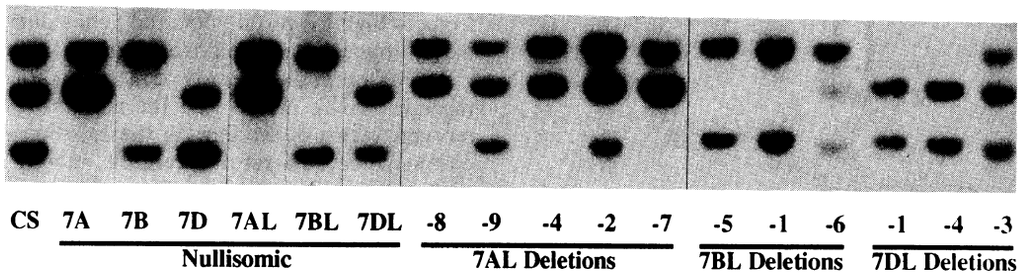

FIG. 2. Southern analysis of cDNA clone 129 to *Eco*RI-digested genomic DNA from the nullisomic and deletion stocks of CS wheat to allocate specific fragments to specific chromosome regions. Part of the gel with a 7AS specific band is not shown.

Another consequence of suppression of recombination around the centromere is that the linear order of 105, 165, and 169 in 7D could not be resolved from genetic data. However, from deletion mapping their physical order is centromere–169–165–105, based on the consensus physical map of group 7.

DISCUSSION

The proposed gametocidal system is well suited for deletion mapping in wheat due to the following features: (*i*) Deletions occur in gametes lacking the alien chromosome and are thus recovered in pure wheat background. (*ii*) Most deletions arise from single breakages with concomitant loss of distal segments as indicated from C-banding analysis. The correspondence of gene order between the genetic and physical maps confirms cytological observations. The reverse order for loci 72 and 129 on chromosome 7B might indicate either a double break and inversion during the production of deletion or a preexisting true inversion. (*iii*) Deletions are stable from generation to generation and broken chromosomes are healed by the addition of telomeric sequences at the physical ends (18). (*iv*) Breakages along a chromosome occur regardless of a euchromatin or heterochromatin region. The deletion lines have been isolated for all 42 wheat chromosomes with well ordered BPs for all chromosomes except 1A (19).

Deletion mapping provides a simple and rapid method to construct cytogenetically based physical maps of RFLP loci. Mapping is done on wheat chromosomes in hemizygous or homozygous conditions. Any probe can be used without the necessity of identifying polymorphism, a particularly significant factor in wheat, where a lack of DNA polymorphism is a major obstacle in generating RFLP maps.

Despite the fact that the three genomes differ in chromosome size and in the amount and distribution of heterochromatin, the relative gene position has been largely conserved. This allows construction of consensus physical maps leading to the allocation of loci across the genomes. Likewise, consensus genetic maps relative to physical landmarks (BPs,

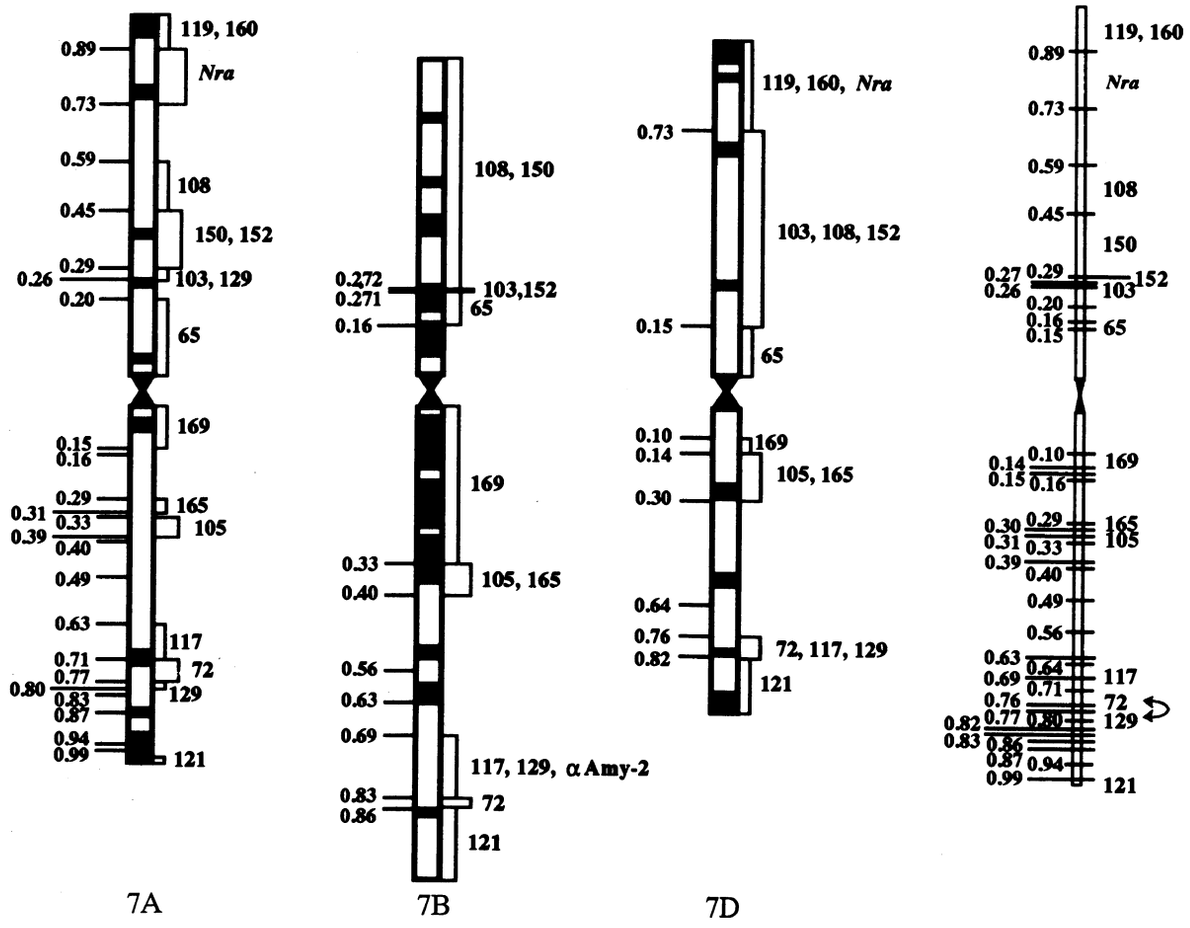

FIG. 3. Physical maps of RFLP loci for wheat chromosomes 7A, 7B, and 7D, and a group 7 consensus map. FL values are shown on the left and locus position is on the right.

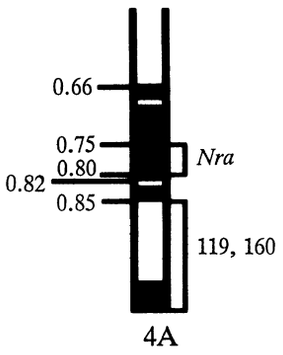

FIG. 4. Physical map of distal 4AL arm of wheat. Note these RFLP loci are present on 7A and 7D (see Fig. 3) but absent in 7B.

C-bands) can be constructed based on a similar relationship between genetic and physical distances for chromosomes 7B and 7D. Consensus genetic maps would permit the allocation of markers to specific chromosome regions across three genomes. For example, many genes are genetically mapped in wheat with respect to the centromere (20). Based on the genetic distances from the centromere, their position on the physical map can be predicted. Deletion mapping would confirm gene allocation such that any mapped gene may be integrated into the consensus cytogenetic maps.

Suppression of recombination in proximal regions of the chromosomes limits the power of recombination mapping in ordering proximal loci. Thus, for proximal regions, physical mapping is more powerful for resolving the order of the loci. However, the resolving power of genetic analysis is extremely high for distally located genes due to high recombination toward the chromosome ends.

The different distribution of recombination along the chromosome length means that the amount of DNA per centimorgan varies depending on the location of the gene on a chromosome. Therefore, caution should be exercised in undertaking chromosome walking on the basis of tight linkage alone.

Physical mapping of RFLP loci opens the potential for integration of genetic, chromosome, and DNA-based maps (long-range restriction maps, sequence data). The allocation of molecular markers in relation to chromosome landmarks leads to the ordering of markers spanning millions of base pairs of DNA and the establishment of molecularly tagged chromosome regions (MTCRs). The DNA size of MTCRs can be estimated based on the DNA content of each chromosome. The total haploid DNA content of wheat is estimated to be 16 billion base pairs of DNA (21), and the total length of the haploid chromosome complement is 235.4 μm (9). Assuming uniform DNA distribution per unit length throughout all chromosomes, and assuming BP values (0.271 and 0.272 on 7BS) are correct, the MTCR marked by loci 103 and 152 would contain 0.85 million base pairs. There are several deletions with proximal BPs on chromosomes 7A, 7B, 7D, and 4AL that identify MTCRs of a few million base pairs (Fig. 3). The MTCRs of up to several million base pairs can be resolved by pulsed-field gel electrophoresis (22, 23). Therefore, MTCRs provide a basis for integration of genetic, chromosome, and DNA-based physical maps. MTCRs also provide opportunities for constructing region-specific targeting libraries either by chromosome dissection and/or microcloning or by subtractive hybridization (24). Furthermore,

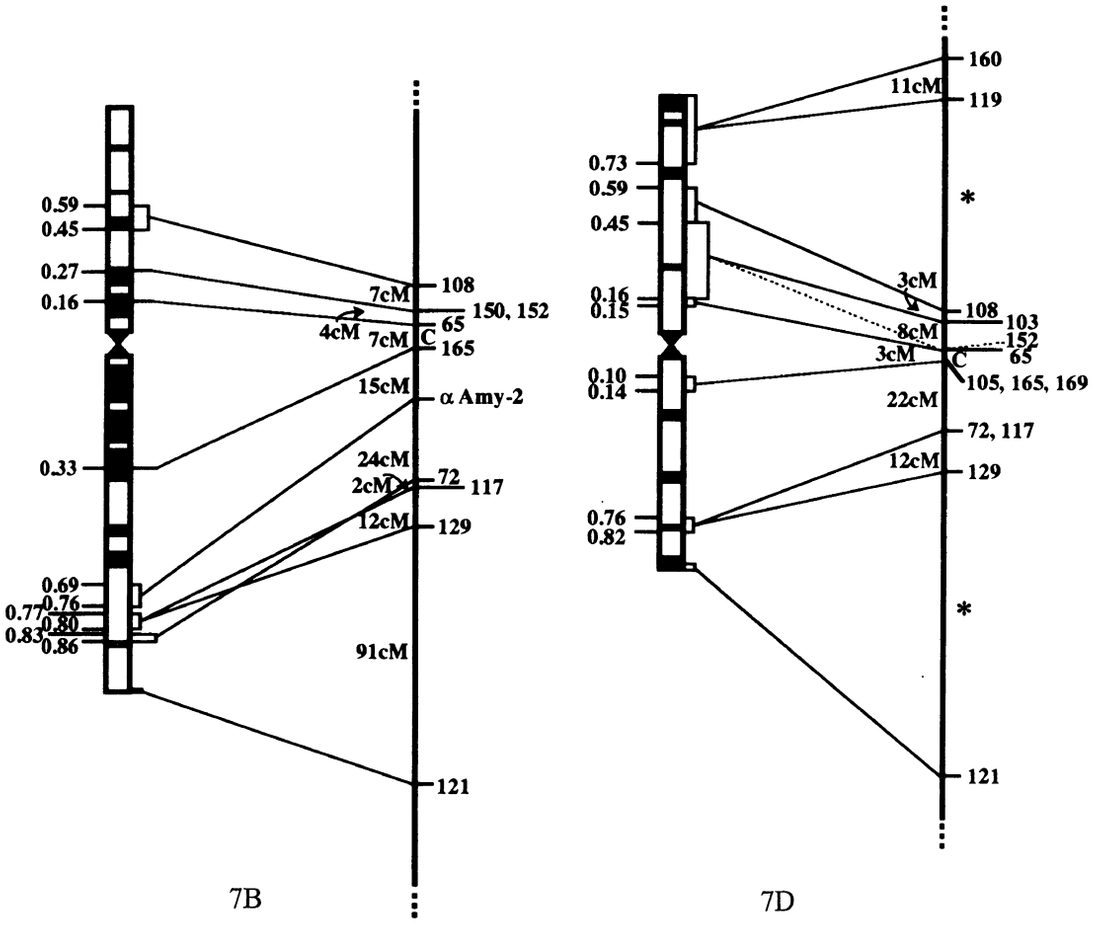

FIG. 5. Comparison between physical and genetic maps of wheat chromosomes 7B and 7D. Physical locations of some loci in 7B and 7D are based on the consensus physical map (see Fig. 3). Genetic maps are based on Chao *et al.* (11). *, Lack of linkage.

MTCRs will be useful in directing chromosome walking/jumping during the cloning process.

Deletion mapping is a powerful technique for constructing a cytogenetically based physical map of the wheat genome. Further physical and genetic mapping of other chromosomes will result in integration of cytogenetic and linkage data into one correlated map of the entire wheat genome. The identification of MTCRs could open the possibility of molecular cloning of a large number of agronomically useful genes previously intractable to molecular analysis.

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