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ORIGINAL PAPER

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SSR-based linkage map with new markers using an intraspecific population of common wheat

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Abstract Simple sequence repeats (SSRs) are valuable molecular markers in many plant species. In common wheat (*Triticum aestivum* L.), which is characteristic of its large genomes and alloploidy, SSRs are one of the most useful markers. To increase SSR marker sources and construct an SSR-based linkage map of appropriate density, we tried to develop new SSR markers from SSR-enriched genomic libraries and the public database. SSRs having (GA)n and (GT)n motifs were isolated from enriched libraries, and di- and tri-nucleotide repeats were mined from expressed sequence tags (ESTs) and DNA sequences of *Triticum* species in the public database. Of the 1,147 primer pairs designed, 842 primers gave accurate amplification products, and 478 primers showed polymorphism among the nine wheat lines examined. Using a doubled haploid (DH) population from an intraspecific cross between Kitamoe and Münstertaler (KM), we constructed an SSR-based linkage map that consisted of 464 loci: 185 loci from genomic libraries, 65 loci from the sequence database including ESTs, 213 loci from the SSR markers already reported, and 1 locus of morphological marker. Although newly developed SSR loci were distributed throughout all chromosomes, clustering of them around putative centromeric regions was found on several chromosomes. The total length of the KM map spanned 3,441 cM and corresponded to approximately 86% genome coverage. The KM map comprised of 23 linkage groups because two gaps of over 50 cM distance remained on chromosome 6A. This is a first report of SSR-based linkage map using single intraspecific population of common wheat. This mapping result suggests that it becomes possible to construct linkage maps with sufficient genome coverage using only SSR markers without RFLP markers, even in an intraspecific population of common wheat. Moreover, the new SSR markers will contribute to the enrichment of molecular marker resources in common wheat.

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Introduction

Simple sequence repeat (SSR) is a variable, co-dominant, and PCR-based molecular marker. Thus far, it has been developed and widely used for the construction of genetic maps, marker-assisted selection (MAS), and in the estimations of the genetic diversity in rice (Cho et al. 2000), maize (Sharopova et al. 2002), and barley (Ramsay et al. 2000). In common wheat (*Triticum aestivum* L.) which harbors three different genomes, namely A, B, and D, SSR markers have already been developed and mapped with an International Triticeae Mapping Initiatives (ITMI) population from an interspecific cross between common and synthetic wheat (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Somers et al. 2004; Song et al. 2005). However, the genome size of common wheat is much larger than that of rice, maize, and barley. The entire genetic distance is considered to correspond to about 4,000 cM (Sourdille et al. 2003). In most cases, MAS against desirable agronomic traits located on the specific chromosomal regions should be carried out in intraspecific populations. With regard to intraspecific populations, map construction would be often tedious because of a lower rate of polymorphism compared to interspecific populations. Groos et al. (2002), Sourdille et al. (2003) and Paillard et al. (2003) tried to construct linkage maps with SSR and restriction fragment length polymorphism

(RFLP) markers in intraspecific populations of common wheat, which spanned a total map length of 2,360, 3,685, and 3,086 cM, respectively. Taking into account that several gaps still remained in their maps even after the SSR and RFLP markers were used, and that the SSR markers tended to cluster near centromeric regions (Röder et al. 1998), the construction of an SSR-based linkage map using single intraspecific population for targeted QTL analysis remains elusive. Therefore, the development of further SSRs as additional marker sources is required to provide an SSR-based linkage map with sufficient genome coverage without using RFLP markers. Röder et al. (1995) estimated that 36,000 (GA)n blocks and 23,000 (GT)n blocks existed in the wheat genome. As a result, a number of undeveloped SSRs will be considered to remain in the genome. In rice, a large number (2,240) of SSR markers have already been developed by utilizing the information of complete genomic sequences (McCouch et al. 2002).

Some methods and sources for the isolation of SSR sequences are applied to develop SSRs in common wheat. These include colony hybridization (Ma et al. 1996; Röder et al. 1998; Pestsova et al. 2000; Song et al. 2002) and the SSR enrichment procedure (Sourdille et al. 2001; Gupta et al. 2002) from genomic DNA. The other source is public databases, which include a very large number of expressed sequence tags (ESTs). EST-derived SSR-containing sequences have been mined and characterized by several groups from public and private wheat EST databases (Eujayl et al. 2002; Kantety et al. 2002; Nicot et al. 2004; Peng and Lapitan 2005). EST-derived SSR markers of common wheat can be utilized not only for genetic mapping (Gao et al. 2004; Yu et al. 2004b), but also comparative analysis in cereals (Yu et al. 2004a). Also, because gene-related entries of rice (Cho et al. 2000) and barley (Becker and Heun 1995) can be used as indication to search SSR sequences in the genome, these sequences in the databases are an important source for the development of SSR markers in common wheat.

We report here the newly developed SSR markers from the genomic libraries and the database including ESTs. We also describe an SSR-based linkage map with new markers in combination to well-characterized SSR markers using an intraspecific doubled haploid (DH) population in common wheat.

Materials and methods

Isolation of SSRs from enriched libraries and public databases

Total DNA was extracted from seedlings of common wheat (*T. aestivum* L.) using a standard cetyltrimethylammonium bromide (CTAB) method and DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Genomic libraries enriched for (GA/CT)n and (GT/CA)n motifs were constructed by Genetic Identification Services (GIS, Chatsworth, CA, USA). Randomly selected clones were sequenced from the two libraries, and all sequences were checked for duplication using Sequencher ver. 4.1.2 (Gene Codes Corp., Ann Arbor, MI, USA). Only unique sequences were selected for the following experiments.

Contigs (25,971) derived from 116,232 ESTs (Ogihara et al. 2003) were subjected to a BLAST search to identify di- and tri-nucleotide SSR-containing sequences with 12 motif types: (GA/CT)n, (GT/CA)n, (AT)n, (CGA/GCT)n, (CAA/GTT)n, (CAT/GTA)n, (GGT/CCA)n, (ATT/TAA)n, (CGT/GCA)n, (CTA/GAT)n, (GGA/CCT)n, and (CTT/GAA)n. Additionally, gene-related sequences of *Triticum* species were extracted from the KOMUGI web site (http://www.shigen.nig.ac.jp/wheat/wheatja.html) to search for SSR-containing sequences deposited in the DDBJ databases.

SSR identification, primer design, PCR conditions, and detection

For all sources, identification and confirmation of repeat number, repeat type, and SSR motifs with more than seven repeats in di-nucleotide motifs and five repeats in tri-nucleotide motifs were performed with the SSR IT program on the Gramene web site (http://www.gramene.org/gramene/searches/ssrtool). Primer pairs were designed for SSR-containing sequences with sufficiently long flanking sequences using the Primer 3 program (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with T_m set from 60 to 65°C. Primers were designed with an optimal length of 23 bases for the amplification of products between 150 and 300 bp in size. The PCR reaction mixture contained 50 ng of template DNA, 2.5 mM Mg^{2+}, 1.5 mM dNTP, 1.5 μM of each primer, and 1 unit of Taq polymerase. This was made up to a total volume of 15 μl. PCR conditions were as follows: 3 min at 94°C, followed by 30 cycles of 40 s at 94°C, 40 s between 50° and 65°C depending on the primer combination, and 1 min at 72°C. The last step was incubation for 7 min at 72°C. The amplification and polymorphism of the PCR products between the parents of the mapping population were confirmed by analyzing the agarose or non-denatured polyacrylamide gels and visualizing with SYBR green I (Cambrex Bio Science, Rockland, ME, USA). Polymorphic markers were used for genetic mapping, and monomorphic markers were used for the evaluation of polymorphism.

Genetic mapping

Ninety-six DH lines from an intraspecific cross between Kitamoe and Münstertaler (KM) were developed from the maize method (Suenaga et al. 1989) and used for the construction of a linkage map. Kitamoe is a Japanese winter wheat variety, and Münstertaler is a line from Switzerland. Polymorphic SSRs between parents of the

population were genotyped and used for map construction, which was carried out with MAPMAKER/EXP 3.0b (Lander et al. 1987). The threshold of LOD scores was set at 3.0, and the genetic distances were calculated with the Kosambi function (Kosambi 1944). A total of 213 published SSR loci were used to assign chromosomes as anchor markers: 104 *gwm* and 17 *gdm* SSR loci from Röder et al. (1998) and Pestsova et al. (2000), 37 *wmc* loci from the Wheat Microsatellite Consortium (Gupta et al. 2002), 48 *barc* loci from the US Wheat and Barley Scab Initiative (http://www.scabusa.org/), and 7 *cfd* loci from Guyomarc'h et al. (2002). Chromosomal assignment was generally conducted based on the position of well-characterized markers on other reference maps. Only groups that included published loci at distances of less than 50 cM were integrated into one linkage block. Linkage groups composed of only new markers were excluded from map construction.

Polymorphic evaluation

Newly developed primers were evaluated for polymorphism in the nine wheat lines: Kitamoe (Japan), Münstertaler (Switzerland), Norin61 (Japan), Haruyokoi (Japan), RL4137 (Canada), Suneca (Australia), AUS1408 (South Africa), Frontana2 (Brazil), and Sumai3 (China).

Results

Development and characterization of new SSRs from genomic libraries and public databases

We tried to isolate SSR-containing sequences from genomic DNAs using two enriched libraries of (GA)n and (GT)n motifs. From these libraries, 2,400 randomly selected clones were sequenced. After eliminating clones with redundancy and without SSRs, 940 (39.2%) clones were identified as having unique SSR-containing sequences with at least seven repeats of (GA)n and (GT)n motifs. To date, 349 and 250 primer pairs have been designed from (GA)n and (GT)n libraries, respectively (Table 1).

SSR-containing sequences were searched from public databases for 12 motifs (see Materials and methods) with the BLAST program. In this study, marker development was conducted from the 25,971 contigs derived from 116,232 ESTs including both the 5′ and 3′ sequences that were developed by the Japanese consortium (Ogihara et al. 2003), and from gene-related sequences of *Triticum* species deposited to the DDBJ database. Totally, 1,104 EST contigs (4.3%) were mined, which contained at least seven repeats in di-nucleotide motifs and five repeats in tri-nucleotide motifs. The EST-derived SSRs with the CAA motif were the most abundant, and the AT motif had the least number of EST contigs (data not shown). Finally, 548 primer pairs could be designed from the EST-derived contigs and gene-related sequences on the DDBJ database. Summarizing the total conversion toward useful molecular markers, 842 primers gave PCR amplification, 478 of which were polymorphic markers with at least two alleles in the nine lines of common wheat (Table 1).

Undesirable PCR amplification showing smear profiles was observed in a high proportion of primers from enriched libraries in contrast to the primers from the database. The rate of nonfunctional primers was 28.7 and 24.8% from (GA)n and (GT)n libraries, respectively. This was much higher than the rate in the database-derived SSRs (Table 1). On the other hand, the rate of markers showing at least one polymorphic character among nine wheat lines was 51.9 and 46.0% from (GA)n and (GT)n libraries, respectively. This was higher than the rate in the database-derived SSRs (Table 1).

SSR-based map construction

A DH population from an intraspecific cross between two common wheat varieties was used for the mapping analysis. In the first screening of polymorphism in published SSR markers, the rate of polymorphism was highest in combinations between KM in our materials (data not shown). Many agronomic traits of importance in our region differed between the lines. These included preharvest sprouting, flowering time, lodging resistance, and disease resistance. Therefore, we selected the DH

Table 1 Development of SSR markers in this study

		Genome libraries				Databases*				Total	%**
		(GA)n	%**	(GT)n	%**	ESTs	%**	Genes	%**		
Primers		349	–	250	–	484	–	64	–	1,147	–
PCR Products	No	18	5.1	28	11.2	68	14.0	10	15.6	124	10.8
	Smear	100	28.7	62	24.8	13	2.7	6	9.4	181	15.8
	Clear	231	66.2	160	64.0	403	83.3	48	75.0	842	73.4
Polymorphism[a]		181	51.9	115	46.0	160	33.1	22	34.4	478	41.7

[a]Number of markers showing at least one polymorphic character among the nine wheat lines
*(GA/CT)n, (GT/CA)n, (AT)n, (CGA/GCT)n, (CAA/GTT)n, (CAT/GTA)n, (GGT/CCA)n, (ATT/TAA)n, (CGT/GCA)n, (CTA/GAT)n, (GGA/CCT)n, (CTT/GAA)n
**Rate of primer pairs to the total number of primer pairs

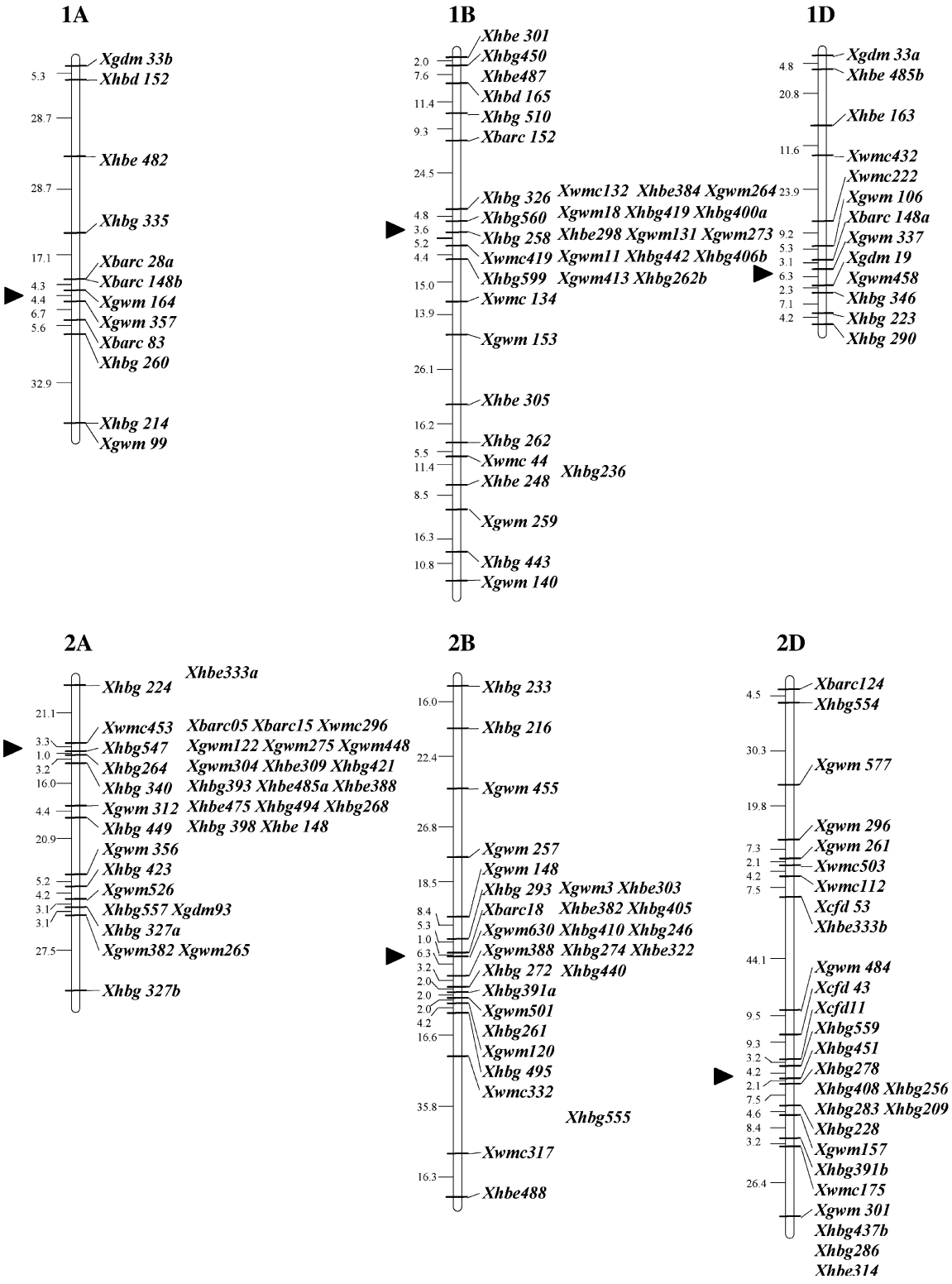


Fig. 1 SSR-based linkage map of 464 loci from an intraspecific DH population of a cross between Kitamoe and Münstertaler in common wheat. Locus names are indicated on the *right side* of each chromosome. Genetic distances (cM) are indicated on the *left side* of each chromosome. SSR loci mapped with LOD >3.0 are integrated in the framework map. The other SSR loci are placed in the most probable intervals. Putative locations of centromeres are indicated by *black arrows* on the *left side* of each chromosome. *hbg* SSR loci from enriched libraries developed in this study. *hbe* SSR loci from ESTs developed in this study. *hbd* SSR loci from DNA sequences of *Triticum* species on the public database developed in this study. *gwm* Röder et al. (1998). *gdm* Pestsova et al. (2000). *wmc* Gupta et al. (2002). *barc* The US Wheat and Barley Scab Initiative (http://www.scabusa.org/). *cfd* Guyomarc'h et al. (2002). *AWN* A morphological marker on 5A segregating with awn or awnless

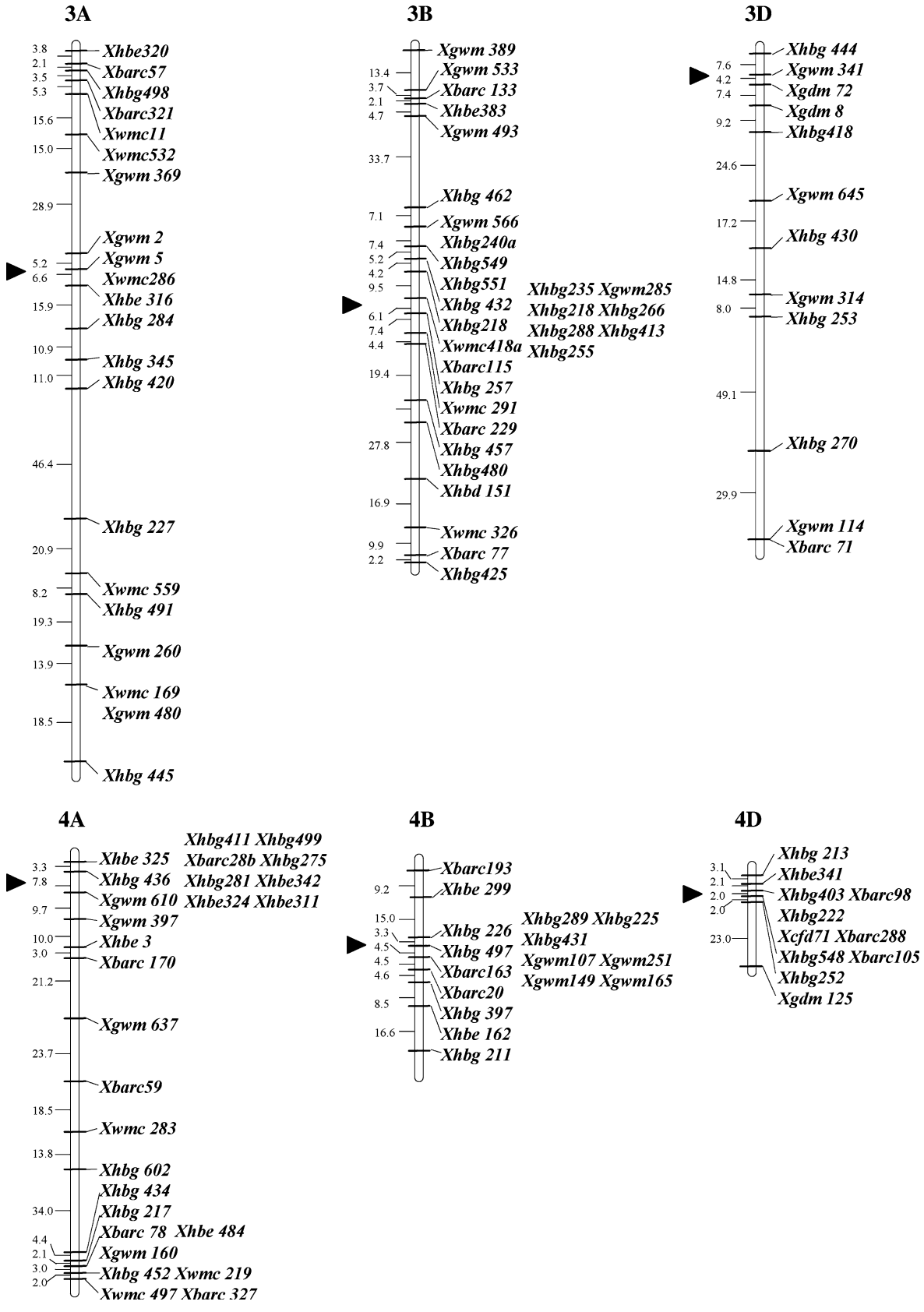

3A
3.8 Xhbe320
2.1 Xbarc57
3.5
5.3 Xhbg498
Xbarc321
15.6
Xwmc11
15.0 Xwmc532
Xgwm 369
28.9
Xgwm 2
5.2 Xgwm 5
6.6 Xwmc286
15.9 Xhbe 316
Xhbg 284
10.9
Xhbg 345
11.0 Xhbg 420
46.4
Xhbg 227
20.9
Xwmc 559
8.2
Xhbg 491
19.3
Xgwm 260
13.9
Xwmc 169
Xgwm 480
18.5
Xhbg 445
3B
Xgwm 389
13.4 Xgwm 533
3.7 Xbarc 133
2.1 Xhbe383
4.7 Xgwm 493
33.7
Xhbg 462
7.1 Xgwm 566
7.4 Xhbg240a
5.2 Xhbg549
4.2
9.5 Xhbg551
Xhbg235 Xgwm285
6.1 Xhbg 432
Xhbg218 Xhbg266
7.4 Xhbg218
Xhbg288 Xhbg413
4.4 Xwmc418a
Xhbg255
19.4 Xbarc115
Xhbg 257
Xwmc 291
Xbarc 229
27.8
Xhbg 457
Xhbg480
Xhbd 151
16.9
Xwmc 326
9.9
Xbarc 77
2.2
Xhbg425
3D
7.6 Xhbg 444
4.2 Xgwm 341
7.4 Xgdm 72
Xgdm 8
9.2 Xhbg418
24.6
Xgwm 645
17.2 Xhbg 430
14.8
Xgwm 314
8.0 Xhbg 253
49.1
Xhbg 270
29.9
Xgwm 114
Xbarc 71
4A
Xhbg411 Xhbg499
3.3 Xhbe 325
Xbarc28b Xhbg275
7.8 Xhbg 436
Xhbg281 Xhbe342
Xgwm 610
Xhbe324 Xhbe311
9.7
Xgwm 397
10.0
Xhbe 3
3.0
Xbarc 170
21.2
Xgwm 637
23.7
Xbarc59
18.5
Xwmc 283
13.8
Xhbg 602
Xhbg 434
34.0
Xhbg 217
Xbarc 78 Xhbe 484
4.4
2.1 Xgwm 160
3.0
Xhbg 452 Xwmc 219
2.0
Xwmc 497 Xbarc 327
4B
Xbarc193
9.2 Xhbe 299
15.0
3.3 Xhbg 226
Xhbg289 Xhbg225
4.5 Xhbg 497
Xhbg431
4.5
Xgwm107 Xgwm251
4.6 Xbarc163
Xgwm149 Xgwm165
Xbarc20
8.5
Xhbg 397
16.6
Xhbe 162
Xhbg 211
4D
3.1 Xhbg 213
2.1 Xhbe341
2.0
Xhbg403 Xbarc98
2.0
Xhbg222
23.0
Xcfd71 Xbarc288
Xhbg548 Xbarc105
Xhbg252
Xgdm 125

Fig. 1 (Contd.)

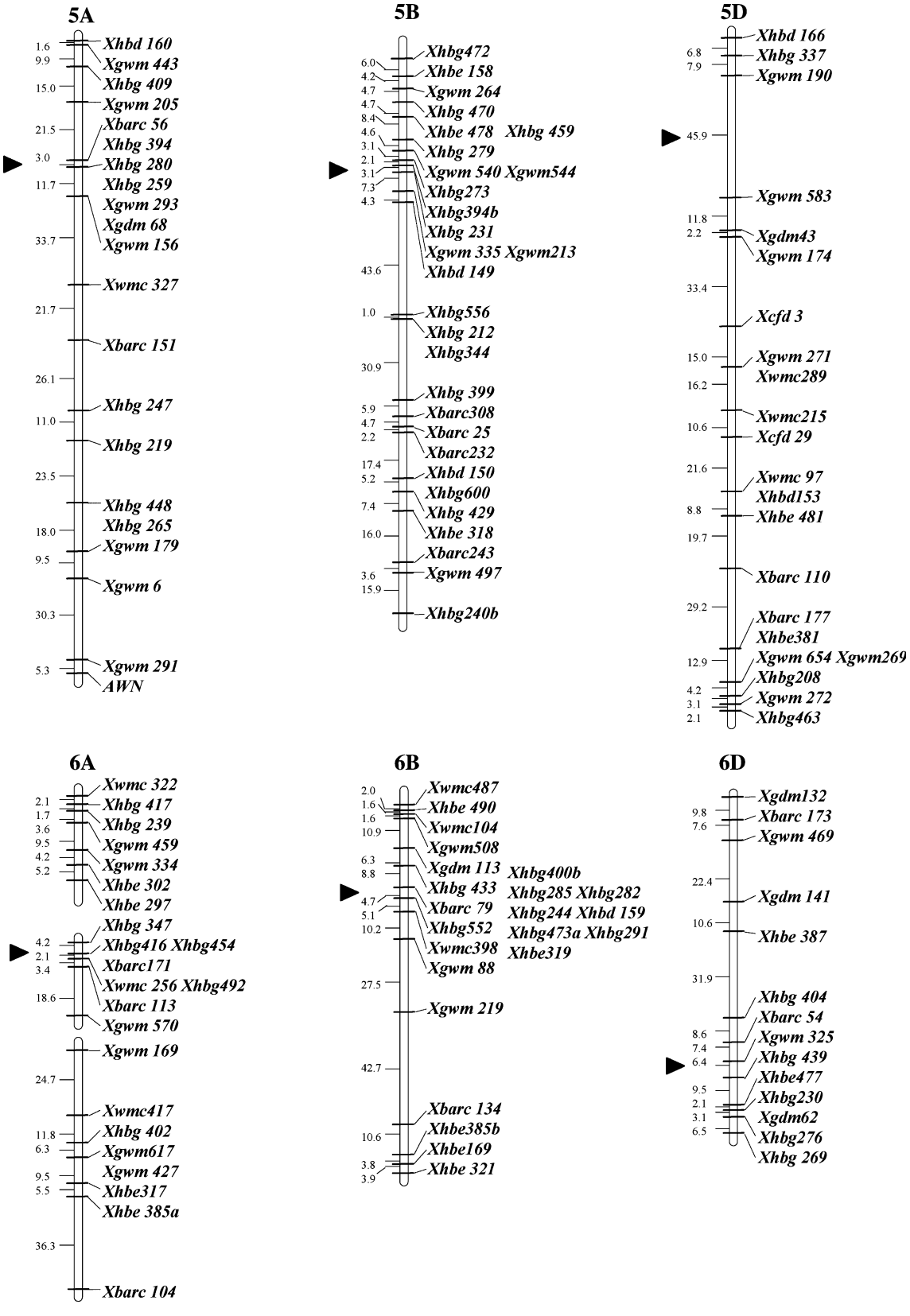
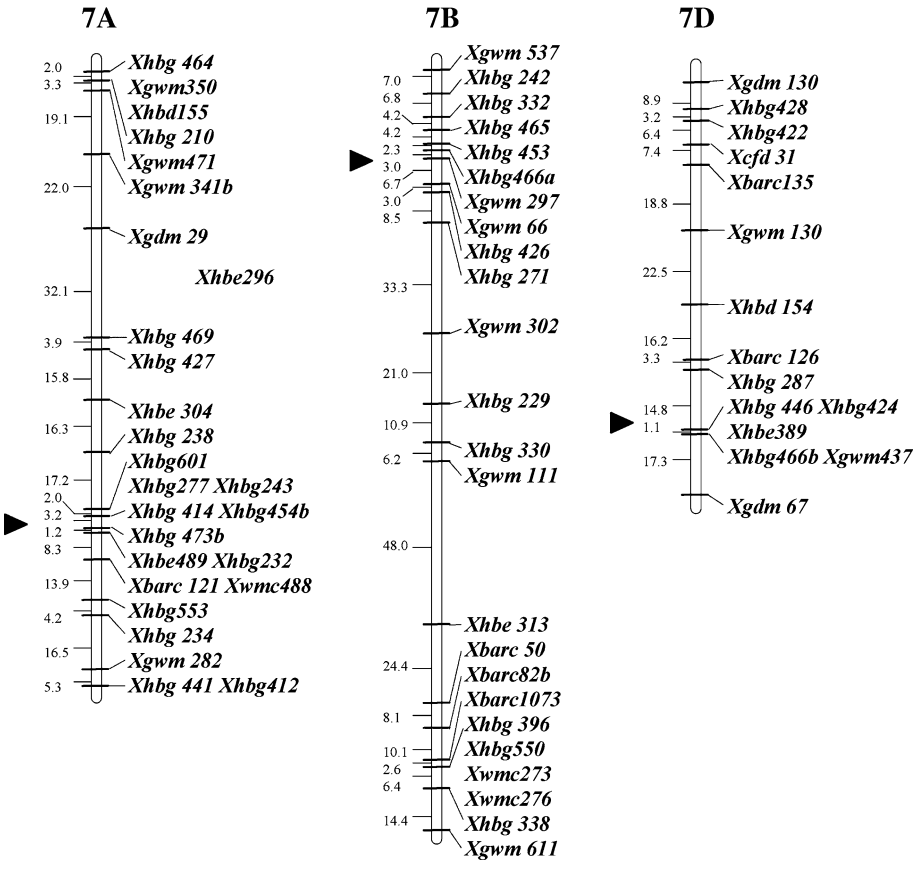

5A
1.6 Xhbd 160
9.9 Xgwm 443
15.0 Xhbg 409
Xgwm 205
21.5 Xbarc 56
Xhbg 394
3.0 Xhbg 280
11.7 Xhbg 259
Xgwm 293
Xgdm 68
33.7 Xgwm 156
Xwmc 327
21.7
Xbarc 151
26.1
Xhbg 247
11.0
Xhbg 219
23.5
Xhbg 448
18.0 Xhbg 265
Xgwm 179
9.5
Xgwm 6
30.3
5.3 Xgwm 291
AWN
5B
6.0 Xhbg472
4.2 Xhbe 158
4.7 Xgwm 264
4.7 Xhbg 470
8.4 Xhbe 478 Xhbg 459
4.6
3.1 Xhbg 279
2.1
3.1 Xgwm 540 Xgwm544
7.3 Xhbg273
4.3 Xhbg394b
Xhbg 231
Xgwm 335 Xgwm213
43.6 Xhbd 149
1.0 Xhbg556
Xhbg 212
Xhbg344
30.9
Xhbg 399
5.9 Xbarc308
4.7
2.2 Xbarc 25
Xbarc232
17.4 Xhbd 150
5.2
Xhbg600
7.4 Xhbg 429
16.0 Xhbe 318
Xbarc243
3.6 Xgwm 497
15.9
Xhbg240b
5D
6.8 Xhbd 166
7.9 Xhbg 337
Xgwm 190
45.9
Xgwm 583
11.8
2.2 Xgdm43
Xgwm 174
33.4
Xcfd 3
15.0 Xgwm 271
Xwmc289
16.2
Xwmc215
10.6 Xcfd 29
21.6
Xwmc 97
Xhbd153
8.8 Xhbe 481
19.7
Xbarc 110
29.2
Xbarc 177
Xhbe381
12.9 Xgwm 654 Xgwm269
Xhbg208
4.2
3.1 Xgwm 272
2.1 Xhbg463
6A
2.1 Xwmc 322
1.7 Xhbg 417
3.6
Xhbg 239
9.5 Xgwm 459
4.2
5.2 Xgwm 334
Xhbe 302
Xhbe 297
Xhbg 347
4.2
2.1 Xhbg416 Xhbg454
3.4
Xbarc171
18.6 Xwmc 256 Xhbg492
Xbarc 113
Xgwm 570
Xgwm 169
24.7
Xwmc417
11.8
Xhbg 402
6.3
Xgwm617
Xgwm 427
9.5
5.5 Xhbe317
Xhbe 385a
36.3
Xbarc 104
6B
2.0 Xwmc487
1.6 Xhbe 490
1.6
Xwmc104
10.9
Xgwm508
6.3 Xgdm 113 Xhbg400b
8.8
Xhbg 433 Xhbg285 Xhbg282
4.7
5.1 Xbarc 79 Xhbg244 Xhbd 159
10.2 Xhbg552 Xhbg473a Xhbg291
Xwmc398 Xhbe319
Xgwm 88
27.5
Xgwm 219
42.7
Xbarc 134
10.6 Xhbe385b
Xhbe169
3.8
3.9 Xhbe 321
6D
9.8 Xgdm132
7.6 Xbarc 173
Xgwm 469
22.4
Xgdm 141
10.6
Xhbe 387
31.9
Xhbg 404
Xbarc 54
8.6
7.4 Xgwm 325
6.4 Xhbg 439
Xhbe477
9.5
2.1 Xhbg230
3.1 Xgdm62
6.5 Xhbg276
Xhbg 269

Fig. 1 (Contd.)

Fig. 1 (Contd.)



population between KM for the SSR-based map construction.

Figure 1 shows the intraspecific SSR-based linkage map (KM map) for common wheat. The chromosome assignment of the SSR loci on the map was performed using the chromosome location of published markers as anchors. SSR-based map construction was developed from 464 segregating loci (1 morphological trait locus), of which 213 loci were already published SSR markers, and 250 loci were newly developed markers. Chromosome distribution of the loci was that 164 (35.4%), 185 (40.0%), and 114 loci (24.6%) were mapped on A, B, and D genomes, respectively. The maximum number of mapped loci on a chromosome was 35 on 1B, and the minimum number was 11 on chromosome 4D (Table 2).

In the newly developed 250 loci, 185 loci were from genome-derived SSRs (designated as *hbg*) and 65 were from public databases included EST-derived SSRs (designated as *hbe*) and SSR-containing sequences of *Triticum* species deposited on the DDBJ database (designated as *hbd*). Most *hbg* loci were isolated from enriched libraries, but only 17 loci were the SSR markers isolated with the PCR technique (unpublished data). Although the newly developed markers were distributed throughout all chromosomes on the KM map, the database-derived SSRs (*hbe* and *hbd*) could not be mapped on chromosome 3D. Of the 250 loci derived from new markers, 89 loci were located on the A genome, 110 loci were on the B genome, and 51 loci were on the D genome. The rate of new SSR loci to total mapped loci on the A, B, and D genomes was 54.3, 59.5, and 44.7%, respectively. In the newly mapped loci, the maximum number of mapped loci on a chromosome was 21 on 1B, and the minimum number was five on chromosome 1A, 1D, and 3D (Table 2).

A clustering of the SSR loci close to putative centromeric regions was found in several chromosomes. SSR loci, in which the published and the new SSR markers were included, tended to form clusters close to putative centromeric regions on chromosome 1B, 2A, 2B, 3B, 4A, 4B, and 6B.

The KM map length spanned 3,441 cM, which corresponded to approximately 86% genome coverage based on the estimation by Sourdille et al. (2003), who suggested that the entire map length of common wheat was about 4,000 cM in the case of an intraspecific population. Two gaps of over 50 cM still remained on chromosome 6A in the KM map, and several chromosome regions harbored few SSR markers compared with other maps. This is due to a lack of markers or unknown linkage blocks. Consequently, the SSR-based KM map integrated 23 linkage groups at this moment (Fig. 1).

Table 2 Number of mapped SSR loci in each chromosome of three genomes

Chromosome	Published SSRs					Total	New SSRs			Total	%***	Total
	gwm	*gdm*	*wmc*	*barc*	*cfd*	1*	*hbg*	*hbe*	*hbd*	2**		(1 + 2)
1A	3	1	0	3	0	7	3	1	1	5	41.7	12
2A	9	1	2	2	0	14	14	6	0	20	58.8	34
3A	5	0	5	2	0	12	7	2	0	9	42.9	21
4A	4	0	3	5	0	12	9	6	0	15	55.6	27
5A	7	1	1	2	0	11	8	0	1	9	45.0	20
6A	6	0	3	3	0	12	7	4	0	11	47.8	23
7A	4	1	1	1	0	7	16	3	1	20	74.1	27
Total on A genome	38	4	15	18	0	75	64	22	3	89	54.3	164
1B	9	0	4	1	0	14	14	6	1	21	60.0	35
2B	8	0	2	1	0	11	13	4	0	17	60.7	28
3B	5	0	3	4	0	12	16	1	1	18	60.0	30
4B	4	0	0	3	0	7	7	2	0	9	56.3	16
5B	6	1	0	4	0	11	13	3	2	18	62.1	29
6B	3	1	3	2	0	9	8	5	1	14	60.9	23
7B	6	0	2	3	0	11	12	1	0	13	54.2	24
Total on B genome	41	2	14	18	0	75	83	22	5	110	59.5	185
1D	3	2	2	1	0	8	3	2	0	5	38.5	13
2D	6	0	3	1	3	13	12	2	0	14	51.9	27
3D	4	2	0	1	0	7	5	0	0	5	41.7	12
4D	0	1	0	3	1	5	5	1	0	6	54.5	11
5D	7	1	3	2	2	15	3	2	2	7	31.8	22
6D	2	3	0	2	0	7	5	2	0	7	50.0	14
7D	3	2	0	2	1	8	5	1	1	7	46.7	15
Total on D genome	25	11	8	12	7	63	38	10	3	51	44.7	114
Total on whole genome	104	17	37	48	7	213	185	54	11	250	54.0	463

*Total number of published SSR loci
**Total number of newly developed SSR loci
***Rate of new SSR loci to total number of all mapped SSR loci (1 + 2)

Discussion

SSR markers play an important role in genetic mapping as anchors and in the MAS of a number of plant species. In common wheat, SSR loci that were developed by Röder et al. (1998) and Gupta et al. (2002) have been mapped on an ITMI population from a cross between Opata 85 and W7984. However, there is still difficulty of its application to a practical breeding program in terms of map density in the case of intraspecific materials such as breeding lines, because the number of mapped loci would be clearly reduced by showing lower polymorphism compared to the case of interspecifc populations.

In order to develop new SSR markers for common wheat, we designed a total of 1,147 primer pairs for SSR-containing sequences from two enriched libraries of (GA)n and (GT)n motifs, and from a public database including an enormous number of ESTs and gene-related sequences of *Triticum* species. The primers derived from the genome libraries provided more polymorphic markers compared to those selected from database (Tables 1, 2), although those produced more frequent smear products after PCR amplification (Table 1). Röder et al. (1998) and Song et al. (2002) also reported the conversion rate to accurate amplification was low in di- and tri-nucleotide repeats derived from wheat genomic libraries. Most primer pairs from the database produced accurate amplification, and the rate was higher than the one from the genome. This may be due to stable annealing of the primers synthesized from the ESTs (*hbe*) and gene-related sequences (*hbd*), because these primers should be located on the low copy sequences around expressed genes.

The newly developed markers could be mapped on all 21 chromosomes of KM map, however, the loci on the D genome were fewer than those on other genomes (Table 2, Fig. 1). In addition, concerning all 464 loci on KM map, the loci on D genome were fewest (24.6%) of three genomes. This agrees with other maps, where fewer markers were found on D genome, especially on chromosome 4D (Paillard et al. 2003; Sourdille et al. 2003). A targeted isolation from a diploid ancestor might be effective in saturating SSR markers on the D genome, as described by Pestsova et al. (2000) and Guyomarc'h et al. (2002).

Recently, a high-density SSR-based consensus map was constructed by integrating several populations including ITMI population (Somers et al. 2004), however, this KM map (464 loci, 3,441 cM) might be a first report of an SSR-based linkage map in single intraspecific population of common wheat. The KM map is comparable with other linkage maps for intraspecific populations. Sourdille et al. (2003) constructed a linkage map with 3,685 cM length that

consisted of 200 anchor markers selected from 659 markers including RFLP and SSR markers using wheat varieties Courtot and Chinese Spring (CtCs). Paillard et al. (2003) reported another intraspecific map that spanned 3,086 cM with RFLP and SSR markers utilizing the Swiss winter wheat varieties Arina and Forno (ArFo). Comparing ArFo map with our KM map, all 48 common SSR loci were located at the same chromosome position and showed perfect colinearity. These results represent that our SSR-based KM map with 250 new loci is consistent with other published map, and the common SSR markers can become anchor ones for the map construction of intraspecific populations. Moreover, the KM map is showing sufficient genome coverage compared to the CtCs and ArFo maps with RFLP and SSR markers. The high polymorphisms of the SSR markers between KM will lead to this successful map construction. The CtCs map included six gaps (>50 cM) on chromosome 4A, 4B, 4D, 5D, and 7D. There were also six gaps (>50 cM) on chromosome 2D, 3A, 3B, 3D, 5B, and 5D on the ArFo map, as was the case with the CtCs map. On the other hand, the KM map had two gaps (>50 cM) on the 6A chromosome. These results suggest that the position of the gaps depends on the materials used. Homozygous regions between mapping parents or lack of SSR marker sources on the regions might lead to the gaps and insufficient coverage in each map. Another prominent feature of the SSR-based genetic map is the significant clustering of the SSR loci around the putative centromeric regions of several chromosomes. The clustering of SSR loci was observed on chromosome 1B, 2A, 2B, 3B, 4A, 4B, and 6B of the KM map (Fig. 1). This observation is also described in the map of SSR markers by Röder et al. (1998) in wheat and Ramsay et al. (2000) in barley, and is likely due to the suppression of recombination around the centromeric regions. Taking into account that the clustering and the gap are found on the SSR-based linkage map of intraspecific population, the further development of SSR markers from whole genomic libraries is thought to be inefficient. To obtain the SSR markers located on targeted chromosome regions where the marker density is low, the development of SSR markers by screening of BAC clones with SSR-containing sequences might be useful as described by Cregan et al. (1999).

In conclusion, mapping results in this study suggest that the construction of linkage maps with sufficient genome coverage with only SSR markers is becoming possible even in intraspecific hexaploid wheat populations, although the availability will depend on mapping populations. Moreover, new set of SSR markers for common wheat were developed in this study. The new SSR markers will contribute to the enrichment of molecular marker resources in common wheat. These PCR-based molecular marker resources can provide to identify QTLs related to agronomically important traits in common wheat.

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