An integrated physical map of 2072 SSRs Loci (gSSR and EST-SSRs) in bread wheat

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ABSTRACT

In bread wheat, as many as ~2,800 genomic SSRs (gSSRs) and ~300 EST-SSRs have been genetically mapped. Of these only ~1,320 gSSRs and no EST-SSRs were physically mapped when we started our studies at Meerut, so that a very large number of genetically mapped/unmapped gSSRs and EST-SSRs were yet to be physically mapped. In our laboratory, we physically mapped as many as ~1,500 SSR loci (~800 gSSR loci + ~700 EST-SSR loci) involving all the 21 wheat chromosomes. We combined our results with all available wheat SSR physical maps and produced an integrated map (with 2072 SSR loci), which had, a maximum of 776 loci (37.45%) on sub-genome B followed by 672 loci (32.43%) on sub-genome D and 624 loci (30.11%) on sub-genome A. However, in this map, the loci mapped within individual chromosome bins cannot be easily ordered. To overcome this problem, we have also initiated a radiation hybrid mapping programme, and initially taken up chromosomes 5B and 7B for further fine mapping.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a segmental allohexaploid with a very large genome (16,000Mb), having >80% repetitive DNA, which is interspersed within the gene-rich and gene-poor regions. These attributes of wheat genome made it difficult to prepare high density molecular maps. Moreover, the recombination rates differ in different parts of individual chromosomes, so that the genetic and physical distances in the maps do not correspond, making it necessary to construct physical maps and then compare them with genetic maps, to make them useful for both marker assisted selection (MAS) and map-based cloning. In bread wheat, as many as ~2,800 gSSRs[1] have already been genetically mapped. Out of these genetically mapped gSSRs, only ~1,320 gSSRs have been physically mapped[2]. In addition to this, physical mapping of as many as 275 EST-SSRs was also recently completed by us[3]. However, despite these earlier studies on physical mapping of wheat SSRs, a very large number of genetically mapped/unmapped gSSRs and EST-SSRs are yet to be physically mapped. In the present study, an additional set of 503 SSRs, including 385gSSRs, 18 HC-SSRs (High C_ot) and 100 MF-SSRs (Methyl-Filtration) were tried for physical mapping leading to successful mapping of 252 SSRs on 311 SSR loci. An integrated physical map using this data with all other available maps was also prepared and analysed.

MATERIAL AND METHODS

In the present study, 21 nullisomic-tetrasomics (NTs), 24 ditelosomics (Dts) and 192 overlapping deletions were used to facilitate physically mapping. The primer sequences of 385 gSSRs (286 wmc, 75 gwm and 24 gdm) were retrieved from GrainGenes (**http://wheat.pw.usda.gov**), and those for additional 118 SSRs (18 HC-SSRs and 100 MF-SSRs earlier detected in our own laboratory) were designed in our own laboratory. All these primers were synthesized on contract by Illumina Inc, USA. DNA isolation and PCR analysis were undertaken as per details described elsewhere[4].

RESULTS AND DISCUSSION

In the present study, as many as 503 SSRs (including 385 gSSRs, 18 HC-SSRs and 100 MF-SSRs) were tried on 21 NT, 24 Dt and 192 overlapping deletion lines. This allowed successful mapping of 252 SSRs at 311 SSR loci, which included 248 gSSRs loci, 11 HC-SSR loci and 52 MF-SSR loci. This information was combined not only with our own earlier published physical maps (involving 313 gSSR loci and 672 EST-SSR loci[1,2]), but also with the physical maps prepared by other[5-7]. As a result, an integrated physical map of wheat genome with 2072 SSR loci became available (Table 1), which is described in this communication. A representative map for chromosome 2B is given in Figure 1 (figures for other 20 chromosomes are available with the authors).

Distribution of loci on three sub-genomes and seven homoeologous groups

Out of 2072 loci that are now available on the integrated SSR physical map, a maximum of 776 loci (37.45%) were mapped on sub-genome B (with the highest DNA content), followed by 672 loci (32.43%) on sub-genome D (with lowest DNA content) and 624 loci (30.11%) on sub-genome A. Among the seven homoeologous groups, a maximum of 345 loci were available on group 2 and a minimum of 226 loci were available on group 4. Chi-square (χ^2) tests were conducted for testing random distribution of loci on three sub genomes, and among seven homoeologous groups. The expected distribution was worked out on the basis of either the relative lengths of chromosomes in μM[8] or on the basis of DNA content; the results were significant (P = <0.05) and suggested non-random distribution of loci both on the three sub-

genomes and among seven homoeologous groups. These results were in agreement with earlier studies [1, 2, 5-7, 9-11].

Redundancy of SSR loci in physical map

Another interesting feature of integrated map is the availability of multiple loci for individual SSRs. Among gSSRs, there were 155 multilocus SSRs (137 gSSRs, 1HC SSRs and 17MF SSRs), which mapped on 248 loci, and among EST-SSRs, there were 275 multilocus SSRs, which mapped on 672 loci. This clearly shows that relative to gSSRs, the mean number of loci per EST-SSR was much higher.

Table1: Distribution of loci (gSSR + EST-SSR) according to their assignment to wheat chromosomes.

	Homoeologous group							
	1	2	3	4	5	6	7	Total
Sub-genome A	81	91	106	77	91	80	98	624
Sub-genome B	125	129	111	72	114	102	123	776
Sub-genome D	97	125	96	77	98	72	107	672
Total	303	345	313	226	303	254	328	2072
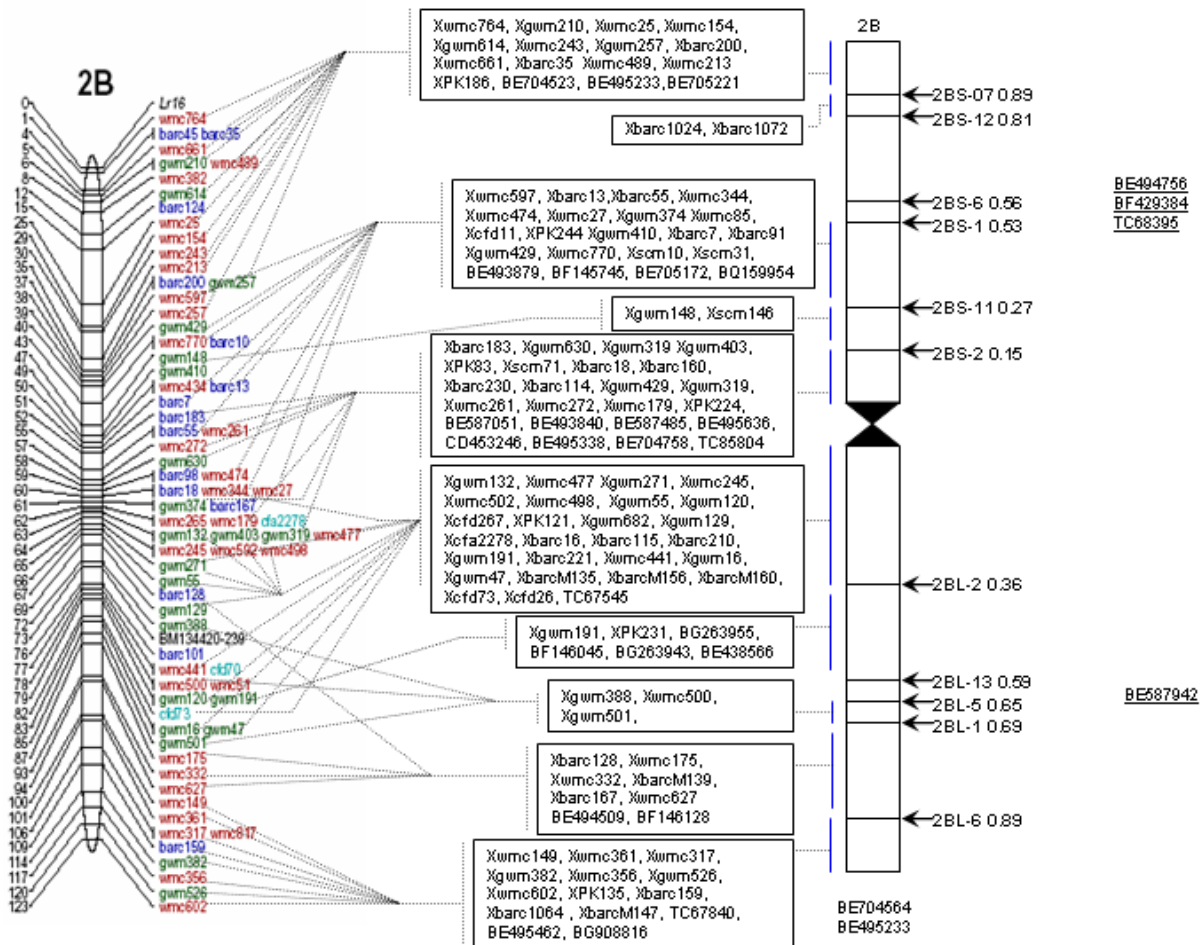
Figure 1.

Integrated physical map of chromosome 2B (on the right) and its comparison with the genetic map (on the left). The EST–SSRs, which could not be assigned to bins and were assigned to chromosome arms only are underlined and given on the right of the physical map and those assigned to chromosomes (with no information about arm) listed at the bottom.

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Comparison of physical maps with the genetic maps

Since majority of SSR loci were available both on the integrated map prepared during the present study and on the earlier published genetic maps[6,10], it was possible to compare positions of these SSR loci on physical and genetic maps. It was noticed that the linear orders of the genetically mapped SSRs are not very different in the integrated physical map. Nevertheless, 75 SSR loci involving chromosomes 1B, 2A, 2B, 3A, 3B, 3D, 4A, 5D, 6B and 7B were physically mapped in regions other than those on the corresponding genetic maps. In no case, an individual SSR locus occupied different chromosomes, not even different arms of the same chromosome.

FUTURE PROSPECTS

To further increase the density of the integrated physical map, we are currently mapping on wheat genome another 132 class I gSSRs belonging to wheat and its relatives (derived from ~14 Mb genomic sequences available at http://www.tigr.org/tdb/e2k1/tae1/info.shtml), and hundreds of gSSRs belonging to *Brachypodium distachyon*. Efforts are also being made to arrange in linear order the SSR loci available in individual bins using the approach of radiation hybrid mapping. Hopefully, the SSR physically mapped to specific chromosome bins in the integrated physical map may be used as anchor points for construction of global physical BAC contig map that is needed for sequencing the entire gene space in wheat genome under the aegis of International Wheat Genome Sequencing Consortium (IWGSC).

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