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An integrated physical map of simple sequence repeats in bread wheat

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Abstract

Physical mapping of DNA-based markers in wheat has been greatly facilitated due to the availability of deletion stocks, which constitute an ideal material for mapping these markers to specific chromosomal regions or bins to create physical landmarks. In the present study, the available physical maps for wheat SSRs were enriched by addition of 128 new SSR loci that belonged to wheat gSSRs and brachypodium gSSRs and EST-SSRs. This led to the development of an integrated physical map of 2,031 wheat SSR loci. A maximum of 765 loci (37.67%) were mapped on sub-genome B followed by the 651 loci (32.05%) on sub-genome D and 615 loci (30.28%) on sub-genome A, thus giving a mean resolution of 7.8 Mb between any two SSR loci. Relative to genomic SSRs (gSSRs), the EST-SSRs of brachypodium showed greater transferability in cv. Chinese Spring. Using 704 SSR loci which were mapped genetically as well as physically, a comparison was made between genetic and physical maps to determine the distribution of recombination frequencies (cM/Mb) in different regions of the wheat genome. Recombination frequencies within the individual bins ranged from 0.01 cM/Mb (low recombination) to 13.16 cM/Mb (high recombination), suggesting an uneven distribution along the chromosomes or chromosome arms. Hopefully, the integrated physical map presented in this communication may prove useful in the currently on-going whole genome sequencing of wheat genome through alignment of BAC contigs. A comparison of integrated physical map with genetic linkage map will also facilitate on-going and future genomics research.

Keywords: Wheat, *Brachypodium distachyon*; SSRs, genetic map; physical map; recombination frequency

Introduction

Bread wheat (*Triticum aestivum* L. em Thell. 2n = 42, AABBDD) is a segmental allohexaploid (genome size, ~16 Gb), having >80% repetitive DNA which is interspersed within the gene-rich regions (GRRs) and gene-poor regions (GPRs). Recombination rates differ in different parts of individual chromosomes and therefore genetic and physical distances in the maps do not correspond well. However, during the last two decades, significant progress has been made in preparing high density molecular maps in wheat. Initially, restriction fragment length polymorphism (RFLP) markers were used for construction of genetic and physical maps. These efforts led to the mapping of ~2000 RFLP loci in the genetic maps and 1,200 RFLP loci in the physical maps (for reviews, see Gupta et al., 1999, 2008a; Hussain and Qamar 2007). Later emphasis shifted to the use of PCR-based microsatellite or simple sequence repeat (SSR) markers for the construction of wheat molecular maps (Stephenson et al., 1998; Roder et al., 1998). The available wheat genetic and physical maps prepared using SSRs contain approximately 3,000 and 2,000 SSR loci, respectively (see Gupta et al., 2008a for review). Sourdille et al (2004) prepared a physical map of 725 genetically mapped SSRs using wheat deletion stocks and also established relationship between the genetic and physical maps. The remaining genetically mapped SSR markers provide an opportunity for their physical mapping and to understand the relationship between the genetic and physical distances between the mapped loci (Erayman et al., 2004; See et al., 2006). It is known that the recombination occurs predominantly in the distal regions of the wheat chromosomes (Erayman et al., 2004). Precise relationship among recombination rate, gene distribution and gene density in wheat chromosomes have not been established except in case of the longest wheat chromosome 3B (995 Mb), where recombination has been examined along the entire length of the chromosome using 102 markers. It was found that the recombination frequency (also known as crossover frequency) increases gradually from proximal region to distal region (Saintenac et al., 2009). In the present study, we prepared an integrated SSR physical map of the whole genome with higher density of markers for all the wheat chromosomes utilizing the available physical maps and the SSR markers that were mapped during the present study. A relationship between the recombination frequency and distribution of SSR markers was also examined by comparing integrated physical maps with available consensus genetic maps using common SSR markers.

Results and Discussion

It is known that bread wheat genome is buffered by polyploidy (triplication of genomic content) that allows it to tolerate the loss of individual chromosomes, arms, and segments (Endo and Gill, 1996). This feature of bread wheat genome and the availability of valuable cytogenetic stocks/lines facilitated the development of a number of physical maps for all the 21 wheat chromosomes (Sourdille et al., 2004; Qi et al., 2004; Goyal et al., 2005; Mohan et al., 2007).

Table 1. Details of the gSSRs and EST–SSRs used and the number of loci physically mapped in wheat (in each case, number of SSRs used for mapping are given in parenthesis).

SSR type	SSR used	Number of SSR loci mapped
Wheat gSSR		
Chromosome 3B specific SSR	154	28 (28)
Random SSR	132	48 (34)
Brachypodium SSR		
gSSR	47	17 (16)
EST-SSR	96	35 (30)
Total	429	128 (108)

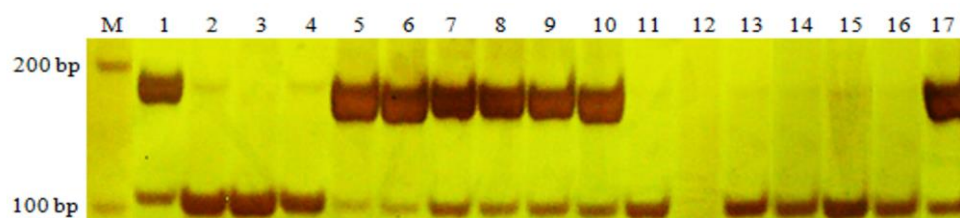

Fig 1. Gel pictures showing physical mapping of PCR amplified 195bp fragment of wheat gSSR locus (mwm152) in distal bin of long arm of chromosome 3B; Lanes, M 100-bp ladder, 1 Chinese Spring (CS), 2 N3BT3A, 3 N3BT3D, 4 Dt3BS, 5 Dt3BL, 6 3BS-5, 7 3BS-10, 8 3BS-2, 9 3BS-8, 10 3BS-3, 11 3BL-2, 12 3BL-8, 13 3BL-3, 14 3BL-9, 15 3BL-10, 16 3BL-7, 17 3BL-11.

During the present study, new SSR loci were physically mapped on wheat chromosomes using wheat gSSRs and *Brachypodium distachyon* (brachypodium) gSSRs and EST-SSRs. The information on the above mapped loci was integrated in the existing physical maps reported in our earlier study (Gupta et. al, 2008b). The integrated physical map and the consensus genetic map were compared for understanding the colinearity between them. These maps were also used to derive information on the distribution of recombination frequencies along the lengths of individual chromosomes. The recombination frequencies were also correlated with the gene-rich regions reported in the past (Sandhu et al., 2001; Erayman et al., 2004).

Physical mapping of gSSRs and EST-SSRs

A total of 429 SSRs (286 gSSRs of wheat; 47 gSSRs + 96 EST-SSRs of brachypodium) were used for physical mapping in wheat using wet-lab approach (Fig. 1). This allowed mapping of 128 SSR loci (76 gSSRs of wheat; 17 gSSRs + 35 EST-SSRs of brachypodium) belonging to 108 SSRs that were distributed on all the wheat chromosomes (Table 1). Remaining 321 SSRs (224 gSSRs of wheat; 31 gSSRs + 66 EST-SSRs of brachypodium) could not be assigned to specific wheat chromosomes because for each of these SSRs, the amplified fragment of the expected size was available in all the nullisomic-tetrasomic lines. We believe that each such SSR was present on more than one homoeologues, exhibiting no polymorphism since assignment of homoeoalleles to specific bins requires polymorphism among three sub-genomes.

Distribution of genomic and EST-SSR loci in wheat genome

A summary of the distribution of SSR loci physically mapped during the present study is given in Table 2. Out of 128 loci mapped, much higher number of loci were mapped on B sub-genome (74 loci = 57.8%) than on the D (33 loci = 25.7%) and the A (21 loci = 16.4%) sub-genomes. This unequal distribution may be attributed to highest DNA content of B sub-genome and the use of a large number of chromosome 3B specific SSRs. Similarly, mapping of higher number of SSR loci on the D sub-genome than on the A sub-genome may be attributed to the use of D sub-genome specific SSRs derived from *Aegilops tauschii*. Among the seven homoeologous groups, 47 SSR loci (maximum) were mapped on group 3 chromosomes particularly on chromosome 3B (due to utilization of 3B specific gSSRs), and only 5 SSR loci (minimum) were mapped on group 4 chromosomes. The minimum number of loci in group 4 may be attributed to low level of polymorphism in group 4 chromosomes (Zhang et al., 2005). The distribution pattern of brachypodium EST-SSR loci in wheat genome was analyzed separately; their distribution on the three sub-genomes and seven homoeologous chromosomes was non-random. Maximum of 22 EST-SSR loci (62.8%) were mapped on chromosomes of B sub-genome and the minimum of 6 loci were mapped on D sub-genome. Among the seven homoeologous groups, group 3 chromosomes had highest number of mapped loci (11 loci) and group 4 had lowest number of mapped loci (2 loci). This distribution of mapped loci followed the distribution of DNA content in the three sub-genomes and the seven homoeologous groups, so that more loci were mapped in the sub-genome/homoeologous groups with relatively higher DNA content and fewer loci were mapped on the sub-genome/homoeologous groups with relatively lower DNA content.

Transferability of* brachypodium *SSRs to wheat

Together with physical mapping, we also evaluated the transferability of brachypodium EST-SSRs and gSSRs to wheat. Eighty nine out of the 96 EST-SSRs (i.e. 92.70% EST-SSRs) gave amplification product when tested in wheat (cv. Chinese Spring); seventy two (80.89%) of these 89 EST-SSRs gave products of the expected size, indicating a high level of cross-species transferability. These results are comparable to the high level of interspecific transferability of EST-SSRs earlier reported in cereals (Gupta et al., 2003; Kumar et al., 2009; Zeid et al., 2010). The success of cross-

Table 2. Distribution of 128 gSSR and EST-SSR loci (mapped during the present study) according to their assignment to wheat chromosomes arranged in a two-way classification.

Homoeologous group Sub-genome	1	2	3	4	5	6	7	Total
A	4	1	1	1	7	4	3	21
B	6	4	41	0	10	4	9	74
D	5	5	5	4	5	5	4	33
Total	15	10	47	5	22	13	16	128

Table 3. Distribution of 2,031 mapped SSR loci (including both gSSR and EST-SSR loci) on the three sub-genomes and seven homoeologous groups in the integrated physical map of bread wheat.

Homoeologous group Sub-genome	1	2	3	4	5	6	7	Total
A	84	86	99	75	92	81	98	615
B	118	133	137	67	115	87	108	765
D	98	122	87	71	98	74	101	651
Total	300	341	323	213	305	242	307	2,031

species amplification and transferability depends on the evolutionary distance between the source and the target species (Rossetto, 2002). Therefore, it was not unexpected that brachypodium EST-SSRs would show high transferability to wheat as both are closely related species and belong to the same subfamily *Pooideae* (Huo et al., 2008). Like EST-SSRs, 40 out of 47 brachypodium gSSRs (85.12%) gave amplification in wheat cv. Chinese Spring and as many as 27/40 (67.50%) brachypodium gSSRs gave products of expected size, indicating a fairly high level of transferability of gSSRs to wheat. However, the level of transferability of brachypodium gSSRs was lower than the transferability of brachypodium EST-SSRs. This is in agreement with earlier reports suggesting higher transferability of EST-SSRs than gSSRs (Varshney et al., 2005; Sim et al., 2009). The higher level of transferability of EST-SSRs than the gSSRs may be attributed to more conserved nature of coding region across the species. The gSSRs relative to EST-SSRs are more genome-specific but less transferable to related species due to their relatively poor conservation across the related species. The potential use of wheat and barley EST-SSRs has also been demonstrated for comparative mapping in wheat, rye and rice (Varshney et al., 2005). These studies suggested that the available EST-SSR markers in one species could be used in related plant species. Thus, brachypodium SSRs are good candidate for the development of conserved orthologous markers for genetic analysis and breeding in wheat.

Integrated physical map of SSRs

An integrated physical map of bread wheat comprising as many as 1,903 SSR loci were made available earlier in our laboratory (Gupta et al., 2008b). During the present study, we integrated a new set of 128 SSR loci into the above map resulting into a physical map containing 2,031 SSR loci (Supplementary Fig. 1a-u). These loci were distributed throughout the wheat genome giving an average resolution of 7.8 Mb between the two loci.

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

Out of 2,031 SSR loci that are now available in the integrated physical map, a maximum of 765 loci (37.67%) were mapped on sub-genome B (with the highest DNA content) followed by the 651 loci (32.05%) on sub-genome D (with the lowest DNA content) and 615 loci (30.28%) on sub-genome A (Table 3). Chi-square (χ^2) analysis for goodness-of-fit was conducted for testing the distribution of loci on three sub-genomes and among seven homoeologous groups. The expected distribution of loci was examined on the basis of DNA content and the physical length of the chromosomes (Gill et al., 1991); the results were statistically significant ($P<0.05$) suggesting non-random distribution of SSR loci on the three sub-genomes. Significantly higher number of loci were mapped on sub-genome B than those expected. Among the sub-genomes A and D, higher number of loci was present on sub-genome D rather than on sub-genome A, which was unexpected in view of the DNA content and relative size of these two sub-genomes. Our results on the distribution of SSR loci agreed with earlier report (Gadaleta et al., 2009). The distribution of SSR loci among the seven homoeologous groups of wheat chromosomes was also non-random ($P< 0.05$). Among the seven homoeologous groups, maximum number of SSR loci (341) was mapped on group 2 chromosomes and minimum number of SSR loci (213) was mapped on group 4 chromosomes. This distribution pattern is in agreement with the distribution of SSR loci in the physical maps reported earlier (Sourdille et al., 2004).

Distribution of SSR loci on individual chromosomes and their arms

On the basis of DNA content and physical length of chromosomes, the distribution pattern of SSR loci on individual wheat chromosome was also non-random ($P << 0.05$). Among the 21 wheat chromosomes, a maximum of 137 and 133 loci were mapped on chromosomes 3B and 2B, respectively, and a minimum of 67 loci were mapped on chromosome 4B. The distribution pattern of SSR loci on the long and short arms was studied in relation to their DNA contents and relative lengths. Over all, more SSR loci were mapped on the long arms (1,211 loci; 59.62%) than on the short arms (773 loci; 38.06%). This distribution pattern followed 38% excess DNA in the long arms than in the short arms (Furata and Nishikawa, 1991). Among the chromosome arms, 5BL is the longest arm and had relatively more mapped loci during the present study. This particular trend was observed in all cases except chromosome arms 2BS, 7AS and 7DS which had more loci than their corresponding long arms, and the chromosome arms 2AS and 2AL where the number of loci mapped on the two arms was same. The SSR loci that were mapped to a chromosome but not to a specific arm were excluded from this analysis. The distribution pattern of EST-SSR and gSSR loci within individual arms was also analysed. The analysis showed that more EST-SSRs were mapped in the distal 60% regions while more gSSRs were mapped in the

proximal 40% regions of the chromosome arms. These results are in agreement with the observations that the gene-rich regions are located in the distal parts of the chromosome arms (Akhunov et al., 2003; Erayman et al., 2004; See et al., 2006).

Multilocus SSRs in integrated physical maps

Even though most wheat SSRs are locus-specific, some SSRs are mapped on multiple loci on different chromosomes belonging to separate genomes of hexaploid wheat. Therefore, the SSRs that were mapped on multiple loci in the integrated physical map were also sorted out during the present study. Among gSSRs, 169 multilocus gSSRs were such, which were mapped on 265 loci, and among the EST-SSRs, there were 279 multilocus SSRs {including the wheat SSRs earlier mapped by Mohan et al. (2007) and of brachypodium EST-SSRs mapped during the present study}, which mapped on 677 loci. This clearly showed that among the multilocus SSRs, the mean number of loci mapped per EST-SSR was much higher than the mean number of loci mapped per gSSR. Further examination of multilocus SSRs also suggested that some of the SSRs were present on homoeologous chromosomes at altered positions. It is thus obvious that although most of the SSRs are locus specific, perhaps homoeoloci and paralogues (non-homoeologous duplicates) of the SSRs are also present in the genome. It is concluded that the frequency of multilocus gSSRs and EST-SSRs in the wheat genome is fairly large and this is mainly attributed to polyploid nature of bread wheat. The exact mechanism of the origin of multilocus SSRs in wheat is not known. However, duplications preceding the evolution of polyploid wheat (Akhunov et al., 2003) and/or the movement of SSR containing transposon like sequences (within and among different chromosomes) may have contributed to the observed nature of multilocus SSRs in bread wheat (Qi et al., 2002; Wicker et al., 2010).

Genetic-physical map comparisons

The integrated SSR physical maps of individual wheat chromosomes prepared during the present study were compared with the SSR consensus genetic maps of bread wheat earlier reported by Somers et al. (2004), by integrating four independent genetic linkage maps (Synthetic/Opata, RL4452/AC Domain, Wuhan/Maringa and Superb/BW278). The consensus genetic map covered 2,569 cM distance and included 1,235 SSR loci. The physical map prepared during the present study and the consensus genetic map had 791 SSR loci in common that were distributed on all the 21 chromosomes of bread wheat. Integrated physical map was compared with consensus genetic map by drawing lines to join the common SSR loci (Supplementary Fig. 1a-u). The number of SSR loci shared between the physical maps prepared during the present study and the available consensus genetic maps included 306 loci from sub-genome B, 248 loci from sub-genome A and 237 loci from sub-genome D. The number of shared loci between the consensus genetic and physical maps of the individual homoeologous groups varied from 79 loci (group 6 chromosomes) to 146 (group 2 chromosomes). A range of 19 SSR loci on 6D (Supplementary Fig. 1r) to 63 SSR loci on 2B (Supplementary Fig. 1e) were shared per chromosome between both the maps. Similarly, the number of genetically mapped SSR loci that were mapped in an individual bin of the physical map ranged from 1 to 15. In majority of cases, the distribution of SSR loci on the individual chromosomes of the integrated physical maps and the consensus genetic maps was nearly similar; although few perturbations of SSR loci between both maps were also noticed (discussed in later section). In the past, comparison between genetic and physical maps (sharing common molecular markers in the genetic and physical maps) were made in a number of crop plants including wheat (Saintenac et al., 2009), rice (Kim et al., 2007), sorghum (Klein et al., 2000), maize (Cone et al., 2002) etc., which proved helpful for anchoring the BAC contigs during alignment, comparative genome analysis and also for map-based gene isolation. Integrated physical map of SSRs, where all the shared SSR markers had consensus order, will accelerate the pace of gene mapping and cloning in bread wheat.

Perturbation of shared SSR loci in genetic and physical maps

Examination of the majority of SSR loci shared by the integrated physical maps and the consensus genetic maps revealed that the linear order (colinearity of mapped SSRs between two maps) of the loci was not very different in the two maps (Supplementary Fig. 1a-u). However, 11% (87/791) SSR loci shared by the physical and genetic maps showed some rare perturbations between the two maps. Such loci were classified into two groups, (i) loci mapped on the same arm (either short or long) of individual chromosome in the genetic and in the physical maps, and (ii) the loci mapped on different arms of individual chromosomes in genetic and physical maps. No case was observed, where an individual SSR locus occupied different chromosomes in integrated physical map (Supplementary Table 1; Supplementary Fig. 1a-u). The perturbations of the loci in the two maps could be attributed to the mapping error, difference in recombination rates, or even to the structural changes in chromosomes in some cases.

Genetic-physical map: recombination distribution

Physical mapping in wheat using deletion stocks provide a fast and efficient method of locating DNA sequences within a specific chromosomal region or bin to create physical landmarks (Qi et al., 2004; Goyal et al., 2005) and to relate these to genetic/recombination maps (Sandhu and Gill 2002). A comparison of the genetic and physical maps allows delineations of the genomic regions with high and low recombination rates (based on genetic distance vs. physical distance) along the length of individual chromosomes (Erayman et al., 2004, Goyal, 2008). During the present study, using SSR loci (704) common to both the genetic and physical maps, estimation of recombination frequency (or crossover frequency) was carried out for bins of all the wheat chromosomes. Bins containing perturbed SSRs were not included in the estimation of recombination frequency. Assuming that the condensation of the DNA is homogeneous all along (from the centromere to the telomere) the short and the long arms of each of the 21 chromosomes of bread wheat (Dvorak et al., 2004), recombination frequencies were separately calculated for each bin of each of the chromosome arm. Within the individual bins, recombination frequencies ranged from 0.01 cM/Mb (low recombination) in the centromeric bins of chromosome arms 3DL (C-3DL1-0.23) and 4DS (C-4DS1-0.53) to 13.16 cM/Mb (high recombination) in the interstitial bin of 5AL (5AL22-0.56-0.57). The recombination frequencies and their distribution were highly uneven along the chromosomes or chromosome

Table 4. Detailed analysis of the comparison between the genetic and physical maps of wheat.

Chrom-osome arm	Fraction length	Size of bin (Mb)	SSRs shared between both maps	Mb/SSR	Distance covered in genetic map (cM)	Recombination frequency (cM/Mb)
1AS	0.86-1.00*	38.50	3	12.83	21	0.55
	C-0.20	55.00	8	6.88	17	0.31
1AL	C-0.47	245.81	3	81.94	3	0.01
	0.47-0.61	73.22	6	12.20	54	0.74
	0.61-1.00*	203.97	4	50.99	55	0.27
1BS	0.80-1.00*	62.80	5	12.56	10	0.16
	C-0.35	109.90	9	12.21	6	0.05
1BL	C-0.32*	171.20	7	24.46	32	0.19
	0.47-0.73	139.10	3	46.37	25	0.18
	0.73-0.82*	48.15	7	6.88	35	0.73
	0.89-1.00*	58.85	9	6.54	50	0.85
1DS	0.70-1.00*	67.20	5	13.44	28	0.42
	C-0.48	107.52	7	15.36	27	0.25
1DL	C-0.18*	68.58	9	7.62	26	0.38
	0.64-1.00	137.16	7	19.59	40	0.29
2AS	0.78-1.00	86.02	9	9.56	46	0.53
	C-0.78	304.98	8	38.12	42	0.14
2AL	C-0.27	137.16	7	19.59	72	0.52
	0.85-1.00*	76.20	5	15.24	81	1.06
2BS	0.89-1.00*	46.42	13	3.57	37	0.80
	0.27-0.53	109.72	11	9.97	23	0.21
	C-0.15	63.30	6	10.55	10	0.16
2BL	C-0.36*	182.16	13	14.01	21	0.12
	0.65-0.69	20.24	3	6.75	13	0.64
	0.69-0.89	101.20	4	25.30	27	0.27
	0.89-1.00	55.66	8	6.96	23	0.41
2DS	0.47-1.00*	167.48	13	12.88	47	0.28
	C-0.33	104.28	8	13.04	22	0.21
2DL	C-0.09	36.99	10	3.70	27	0.73
	0.66-0.69	12.33	3	4.11	16	1.30
	0.69-1.00	127.41	8	15.93	19	0.15
3AS	0.45-1.00	198.00	6	33.00	45	0.23
	C-0.13	46.80	6	7.80	5	0.11
3AL	C-0.21	98.28	7	14.04	14	0.14
	0.42-0.85*	201.24	4	50.31	25	0.12
	0.85-1.00*	70.20	10	7.02	50	0.71
3BS	0.87-1.00*	56.16	7	8.02	57	1.01
	0.56-0.78*	95.04	2	47.52	24	0.25
	C-0.20	86.40	12	7.20	13	0.15
3BL	C-0.22	123.42	8	15.43	48	0.39
	0.81-1.00*	106.59	9	11.84	51	0.48
3DS	0.59-1.00	131.61	11	11.96	22	0.17
	C-0.31	99.51	6	16.59	28	0.28
3DL	C-0.23	103.27	2	51.64	1	0.01
	0.23-0.81	260.42	5	52.08	13	0.05
	0.81-1.00*	85.31	5	17.06	24	0.28
4AS	0.76-1.00	76.08	6	12.68	7	0.09
4AL	C-0.43	231.77	11	21.07	32	0.14
	0.43-0.59	86.24	5	17.25	42	0.49
	0.80-1.00	107.80	15	7.19	41	0.38
4BS	C-1.00	391.00	11	35.55	27	0.07
4BL	C-0.10	43.00	8	5.38	7	0.16
	0.95-1.00*	21.50	12	1.79	27	1.26
4DS	0.82-1.00*	41.58	4	10.40	30	0.72
	C-0.53*	122.43	2	61.22	1	0.01
4DL	C-0.09	37.44	9	4.16	24	0.64
	0.70-1.00*	124.80	6	20.80	24	0.19
5AS	0.75-0.97*	64.90	7	9.27	29	0.45
	C-0.40*	118.00	8	14.75	22	0.19
5AL	C-0.33	175.56	10	17.56	32	0.18
	0.33-0.56	122.36	2	61.18	24	0.20
	0.56-0.57*	05.32	4	1.33	70	13.16

	0.68-0.78	53.20	3	17.73	28	0.53
	0.82-0.87	26.60	2	13.30	1	0.04
	0.87-1.00*	69.16	5	13.83	17	0.25
5BS	0.81-1.00*	55.10	4	13.78	27	0.49
	0.71-0.81	29.00	3	9.67	42	1.45
	0.56-0.71*	43.50	2	21.75	2	0.05
	C-0.13	37.70	9	4.19	7	0.19
5BL	C-0.59	342.20	7	48.89	58	0.17
	0.59-0.75*	92.80	2	46.40	79	0.85
	0.75-0.79*	23.20	5	4.64	79	3.41
	0.79-0.82*	17.40	2	8.70	41	2.36
	0.82-1.00	104.40	11	9.49	60	0.57
5DS	0.78-1.00	56.76	5	11.35	16	0.28
	C-0.22	56.76	7	8.11	12	0.21
5DL	C-0.60	294.00	3	98.00	35	0.12
	0.60-0.69*	44.10	13	3.39	57	1.29
	0.76-1.00*	117.60	13	9.05	51	0.43
6AS	0.67-1.00*	110.88	2	55.44	2	0.02
	0.35-0.67*	107.52	3	35.84	22	0.20
	C-0.35	117.60	5	23.52	8	0.07
6AL	C-0.42	154.98	9	17.22	15	0.10
	0.88-1.00	44.28	8	5.54	73	1.65
6BS	0.76-1.00*	99.60	9	11.07	27	0.27
	0.46-0.76	124.50	4	31.13	7	0.06
	C-0.25*	103.75	4	25.94	5	0.05
6BL	C-0.13	64.74	8	8.09	5	0.08
	0.79-1.00*	104.58	9	11.62	37	0.35
6DS	0.79-1.00*	68.04	8	8.51	25	0.37
	C-0.45*	145.80	2	72.90	3	0.02
6DL	C-0.07	27.23	5	5.45	22	0.81
	0.28-0.66	147.82	2	73.91	1	0.01
7AS	0.89-1.00*	44.77	10	4.48	34	0.76
	0.73-0.89*	65.12	3	21.71	29	0.45
	0.59-0.73*	56.98	3	18.99	15	0.26
	C-0.21*	85.47	5	17.09	44	0.51
7AL	C-0.18	73.26	12	6.11	42	0.57
	0.90-1.00	40.70	6	6.78	19	0.47
7BS	0.27-1.00	262.80	7	37.54	55	0.21
	C-0.16*	57.60	5	11.52	59	1.02
7BL	C-0.24*	129.60	13	9.97	58	0.45
	0.24-0.40	86.40	4	21.60	16	0.19
	0.86-1.00	75.60	11	6.87	84	1.11
7DS	077-1.00	79.58	9	8.84	32	0.40
	0.61-0.77	55.36	2	27.68	9	0.16
	C-0.14*	48.44	8	6.06	52	1.07
7DL	C-0.15*	57.15	13	4.40	91	1.59
	0.61-1.00*	148.59	9	16.51	66	0.44

*Gene rich regions (Erayman et al., 2004)

arms and were severely suppressed in the centromeric regions except in case of C-1AS, C-1DS and C-1DL, C-2AL, C-2DL, C-3BL, C-3DS, C-4AL, C-4DL, C-6DL, C-7A, C-7B and C-7D (Table 4 and Supplementary Fig. 2a-g). Suppression of recombination in the centromeric regions was also reported in earlier studies in wheat (Akhunov et al., 2003; Saintenac et al., 2009). In addition, low recombination frequency was observed in most distal bins of a few chromosomes e.g. 1A, 1B, 2A, 2D, 3D, 4A, 4D, 5B, 5D, 6A, 6D, 7A, 7B and 7D, and average recombination rate was observed in interstitial bins. These observations strongly suggest that the recombination frequency (crossing over) is not uniform from the centromere to the distal end as previously thought. However, earlier studies in wheat indicated that the recombination occurs mainly in the distal regions of the chromosome with a gradient from the centromere to the telomere (Erayman et al., 2004; See et al., 2006; Saintenac et al., 2009). With respect to the above, it has been generalized for cereals (including wheat), that highly recombinogenic regions are found in the distal 20-30% of the chromosome arms (Akhunov et al., 2003). In the present study, we compared our results with those reported by Erayman et al. (2004) and demarcated the gene rich regions/bins (Table 4). The variable recombination rates within each of the different gene rich regions were observed in the distal half of the chromosomes. On the other hand, recombination rates in some proximal gene rich regions were higher than in distal gene rich regions i.e. C-7DS1-0.14 and C-7DL6-0.15 in chromosome 7D. More accurate estimation of recombination frequency and distribution along the chromosomes can be studied in wheat on the availability of genomic sequence scaffolds. Saintenac et al. (2009) estimated recombination rate very precisely by using markers anchoring contigs along chromosome 3B and observed that recombination gradient

Table 5. A list of cytogenetic stocks used for physical mapping of SSRs in wheat

S. No.	Name of cytogenetic stock	Number of stocks		Source
		Available	Used	
1.	Nullisomic-tetrasomic lines (NT)	42	42	Dr. B. S. Gill, Kansas State University, USA
2.	Ditelocentric (Dt) lines	42	24	Dr. B. S. Gill, Kansas State University, USA
3.	Deletion lines	332	192	Dr. T. R. Endo, Kyoto University, Kyoto, Japan

Table 6. Details of the SSRs used for physical mapping in wheat.

SSR type	Number of SSRs	Source
Wheat		
mwm (3B specific genomic SSRs)	154	NCBI database (http://www.tigr.org/tdb/e2k1/tae1/info.shtml)
mwm (other genomic SSRs)	132	NCBI database (http://www.tigr.org/tdb/e2k1/tae1/info.shtml)
Brachypodium		
mbm (genomic SSRs)	47	Brachypodium resource page (http://brachypodium.pw.usda.gov/SSR/)
bde (EST-SSR)	96	Texas AgriLife Research, Texas, USA
Total	429	

mwm, Meerut wheat microsatellites; mbm, Meerut brachypodium microsatellite; bde, *Brachypodium* EST-SSRs.

from the centromere to telomere on both arms with a suppression of crossing over in 27% of the chromosome in the proximal regions.

Materials and Methods

Cytogenetic stocks

Different cytogenetic stocks used during the present study are listed in Table 5. All aneuploid stocks including nullisomic-tetrasomic (NT) lines, ditelocentric (Dt) lines (except N7DT7B and Dt4AL) and also the terminal deletion lines were available in the genetic background of common wheat cv. Chinese Spring (CS) (Endo and Gill, 1996). The NT line N7DT7B is in the background of cv. Thatcher and the Dt4AL is in the background of *Triticum durum*/*Aegilops tauschii*. The details of the deletion stocks are available elsewhere (Endo and Gill, 1996).

SSRs used for physical mapping

Wheat SSRs

About 14 Mb of genomic sequences of wheat and its wild relative (*Aegilops tauschii*) were available in the NCBI databases (http://www.tigr.org/tdb/e2k1/tae1/info.shtml). These sequences mainly corresponded to Genome Survey Sequences (GSS) and their chromosomal locations were largely unknown (Paux et al., 2006). These sequences were used for mining of SSRs and for designing of their primers for physical mapping in wheat (Table 6).

Brachypodium SSRs

Two hundred SSR primers that were designed from brachypodium genomic sequences (derived from BAC-end sequences) were available at brachypodium resource page (http://brachypodium.pw.usda.gov/SSR/). Non-redundant 47 primer pairs of class I SSRs were randomly chosen for the present study. Aliquots of 96 brachypodium EST-SSR primer pairs were kindly supplied by Dr. Azhaguvel Perumal, Texas Agri Life Research, Texas A&M University, Amarillo, USA. The brachypodium genomic SSRs and EST-SSRs were used for physical mapping in wheat during the present study using wet-lab approach (Table 6).

DNA extraction and PCR analysis

The genomic DNA from all cytogenetic stocks listed in Table 5 was isolated following modified CTAB method described elsewhere (Prasad et al., 2000). Primers mainly synthesized by Illumina Inc. USA. PCR amplification was carried out using DNA Master Cycler, Eppendorf, Germany. The amplified products were resolved on 10% PAGE following silver staining (Tegelstrom, 1992). After staining the gels, data on the presence/absence of the band of expected size was recorded in each case.

Methodology for physical mapping of SSRs through wet-lab

Nullisomic-tetrasomic (NT) and ditelocentric (Dt) lines were used to assign each SSR to individual chromosome and chromosome arm, respectively. The concerned SSR was then mapped to a specific bin using terminal deletion stocks belonging to the relevant chromosome arm. The absence of SSR marker in a particular deletion stock lacking a specific deleted segment suggested that the SSR is located within the deleted segment (Fig. 1). Using the above principle, an individual SSR (genomic/EST) locus was assigned to a chromosome, an arm, or a bin according to whether or not the expected PCR amplified product was obtained when genomic DNA from particular cytogenetic stocks was used as a template. In this manner, the SSRs were mapped to specific bins (regions) of chromosome arms and physical maps were prepared.

Statistical tests for random distribution of mapped loci

On the basis of physical size (in μM) of chromosomes, chromosome arms (Gill et al., 1991) and DNA contents for chromosomes, chromosome arms were used to calculate the expected number of SSR loci under the assumption of random distribution among chromosomes/chromosome arms and among three sub-genomes. The χ^2 test for goodness-of-fit was used to test the random distribution of SSR loci among chromosomes, chromosomes arms, three sub-genomes and seven homoeologous groups of bread wheat.

Available mapped SSRs for integrated physical map

In wheat, a total of 1,344 SSR loci [including 313 gSSR loci (Goyal et al., 2005), 672 EST-SSRs loci (Mohan et al., 2007) and 359 gSSR loci (Balyan et al., 2008)] were earlier

physically mapped in our laboratory. Together with the above SSR loci, 559 gSSR loci that were physically mapped by Roder et al. (1998), Sourdille et al. (2004) and Song et al. (2005) were also used for construction of an integrated physical map during the present study.

Computation of recombination frequency

With a view to compute the recombination frequency for each bin, the genetic distance in cM between SSRs and their location in a bin were determined from published wheat genetic (Somers et al., 2004) and physical (Gupta et al., 2008b) maps. The recombination frequency was calculated by dividing the length of genetic sub-region (bin) in cM by the corresponding physical sub-region (bin) in Mb. The resulting fraction (cM/Mb) within each bin was determined for all bins distributed on different chromosomes.

Conclusions

Construction of physical maps using cytogenetically characterized deletion lines is a powerful approach in bread wheat. The specific combination of features including mapping of new markers, enhanced genome-wide coverage and relatively high resolution of the integrated SSR physical maps represent a useful resource for genetic and genomic analysis. Integrated physical map also aid in determining physical distances between linked markers and traits as well as map-based cloning of agriculturally important genes. Further, the integrated physical maps of SSRs may be used as anchor point for the construction of global BAC contigs map needed for sequencing of individual wheat chromosomes under the aegis of International Wheat Genome Sequencing Consortium (IWGSC).

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