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A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags

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Abstract Bread wheat (*Triticum aestivum* L.) is a hexaploid species with a large and complex genome. A reference genetic marker map, namely the International Triticeae Mapping Initiative (ITMI) map, has been constructed with the recombinant inbred line population derived from a cross involving a synthetic line. But it is not sufficient for a full understanding of the wheat genome under artificial selection without comparing it with intervarietal maps. Using an intervarietal mapping population derived by crossing Nanda2419 and Wangshuibai, we constructed a high-density genetic map of wheat. The total map length was 4,223.1 cM, comprising 887 loci, 345 of which were detected by markers derived from expressed sequence tags (ESTs). Two-thirds of the high marker density blocks were present in interstitial and telomeric regions. The map covered, mostly with the EST-derived markers, approximately 158 cM of telomeric regions absent in the ITMI map. The regions of low marker density were largely conserved among cultivars and between homoeologous subgenomes. The loci showing skewed segregation displayed a clustered distribution along chromosomes and some of the segregation distortion regions (SDR) are conserved in different mapping populations. This map enriched with EST-derived markers is important for structure and function analysis of wheat genome as well as in wheat gene mapping, cloning, and breeding programs.

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Introduction

Bread wheat (*Triticum aestivum* L.) is the most widely grown crop in the world and feeds 40% of the world's population. It is a hexaploid species with a nuclear genome consisting of seven homoeologous groups of chromosomes. Each group includes three chromosomes from each of the three homoeologous subgenomes (A, B, and D). Using a recombinant inbred line population derived by crossing the synthetic hexaploid line W7984 with Opata 85, a reference genetic map (International Triticeae Mapping Initiative, ITMI) of the wheat genome containing more than 2,400 loci was constructed; the loci were mainly detected by restriction fragment length polymorphism (RFLP) markers (Nelson et al. 1995a, b, c; Van Deynze et al. 1995; Marino et al. 1996), genomic simple sequence repeat (gSSR) markers (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Song et al. 2005; Hayden et al. 2006), and expressed

sequence tag (EST)-derived SSR markers (eSSR markers) (Gao et al. 2004; Nicot et al. 2004; Yu et al. 2004). Recently, a few wheat genome maps were constructed using populations derived from intervarietal crosses for mapping genes with agronomical and adaptive significance (Paillard et al. 2003; Sourdille et al. 2003; Quarrie et al. 2005; Suenaga et al. 2005; Torada et al. 2006); all of these maps span over 3,000 cM. Although some of these maps have as many as six gaps larger than 50 cM, they show good consistency with the ITMI map and are complementary to one another in regional coverage. These maps improve understanding of the wheat genome and permit the localization of genes related to target traits such as yield, quality, and resistance to biotic and abiotic stresses. However, because of the low level of polymorphism among wheat cultivars (Bryan et al. 1999), these intervarietal maps are usually low in marker density and incomplete in their genome coverage.

ESTs represent partial complementary DNA (cDNA) sequences from expressed genes. Determining the order of ESTs along the chromosomes through genetic mapping would increase the marker density of genome maps, complement the sequence-based EST bin maps (Qi et al. 2004), facilitate genomic information transfer from related species, and directly target parts of the genome that could aid the identification of genes of agronomical importance. In addition to eSSR markers, EST-derived sequence-tagged-site markers (eSTS) have been extensively explored in cereal crops such as rice (Harushima et al. 1998), maize (Davis et al. 1999), and barley (Stein et al. 2007). They are derived from various tissues at different growth stages or under different treatments. More than 1 million wheat ESTs (NCBI EST database, 2007, 159th release) are currently available. Thus, construction of an EST marker-rich genetic map of wheat is feasible.

In this study, we report a high-density intervarietal map of the wheat genome enriched with newly developed eSSR and eSTS markers using a recombinant inbred line (RIL) population developed by crossing two bread wheat varieties Nanda2419 and Wangshuibai.

Materials and methods

Plant materials

The mapping population used in this study is a set of 136 F_7 recombinant inbred lines developed by single-seed descent from an intervarietal cross between bread wheat varieties, Nanda2419 and Wangshuibai. In addition, a set of Chinese Spring nulli-tetrasomic lines was used for chromosomal assignment of some polymorphic markers.

Marker development and analysis

The gSSR markers, eSSR markers, eSTS markers, STS-RFLP markers as well as some 10-oligo-mer randomly amplified polymorphic DNA (RAPD) markers were used in map construction (Table 1). SCAR marker S1021 was converted from a RAPD marker. The EST-derived markers included 43 markers from cnl, ksum (Yu et al. 2004), and sts series (Liu and Anderson 2003; Liu et al. 2006); 320 eSSR markers (MAG464-624 and MAG792-953, synthesized based on primer sequences derived from ESTs for putative transcription factors and signal transduction factors published by Triticeae EST-SSR Coordination, http://wheat.pw.usda.gov/ITMI/2002/EST-SSR/), and 2,293 new markers developed in the authors' lab.

Among the new developed markers, 138 were eSSR marker type and 2,155 were eSTS marker type. They were designed based on 1,637 putatively *Fusarium* infection-inducible ESTs from seven cDNA libraries of Sumai No. 3 spikes (http://www.ncbi.nlm.nih.gov) and 656 other ESTs. With these ESTs, we used the Perl script MISA program (http://pgrc.ipk-gatersleben.de/misa/misa.html) to screen for SSRs containing 2–5 repeat motifs with a run of at least 16 nucleotides. All primers were designed with the program MacVector 8.1 (Accelrys, UK) or Primer 3 (http://www.basic.northwestern.edu/biotools/Primer3.html). Moreover, 68 STS-RFLP markers were converted from RFLP probes mapped on the ITMI map, and STS markers were developed for six known genes, including *TaGST-zeta*, *TaLTP3*, *TaHO* and *TaPGAM* from the authors' lab, which encode for glutathione S-transferase, lipid transfer protein, hydroxylase and phosphoglycerate mutase, respectively, and *Vrn-A1* (Yan et al. 2003) and *Wx-A1b* (Vrinten et al. 1999). The primer sequences, expected product sizes, chromosome locations and other related information for the new markers that detected polymorphism between the mapping parents is provided in Table S1 of the online Electronic Supplementary Materials.

DNA was extracted according to Ma and Sorrells (1995). Polymerase chain reaction (PCR) for RAPD was performed in a PE9600 thermal cycler (Perkin-Elmer, Norwalk, CT, USA) programmed for 3 min at 96°C, followed by five cycles of 40 s at 96°C, 50 s at 35°C, and 1 min at 72°C; 40 cycles of 40 s at 94°C, 50 s at 36°C, and 1 min at 72°C; and 5 min at 72°C. SSR and STS amplification was performed following the procedure of Ma et al. (1996). The PCR products were separated in 8% non-denaturing polyacrylamide gels (Acr:Bis = 19:1 or 39:1) at room temperature in vertical gel apparatus (170 × 150 × 1.0 mm or 105 × 185 × 1.0 mm) with 1 × TBE buffer and visualized by silver staining (Bassam et al. 1991).

Linkage mapping

The genetic linkage map was constructed with MAP-MAKER/EXP Version 3.0 (Lincoln et al. 1992). Loci were grouped using the criteria LOD = 3.0 and recombination fraction = 0.15. Linkage groups belonging to the same chromosomes were integrated using the criteria LOD = 3.0 and recombination fraction = 0.40. Multipoint analysis was used to construct the framework map. The order of "Framework" loci was determined using the "order" command with a LOD threshold of 3.0 and checked using the "ripple" command. Other markers were placed on the framework map using the "try" and "ripple" commands. The maps were drawn with MAPMAKER Version 2.0 (Lander et al. 1987), with the recombination distances calculated with the Kosambi mapping function (Kosambi 1944). Double crossovers between closely linked markers were checked to avoid misscoring. The segregation fit of each locus to a 1:1 ratio was examined using the chi-square test. A region with at least three adjacent loci showing significant segregation distortion ($P < 0.05$) was defined as the segregation distorted region (SDR) (Paillard et al. 2003).

In order to evaluate the synteny relationship and regional coverage between the Nanda2419–Wangshuibai (NW) map and the ITMI map, data for 1,592 markers were downloaded from the GrainGenes website to reconstruct the ITMI map. The framework marker order was determined by referring to Song et al. (2005). Other markers were placed on the framework map using the "try" and "ripple" commands. Data for 154 markers were not included in the map because they considerably extended the map length when placed into the linkage groups.

Results

Polymorphic survey of the mapping parents

We surveyed the mapping parents Nanda2419 and Wangshuibai with 4,180 PCR-based markers, including RAPD, gSSR, eSSR, eSTS, and STS-RFLP markers, in addition to known genes (Table 1). Seven hundred eighty-five of these markers (18.8%) detected polymorphism. The efficiencies of gSSR, eSSR, eSTS, STS, and RAPD markers in detecting polymorphism between the parents varied from 9.9% with eSSR to 35.5% with gSSR. Most SSR markers detected codominant loci, while 66% of the eSTS markers detected dominant loci.

For the newly developed 2,293 EST markers, only 298 (13.0%) gave no amplification or produced smear products (Table 2). The reasons that led to the unsuccessful amplification could be mostly attributed to poor EST sequence quality or sequence polymorphism between different genotypes, since re-designing primers could often alleviate this kind of problem (Z Ma and H Yi, unpublished data). A little higher level of successful amplification was achieved for the eSSR markers than for the eSTS markers; however, both types of markers detected a similar level of polymorphism with an average polymprohism detection rate of 12.8% (Table 2), which was two and three times lower than those for the surveyed STS-RFLP markers and gSSR

Table 1 Types of markers surveyed and the polymorphism detection rates

Type	Code	Number	Polymorphism rate (%)[a]	Source
RAPD	af; ag; ba bb; bc; bd be; bg; bh; s	198	12.7	Invitrogene, USA
gSSR	barc	206	35.5	Song et al. (2005)
	gwm	230		Röder et al. (1998)
	wmc	593		Wheat Microsatellite Consortium (http://wheat.pw.usda.gov)
	cfa; gpw	39		Sourdille's lab (http://wheat.pw.usda.gov)
	cfd	109		Guyomarc'h et al. (2002)
	gdm	64		Pestsova et al. (2000)
	psp	17		Bryan et al. (1997)
eSSR	cnl; ksum	27	9.9	Yu et al. (2004)
	mag	320		Triticeae EST-SSR coordination (http://wheat.pw.usda.gov/ITMI/2002/EST-SSR/)
	mag	138		Authors' lab
eSTS	sts	16	11.4	Liu and Anderson (2003) and Liu et al. (2006)
	mag	2,155		Authors' lab
STS	sts-RFLP	68	25.0	Authors' lab, GrainGenes website (http://wheat.pw.usda.gov)

[a] Vs total number of markers surveyed for each category

Table 2 Amplification of Nanda2419 and Wangshuibai genomic DNA with the newly developed EST markers

Marker type	Number	Number (%) of markers with			
		No amplification	Smear products	With clear bands	Detecting polymorphism[a]
eSSR	138	10 (7.2)	1 (0.7)	127 (92.0)	17 (13.4)
eSTS	2,155	273 (12.7)	14 (0.6)	1,868 (86.7)	239 (12.8)
Total	2,293	283 (12.3)	15 (0.7)	1,995 (87.0)	256 (12.8)

[a] % markers detecting polymorphism was vs markers producing clear bands

markers (Table 1). Interestingly, among markers producing clear bands, only 12.5% eSSR markers, but 33.4% eSTS markers produced a single strand. All eSSR primer pairs and 93.4% eSTS primer pairs that produced clear bands amplified products within the expected size ranges or larger than the expected sizes.

Among the gSSR marker loci surveyed, 243 had been previously mapped to the A subgenome, 272 to the B subgenome and 334 to the D subgenome, with 39.5, 40.4, and 30.5% of them detecting polymorphism, respectively. Consistent with other mapping studies of wheat, the D subgenome had the lowest level of polymorphism. Less than 25% of the surveyed markers previously mapped to chromosomes 4D, 6A, and 6D detected polymorphism; this was one of the lowest rates. For the published intervarietal maps, chromosomes 4D and 6D usually had the fewest mapped markers. It seems that these two chromosomes are best conserved in varieties. Chromosomes 4B and 5B were most polymorphic between Nanda2419 and Wangshuibai among all the chromosomes; 54.6 and 50.0% of the surveyed markers previously mapped to these two chromosomes, respectively, detected polymorphism. Over 40% of markers surveyed for each of the chromosomes 1A, 2A, 2D, 3A, 3B, 4B, 5A, 5B, 6B, and 7A was polymorphic.

Most of the SSR and STS markers detected a single locus, but 86 of them detected two or more loci. One marker, GWM497, identified five polymorphic loci. The multiple polymorphic loci detected by a marker were located, in approximately half of these cases, in the same homoeologous group as shown in the mapping construction.

Genetic mapping

We obtained population data of 908 marker loci detected by the polymorphic markers. The data in dominant loci were verified with internal control. After excluding 11 loci that could not be placed into linkage groups and 10 loci that considerably extended the map length when placed into the linkage groups, the chromosome-based marker linkage maps were established. Chromosome assignment of the linkage maps was determined using the known mapping positions of gSSR markers and nulli-tetrasomic mapping of some markers. To save space, the maps showing order and

location of markers and other related information were provided in Fig. S1 as online Electronic Supplementary Materials.

This Nanda2419–Wangshuibai (NW) map comprises 887 marker loci, including 542 detected by anonymous markers and 345 detected by EST-derived markers (273 by the new EST markers), targeting at least 794 discrete positions in the wheat genome. It covered 4,223.1 cM of the wheat genome, excluding the seven >40 cM gaps distributed on chromosomes 2A, 2D, 3D, 4D, and 6D (these gaps were also excluded from later analysis, unless specifically indicated) (Fig. S1 and Table 3). The A, B, and D subgenome maps spanned 1,478.3, 1,510.4, and 1,234.4 cM, respectively. This coverage is proportional to the respective subgenome size estimated by Gill et al. (1991). However, the genetic markers did not evenly distribute across the subgenomes. Twice as many loci mapped to the B subgenome versus the D subgenome and the B subgenome had a much higher marker density compared with the A and D subgenomes (Table 3). There was also great variability in the number of loci mapping to individual chromosomes, which, however, was not proportional to the individual chromosome lengths ($\chi^2 = 128.2$, $P \leq 0.0001$), suggesting that the polymorphism level varied between different chromosomes.

Marker density

In the NW map, the marker density of individual chromosomes ranged from 2.2 markers/20 cM for 5D to 7.8 markers/20 cM for 6B (Table 3). The EST-derived markers significantly increased the marker density of chromosomes 4A, 6B, 7A, and 7B (Fig. S1), where the number of loci mapped by EST-derived markers exceeded that mapped by anonymous markers. The marker density also varied within the individual chromosomes. In 30 regions, distributed on all chromosomes but 1B, 1D, 4D, 5D, 6D, and 7D, more than eight loci were mapped per 20 cM (Fig. S1). Sixteen of these regions with a high marker density occurred in interstitial regions and four occurred near the telomeres. The EST-derived marker loci accounted for 46.2% of the loci in these high marker density regions, whereas they accounted for only 38.9% in the map as a whole. Particularly

Table 3 Distribution of mapped markers on 21 wheat chromosomes in the NW map

Subgenome	Chromosome	Number of loci	Length (cM)	Density (markers/20 cM)
A	1A	33	197.3	3.3
	2A	50	208.7	4.8
	3A	41	210.7	3.9
	4A	31	197.8	3.1
	5A	49	250.4	3.9
	6A	15	110.5	2.7
	7A	71	302.9	4.7
	Subtotal	290	1,478.3	3.9
B	1B	40	218.5	3.7
	2B	70	266.7	5.2
	3B	69	233.1	5.9
	4B	30	136.2	4.4
	5B	71	294.2	4.8
	6B	57	146.9	7.8
	7B	62	214.8	5.8
	Subtotal	399	1,510.4	5.3
D	1D	21	151.0	2.8
	2D	61	236.9	5.1
	3D	28	128.3	4.4
	4D	18	140.3	2.6
	5D	28	257.2	2.2
	6D	14	73.7	3.8
	7D	28	247.0	2.3
	Subtotal	198	1,234.4	3.2
Total		887	4,223.1	4.2

significant was the 17 cM *Xgdm19.2–Xmag4089* interstitial interval on chromosome 2D, where 17 EST-derived marker loci were mapped (Fig. S1).

Regions of low marker density or gaps occurred on almost every chromosome, most of which were present in subgenomes A and D. Among the seven large gaps, five were in the D subgenome. It appeared that these gap regions are conserved among the three subgenomes of common wheat, for example, the 47.4-cM gap on 2DS and the short arm of 2A, the 43.8-cM gap on 3DS and the 27.0-cM gap on 3AS, the 33.4-cM gap on 5DL and the 23.7-cM gap on 5AL, the 40.5- and 42.0-cM gaps on 6D and the 35.1- and 30.3-cM gaps on 6A, and the 35.6-cM gap on 7DL and the 26.4-cM gap on 7AL (Fig. S1). The one gap greater than 50 cM flanked by *Xsts-cdo1312* and *Xmag2944* on 4D was also homoeologous to the 24.5-cM gap region flanked by *Xwmc413* and *Xmag1682* on 4B.

Segregation distortion regions

The majority of mapped loci in the NW map segregated in the expected 1:1 ratio in the population. However, segregation of 162 mapped loci, over half of which are inherited in a codominant manner, significantly deviated from this ratio ($P < 0.05$). Approximately 70% of the dominant loci showing segregation distortion (SD) were mapped by eSTS markers. The SD loci clustered in 15 regions, distributed on chromosomes 1B, 2A, 2B, 2D, 3A, 4D, 5A, 6B, 6D, and 7B (Fig. S1), in ten of which the Wangshuibai alleles were favored. All seven homoeologous groups have been involved in SD, however, nearly half of the SDRs were identified in the B subgenome and the D subgenome ranked second in the number of SDRs. The two adjacent 4D SDRs skewed towards the opposite parents, and as a result, might have extended the distance between them (Fig. S1). *Xmag1652*, which flanks SDR-4D1 and is closest to the telomere of the short arm, exhibited the most skewed segregation among the loci mapped to this region, with 78% of the RILs having the Nanda2419 allele.

Discussion

We have presented a 4,223.1 cM intervarietal genetic marker map of wheat using the Nanda2419 × Wangshuibai RIL population. Of the two mapping parents, Nanda2419 is

a selection from Italian wheat variety Mentana and has been widely grown in China. It could be found in the pedigrees of more than 110 Chinese cultivars and therefore was viewed as one of key breeding parents in China's wheat breeding programs. Wangshuibai is an indigenous germplasm originating in Jiangsu, China, best known for its excellent scab resistance (Lin et al. 2004, 2006). The different geographical origins of both parents did not lead to a high level of polymorphism. The polymorphism level detected between them by gSSR markers was 9.5% lower than that reported by Suenaga et al. (2005) between cultivars Fukuho-komugi and Oligoculm, and 25.5% lower than that reported by Paillard et al. (2003) between winter cultivars Arina and Forno. Between 'Opata 85' and the synthetic wheat 'W7984', as high as 80% of the SSR primer pairs detected polymorphism (Röder et al. 1998). In our results, the newly developed eSTS markers and eSSR markers had similar polymorphism detection efficiency (Table 2). However, we were able to raise the efficiency for eSTS markers from 118 ESTs or EST contigs to 34.7% by designing primers near the 5′ or 3′ untranslated regions (unpublished data).

The NW map was constructed with only PCR-based markers. There are 30 intervals with a high marker density in the map, with 20 of which mapped to the interstitial or telomerical regions where genes often cluster (Qi et al. 2004). Since the EST-derived markers represented nearly half of the loci mapped to these regions, this map would be especially useful for mapping traits with functional markers. This map conforms well to other published intervarietal maps according to the positions and orders of commonly mapped markers. As far as we know, among the intervarietal maps, the NW map has the best genome coverage, a higher average marker density, less gaps, and is 275 cM larger than the next best map, the 3,948-cM FkO map (Suenaga et al. 2005), which includes 11 > 40 cM gaps. Efforts were made to close or minimize the gaps by surveying STS markers converted from RFLP markers mapped to these regions or ESTs mapped to the corresponding deletion bins or chromosomes besides the gSSR markers found in the published maps. However, we were unable to substantially close the gaps due to the lack of polymorphism. For instance, in the 73.9-cM region between *Xwmc312* and *Xgwm99* on chromosome 1A, only two of the 13 surveyed markers detected polymorphism; in the 30.3-cM gap flanked by *Xbarc113* and *Xgwm169* on chromosome 6A and in the 40.5-cM gap flanked by *Xgwm469* and *Xwmc773* on chromosome 6D, none of the surveyed markers (14 and 15, respectively) detected polymorphism (Fig. S1).

To compare the NW map and the ITMI map, a 5,758.7 cM ITMI map was constructed (see the online Electronic Supplementary Materials in Fig. S2) with all markers publicly available. By aligning 185 common markers mapped in both maps, we found only 20 intervals that were inconsistent between the two maps. These inconsistencies were often involved in small intervals, and 13 of them resided in regions surrounding the centromeres, for instance, those on chromosomes 2A and 5B. Thus, they might have resulted from inaccuracy in ordering loci due to lack of recombination. Another possible reason for these inconsistencies is structural change in the chromosomes. *Xgwm18* and *Xgwm246.1* are two tightly linked loci on chromosome 1B. They have the same orientation in both the NW map and the Kitamoe–Münstertaler (KM) map (Torada et al. 2006), with *Xgwm18* located on 1BS and *Xgwm246.1* located on 1BL, as shown by deletion mapping (Sourdille et al. 2004). However, in the ITMI map, these loci were mapped to the short arm in a reversed order and with a genetic distance of 60.3 cM. Similar inconsistencies were also observed between the ITMI map and other intervarietal maps (Cadalen et al. 1997; Paillard et al. 2003).

Even though the NW map was more than 1,500 cM shorter than the ITMI map in genome coverage, it only lacked coverage of the terminal regions of 2AS, 3AL, 6AL, 6BL, and 6DL that totaled 247.8 cM in the ITMI map. Furthermore, it covered approximately 158 cM that were absent in the ITMI map, including the terminal regions of 2BS, 4BL, 5AS, and 5BL (Fig. S2). Moreover, some regions in the NW map, such as the distal portions of chromosomes 4AL, 5BL, 7AL, and 7BL, had an even better marker coverage or a higher resolution. Thus, these two maps complement each other well. In most cases, the regions with a better coverage in the NW map were rich in EST-derived markers.

Interestingly, we found that regions of low marker density were largely conserved among cultivars and between homoeologous subgenomes, as shown in the NW map and other published intervarietal maps. All 13 > 30 cM gaps in the NW map were corresponding to the low marker density regions in the ArFo (Paillard et al. 2003), CSSQ1 (Quarrie et al. 2005), and KM (Torada et al. 2006) intervarietal maps, even though the corresponding gap size varied between them (Table 4). This kind of conservation could be due to the low level of polymorphism in the corresponding regions, since these regions in the ITMI map, whose mapping parents include a synthetic wheat line, had a much higher marker density (Table 4).

In the NW map, 15 intervals showed SD, involving 10 of the 21 chromosomes. As in this study, Groos et al. (2002) reported SDRs on chromosomes 2A, 2B, and 2D, even though the correspondences in locations between both studies could not be determined. The corresponding regions of the two 2B SDRs showed SD in the CtCS and ArFo maps (Sourdille et al. 2003; Paillard et al. 2003). SDRs on the short arm and the pericentromeric region of chromosome 4D have been identified in the CSSQ1 (Quarrie et al. 2005)

Table 4 Comparison of gaps greater than 30 cM in the NW map to corresponding intervals in four other genetic maps of wheat

Gaps in the NW map[a]			Corresponding interval distance (cM)			
Flanking markers	Location	Interval size (cM)	ArFo	CSSQ1	KM	ITMI
Xwmc667–Xmag3253	2AS1	40.4	44.0	24.8	–[b]	13.1
Xwmc602–Xwmc390.2	2AS2	44.4	23.0	10.8	21.1	14.1
Xmag277–Xbarc168	2DS	47.4	35.0	55.5	44.1	13.3
Xmag3616–Xmag2120	2DL	35.8	25.0	–	–	16.0
Xcfd79.1–Xgwm341	3DS	43.8	27.0	41.2	–	20.0
Xsts-cdo1312–Xmag2944	4DL	>50.0	–	75.0	–	13.4
Xgwm639.1–Xbarc320	5DL	33.4	37.0	18.1	33.4	17.7
Xmag1053–Xmag1470	6AS	35.1	35.0	59.3	>50.0	11.5
Xbarc113–Xgwm169	6AL	30.3	46.0	17.6	>50.0	21.4
Xgwm469–Xwmc73	6DS	40.5	43.0	43.1	21.6	8.6
Xmag1459–Xbarc96	6DL	42.0	–	43.7	–	13.0
Xcfd31–Xmag1132.2	7DS	36.8	33.0	31.3	31.9	17.7
Xpsp3123–Xgwm428	7DL	35.6	47.0	27.3	–	12.0

[a] The corresponding positions in other published maps were determined according to common markers or centromeres

[b] Region not mapped in the corresponding map

and FkO (Suenaga et al. 2005) double haploid populations, in a wheat × *T. spelta* RIL population (Messmer et al. 1999), and in an *Aegilops tauschii* F_2 population (Faris et al. 1998). The SDR-5A corresponding region has been involved in SD in four different populations (Messmer et al. 1999; Peng et al. 2000; Groos et al. 2002; Liu et al. 2005). Campbell et al. (1999), Sourdille et al. (2003), and Liu et al. (2005) reported distorted segregation in the region corresponding to SDR-6B (Fig. S1). SD in the regions corresponding to SDR-6D1 and SDR-7B1 were reported, respectively, by Suenaga et al. (2005) and Messmer et al. (1999). Since over 80% of the loci segregated normally in the NW population, physiological conditions are unlikely the reason of SD. Genetically, both gametophytic genes and mutants unfavorable to growth and reproduction could cause skewed segregation of linked alleles. But deleterious mutants cannot be maintained in cultivars as breeders would select against them. In RIL populations, genes for sterility would also cause SD. Eleven gametophytic genes have been detected in rice (Kinoshita 1991) and five in maize (Coe and Polacco 1995); some of these genes are associated with SD (Harushima et al. 1996; Lu et al. 2002). To our knowledge, no gametophytic genes have been isolated or mapped in wheat, but the fact that gametocidal factors in heterozygous and hemizygous states from *Aegilops* relatives of wheat would affect the function of gametes without the gametocidal alleles was well documented (Endo 1990). We assume that at least some of the SDRs identified in this study involve gametophytic genes.

The NW map provides a supplement to the previously published genetic maps and could find wide application in structure and function analysis of the wheat genome, as well as in gene mapping, cloning, and wheat breeding (Lin et al. 2004, 2006; Ma et al. 2007). The use of PCR markers makes this map especially useful for information exchange between different labs. Even though EST-derived markers usually detect less polymorphism than do genomic markers, such as gSSR markers, their mapping could provide invaluable information for exploration of the expressed portion of the wheat genome and functional analysis of traits of interest. In this study, we identified some EST markers clustered in QTL intervals for resistance to wheat Fusarium head blight (FHB) such as *QFhi.nau-2D1* (Lin et al. 2006) and *QFhs.nau-6B* (Lin et al. 2004), probably because the ESTs from *F. graminearum*-induced spike cDNA libraries were used in the marker development. Some of these ESTs displayed differential expression patterns between the FHB-resistant Wangshuibai and the FHB-susceptible Nanda2419 (unpublished data) and thus may serve as candidate genes. EST markers linked to genes of interest could also be used to demarcate orthologous gene regions in the genomes of rice and *Brachypodium distachyon*, two model species of cereal crops, which in turn would aid identification of candidate genes or development of new markers for fine mapping.

References

Bassam BJ, Gaetano-Anollé G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Bryan GJ, Stephenson P, Collins A, Kirby J, Smith JB, Gale MD (1999) Low levels of DNA sequence variation among adapted genotypes of hexaploid wheat. Theor Appl Genet 99:192–198

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell. and comparison with a map from a wide cross. Theor Appl Genet 94:367–377

Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. Crop Sci 39:1184–1195

Coe EH, Polacco M (1995) Gene list and working maps. Maize Genet Coop News Lett 694:157–191

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, Xu G, Polacco M, Koster L, Melia-Hancock S, Houchins K, Chao S, Coe EH Jr (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics 152:1137–1172

Endo TR (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. Jpn J Genet 65:135–152

Faris JD, Laddomada B, Gill BS (1998) Molecular mapping of segregation distortion loci in *Aegilops tauschii*. Genetics 149:319–327

Gao LF, Jing RL, Huo NX, Li Y, Li XP, Zhou RH, Chang XP, Tang JF, Ma ZY, Jia JZ (2004) One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. Theor Appl Genet 108:1392–1400

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet D (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread-wheat cross. Theor Appl Genet 104:39–47

Gupta K, Balyan S, Edwards J, Isaac P, Korzun V, Roder M, Gautier MF, Joudrier P, Schlatter R, Dubcovsky J, De La Pena C, Khairallah M, Penner G, Hayden J, Sharp P, Keller B, Wang C, Hardouin P, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Harushima Y, Kurata N, Yano M, Nagamura Y, Sasaki T, Minobe Y, Nakagahra M (1996) Detection of segregation distortions in an *indica-japanica* rice cross using a high-resolution molecular map. Theor Appl Genet 92:145–150

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Hayden MJ, Stephenson P, Logojan AM, Khatkar D, Rogers C, Elsden J, Koebner RM, Snape JW, Sharp PJ (2006) Development and genetic mapping of sequence-tagged microsatellites (STMs) in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1271–1281

Kinoshita T (1991) Report of the committee on gene symbolization, nomenclature and linkage group. Rice Genet Newsl 8:2–37

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lin F, Kong ZX, Zhu HL, Xue SL, Wu JZ, Tian DG, Wei JB, Zhang CQ, Ma ZQ (2004) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. I. Type II resistance. Theor Appl Genet 109:1504–1511

Lin F, Xue SL, Zhang ZZ, Zhang CQ, Kong ZX, Yao GQ, Tian DG, Zhu HL, Li CJ, Cao Y, Wei JB, Luo QY, Ma ZQ (2006) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. II: Type I resistance. Theor Appl Genet 112:528–535

Lincoln SE, Daly MJ, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP Version 3.0. Technical Report, 3rd edn. Whitehead Institute, Cambridge, MA

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to *Fusarium* head blight in wheat. Funct Integr Genomics 6:83–89

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

Lu H, Romero-Severson J, Bernardo R (2002) Chromosomal regions associated with segregation distortion in maize. Theor Appl Genet 105:622–628

Ma ZQ, Röder MS, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Ma ZQ, Sorrells ME (1995) Genetic analysis of fertility restoration in wheat using restriction fragment length polymorphisms. Crop Sci 35:1137–1143

Ma ZQ, Zhao DM, Zhang CQ, Zhang ZZ, Xue SL, Lin F, Kong ZX, Tian DG, Luo QY (2007) Molecular genetic analysis of five spike-related traits in wheat using the RIL and immortalized F_2 populations. Mol Genet Genomics 277:31–42

Marino CL, Nelson JC, Lu YH, Sorrels ME, Leroy P, Lopes CR, Hart GE (1996) RFLP-based linkage maps of the homoeologous group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39:359–366

Messmer MM, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of a wheat × spelt cross. Theor Appl Genet 98:1163–1170

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995a) Molecular mapping in bread wheat. Homoeologous group-2. Genome 38:516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995b) Molecular mapping in bread wheat. Homoeologous group-3. Genome 38:525–533

Nelson JC, Van Deynze AE, Sorrells ME, Lu YH, Atkinson M, Bernard M, Leroy P, Faris J, Anderson JA (1995c) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5 and 7. Genetics 141:721–731

Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P (2004) Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). Theor Appl Genet 109:800–805

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). Theor Appl Genet 107:1235–1242

Peng JH, Korol AB, Fahima T, Röder MS, Ronin YI, Li YC, Nevo E (2000) Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NL, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS (2004) A chromosome bin map of 16,000 EST loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixer MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823–839

Suenaga K, Khairallah M, William HM, Hoisington DA (2005) A new intervarietal linkage map and its application for quantitative trait locus analysis of "gigas" features in bread wheat. Genome 48:65–75

Torada A, Koike M, Mochida K, Ogihara Y (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor Appl Genet 112:1042–1051

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group-1 chromosomes of *Triticeae* species and their relation to chromosomes in rice and oat. Genome 38:45–59

Vrinten P, Nakamura T, Yamamori M (1999) Molecular characterization of waxy mutations in wheat. Mol Gen Genet 261:463–471

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yu JK, Dake TM, Singh S, Benscher D, Li W, Gill B, Sorrells ME (2004) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. Genome 47:805–818