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Development and mapping of microsatellite (SSR) markers in wheat

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Abstract Microsatellite DNA markers are consistently found to be more informative than other classes of markers in hexaploid wheat. The objectives of this research were to develop new primers flanking wheat microsatellites and to position the associated loci on the wheat genome map by genetic linkage mapping in the ITMI W7984 × Opata85 recombinant inbred line (RIL) population and/or by physical mapping with cytogenetic stocks. We observed that the efficiency of marker development could be increased in wheat by creating libraries from sheared rather than enzyme-digested DNA fragments for microsatellite screening, by focusing on microsatellites with the $[ATT/TAA]_n$ motif, and by adding an untemplated G-C clamp to the 5′-end of primers. A total of 540 microsatellite-flanking primer pairs were developed, tested, and annotated from random genomic libraries. Primer pairs and associated loci were assigned identifiers prefixed with BARC (the acronym for the USDA-ARS Beltsville Agricultural Research Center) or Xbarc, respectively. A subset of 315 primer sets was used to map 347 loci. One hundred and twenty-five loci were localized by physical mapping alone. Of the 222 loci mapped with the ITMI population, 126 were also physically mapped. Considering all mapped loci, 126, 125, and 96 mapped to the A, B, and D genomes, respectively. Twenty-three of the new loci were positioned in gaps larger than 10 cM in the map based on pre-existing markers, and 14 mapped to the ends of chromosomes. The length of the linkage map was extended by 80.7 cM. Map positions were consistent for 111 of the 126 loci positioned by both genetic and physical mapping. The majority of the 15 discrepancies between genetic and physical mapping involved chromosome group 5.

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Introduction

DNA markers are required for the creation of genetic maps that can be used to discover the position of genes or quantitative trait loci (QTLs) controlling important traits. The same markers can be used to incorporate genes and QTLs into improved cultivars via marker-assisted selection (MAS). Also, markers tightly linked to important genes provide the starting point for map-based cloning of those genes. In bread wheat, the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD) marker systems have detected only low levels of intraspecific polymorphism (Penner et al. 1995; Paull et al. 1998; Kim and Ward 2000; Hazen et al. 2002). In contrast, microsatellite markers are consistently found to be highly polymorphic, easily visualized, stable, and co-dominant (Akkaya et al. 1992; Röder et al. 1995; Powell et al.

1996; McCouch et al. 1997; Song et al. 1999, 2004). The development of informative microsatellite markers in wheat is difficult and time-consuming due to its large genome size, polyploidy, and the high level of repetitive sequences in its genome. Approximately 570 publicly available wheat microsatellite primer sequences have been reported (Devos et al. 1995; Korzun et al. 1997; Röder et al. 1998a, b; Pestsova 2000a, b, c; Salina et al. 2000; Song et al. 2002; Gao et al. 2004, Nicot et al. 2004), which is a small number relative to the genome size of wheat, the latter estimated to be 16,000 Mbp/1C (Arumuganathan and Earle 1991). A map based on recombinant inbred lines (RILs) from the cross W7984 × Opata 85 [generally referred to as the ITMI (International Triticeae Mapping Initiative) map; see http://wheat.pw.usda.gov/ggpages/maps.html] contained a total of 1,214 markers, including 944 loci detected by 722 RFLP probes, 261 loci amplified by 223 microsatellite primer pairs, and nine other markers before the addition of markers reported herein. There are 121 gaps that are longer than 10 cM. Although there is an average of one marker every 2.8 cM (Kam-Morgan and Gill 1989), the relatively low likelihood that a specific marker locus will be polymorphic in any given single cross dictates the need for much greater marker density.

Wheat is one of few crops suited for cytogenetic mapping. In common wheat, the mapping of markers of the chromosome, arm, and sub-arm has been possible through the use of nulli-tetrasomic (Sears 1966), ditelosomic (Sears and Sears 1978), and deletion lines (Endo and Gill 1996). Using aneuploid stocks, any markers can be used without the necessity of identifying polymorphism, a particularly significant factor in wheat with its low level of DNA polymorphism. These aneuploid stocks have been used to develop cytogenetically based physical maps of the wheat homoeologous groups (Werner et al. 1992; Gill et al. 1993, 1996; Kota et al. 1993; Hohmann et al. 1994; Delaney et al. 1995a, b; Mickelson-Young et al. 1995), to determine the chromosome locations of genes (Hart et al. 1993; Qi and Gill 2001) and of molecular markers (Sorrells et al. 1991; Plaschke et al. 1996; Anderson et al. 2001). These data have provided a wealth of information on the physical positions of genes controlling phenotypic traits and many aspects of wheat chromosome structure and function (Endo and Gill 1996).

Microsatellites are commonly assumed to be abundant in pericentromeric DNA and rare in the euchromatin arms (Areshchenkova and Ganal 1999; The Arabidopsis Genome Initiative 2000). Alternatively, others have suggested that microsatellite sequences are generated by chance and therefore occur randomly throughout the genome (Levinson and Gutman 1987). More recently, Morgante et al. (2002) reported that microsatellites are more abundant in transcribed regions. The usefulness of genetic markers for map-based cloning and MAS is dependent on their proximity to the genes. Thus, it is important to examine the distribution of genes or QTLs versus that of microsatellites.

This paper presents information pertinent to the efficient development of 540 new, public domain microsatellite-amplifying primer pairs. This set of primer pairs and associated marker loci bear the prefix BARC, reflective of their origin at the Beltsville Agricultural Research Center. We also report the genetic and/or physical location of 347 loci associated with 315 of the BARC primer pairs. In addition, the distribution of microsatellites in reference to known genes and QTLs is assessed.

Materials and methods

Wheat library construction, screening, and DNA sequencing

Various libraries were constructed for microsatellite isolation. One group of libraries was constructed by digesting genomic DNA of wheat cv. Chinese Spring (CS) with one of the following sets of four to six restriction enzymes (set 1: *Bsr*BI, *NIa*IV, *Cac*8I, *Nae*I, *Ehe*I; set 2: *Dra*I, *Msc*I, *Psh*AI, *Ava*II, *CIa*I; set 3: *Nru*I, *Msc*I, *Rsa*I, *Alu*I, *Hin*cII; set 4: *Alu*I, *Msp*A1I, *Fsp* I, *Dpn*II, *Pme*I, *Sna*BI; set 5: *Hae*III, *Eco*RV, *Pvu*II, *BstU*I; set 6: *Sfo*I, *Dpn*I, *PmI*I, *Xmn*I; set 7: *Fnu4H*I, *Pae*R7I, *Mfe*I, *Nci*I.) The enzymes in each set were used together in a single reaction. DNA digested from each reaction was then used to construct a genomic library for screening. Another library was constructed by shearing CS DNA using a nebulizer followed by size selection by means of electrophoresis on a 1% agarose gel. Sheared DNA was treated with mungbean exonuclease or T4 DNA polymerase to create blunt ends. DNA fragments in ranging from 400 bp to 750 bp in size were isolated from the agarose gels using Geneclean II (Bio101, La Jolla, Calif.), and the purified DNA fragments were ligated into the *Sma*I site of pBluescript (Stratagene, La Jolla, Calif.). A third library was enriched for microsatellite-containing sequences using a procedure described in the literature (Ostrander et al. 1992; Pulido and Duyk 1994; Song et al. 2000), and an additional set of libraries was enriched for microsatellites by Genetic Identification Services (GIS; Chatsworth, Calif.).

Clone selection and re-screening were conducted as described by Cregan et al. (1994). Following screening, wheat genome inserts from colonies carrying putative microsatellite-containing clones were either amplified directly by PCR or purified with the QIAwell Plasmid kit (Qiagen, Valencia, Calif.) after culture in LB liquid medium and lysis. Clones or PCR products were subsequently sequenced on an ABI PRISM 377 DNA sequencer following labeling reactions using a dRhodamine Terminator Cycle Sequencing kit (Perkin-Elmer Applied Biosystems, Foster City, Calif.), and the sequences assembled and analyzed using Autoassembler

3.1.2 (Applied Biosystems). For the purpose of identifying sets of clones with similar but not identical sequences, the ASSEMBLY SETUP of AUTOASSEMBLER software (Perkin-Elmer) was set as follows: minimum overlap, 30 bp; percentage error, 50%.

Primer design and evaluation

Primer sets were designed for those clones with tetra-, tri-, or di-nucleotide repeat units using the software OLIGO 5.0 (National Biosciences, Plymouth, Minn.) (Song et al. 2002, 2004). An untemplated GC clamp (Sheffield et al. 1989) was added to the 5′ ends of approximately 82% of the primer pairs designed. The GC clamp generally consisted of GCG, although in some cases, GGG or CCC was added to the 5′ end. The primers were synthesized by BioServe Biotechnologies (Laurel, Md.). Each primer pair was first examined in amplification reactions using CS, Opata 85, and W7984 genomic DNA as templates. The reaction mix contained 100 ng template DNA, 1.5 m *M* Mg^{+2}, 1.5 m *M* of dNTPs, 1.5 μM of each primer, 1 µl 10× buffer, 1.25 U *Taq* DNA polymerase, 0.1 µl 1.11×10^{14} Bq/mmol α-[^{32}P]dATP in a total volume of 10 µl. The PCR cycling conditions were: 3 min at 95°C, followed by 30 cycles of 40 s at 94°C (denaturation), 40 s at the optimized annealing temperature (annealing), and 1 min at 72°C (extension). After cycling, the reactions were incubated at 72°C for 10 min. The [^{32}P]-labeled PCR products were analyzed on a 6% DNA sequencing gel with 30% formamide followed by autoradiography. Primer pairs which yielded simple band patterns were given sequentially numbered identifiers with the prefix BARC.

Genetic mapping

A total of 222 BARC microsatellite loci shown to be polymorphic in the ITMI population were used for genetic mapping. The protocol adopted to name the loci amplified by the BARC primer pairs was to combine the prefix Xbarc with the primer pair identification number (ID). The positions of the Xbarc loci on the genetic map were identified by analyzing RILs from a mapping population employed by others for the purpose of linkage mapping; this mapping population is generally referred to as the ITMI population because of its origins in the International Triticeae Mapping Initiative (Nelson et al. 1995a; Van Deynze et al. 1995; Marino et al. 1996; Röder et al. 1998b). The population's parents are Opata 85 and W7984, the latter bing a synthetic allohexaploid created from a cross between Altar 84 (a cultivar of *Triticum dicoccoides* var. *durum*) and *Aegilops tauschii*, accession CI = 18 WPI 219 (PR88-89) (Nelson et al. 1995b). RILs 1–59 and 61–84 of the ITMI population were used for the segregation analysis in this study. RIL banding patterns for BARC primer pairs were determined with the electrophoresis system described by Wang et al. (2003). These data were combined with RIL allele states for 1,184 loci, including 915 RFLPs (Nelson et al. 1995a, b, c, Van Deynze et al. 1995), 258 microsatellites (Röder et al.1998b), and 11 others obtained from the Graingenes website (http://wheat.pw.usda.gov/ggpages/maps.shtml).

Preliminary linkage analysis and map construction were conducted through an iterative process involving MAPMAKER 3.0B (Lincoln and Lander 1993) and JOINMAP 3.0 (Van Ooijen and Voorrips 2001). Final map construction was conducted with JOINMAP 3.0. In some cases, physical map information was employed to order loci.

Physical mapping

Physical mapping was carried out with 432 BARC loci, including 222 that were employed in genetic mapping. Using the conditions described by Song et al. (2002), PCR amplification with the BARC primers was carried out initially on a full set of nulli-tetrasomic lines and subsequently on ditelosomic lines to assign bands generated by a given BARC primer pair to a chromosome arm. A panel of 117 homozygous chromosome deletion lines of CS was used for sub-arm localization of marker loci (the names and order of chromosome deletion breakpoints used in this study are listed in the electronic supplementary material of this article). The generation and nomenclature of deletion stocks are described by Endo and Gill (1996) and Qi et al. (2003).

Statistical simulation of the number of Xbarc microsatellite loci associated with genes

A simulation analysis was undertaken to determine if microsatellite loci were distributed randomly relative to genes already positioned on the genetic map. Xbarc microsatellite loci and genes were randomly assigned to genome positions based upon a genome length of 2,654 cM, and the number of marker loci with a distance of less than 5 cM from one or more genes was counted. The simulation was performed 5,000 times, and the mean and standard deviation of the number of markers within 5 cM of a gene was calculated.

Results

SSR primer pair development and information

Effect of four motifs on the level of polymorphism

Primers were designed to sequences containing the dinucleotide motifs $[CT/GA]_n$ or $[CA/GT]_n$, the trinucleotide motif $[ATT/TAA]_n$, and the tetra-nucleotide $[TAGA/ATCT]_n$ motif. The rates of polymorphism were 28%, 24%, 36%, and 28% for $[CT/GA]_n$, $[CA/GT]_n$, $[ATT/TAA]_n$, and $[TAGA/ATCT]_n$, respectively, among

Opata 85, W7984, and CS. Although the polymorphism rate of $[CA/GT]_n$ and $[CT/GA]_n$ were similar, $[CT/GA]_n$ was present at a much higher frequency in the genome. Loci containing the $[ATT/TAA]_n$ motif had a significantly higher polymorphism rate ($P < 0.05$) than those with the dinucleotide $[CT/GA]_n$ and $[CA/GT]_n$ and the tetra-nucleotide $[TAGA/ATCT]_n$motifs.

Effect of the 5′ G-C clamp on PCR amplification

The numbers of primers that amplified discrete products, no product, or multiple products are listed in Table 1. Among the primers with no G-C clamp, 45% amplified discrete products, while 55% amplified with no product or multiple products. In contrast, for primers with G-C clamps, the corresponding percentages were 56% and 44%. Thus, the addition of an untemplated G-C clamp to one or both primers gave a greater proportion of PCR primer pairs that yielded a single discrete product (contingency $\chi^2 = 4.7$, $P < 0.10$).

Plasmid libraries created from sheared and enzyme-digested fragments

Libraries with insert DNA either digested with enzymes or sheared with the nebulizer were screened for the presence of simple sequence repeat markers (SSRs). Sixty-one similar and 218 unique sequences were observed from the sheared library; their counterparts from the libraries derived from enzyme digestion were 290 and 184, respectively. Sets of similar sequences as determined via analysis with AUTOASSEMBLER contained a mean of two sequences in those derived from the sheared library and five sequences in sets from the restriction enzyme-derived library. Typical sets of similar SSR-containing sequences from the enzyme-derived library are presented in Fig. 1. The difference in the rate of unique sequences between the two libraries was clear. A large portion of the DNA fragments originating from the restriction enzyme-derived libraries was apparently from homeologous or duplicated regions.

Functionality of primers

The percentages of primer pairs that amplified the expected size product in CS were 53%, 53%, 55%, and 56% for the $[CA/GT]_n$-, $[CT/GA]_n$-, $[ATT/TAA]_n$- and $[TAGA/ATCT]_n$- motif-containing fragments, respectively. A total of 540 primer pairs were developed. Of these, 360 were polymorphic among CS, Opata 85 and W7984, and 206 were polymorphic between Opata 85 and W7964. Primer information for each marker is available in the online Electronic Supplementary Materials and at the GrainGenes website.

Table 1 Number of primers with or without a 5′ G-C clamp and the impact of the 5′ G-C clamp on PCR amplification

	Both primers with a 5′ GCG clamp	One primer with a 5′ GCG clamp	Neither primer with a GCG clamp
Amplified discrete products	196 (56%)	185 (56%)	67(45%)
Null or multiple products	154 (44%)	146 (44%)	79 (55%)
Total	350	331	146

Genetic mapping of Xbarc loci

We added 222 Xbarc loci to the ITMI linkage map. Fourteen were placed distal to previously mapped loci on ten chromosome arms (1AL, 1BL, 1DL, 2AS, 3DL, 4AS, 4BS 5AS, 5DS, and 7AL). The resulting increase in length of these chromosomes totaled 80.7 cM. Twenty-three Xbarc loci mapped to gaps greater than 10 cM in the map based on pre-existing markers. Sixteen of the primer pairs amplified more than one fragment. Of these 16, four loci (Xbarc23, Xbarc229, Xbarc353, and Xbarc1138) mapped on different regions of the same chromosome, five (Xbarc119, Xbarc121, Xbarc124, Xbarc204 and Xbarc321) mapped on homoeologous chromosomes, and seven (Xbarc48, Xbarc197, Xbarc292, Xbarc308, Xbarc315, Xbarc340, and Xbarc361) mapped on non-homeologous chromosomes. The genetic and physical positions of all Xbarc loci are indicated in the online Electronic Supplementary Materials and at the GrainGenes website.

The total number of marker loci in the resulting genetic map is 1,406, with a total length of 2,654 cM. The map contains 22 gaps larger than 10 cM and two gaps larger than 20 cM.

Physical mapping

A total of 255 primer pairs showing polymorphism among CS, Opata 85 and W7964 and 177 that were monomorphic were selected for physical mapping. Bands from 243 primer pairs in the polymorphic group could be physically mapped. Of these, 25 were mapped only to a chromosome, 11 only to a chromosome arm, and 188 to segments of chromosome arms using deletion lines. Nineteen loci (Xbarc11, Xbarc20, Xbarc32, Xbarc93, Xbarc105, Xbarc114, Xbarc115, Xbarc124, Xbarc144, Xbarc155, Xbarc156, Xbarc162, Xbarc176, Xbarc193, Xbarc204, Xbarc210, Xbarc221, Xbarc231, and Xbarc235) were mapped to multiple genomes, and 12 loci could not be mapped to a discrete position. Eighty-nine markers from the monomorphic group were mapped, of which 19 mapped only to a chromosome, nine to a chromosome arm, and the remaining 61 were positioned using deletion lines. Those markers that were only positioned to chromosomes or chromosome arms are not reported on the GrainGenes website but are listed in Table 2. Twenty-four markers were mapped to more than one genome, while 88 could not be mapped.

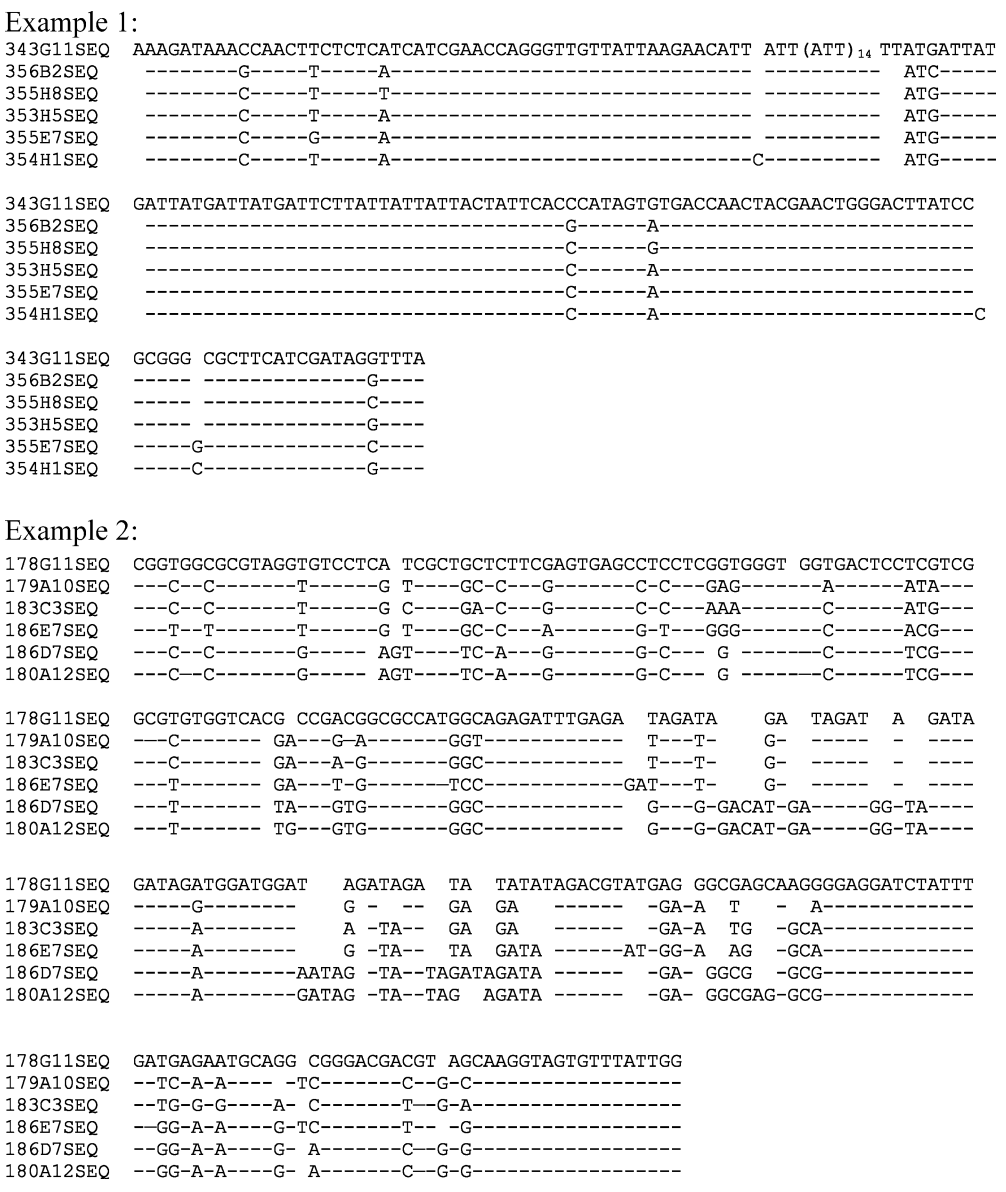

Fig. 1 Two examples of assembled sequence contigs using AUTOASSEMBLER software and applying the criteria of a 30-bp minimum overlap and a percentage error of 50%

The proportion of primer pairs from the monomorphic group from which no mappable band was amplified (88/177 = 50%) was much higher than in the polymorphic group (12/255 = 5%).

Physical versus genetic maps

Some microsatellite markers amplified two or three polymorphic products and, consequently, were mapped to more than one position in the genome. Because of this, 315 microsatellite primer pairs yielded 347 marker loci as a result of physical mapping, genetic mapping, or both. Of these, 126, 125, and 96 were positioned on the A, B, and D genomes, respectively (Table 3). The distribution of markers on each chromosome was significantly different from a uniform distribution ($\chi^2 = 24.8, P < 0.05$, with χ^2 calculated on the basis of chromosome lengths). Among the 126 markers that were positioned by means of both physical and genetic mapping, 121 were mapped on the same chromosome by both methods. Of these, ten loci (Xbarc23, Xbarc32, Xbarc57, Xbarc65, Xbarc85, Xbarc122, Xbarc134, Xbarc186, Xbarc202, and Xbarc225) were mapped on different arms of the same chromosome. The order of the remaining markers as determined by physical and genetic mapping was generally consistent. There were five cases of inconsistencies with respect to the positioning determined by the two mapping approaches; three of the inconsistencies were confined to chromosome 5 (Table 4).

The combined physical and genetic map provided in the Electronic Supplementary Materials contains a total of 1,531 loci, including 915 RFLP, 258 pre-existing SSR, 347 new SSR markers, and 11 others (Table 3).

Table 2 Microsatellite markers that were only physically mapped either to a chromosome or a chromosome arm

Chromosome	Markers on chromosome	Markers on short arm	Markers on long arm
1A	Xbarc119, Xbarc209, Xbarc1168	Xbarc150	
1B	Xbarc116, Xbarc119, Xbarc1094, Xbarc1110, Xbarc1115, Xbarc1134, Xbarc1154, Xbarc1168	Xbarc1159	
1D	Xbarc27, Xbarc112, Xbarc1150		Xbarc1042, Xbarc1108
2B	Xbarc116, Xbarc1154	Xbarc35, Xbarc1142	Xbarc150, Xbarc1027
2D	Xbarc103, Xbarc112, Xbarc235, Xbarc1153	Xbarc1070	
3A	Xbarc112, Xbarc215, Xbarc1159		Xbarc150
3B	Xbarc112, Xbarc131, Xbarc234		
3D	Xbarc226, Xbarc1161	Xbarc150, Xbarc132	Xbarc1162
4A	Xbarc190, Xbarc216, Xbarc233, Xbarc1070		
4B	Xbarc1142		
4D	Xbarc27, Xbarc1145		
5D	Xbarc234, Xbarc1097, Xbarc1117		Xbarc1002, Xbarc1018
6A	Xbarc103,		
6B	Xbarc112, Xbarc136, Xbarc185, Xbarc1117	Xbarc14	
6D	Xbarc112, Xbarc1145, Xbarc1087		
7A	Xbarc103, Xbarc112, Xbarc157, Xbarc1004		
7B	Xbarc112, Xbarc255, Xbarc258, Xbarc1082		
7D	Xbarc235		

Microsatellite loci in close proximity to genes

Analyses of consensus maps and concurrent research in other laboratories (Van Deynze 1994; McGuire and Qualset 1996; Dubcovsky et al. 1997; Peng et al. 1999; Tranquilli et al. 1999; Anderson et al. 2001; Zhou et al. 2002a, b; Czembor et al. 2003; Leonova et al. 2003; Lin et al. 2004; Paillard et al. 2004; Singh et al. 2004; Steiner et al. 2004; Zhu et al. 2004) indicate that at least 40 of the 222 Xbarc loci reported here are in close proximity

Table 3 Number of new BARC markers mapped to each chromosome

Chromosome	Pre-existing marker loci			New SSR			
	SSR	RFLP	Other	Physical positioned only	Genetically and physically mapped	Genetically mapped	Total
1A	8	39	1	12	6	10	16
1B	17	52	2	10	6	10	14
1D	5	26	3	9	4	10	15
2A	30	55		9	3	10	16
2B	19	57		24	8	12	28
2D	12	44	1	9	5	9	13
3A	9	68	1	18	7	15	26
3B	16	64	2	16	6	9	19
3D	12	40		6	3	7	10
4A	9	45		12	7	12	17
4B	11	34		10	3	4	11
4D	6	18		9	2	7	14
5A	17	40		18	12	17	23
5B	16	48		19	11	14	22
5D	16	29		6	6	13	13
6A	7	60		9	5	7	11
6B	12	48	1	10	5	7	12
6D	3	46		13	9	12	16
7A	10	43		13	6	10	17
7B	16	39		16	11	14	19
7D	7	20		12	10	13	15
Total	258	915	11	260	135	222	347

Table 4 Five microsatellite markers that mapped at different locations based on deletion mapping and genetic mapping in the ITMI population

Locus	Physical mapping using Chinese Spring deletion lines	Linkage mapping using ITMI population
Xbarc25	1AS	3AS
Xbarc180	6BL	5AS
Xbarc216	4A	5BS
Xbarc219	7BL	2DS
Xbarc230	2BS	5AL

(< 5 cM) to 32 genes and/or QTLs. If we assume that genes and markers are uniformly distributed on chromosomes, approximately 27 of the 222 Xbarc markers would be expected to map within 5 cM of a gene with a standard deviation of 4.98 cM based upon the simulation analysis. The number of loci (40) within 5 cM of any given gene exceeded the number that would be expected by chance alone ($P < 0.05$).

Discussion

Efficiency of marker development

Restriction enzyme- versus nebulizer-derived genomic library

There was a marked difference in the spectrum of DNA fragments derived from the two types of libraries, and this difference had a large impact on the success of SSR marker development. The results indicate that microsatellite-containing clones derived from sheared DNA are much less likely to contain sets of clones with similar or nearly identical sequences. We observed that while very few sequences were identical in the libraries constructed by the two methods, there were significantly higher frequencies of similar sequences among SSR-containing clones in the library derived from enzyme digestion. Typical examples of such sets of similar sequences from the restriction enzyme-derived library are shown in Fig. 1. The likely explanation for this result is that conserved restriction sites in duplicated and homoeologous regions yielded clones with very similar but not identical sequences. In contrast, the physically sheared library yielded a random sampling of genomic fragments. It may be possible to develop locus-specific PCR primers to many of the sequences within the sets such as those shown in Fig. 1.

Conversion of di-, tri-, tetra-nucleotide microsatellite-containing sequences to useful markers

The conversion of microsatellite-containing sequences into useful markers is sometimes quite difficult, especially in species with large genomes. Röder et al. (1998b) reported that only one of six microsatellite-containing clones yielded a functional marker; the remaining primer pairs either gave poor amplification or produced a complex pattern with multiple bands. A similarly low conversion rate from SSR-containing sequences to useful markers has been observed in pine (Smith and Devey 1994; Kostia et al. 1995) and spruce (Pfeiffer et al. 1997). The low conversion rates of primer pairs to useful markers in these latter two species was probably due to the high level of repetitive DNA sequences or to similar sequences in homoeologous genomes. In the present study, primers were designed to the sequences containing dinucleotide repeats $[CT/GA]_n$ or $[CA/GT]_n$, the tri-nucleotide motif $[ATT/TAA]_n$, and the tetra-nucleotide $[TAGA/ATCT]_n$. The rate of polymorphic markers was 28%, 24%, 36%, and 28% for $[CT/GA]_n$, $[CA/GT]_n$, $[ATT/TAA]_n$ and $[TAGA/ATCT]_n$, respectively. A previous investigation indicated that the $[ATT/TAA]_n$ motif was superior to all other trinucleotide repeats for the successful development of polymorphic microsatellite markers (Song et al. 2002). The current data strongly suggest that the $[ATT/TAA]_n$ motif is also superior to the two most commonly used dinucleotide motifs and one tetra-nucleotide motif in terms of the rate with which polymorphic marker loci can be developed. Although dinucleotide repeats are the most commonly used class of microsatellite markers in plants, dinucleotide-based loci often prove difficult to genotype due to a high frequency of strand-slippage artifacts (Levinson and Gutman 1983). In contrast, tri- and tetra-nucleotide repeat-based markers generally produce a higher proportion of discrete PCR products as a result of a reduced level of strand-slippage artifacts (Gastier et al. 1995).

Efficiency of adding an untemplated G-C clamp to the 5′ end of primers

The data showed that the arbitrary addition of a clamp such as a GCG, CCC, or GGG to the 5′ end of one or both primers improved the success rate of PCR amplification, especially with respect to reducing the number of primers that did not amplify a PCR product.

Mapping

In relation to RFLPs, SSR markers are generally more genome specific. This avoids the ambiguity that sometimes results with RFLP probes that hybridize to two or more positions in the wheat genome. Among the 255 new polymorphic SSR markers reported here, only 20 (7.8%) markers were genetically mapped to two or more genome positions, a proportion similar to that found in rice (McCouch et al. 2002). This is in contrast to the results obtained with RFLP markers by Nelson et al. (1995a, b, c) and Van Deynze et al. (1995): of 722 RFLP markers, 222 (31%) mapped to more than two positions.

One hundred primer pairs did not yield bands which could be physically mapped to a chromosome; of these, 88% were markers that were not polymorphic among the very diverse genotypes: Chinese Spring, Opata 85 and W7984. In contrast, of the 255 markers that were polymorphic for which physical mapping was attempted, only 12 could not be unambiguously positioned on a chromosome. It is our assumption that the loci that could not be physically mapped amplified homoeologous sites with identical or very similar PCR fragment sizes or amplified from cytoplasmic organelle DNA.

Deletion mapping provides a simple and rapid method to construct cytogenetically based physical maps. Markers can be used without the necessity of identifying polymorphism, a particularly significant factor in a species in which the lack of DNA polymorphism is a major obstacle. Recombination suppression in proximal regions of the chromosomes limits the power of recombination mapping in ordering proximal loci (Gill et al. 1993, 1996; Delancy et al. 1995a, b). The amount of DNA per centiMorgan generally increases from the telomeres to the centromere, with the exception of recombination 'hot spots'. For proximal regions, physical mapping is more powerful for resolving the order of loci (Werner et al. 1992). Physical mapping with genetic mapping information could help in the analysis of the physical distribution of recombination within each chromosome region and the actual physical distances between loci. In the present investigation, there were a few discrepancies between the physical and genetic mapping of Xbarc loci. However, 5 of the 126 markers mapped by both methods were mapped on different chromosomes and 11 of these 126 markers were mapped on different arms of the same chromosome. Similar discrepancies between the two mapping methods have been reported previously (Werner et al. 1992; Gill et al. 1993, 1996; Kota et al. 1993; Hohmann et al. 1994; Delaney et al. 1995a, b; Mickelson et al. 1995). These might indicate that cytological abnormalities such as translocation, inversion of chromosome segments, or multiple deletions may have occurred during the development of the CS deletion stocks. Alternatively, there may be cytological abnormalities in the ITMI parents.

Microsatellites located in close proximity to genes

Based on the relative positions of the Xbarc markers mapped in this study to the positions of known wheat genes reported in the literature by Van Deynze et al. (1995), McGuire and Qualset (1996), Dubcovsky et al. (1997), Peng et al. (1999), and Tranquilli et al. (1999), a number of Xbarc loci were determined to be in close proximity to genes.

Homeologous group 1 chromosomes. Markers Xbarc119 and Xbarc263 were close to the region with resistance to leaf rust, powdery mildew diseases (*Pm3*), and preharvest sprouting (*Qphs*) at the end of short arm of chromosome 1A. Xbarc137, Xbarc174, Xbarc181, Xbarc187, Xbarc240, and Xbarc302 were close to the regions with resistance to leaf rust, stem rust, and yellow rust (*Sr14*, *Sr18*, and *Lr33*). Xbarc61 was close to a region affecting gluten strength (glutenin subunits) on 1B. Xbarc149 was close to the region affecting 1,000 kernel weight and stem rust resistance genes at the end of 1D. Xbarc99, Xbarc169, Xbarc229, and Xbarc1090 were at a position flanking stem and leaf resistance genes near the centromere of 1D.

Homeologous group 2 chromosomes. Xbarc15, Xbarc220, and Xbarc309 were located at the centromere regions of chromosome 2A, close to a stem rust resistance gene (*Sr21*). Xbarc18, Xbarc91, and Xbarc160 were close to leaf and stem rust resistance genes (*Sr19*, *Lr23*, and *Lr13*). Xbarc16, Xbarc128, Xbarc114, Xbarc115, Xbarc167, Xbarc210, Xbarc221, Xbarc1135, and Xbarc1156 mapped near leaf and stem rust resistance genes (*Sr9*, *Yr7*, and *Yr5*) at the centromeric region of 2B. Xbarc124 was close to resistant genes *Lr41*, *Lr39*, and a leaf resistant gene from *Agilops cylindrica* (Singh et al. 2004).

Homeologous group 3 chromosomes. Markers Xbarc68, Xbarc75, Xbarc87, Xbarc102, Xbarc133, Xbarc147, and Xbarc218 were at the flanking region of a major scab resistance gene on chromosome 3BS. Anderson et al. (2001) reported that two markers, Xbarc87 and Xbarc73, flanked a major scab resistance gene in a Sumai 3/Stoa population.

Homeologous group 5 chromosomes. Xbarc92, Xbarc151, and Xbarc1182 on chromosome 5A were close to a vernalization response gene (*Vrn1*). Xbarc130 was located at the end of the short arm of 5D adjacent to a gene controlling endosperm texture, i.e., the softness of the grain (*Ha*). Xbarc44 and Xbarc93 were located in the region of the vernalization response gene (*Vrn3*). Xbarc140 and Xbarc59 were linked with *Vrn-B1* on 5BL (Leonova et al. 2003).

Homeologous group 6 chromosomes. Xbarc024 was close to a QTL controlling resistance to *Fusarium* head blight on 6B (Lin et al. 2004).

Homeologous group 7 chromosomes. Xbarc125 and Xbarc126 were close to the region of a red/purple coleoptile gene (*Rc3*), while Xbarc154 was close to a gene controlling leaf rust resistance (*Lr34*) on 7D.

Other linkages of Xbarc SSR markers to genes have been reported by Zhou et al. (2002a, b), Czembor et al. (2003), Paillard et al. (2004), Steiner et al. (2004), and Zhu et al. (2004).

Even though a total of 112 genes have been positioned on the composite wheat linkage map of the GrainGenes database, most of the genes were not incorporated into the ITMI maps due to the lack of

sufficient information to link the genes to an unambiguous map position. Thus, only 32 of the 112 genes can be definitively positioned on the current map. Our observation of at least 40 SSR markers within 5 cM of these 32 genes significantly exceeded the number expected by chance. This result contradicts the assumption that microsatellites preferentially occur in pericentric regions or other gene-poor regions with repetitive DNA. In contrast, it supports the findings of Morgante et al. (2002), who reported that microsatellites are present at a higher frequency in transcribed regions than in genomic DNA, and suggests that SSR markers should be particularly useful for investigating gene-rich regions of the genome.

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Electronic Supplementary Material

(PDF 54 KB)(PDF 180 KB)(PDF 85 KB)

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