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Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.)

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Abstract Because of polyploidy and large genome size, deletion stocks of bread wheat are an ideal material for physically allocating ESTs and genes to small chromosomal regions for targeted mapping. To enhance the utility of deletion stocks for chromosome bin mapping, we characterized a set of 84 deletion lines covering the 21 chromosomes of wheat using 725 microsatellites. We localized these microsatellite loci to 94 breakpoints in a homozygous state (88 distal deletions, 6 interstitial), and 5 in a heterozygous state representing 159 deletion bins. Chromosomes from homoeologous groups 2 and 5 were the best covered (126 and 125 microsatellites, respectively) while the coverage for group 4 was lower (80 microsatellites). We assigned at least one microsatellite in up to 92% of the bins (mean 4.97 SSR/bin). Only a few discrepancies concerning marker order were observed. The cytogenetic maps revealed small genetic distances over large physical regions around the centromeres and large genetic to physical map ratios close to the telomeres. As SSRs are the markers of choice for many genetic and breeding studies, the mapped microsatellite loci will be useful not only for deletion stock verifications but also for allocating associated QTLs to deletion bins where numerous ESTs that could be potential candidate genes are currently assigned.

Keywords Microsatellites · Genetic map · Deletion lines · Comparative mapping

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Introduction

Among the Poaceae, the common or bread wheat (*Triticum aestivum* L.) occupies a unique position due to its allopolyploidy and large genome size (1C =16,000 Mb compared to 1C =430 Mb in rice). During the last decade, wheat reference genetic maps with 300 to over 1,000 loci were constructed mainly based on RFLP markers and mapping populations derived from "wide crosses" involving a synthetic and a cultivated wheat (Devos and Gale 1993; Van Deynze et al. 1995; Nelson et al. 1995a, 1995b, 1995c; Marino et al. 1996), interspecific *T. spelta* × *T. aestivum* crosses (Liu and Tsunewaki 1991; Messmer et al. 1999) and inter-varietal crosses (Cadalen et al. 1997; Groos et al. 2002). Because RFLP markers exhibited low levels of polymorphism, especially for D-genome chromosomes (Chao et al. 1989; Kam-Morgan et al. 1989), the maps were enriched by Simple Sequence Repeats (SSRs or microsatellites), a class of markers that are co-dominant, locus-specific and suitable for detecting a higher level of polymorphism between closely related wheat varieties (Röder et al. 1995; Plaschke et al. 1995) opening exciting prospects for marker-assisted selection. About 400 microsatellite loci randomly distributed throughout the genome were genetically mapped on the wheat reference mapping population (Röder et al. 1998a; Stephenson et al. 1998). Sourdille et al. (2001a) reported the mapping of 337 microsatellite loci derived from A, B or D genome diploid ancestors on the whole genome of wheat using reference and inter-varietal populations. The isolation and development of microsatellite markers specifically derived from *Aegilops tauschii* significantly

improved the D genome coverage of the existing wheat microsatellite map by 55 (Pestsova et al. 2000) and 100 new loci (Guyomarc'h et al. 2002).

In wheat, the limitations of the large genome size and lack of polymorphism (Chao et al. 1989) can be overcome by targeted mapping made possible by the isolation of more than 400 deletion lines for the 21 chromosomes of wheat (cv Chinese Spring; Endo 1988; Werner et al. 1992; Gill and Gill 1993; Endo and Gill 1996). This unique material was used to physically map RFLP probes onto sub-arm chromosomal regions for homoeologous group 1 (Kota et al. 1993; Gill et al. 1996a; Tsujimoto et al. 1999; Ma et al. 2001), 2 (Delaney et al. 1995a), 3 (Delaney et al. 1995b; Ma et al. 2001), 4 (Mickelson-Young et al. 1995), 5 (Gill et al. 1996b), 6 (Gill et al. 1993a; Weng et al. 2000) and 7 (Werner et al. 1992; Hohmann et al. 1995a, 1995b). Röder et al. (1998b) physically mapped a set of microsatellites on chromosomes of the homoeologous group 2. Zhang et al. (2000) saturated the1BS satellite region with AFLP markers. The deletion mapping strategy has allowed the delineation of chromosomal regions for some important genes like *Ph1* on 5BL (Gill et al. 1993b), *Vrn1* on 5AL (Sarma et al. 1998), *Ha* on 5DS (Sarma et al. 2000) and *Q* on 5AL (Kojima et al. 2000; Faris et al. 2002). More recently, a subset of deletion lines was used to construct a chromosome bin map of wheat for EST loci (http://wheat.pw.usda.gov/wEST/).

In the present paper, we report on the establishment of genetic-physical map relationships in wheat using a set of deletion lines through the anchoring of microsatellites mapped on two wheat populations (a wheat reference population with more than 2,000 markers and an inter-varietal population with 660 markers). A cytogenetic map was constructed for the 21 chromosomes of wheat. Several aspects of its applications in genetic and breeding studies are also presented in this paper.

Materials and methods

Plant material

A set of 84 wheat deletions lines, characterized by terminal deletions, was used at INRA. The lines were chosen according to their ease in multiplying and to the presence of heterozygous and interstitial deletions (Qi et al. 2002). The complete set of 101 lines was used at Kansas State University. The length of each deletion bin was measured as a percentage of the chromosome arm missing. The nomenclature for the characterization of each deletion line was as follows : chromosome arm-number of the line-percentage of the arm present. For example line 3AL3-0.42 is the line number 3, located on the long arm of chromosome 3A and where 42% of the arm is present. Each bin is then defined as the name of the proximal line and the percentage of arm present in the next line. For example bin 7DL2-0.61-0.82 is the bin located between lines 7DL2-0.61 and 7DL3-0.82. Structural description and deletion nomenclature are given in Endo and Gill (1996) and Qi et al. (2002). For each line, three to five seeds were sown, each plant being characterized individually. A set of 19 nulli-tetrasomic (NT) lines and 35 ditelosomic (DT) lines (kindly provided by Dr Steve Reader, John Innes Centre, United Kingdom) was used for chromosomal and arm assignment of markers. Plant DNA was extracted from young leaves using the CTAB method (Rogers and Bendich 1985). Two wheat mapping populations were used for the establishment of genetic-physical map relationships: first, the reference population of the International Triticeae Mapping Initiative (ITMI map) derived by single seed descent (F_8) from the cross between W-7984, a synthetic amphi-hexaploid wheat, and the Mexican variety Opata 85 from CYMMYT (Van Deynze et al. 1995); second, the doubled haploid inter-varietal mapping population CtCS derived from an F1 cross involving the French variety Courtot (Ct) and Chinese Spring (CS; Cadalen et al. 1997).

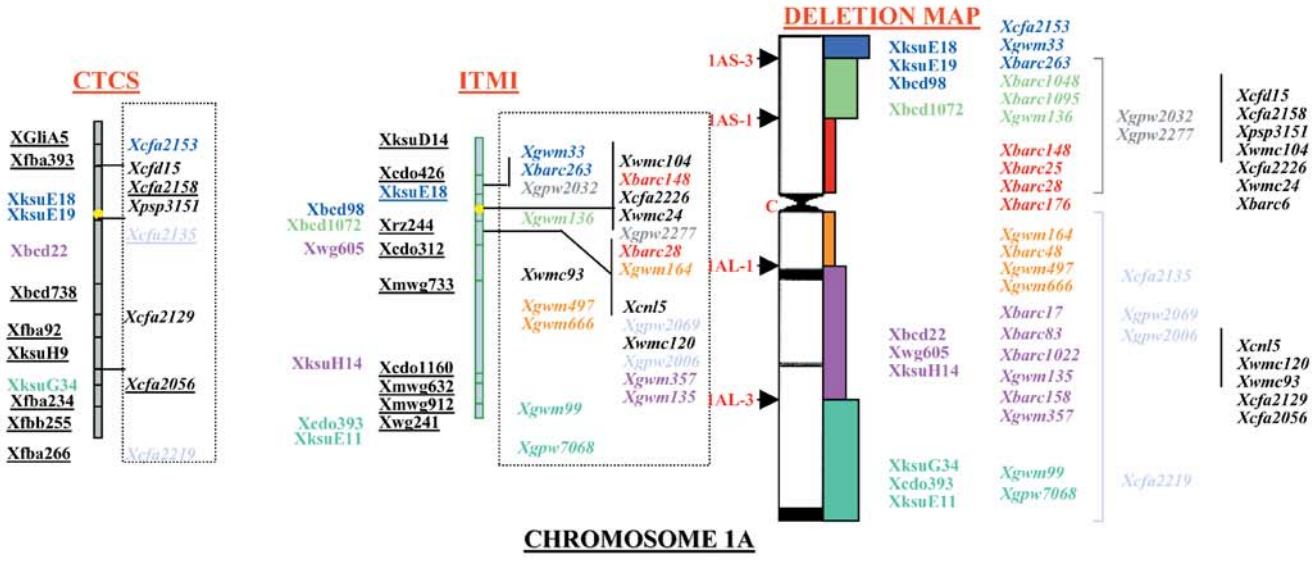
Microsatellite analysis:

Different sources of microsatellites were used: either bread wheat (*Xgwm*: Röder et al. 1998a, 1998b; *Xbarc*: Cregan and Song, http://www.scabusa.org; *Xksu*: Gill, Li and Singh, unpublished data) or A, B and D genome diploid donors (*Xcfa*, *Xcfd, Xgpw*: Sourdille et al. 2001a; Guyomarc'h et al. 2002). PCR reactions were performed in a final volume of 20 µl in a PTC-225 MJ Research tetrad thermocycler as described in Guyomarc'h et al. (2002). The acrylamide gels were silver-stained following the protocol from Tixier et al. (1997). In a preliminary step, we identified shared RFLP and SSR markers between genetic and deletion bin maps from published data (Van Deynze et al. 1995; Nelson et al. 1995a, 1995b, 1995c; Marino et al. 1996; Cadalen et al. 1997; Graingenes database). From 2,552 RFLP mapped loci, 73 shared markers were recorded between the published ITMI and deletion maps and 38 between the published CtCS and deletion maps. At INRA Clermont-Ferrand, this initial framework cytogenetic map was used to select 348 microsatellites from genetic maps for deletion bin mapping. All the deletion breakpoints characterizing a particular chromosomal arm were analyzed with a set of microsatellites identifying the same chromosomal arm on the ITMI and CtCS maps. At Kansas State University, 377 additional microsatellites were tested on the whole set of deletion lines and mapped into deletion bins. These two sets of data were used to construct cytogenetic maps for the seven homoeologous groups of wheat.

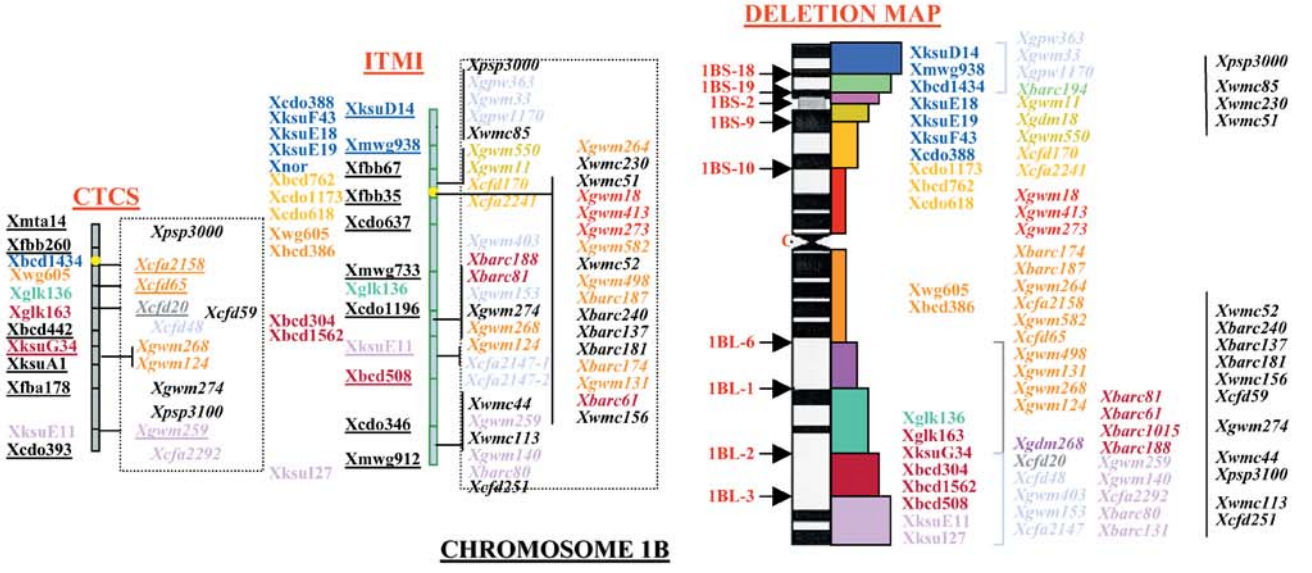
Results

Genetic-physical map relationships

The cytogenetic maps for the seven homoeologous groups are shown in Figs. 1, 2, 3, 4, 5, 6 and 7. A range of 22 to 47 microsatellites per chromosome were shared between genetic and deletion bin maps. Only a few discrepancies were observed between genetic and physical maps. Most of them were observed close to the centromeres where the density of markers is important, the genetic distances very short and it is difficult to order the loci accurately. Other differences could be due to previously undetected interstitial deletions (Qi et al. 2002). Up to 92% of the bins were characterized by at least one microsatellite marker. Only some of the short (less than 10% of the chromosome-arm length) or some very distal bins lacked assigned microsatellite loci. A range of 1 to 16 SSR (mean 4.97 SSR/bin) were assigned to the characterized bins, the bins with best coverage were located on chromosomes 2B (C-2BL2-0.36) and 3A (C-3AL3-0.42).



CHROMOSOME 1A



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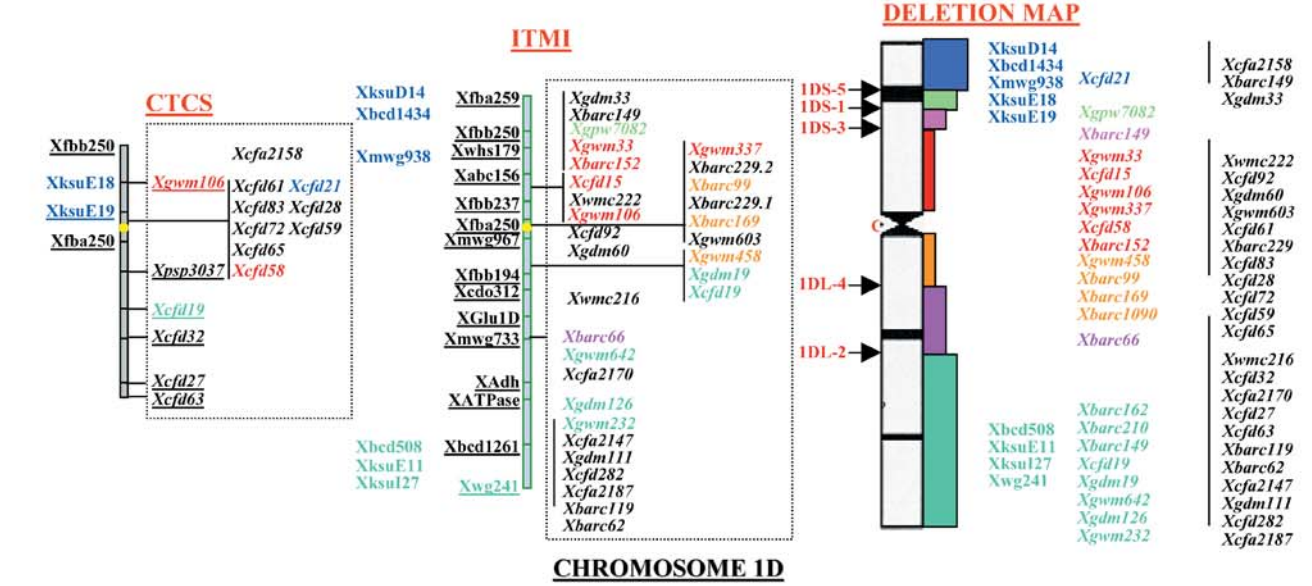

CHROMOSOME 1D

Homoeologous group 1

Eighty-seven microsatellite loci were tested on the three homoeologues, 28 on 1A, 37 on 1B and 22 on 1D (Fig. 1). Chromosomes 1A, 1B and 1D were characterized by 6, 11 and 7 bins, respectively. The markers were non-randomly distributed on chromosomes 1A and 1D. Clusters were observed that were interspersed by regions of low marker density. On chromosome arm 1AS, ten markers physically mapped in the distal bins 1AS3-0.86-1.00 and 1AS1-0.47-0.86 genetically encompassed the entire arm (>40 cM). On the contrary, four markers were assigned to the bin C-1AS1-0.47, of which two markers (*Xbarc148* and *Xbarc28*) nearly cosegregated with the centromere. Similarly, on chromosome arm 1DL, eight microsatellite loci mapped in bin 1DL2-0.41-1.00 covered up to 80 cM on the ITMI genetic map (Fig. 1). This confirms the low recombination frequency close to the centromeres compared to the telomeres. No microsatellite was allocated to three bins in chromosome 1B, two small deletions on the satellite region of the short arm (1BSsat-0.31 and 1BSsat19-0.31-0.50) and one on the long arm (1BL1-0.47-0.69).

Homoeologous group 2

One hundred and twenty-six microsatellite loci were placed on this homoeologous group, 35 on 2A, 47 on 2B and 44 on 2D (Fig. 2). Chromosomes 2A, 2B and 2D were characterized by four, eight and six bins, respectively. An inversion was noticed on chromosome 2D between ITMI and CtCS maps: the fragment between locus *Xgwm249-2D* and *Xcfd56-2D* was inverted on the CtCS map compared to the ITMI and deletion maps. *Xgwm249-2D* mapped distally on the CtCS map while this same locus cosegregated with markers located close to the centromere on the ITMI map. This suggests that a chromosomal rearrangement was present on chromosome 2D in the F1 between Courtot and Chinese Spring. All the bins were tagged with markers for this homoeologous group. However, this group bears only 18 bins and was the best saturated (126 SSRs).

Homoeologous group 3

Ninety-eight microsatellite loci were tested for this group, 32 on 3A, 33 on 3B and 33 on 3D (Fig. 3). Chromosomes 3A, 3B and 3D were characterized by six, eight and six bins, respectively. Clusters of microsatellites were detected on chromosome arm 3DS where 12 loci mapped in the distal bin 3DS6-0.55-1.00 while they were genetically mapped on a fragment length of up to 75 cM. All the bins were characterized by at least one microsatellite although on the long arm of chromosome 3D, six loci could not be attributed to a precise bin. Locus *Xgwm456* was found to cosegregate with the centromere of chromosome 3D.

Homoeologous group 4

Eighty microsatellite loci were tested on the three homoeologues, 22 on 4A, 29 on 4B and 29 on 4D (Fig. 4). Chromosomes 4A, 4B and 4D were characterized by nine, seven and eight bins, respectively. This homoeologous group had the least number of SSR loci. Only 105 SSR loci were mapped on the three chromosomes of this group compared to 183 mapped SSR loci on homoeologous group 2 and 187 on group 5. Four bins of this group were devoid of SSR loci (4AS4-0.63-0.76, 4AL12-0.43-0.59, 4BS4-0.37-0.57 and 4DS3-0.67-0.82). Two recombination hot spots were detected on the long arms of chromosomes 4B and 4D for the CtCS population. The one on 4BL is probably located distally between loci *XksuH11-4B* and *Xcdo1312-4B* in the bin 4BL5-0.86-1.00. These two loci were not genetically linked (% recombination θ>50%). The one on 4DL is located between *Xfba211-4D* and *Xcfd84-4D*, two loci which were found to be genetically independent (θ>50%), in the bin C-4DL9-0.31. It was surprising to detect a recombination hot spot in a centromeric region where recombination is reported to be lower compared to the telomeric regions.

Homoeologous group 5

One hundred and twenty-five microsatellite loci were detected on the three homoeologous chromosomes, 42 on 5A, 43 on 5B and 40 on 5D. Chromosomes 5A, 5B and 5D were characterized by 9, 11, and 8 bins, respectively. Only bin 5DS5-0.67-0.78 was not marked by an SSR. Genetic and physical distance ratios differed depending on the region of the chromosome. For example, on chromosome 5A, 40 cM around the centromere represented 40% of the short arm and nearly 60% of the long arm. On the contrary, on the long arm of the same chromosome, nearly 60 cM including the telomeric region represented only 13% of the chromosome arm. On

Fig. 1 Comparison between genetic and physical maps of wheat: homoeologous group 1. On the *left* is the Courtot × Chinese Spring map (Cadalen et al. 1997) and on the *right* is the ITMI map derived from the cross between W7984 and Opata (Van Deynze et al. 1995). C-bands on the chromosomes are drawn to scale. The breakpoints of the various deletions are indicated with *arrows*. Anchor markers of the genetic maps are *underlined*. RFLP markers are *on the left side of the chromosomes* and microsatellites are *italicized and placed on the right* according to their more likely position. *Dotted lines* on the chromosomes indicated genetic distances >50 cM. *Coloured markers* were those tested on the deletion lines and are linked to the corresponding deletion bin. When it was impossible to discriminate between two or more bins or when microsatellites were assigned on an entire arm, this was indicated with a corresponding *coloured bar*. Approximate position of the centromeres are indicated with a *circle* or with a *constriction* for C-banded chromosomes. Approximate physical position of the unassigned microsatellites (in *black*) is indicated on the *right* of each deletion map

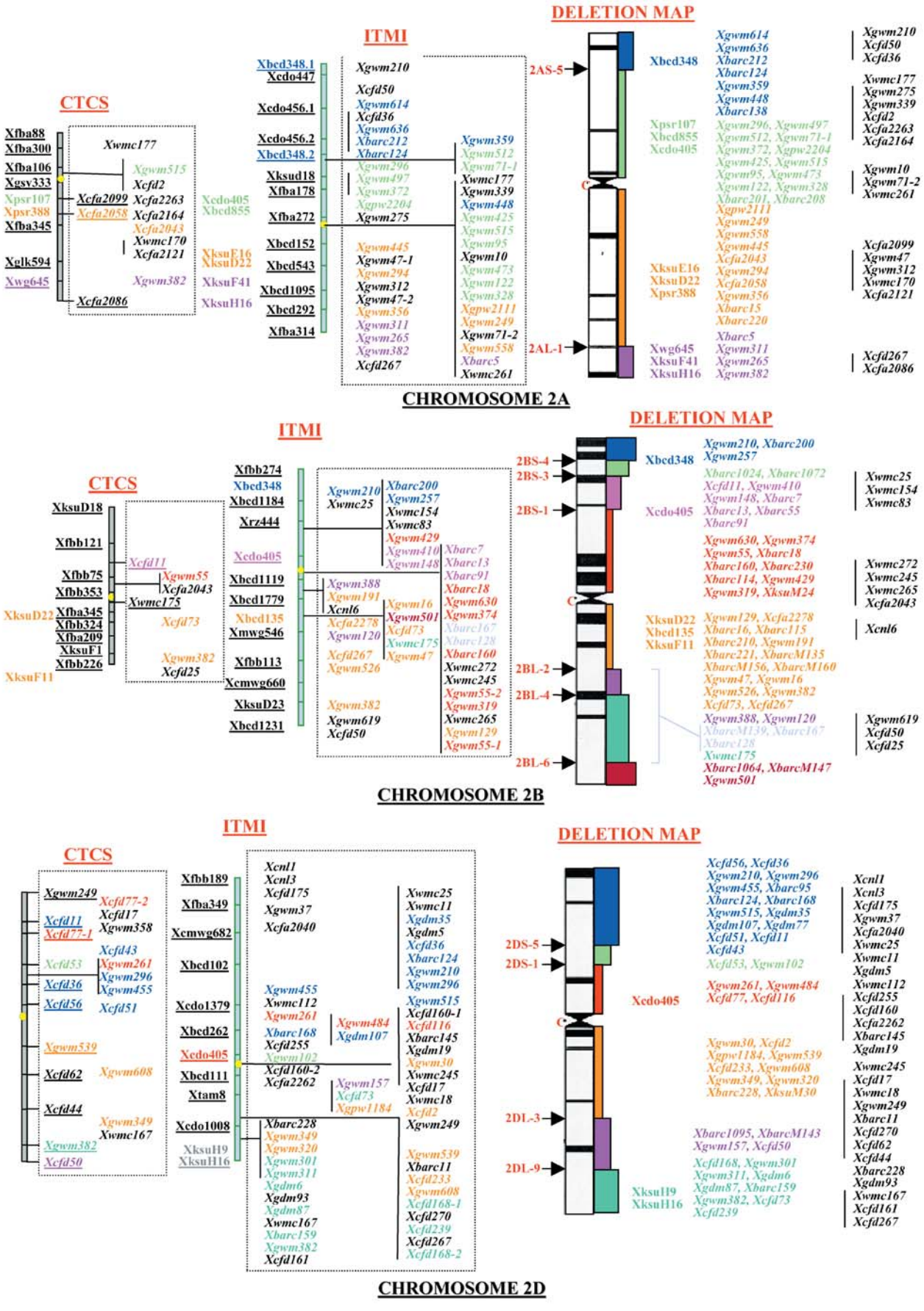

Fig. 2 Comparison between genetic and physical maps of wheat: homoeologous group 2. For details see Fig. 1

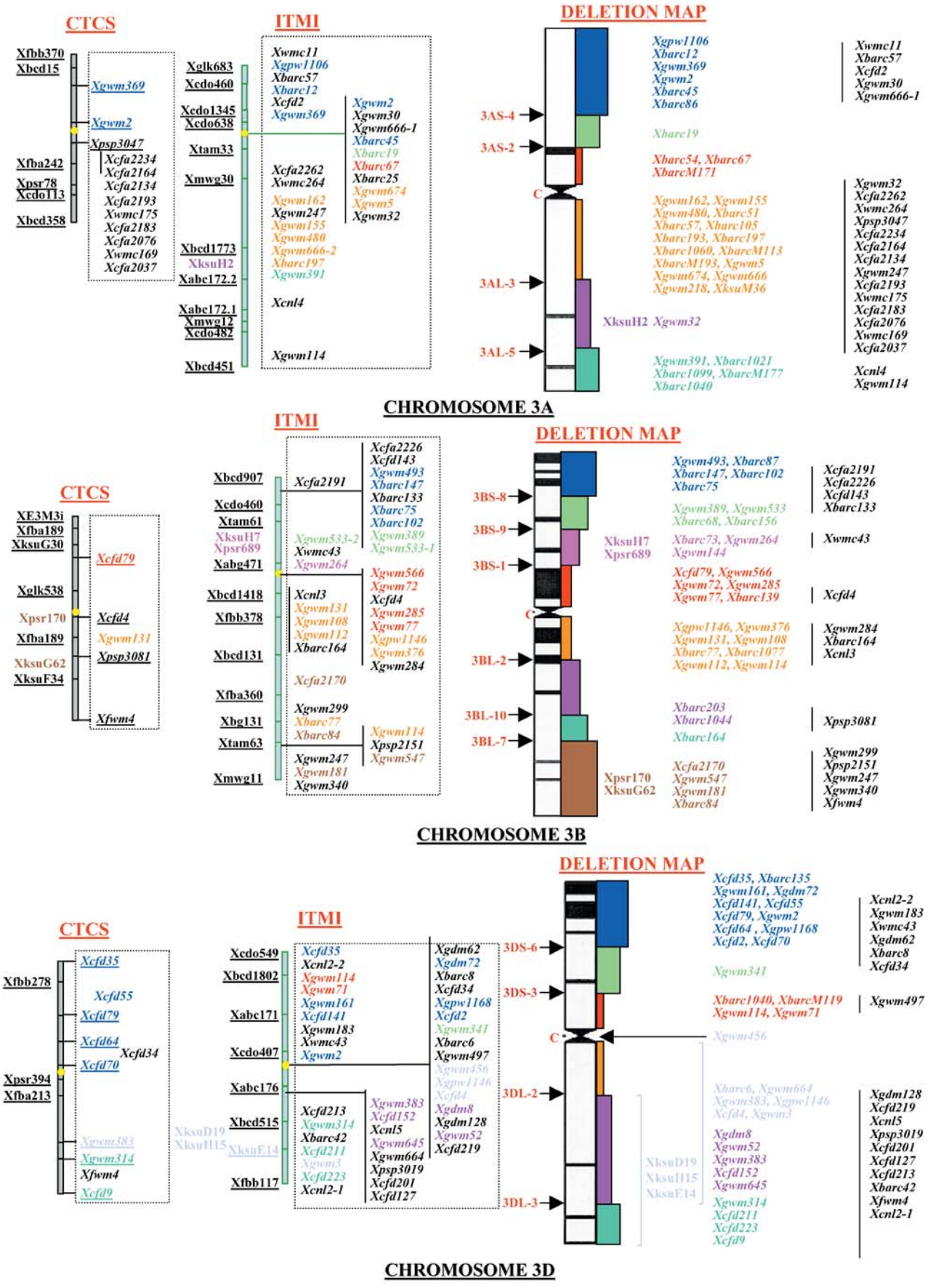

Fig. 3 Comparison between genetic and physical maps of wheat: homoeologous group 3. For details see Fig. 1

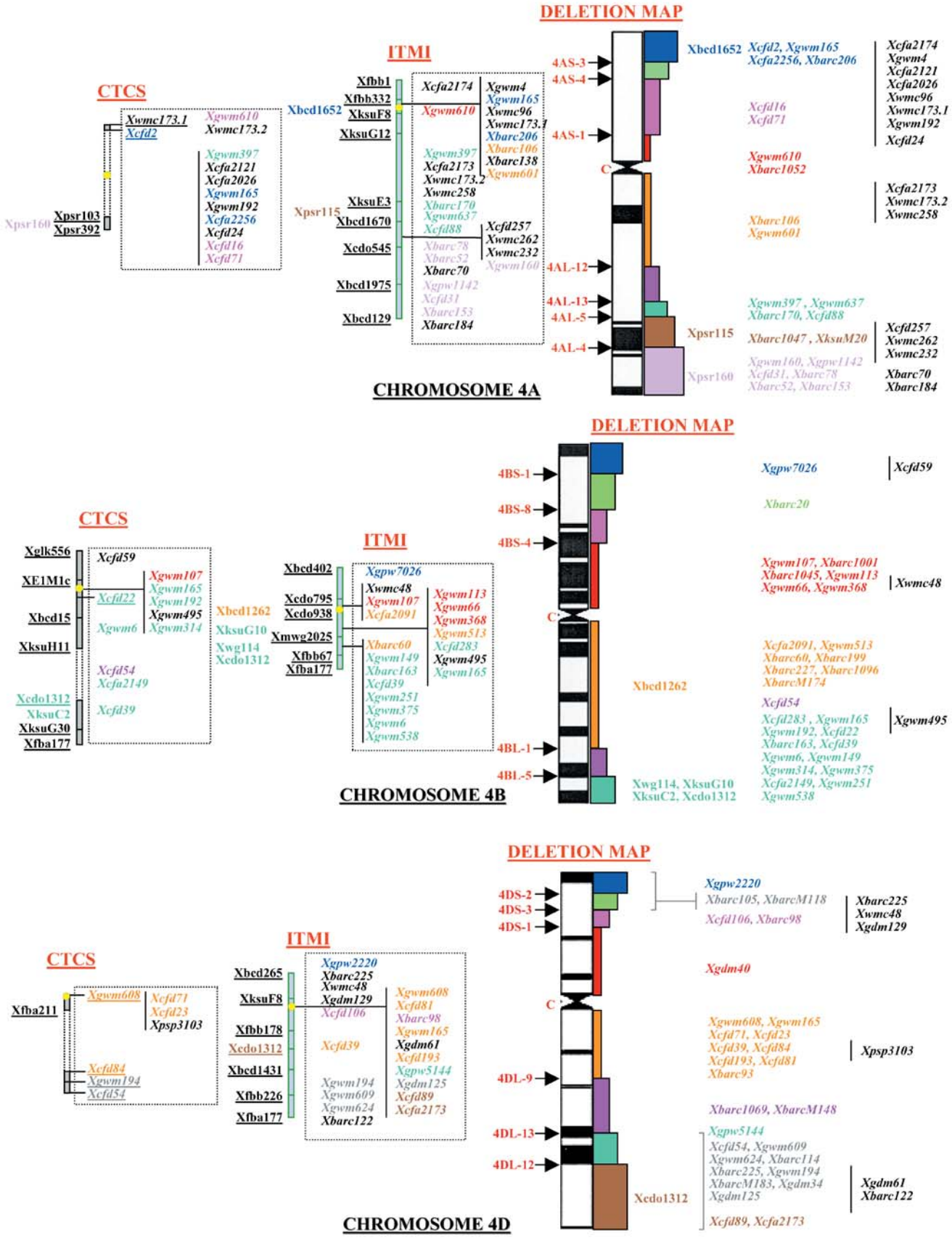

Fig. 4 Comparison between genetic and physical maps of wheat: homoeologous group 4. For details see Fig. 1

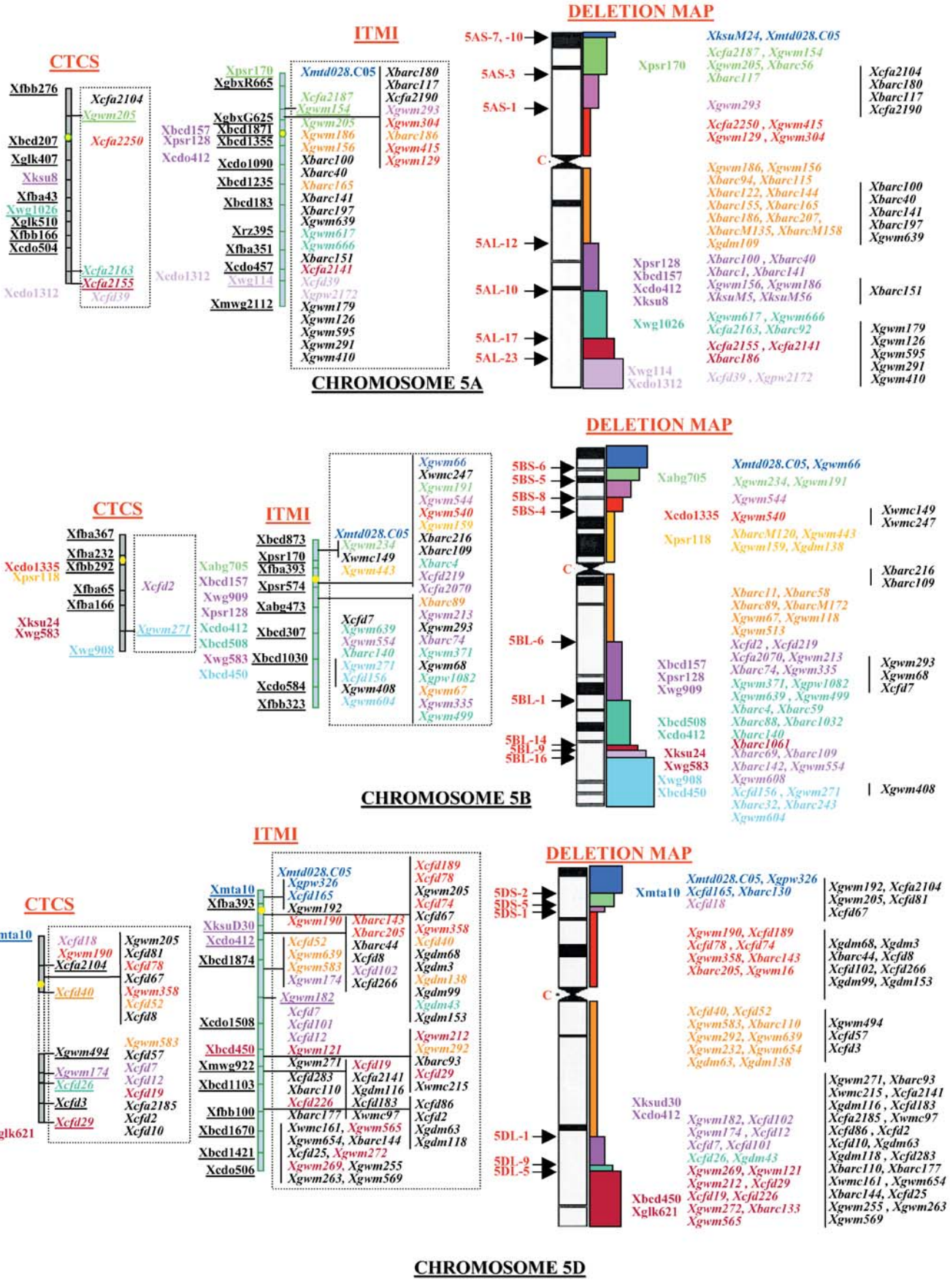

Fig. 5 Comparison between genetic and physical maps of wheat: homoeologous group 5. For details see Fig. 1

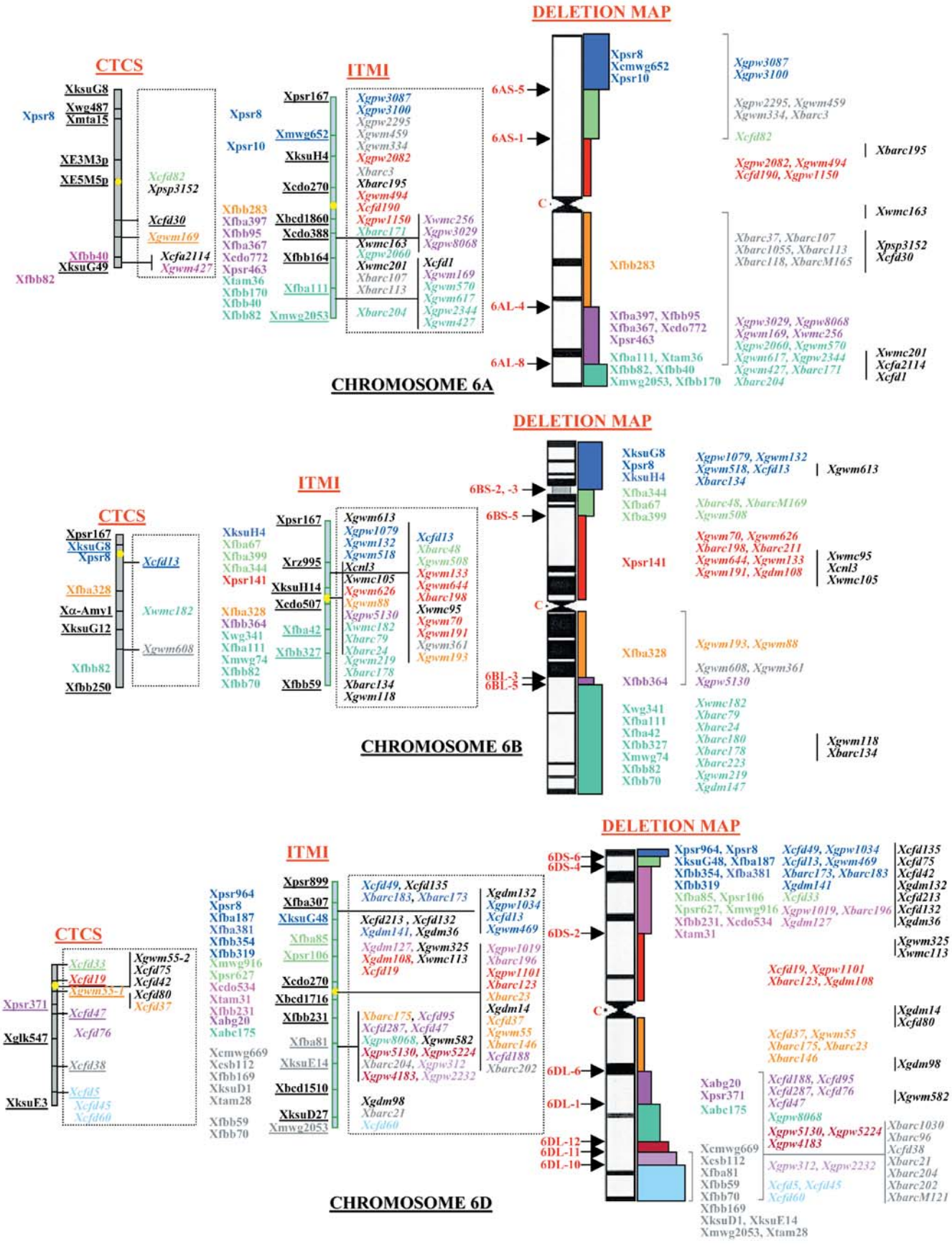

Fig. 6 Comparison between genetic and physical maps of wheat: homoeologous group 6. For details see Fig. 1

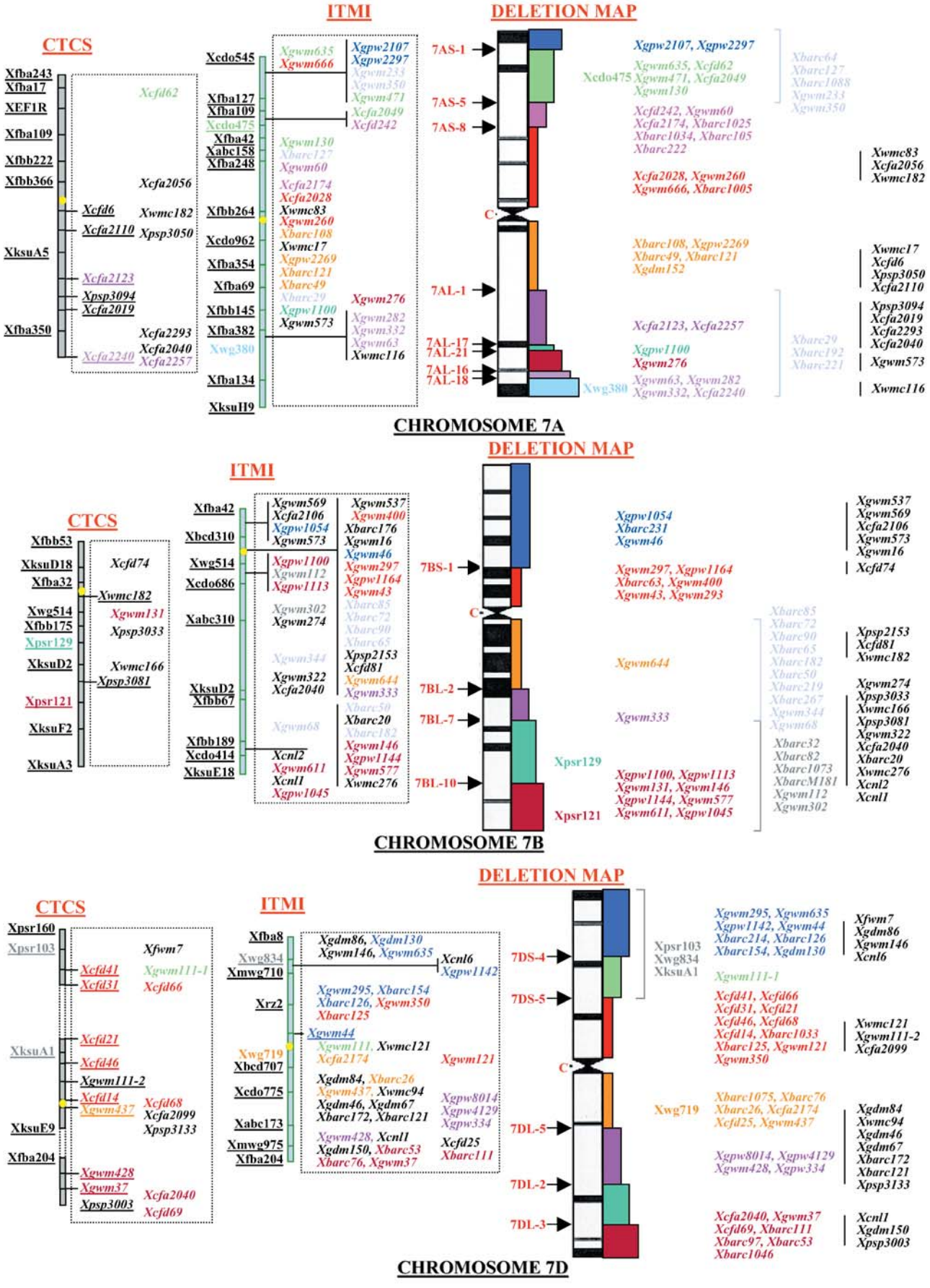

Fig. 7 Comparison between genetic and physical maps of wheat: homoeologous group 7. For details see Fig. 1

chromosome 5D, the bin C-5DL1-0.60 represented 60% of the long arm. This region included loci *Xcfd40* and *Xgwm583* (Fig. 5) which were found to be genetically independent (θ>50%) on the CtCS map suggesting the occurrence of a recombination hot spot in this cross in bin C-5DL1-0.60.

Homoeologous group 6

Ninety-eight microsatellite loci were located on the three homoeologues, 28 on 6A, 29 on 6B and 41 on 6D (Fig. 6). Chromosomes 6A, 6B and 6D were characterized by 6, 6 and 10 bins, respectively. No microsatellites were physically mapped in the bin C-6AL4-0.55. However, some of the *Xbarc* loci that could not be assigned precisely in this region (in grey) could be located in this bin. Seven loci covering 50 cM were assigned to bin 6DS6-0.99-1.00 confirming high rates of recombination in the telomeric regions. On the contrary, eight loci mapping in a cluster close to the centromere on chromosome 6B were all assigned to bin C-6BS5-0.76 covering half of the short arm of this chromosome, confirming low rates of recombination in the proximal regions.

Homoeologous group 7

One hundred and eleven microsatellite loci were placed on this homoeologous group, 39 on 7A, 35 on 7B and 37 on 7D (Fig. 7). Chromosomes 7A, 7B and 7D were characterized by ten, six and seven bins, respectively. Three bins were without SSR markers (7AL18-0.90-1.00, 7BL7-0.48-0.78 and 7DL2-0.61-0.82). Two recombination hot spots were detected on chromosome 7D for the CtCS population, one on the short arm between loci *Xcfd31-7D* and *Xcfd21-7D* located proximal to the centromere in bin C-7DS5–0.36 but found to be genetically independent (θ>50%), the other between *XksuE9-7D* and *Xfba204-7D* located either in bin 7DL5-0.30-0.61 or bin 7DL2-0.61-0.82.

Distribution of microsatellites and recombination

The microsatellite loci were found to be relatively evenly distributed along the chromosome length. We detected 21% of the SSR loci in the distal bins covering 20% of the physical length of the genome, and 36% of the proximal loci in the proximal bins covering 41% of the physical length. However, in some cases and using the centromere as reference, more loci appeared to be located in the distal regions as compared to the proximal. As an example, on the long arm of chromosome 5D (Fig. 5) and according to the ITMI genetic map, 29 out of the 79 microsatellite loci (37%) of the whole chromosome presumably mapped to the distal region that encompassed only 24% of the arm (bin 5DL5-0.76-1.00). On the contrary, only one locus was located in bins 5DS1-0.63-0.67 and 5DS5-0.67-0.78 on the short arm of this same chromosome. This may be explained by the fact that these two bins covered only 15% of the length of the short arm. Concerning recombination, as expected, we found huge discrepancies between telomeric and centromeric regions: 44% of the genetic linkage map (1,722/3,876 cM) was located in only the distal 20% of the physical length of the genome (ratio genetic %/physical % =2.20). On the contrary, 13.7% of the map (530/3,876 cM) was located in the proximal 41% of the genome (ratio 0.33). However, some differences were observed: in the distal bins 5DS2-0.78-1.00 and 7AS1-0.89-1.00 the genetic/physical ratios were only 1.09 and 1.08, respectively, indicating a low recombination frequency while in the proximal bins C2DS1-0.33 and C6AS1-0.35, the ratios were 1.33 and 1.63, respectively, suggesting higher recombination frequencies than expected.

Discussion

Genetic-physical relationships

It has been demonstrated that microsatellites are powerful molecular markers in wheat because of their high degree of polymorphism (Röder et al. 1998a, Stephenson et al. 1998) and also because of their high locus specificity compared to RFLP markers (Sourdille et al. 2001b, Guyomarc'h et al. 2002). Thus, they constitute a major tool for establishing genetic/physical relationships. Here, we used 725 microsatellites in order to identify shared markers between genetic and physical maps of bread wheat. This is very important because establishing such relationships is a prerequisite to positional cloning of important agronomical genes. Consistent with previous reports (Dvorak and Chen 1984; Werner et al. 1992; Gill et al. 1996a, 1996b; Kota et al. 1993), we found uneven distribution of recombination. Recombination hot spots are more frequent close to the telomeres than the centromeres. This is presumably because recombination occurs close to or even within the genes (for a review see Schnable et al. 1998), and as gene-rich regions are more numerous near the telomeres, more recombination occurs in these regions. Also, the gene-rich regions are expected to be highly decondensed which makes them more accessible to recombination factors compared to proximal heterochromatic regions containing highly repetitive sequences (Faris et al. 2000). However, unexpected results were observed. Nelson et al. (1995c) constructed a genetic map of chromosome 5D that was ~180 cM in length. The CtCS map was made of two blocks representing only ~120-cM length, separated by a recombination hot spot. The most proximal markers of each block, *Xcfd40* and *Xgwm494*, are theoretically separated by around 40 cM. They thus should have been genetically linked. This hot spot occurred in a proximal region where recombination is not supposed to be so frequently observed. On the contrary, in the distal bin 5DS2-0.78-1.00 located on the same chromosome, the genetic/physical ratio was only

1.09 which was two times less than expected, indicating a low recombination frequency in this region. In yeast, intragenic recombination frequencies were shown to be associated with specific short DNA sequences required for recombination hot spot activities (Smith 1994). The activity of these sequences depends on binding-specific transcription factors and/or to chromatin structure that allow hypersensitivity to nucleases (Fox et al. 1997; Mizuno et al. 1997). Occurrence of abnormal hot spots or lack of recombination may results from a dysfunction of one of these transcription factors or from a modification of the chromatin structure in these regions. Higher resolution mapping or eventually partial sequencing of these regions will provide definite answers.

Distribution of microsatellites

We used 725 microsatellite loci located on the seven homoeologous groups. This is less than the 1,951 loci described by Qi et al. (2002) but microsatellite loci have the advantage of being chromosome-specific. Deletion mapping in wheat only requires polymorphism between genomes. However, using RFLP probes, it is still possible that some loci cannot be identified because of lack of intergenomic polymorphism. One way to remedy this problem would be to use alternate restriction enzymes but this is time consuming and expensive. Moreover, some probes frequently detect more than one fragment mapping in the same deletion bin. In these cases, it is difficult to determine if each fragment corresponds to a locus or if such a pattern is due to the presence of a restriction site within the locus. All of these problems can be avoided since microsatellites generally give only one amplification product which can be easily attributed to only one bin. In addition, these markers are easy to automate and a lot of data can be produced rapidly. Only four microsatellite loci were detected in bins C-4AS1-0.20 and C-4AL12-0.43 from chromosome 4A, surrounding the centromere, while at least eight were genetically mapped in its neighbourhood. This suggests that genetic mapping only gives an indication of the relative position of the markers with each other. Frequently, markers genetically located close to the centromeres are not physically linked. This is probably due to the lack of recombination in these regions (see later). However, we cannot exclude the possibilities that only few microsatellites are located in the centromeric region or that SSRs located close to centromeres exhibit only a very low level or even no polymorphism.

Microsatellites are also known to evolve faster than coding sequences depending on the type of motif (Ellegren et al. 1995), the structure of the alleles (Brinkmann et al. 1998) or the number of repeats (Wierdl et al. 1997; Kruglyak et al. 1998; Schlötterer et al. 1998). On the contrary, coding sequences are less prone to mutations since the latter frequently may lead to a loss of function. This emphasises the need for extracting microsatellites from different genomic regions that may be under different selective forces. Traditionally, SSRs are isolated from genomic clones produced using various pre- or post-cloning procedures to create enriched libraries. However, significant efforts have recently been placed on generating substantial EST databases for plant species including wheat (GrainGenes). Even if only 8–9% of the ESTs are bearing a microsatellite (Gandon et al. 2002) and even if only 25% are giving polymorphic products (Gandon et al. 2002; Eujayl et al. 2002) EST-SSRs and genomic SSRs will constitute a tool of choice to study the variation of polymorphism between coding and non-coding regions, and between telomeric and centromeric regions. Such analyses will also enhance the value of EST-SSRs in marker-assisted selection, comparative genetic analysis and for exploiting wheat genetic resources by providing a more direct estimate of functional diversity.

Deletion mapping is a powerful technique for constructing a cytogenetically based physical map of the wheat chromosomes. Further physical and genetic mapping will result in integration of cytogenetic and linkage data into a unique correlated map of the entire wheat genome including breaking points, RFLP and microsatellite loci, ESTs and also the BAC clones that are now under development (B. Chalhoub, personal communication). The identification of molecularly tagged chromosome regions will open the possibility of molecular cloning of numerous agronomically useful genes that were previously intractable to classical molecular analysis.

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