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Z. H. Liu · J. A. Anderson · J. Hu · T. L. Friesen
J. B. Rasmussen · J. D. Faris

A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci

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Abstract Efficient user-friendly methods for mapping plant genomes are highly desirable for the identification of quantitative trait loci (QTLs), genotypic profiling, genomic studies, and marker-assisted selection. SSR (microsatellite) markers are user-friendly and efficient in detecting polymorphism, but they detect few loci. Target region amplification polymorphism (TRAP) is a relatively new PCR-based technique that detects a large number of loci from a single reaction without extensive pre-PCR processing of samples. In the investigation reported here, we used both SSRs and TRAPs to generate over 700 markers for the construction of a genetic linkage map in a hard red spring wheat intervarietal recombinant inbred population. A framework map consisting of 352 markers accounted for 3,045 cM with an average density of one marker per 8.7 cM. On average, SSRs detected 1.9 polymorphic loci per reaction, while TRAPs detected 24. Both marker systems were suitable for assigning linkage groups to chromosomes using wheat aneuploid stocks. We demonstrated the utility of the maps by identifying major QTLs for days to heading and reduced plant height on chromosomes 5A and 4B, respectively. Our results indicate that TRAPs are highly efficient for genetic mapping in wheat. The maps developed will be useful for the identification of quality and disease resistance QTLs that segregate in this population.

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Z. H. Liu · J. B. Rasmussen
Department of Plant Pathology, North Dakota State University, Fargo, ND 58105, USA

J. A. Anderson
Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

J. Hu
Sunflower Research Unit, Northern Crop Science Laboratory, Red River Valley Agricultural Research Center, Fargo, ND 58105, USA

T. L. Friesen · J. D. Faris (✉)
USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Laboratory, Red River Valley Agricultural Research Center, Fargo, ND 58105, USA
E-mail: farisj@fargo.ars.usda.gov
Tel.: +1-701-2391339
Fax: +1-701-2391369

Introduction

DNA markers are used for a wide range of purposes in genetic studies including genetic linkage and comparative mapping, positional cloning, genotypic profiling, marker-assisted selection, and the detection of quantitative trait loci (QTLs). While there are a number of different methods for visualizing DNA markers, most are based on the PCR. Random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) use short random oligonucleotides as primers to amplify genomic DNA sequences, but due to the unpredictable behavior of the short primers, RAPDs tend to have low repeatability. Amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) have been used more extensively and are efficient in amplifying a large number of fragments from a single PCR reaction. However, the AFLP technique requires extensive pre-PCR sample processing, including restriction digestion followed by the ligation of adapters and pre-amplification, which makes it more laborious. Microsatellites, or simple sequence repeats (SSRs) (Beckman and Weber 1992; Wang et al. 1994) are widely used because they are user-friendly, highly polymorphic, and usually locus-specific, but they are less efficient for the rapid mapping of whole plant genomes due to the number of PCRs needed to generate a large number of marker loci.

Recently, Hu and Vick (2003) developed a new marker technique known as target region amplified polymorphism (TRAP), which is a rapid and efficient PCR-based technique that employs two 18-mer primers. One "fixed" primer is designed from a known expressed

sequence tag (EST), while the other primer is arbitrary with either an AT- or GC-rich core to anneal with an intron or exon, respectively. Xu et al. (2003) used TRAPs to characterize genetic stocks of tetraploid wheat (*Triticum turgidum* L., $2n=4$, $x=28$, AABB genomes) and found that a large number of chromosome-specific markers could be generated with this technique. The results indicated that TRAPs might be suitable for rapidly mapping the wheat genome.

The first linkage maps in hexaploid wheat (*T. aestivum* L., $2n=6x=42$, AABBDD genomes) were constructed using restriction fragment length polymorphism (RFLP) markers, which are relatively inefficient due to the limited number of loci and the low level of polymorphisms detected (Chao et al. 1989; Liu and Tsunewaki1991; Devos et al. 1992, 1993; Xie et al. 1993; Nelson et al. 1995a, b, c; Van Deynze et al. 1995; Marino et al. 1996). Due to the narrow genetic base of wheat, it can be particularly difficult to detect polymorphism in populations derived from intraspecific or intervarietal crosses (Anderson et al. 1993; Cadalen et al. 1997; Messmer et al. 1999).

More recently, SSRs have been a marker of choice for genetic mapping in wheat because they display higher levels of polymorphism, even between wheat varieties (Plaschke et al. 1995; Röder et al. 1995; Ma et al. 1996; Bryan et al. 1997). SSRs have been used extensively for developing wheat genetic linkage maps (Röder et al. 1998b; Pestsova et al. 2000; Gupta et al. 2002; Song et al. 2002) and for identifying agronomically important genes and QTLs (Groos et al. 2002; Paillard et al. 2003; Sourdille et al. 2003; Somers et al. 2004).

The Brazilian wheat variety BR34 is resistant to multiple diseases but has poor grain end-use quality, while the North Dakota variety Grandin is susceptible to most diseases but has exceptional quality for applications requiring strong gluten, including yeast-leavened products. In the investigation reported here, we assessed the feasibility of TRAP markers in wheat by using them in combination with SSRs to construct a genetic linkage map in a recombinant inbred (RI) population derived from Grandin/BR34. We also demonstrated the utility of the maps by identifying QTLs associated with days to heading and plant height.

Materials and methods

Plant material

We developed a population of RI lines from the North Dakota State University (NDSU) hard red spring wheat (HRSW) variety*Grandin* (PI 531005) (female) and the Brazilian variety BR34 (male) (see Bered et al. 2002 for pedigree). The mapping population consisted of 118 $F_{7:9}$ RI lines derived from a single F_1 plant. Common wheat Chinese Spring (CS) nullisomic-tetrasomic (NT) lines (Sears 1954, 1966), where a pair of missing chromosomes is partially compensated by an extra pair of homoeologous chromosomes, were used to assign markers to individual chromosomes. Chinese Spring ditelosomic (Dt) lines (Sears and Sears 1978), where a pair of chromosome arms is missing, were used to estimate positions of centromeres.

Seeds of the parents and individuals of the RI population were pre-germinated and grown in 30-cm pots containing SB100 professional grow mix (Sungrow Horticulture, Dellevue) in the greenhouse at an average temperature of 21°C under a 12/12-h photoperiod. Three replicates consisting of the parents and entire RI population were grown in a completely randomized design for evaluation of plant height and days to heading (see below).

Generation of markers

Total genomic DNA was isolated from all genotypes using the method described by Faris et al. (2000). The DNA concentration was adjusted to 30–50 ng/μl for PCR. Six hundred SSR primer pairs, of which 432 were BARC (Song et al. 2005), 121 were GWM (Röder et al. 1998a, 1998b), 41 were GDM (Pestsova et al. 2000), and six were CFA (Sourdille et al. 2003), were used to screen the parents for polymorphism. PCR reactions were performed as described by Röder et al. (1998b). In some cases, we multiplexed as many as three SSR primer pairs in a single PCR reaction based on annealing temperature and amplicon size. PCR products were separated on 6% denaturing polyacrylamide gels, stained with SYBR Green II (Sigma, St. Louis, Mo.), and scanned using a Typhoon 9410 variable mode imager (Molecular Dynamics, Ithaca, N.Y.).

For TRAP marker analysis, PCR reactions were multiplexed by using a fixed primer (Table 1) in combination with two random primers (Table 2) 5′-end labeled with different dyes (IR dye 700 or IR dye 800). Fixed primers were designed from mapped wheat EST sequences (http://www.ncbi.nlm.nih.gov) using the program PRIMER3 (Rozen and Skaletsky2000) as described in Xu et al. (2003). The selection of ESTs was done by choosing a single low-copy EST from each homoeologous chromosome arm, e.g., 1AS, 1BS, and 1DS. Fourteen fixed primers were each multiplexed with random primers T04 and T14 and one of the same fixed primers was multiplexed with T03 and T13, for a total of 15 PCR reactions. In addition, a primer (Telor) based on a conserved telomeric repeat (Table 1) was used in combination with the eight random primers shown in Table 2. PCR was carried out in a 20-ul reaction volume containing 200 ng genomic DNA, 2 μl 10× reaction buffer, 1.6 μl 25 m MMgCl$_2$, 100 m MdNTPs, 0.3 pmol each of 700- and 800-IR dye labeled primers, 10 pmol of the fixed primer, and 1.5 U *Taq* DNA polymerase (Clontech, Palo Alto, Calif.). The PCR was performed by initially denaturing the template DNA at 94°C for 2 min, followed by five cycles at 94°C for 45 s, 35°C for 45 s, and 72°C for 1 min, then by 35 cycles at 94°C

Table 1 Fixed primer designations, source EST accession numbers, and primer sequences used to generate TRAP markers in the Grandin × BR34 recombinant inbred population

Fixed primer[a]	Wheat EST accession no. (and chromosome location)	Fixed primer sequence	Random primer	Number of markers (mapped)	TRAP marker designations	Chromosome locations[b]
W01	BE637568 (1AS, 1BS, 1DS)	ATCCATCATCTCCAGAGC	T03,T13	11(10)	*Xfcp3-Xfcp13*	**1B**(2), 2B(3), 3B, 4A(2), 4B, 5A
			T04,T14	34(32)	*Xfcp14-Xfcp46, Xfcp392*	**1A,1B**(5), 2A(2), 2B, 2D(2), 3B(3), 4A(2), 5A, 5B(4), 5D, 6A(2), 6B, 7B(6), 7D
W05	BF200980 (1AL, 1BL, 1DL)	GCCTGAAGAAGGAGTTCA	T04,T14	17(16)	*Xfcp47-Xfcp64*	**1B**(4), 2A, 2B(2), 2D(2), 3B(5), 6A, 6B
W09	BE425566 (2AL, 2BL, 2DL)	TATCGTCACTTACGCCAG	T04,T14	20(18)	*Xfcp105-Xfcp127*	1A, 1B(3), **2A, 2D**,3A(2), 3B(3), 4B(2), 6B(3), 7A, 7B
W11	BE426431 (2AS, 2BS, 2DS)	GAAACTTCCAGTTACCCG	T04,T14	39(36)	*Xfcp65-Xfcp104*	1A(5), 1B(6), **2B**(3), 3A(3), 3B(3), 4A, 4B(2), 5B(3), 6A(2), 6B(3), 6D, 7A, 7B, 7D(2)
W13	BE406551 (3AL, 3BL, 3DL)	GGTGAAAGAGTTTCCGAC	T04,T14	26(24)	*Xfcp160-Xfcp191*	1B(3), 1D, 2A, **3A**(2), **3B**(2), 4A, 4B, 5A, 5B(3), 6B(4), 6D, 7A(2), 7B, 7D
W15	BE426356 (3AS, 3BL, 3DL)	GGAGGATCATGACCAGTT	T04,T14	27(25)	*Xfcp128-Xfcp159*	1B(2), 1D, 2A, 2B(3), **3A**(2), 3B(2), 4A, 4B, 5A(2), 5B, 6A, 6B(2), 7A(3), 7B(3)
W17	BE406618 (4AL, 4BL, 4DL)	AGTACAGCTTCAGCAACG	T04,T14	33(29)	*Xfcp206-Xfcp238*	1B(3), 2B(5), 2D(2), 3B(2), **4A**(2), **4B**, 5A(2), bf 5B(2), 6A(3), 6B, 7A(2), 7B(3), 7D
W19	BE490658 (4AS, 4BS, 4DS)	TCATGCCCAGTGATACCT	T04,T14	11(8)	*Xfcp192-Xfcp205*	1B(2), 2B, 3A, 3B(2), 5D, 6A
W21	BE444334 (5AL, 5BL, 5DL)	ATATGTTGGTCTGGCTCC	T04,T14	19(19)	*Xfcp261-Xfcp279*	1A(2), 1B(2), 2A, 2B, 3B, 4A(3), **5B**(2), 6B(5), 7B(2)
W23	BF145871 (5AS, 5BS, 5DS)	ATAAGCTGCCTCCAGAAG	T04,T14	22(21)	*Xfcp239-Xfcp260*	1A(4), 3A, 3B(4), 4A, **5A**(3), 6A, 6B(4), 7B(3)
W25	BE490226 (6AL, 6BL, 6DL)	CACTCATTCAGGGGTCTT	T04,T14	32(31)	*Xfcp294-Xfcp325*	1A, 1D, 2A, 2B(2), 3A(5), 3B(4), 4A, 4B, 5A(3), 5B, **6A**(3), **6B**(5), 7A(2), 7B
W27	BF483150 (6AS, 6BS, 6DS)	TGGATGTGGAGTCATCTG	T04,T14	14(14)	*Xfcp280-Xfcp293*	1B(2), 2B(3), 2D, 3B(3), 5A, 5B, **6A**, **6B**, 7B
W30	BE403624 (7AL, 7BL, 7DL)	TCAGGTCTAACGTCACCA	T04,T14	31(30)	*Xfcp347-Xfcp377*	1A, 2A(2), 2B(6), 2D(2), 3A, 3B(5), 4A(4), 5A, 5B(3), 6B(3), **7B,7D**
W31	BE446622 (7AS, 7BS, 7DS)	ACCAAGACAGGAATGACC	T04,T14	21(18)	*Xfcp326-Xfcp346*	1A, 1B, 2A, 2B(5), 3B, 4A, 5B, 6A, 6B(2), **7A(2),7B(2)**
Telor	–	AACCCTAACCCTAAACC	Multiple[c]	56(14)	*Xfcp378-Xfcp391*	2A, 2B, 3A(2), 3B(2), 4A(2), 4B, 5A(2), 5B, 6A, 7A
Total				413(345)		

[a]With the exception of W01 and W05, all fixed primers were designed from mapped wheat ESTs by Xu et al. (2003)
[b]Chromosomes in bold indicate the location of the EST used to design the corresponding fixed primer
[c]The telor primer was run in combination with all random primers listed in Table 2

Table 2 Designations and sequences of random primers used for generating TRAP markers

Random primer	Sequence	5′-End-label
T03	CGTAGCGCGTCAATTATG	700-IR
T04	CGTAGTGATCGAATTCTG	700-IR
T05	CTAGTTCATCTAATTCAT	700-IR
Sa12	TTCTAGGTAATCCAACAACA	700-IR
Sa14	TTACCTTGGTCATACAACATT	700-IR
Ga5	GGAACCAAACACATGAAGA	800-IR
T13	GCGCGATGATAAATTATC	800-IR
T14	GTCGTACGTAGAATTCCT	800-IR

for 45 s, 50°C for 45 s, and 70°C for 1 min, and a final extension step of 72°C for 7 min. Loading dye was then added, and 1 μl of each sample was loaded onto a 6.5% polyacrylamide gel in a Li-Cor Global DNA Sequencer (Lincoln, Neb.), and electrophoresis was conducted at 1,500 V for 3.5 h. TRAP markers were given the designation *Xfcp* in accordance with the reserved laboratory designations of J.D. Faris (http://wheat.pw.usda.gov/ggpages/Lab.Designators.html).

The *Tsn1* gene conditions sensitivity to Ptr ToxA, a host-selective toxin produced by the wheat tan spot pathogen (*Pyrenophora tritici-repentis*). This gene was previously mapped to the long arm of chromosome 5B using RFLP markers (Faris et al. 1996; Haen et al. 2004). Reactions of the RI lines to Ptr ToxA were evaluated as described in Haen et al. (2004), and we used *Tsn1* as a marker. In addition, one sequence-tagged-site (STS) marker developed from the RFLP clone KSUD14 (Gill et al. 1991) was included.

Marker analysis and linkage mapping

Segregation ratios of all markers were tested for fit to the expected 1:1 ratio by Chi-squared analysis using the computer programQGENE(Nelson 1997). The computer program MAPMAKER V2.0 for Macintosh (Lander et al. 1987) was used to perform linkage analysis with the Kosambi mapping function (Kosambi1944). First, linkage groups were identified using the "two-point/group" command with a minimum LOD = 3.0 and a maximum θ = 0.40. This resulted in several very large linkage groups, which were then re-grouped individually with a minimum LOD = 10.0. The most plausible order of markers within linkage groups was determined using FIRST ORDER and RIPPLE (LOD > 3.0) commands. Markers that did not RIPPLE at an LOD > 3.0 were temporarily eliminated to establish a marker order significant at LOD > 3.0. Eliminated markers were added back to linkage groups using the TRY command. Markers mapping at an LOD ripple command. Positions of centromeres on the maps were estimated by evaluating selected SSR and TRAP markers on CS Dt lines or using information from previously published maps.

QTL analysis

Days-to-heading was recorded for each plant as the number of days from transplanting to emergence of the first spike. Plant height was measured from the base of the plant to the tallest spike at maturity. In addition, data were collected for plant height and days to heading during one and two seasons in the field, respectively.

A subset of 250 of the most informative markers giving the most complete genome coverage and spaced approximately 10–20 cM apart was used to detect QTLs associated with plant height and days to heading using the program MAP MANAGER QTX (Manly et al. 2001). Composite interval-regression mapping (Haley and Knott1992) was performed to evaluate marker intervals putatively associated with trait phenotypes. To determine the critical LOD threshold, we executed a permutation test with 5,000 permutations. A LOD threshold of about 3.0 in this RI population yields an experiment-wise significance level of 0.05.

Results

SSR marker analysis

From 600 pairs of SSR primers screened for polymorphism between the parents, 175 (30%) primer pairs, including 140 BARC, 23 GWM, 11 GDM and 1 CFA, identified 328 polymorphic marker loci. The number of loci detected per primer pair ranged from one to seven, with an average of 1.9. Another 12 SSR loci (9 BARC, 1 GWM, and 2 GDM) were polymorphic between the parents but did not segregate among the RI lines of population. All RI lines were homozygous for BR34 alleles at these loci, and it was later revealed by NT analysis that these loci all belonged to chromosome 3D. Therefore, chromosome 3D did not segregate in the population, and all RI lines apparently possessed a pair of 3D chromosomes from BR34 (see Discussion).

TRAP marker analysis

A total of 413 TRAP markers were obtained. The entire population was immediately screened for TRAP markers, and the parents were not screened for prior polymorphism. From 15 TRAP PCR reactions that employed fixed EST-based primers in combination with random primers, we generated 357 markers. Each PCR reaction, which contained one fixed and two random primers, produced from 11 to 39 TRAP markers, with an average of 24 markers per reaction (Table 1). The IR 700 and IR 800 images generated by the Li-Cor system showed about 80–100 amplified fragments each (Fig. 1) for a total of about 160–200 fragments visualized for each PCR reaction. Therefore, approximately 13–16% of the amplified fragments generated by the TRAP technique were polymorphic markers. Most TRAP

markers were dominant in nature, and only 29 co-dominant markers were observed.

In an effort to target markers to telomeric regions, we constructed a primer (Telor) based on a conserved telomeric repeat and used it in combination with eight different random primers (Table 2). A total of 56 markers were generated, of which only 14—those that either mapped to the ends of linkage groups or elsewhere at an LOD > 3.0—were retained in our dataset.

Map construction

A total of 743 markers were obtained, including 413 TRAPs, 328 SSRs, one STS, and one morphological marker. Markers that were unlinked or formed small linkage groups that could not be assigned to chromosomes were eliminated from the data set, leaving 646 markers (345 TRAPs, 299 SSRs, 1 STS, and 1 morphological marker) that were used to assemble genetic linkage maps. The number of markers per chromosome (excluding chromosome 3D) ranged from four on chromosome 4D to 68 on chromosome 3B (Fig. 2, Table 3). The B-genome chromosomes had the most markers (357), with an average of 51 markers per chromosome. The A genome had 205 markers, for an average of 29 per chromosome, and the D genome had 84 markers, for an average of 14 per chromosome (excluding 3D).

A total of 352 "anchor" markers mapped at an LOD > 3.0 provided a basic map of significance for calculating recombination frequencies and centiMorgan distances. The basic map accounted for a total length of 3,045.8 cM, with an average density of one marker per 8.7 cM (Fig. 2, Table 3). The B genome was the most dense in containing 172 anchor markers that accounted for 1,149.7 cM of genetic distance (6.7 cM per marker). The A-genome basic maps spanned 1,140.8 cM with 114 anchor markers (10.0 cM per marker), and the D-genome basic maps were less dense, with 66 anchor markers accounting for 755.3 cM (11.4 cM per marker). The lengths of individual chromosomes ranged from 40 cM for chromosome 4D to 272.7 cM for chromosome 3B (Fig. 2).

For the most part, both SSRs and TRAPs were distributed randomly along the maps. However, obvious clusters of markers existed on chromosomes 1BS, 2BL, 4AL, 5AS, 5BL, 6BL, and 7BL (Fig. 2). These clusters

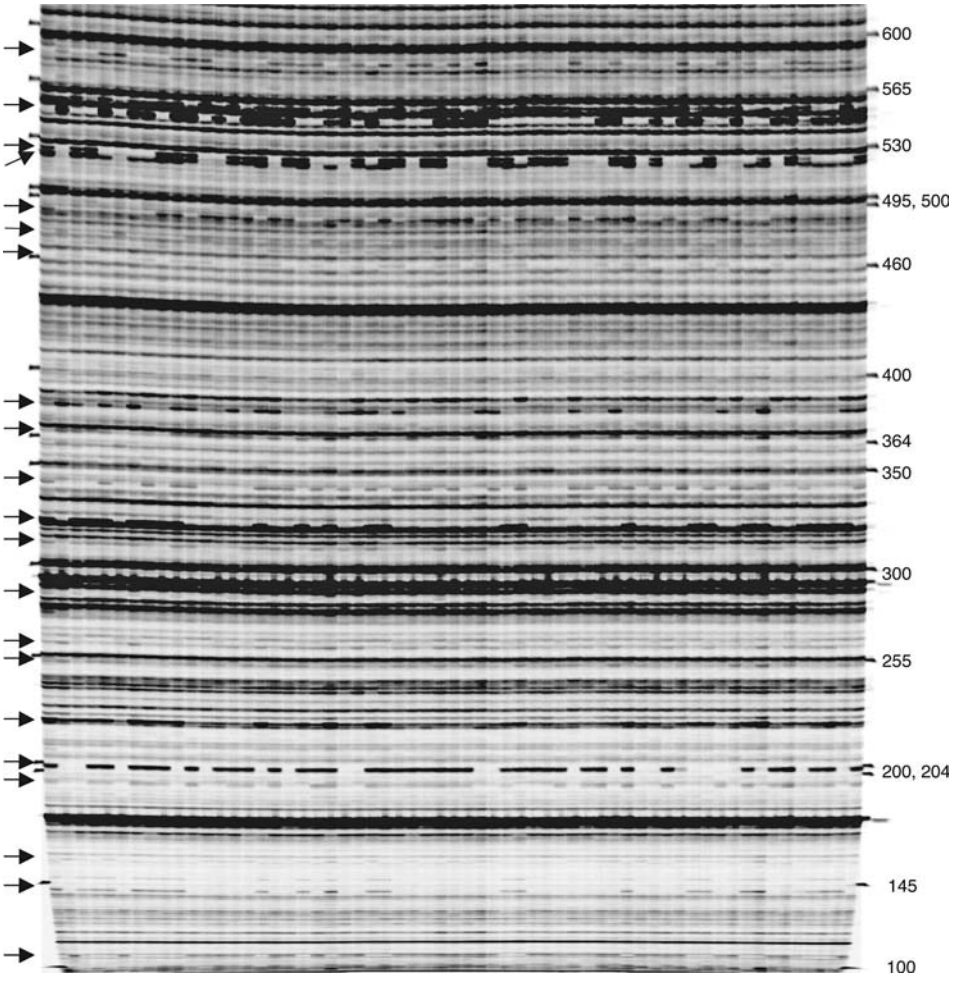

Fig. 1 Polyacrylamide gel showing TRAP fragments generated in the Grandin × BR34 recombinant inbred population using primers W31 and T14. The *arrows* indicate the 21 mapped polymorphic fragments generated by this primer combination. A molecular size marker (base pairs) is to the *right* of the image

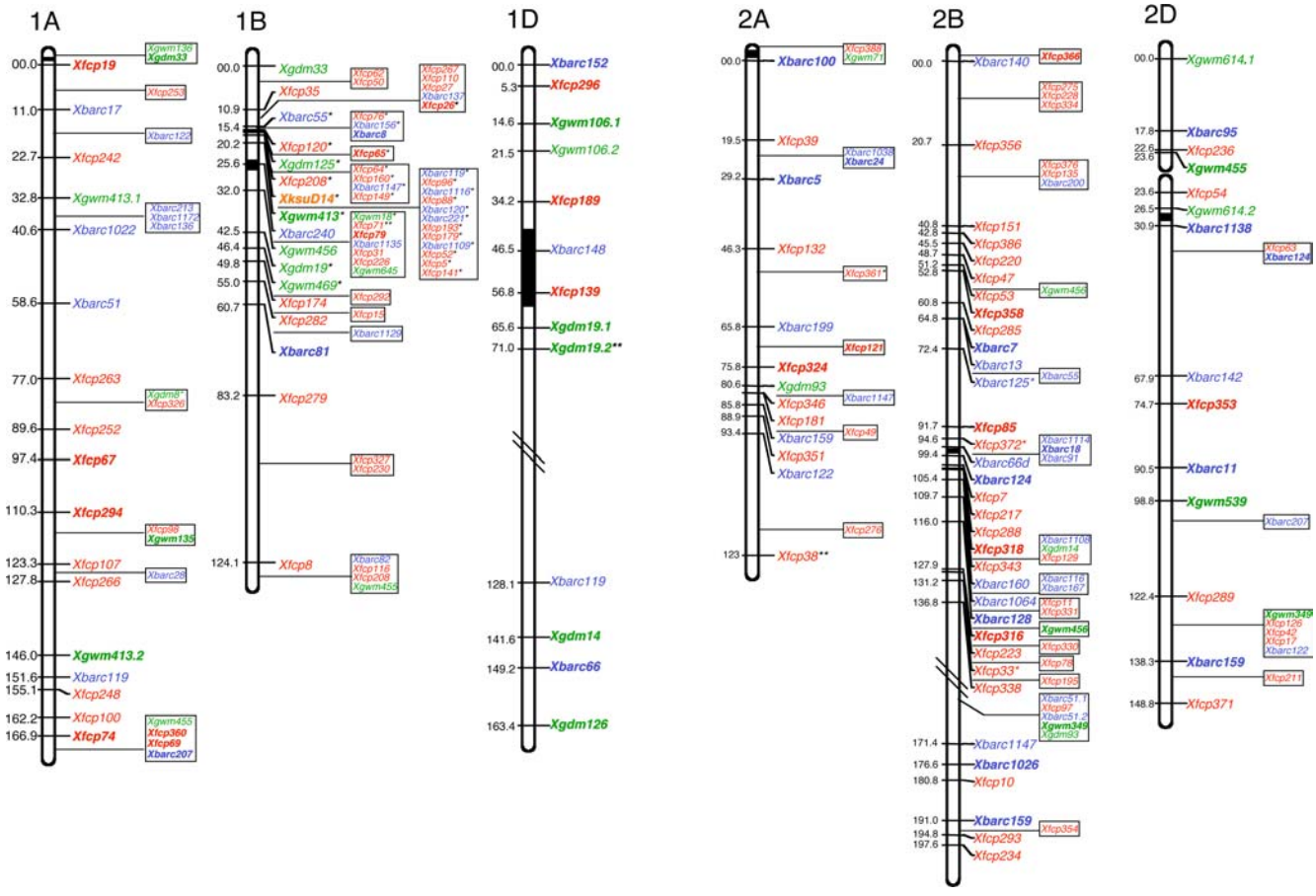

Fig. 2 Genetic linkage maps constructed in the RI population derived from the hard red spring wheat varieties Grandin and BR34. Anchor markers, mapped at an LOD > 3.0, are shown with *lines* drawn across the chromosome. Markers mapping at an LODred TRAP, *blue*BARC, *green* GWM/GDM, *purple* CFA, *orange*STS, *black* *Tsn1*. Markers in *bold* were assigned to chromosomes using the CS aneuploids. *Double diagonal lines* through chromosomes indicate grouping at an LOD asterisks (*$P < 0.05$, **$P < 0.01$). Approximate positions of centromeres are indicated by *black regions* on the chromosomes

consisted of many markers tightly linked with each other, which made it difficult to establish the correct order at the applied LOD threshold. Six of the TRAP markers generated with the Telor primer (*Xfcp388-2A*,*Xfcp391-3A*, *Xfcp383-4A*, *Xfcp390-5A*, *Xfcp387-6A*, and *Xfcp378-7A)* mapped to the ends of linkage groups and possibly represent chromosome ends (Fig. 2). The remaining eight markers (*Xfcp386-2B*, *Xfcp382-3A*, *Xfcp384-3B*, *Xfcp385-3B*, *Xfcp389-4A*, *Xfcp381-4B*, *Xfcp379-5A*, and *Xfcp380-5B*) did not map to the ends of linkage groups and may have resulted from fragments amplified by only the random primer in conjunction with itself.

The B-genome chromosomes had the highest frequency of polymorphism among the three genomes and possessed over one-half (55%) of the markers (Table 3). About one-third of all markers mapped to the A genome, but only 13% of the markers mapped to D-genome chromosomes. However, one-half of all SSR markers and 61% of TRAP markers detected loci on B-genome chromosomes, and while 20% of SSR loci mapped to the D genome, only 6% of TRAPs did the same. This indicates that SSRs were more efficient in detecting polymorphisms on D-genome chromosomes than were TRAPs.

Of the markers used for mapping, 128 (18%) showed segregation ratios that deviated from the expected 1:1 ratio. About one-half (68) of these markers occurred in clusters on chromosomes 1BS and 6BL (Fig. 2), while the remaining markers with distorted segregation ratios were distributed on all of the other chromosomes with the exception of 2D, 4A, 4D, and 5D.

Aneuploid analysis

A total of 109 SSR primer sets were tested on the CS NT and/or Dt lines, which allowed us to assign 99 SSR loci to individual chromosomes. In addition, eight TRAP PCR reactions were also performed on the CS aneuploid lines, thereby allowing 63 TRAP loci to be assigned to chromosomes (Figs. 2 and 3).

QTL analysis

BR34 and Grandin differ in ear emergence time by about 14 days, but days to heading ranged from 38 days to

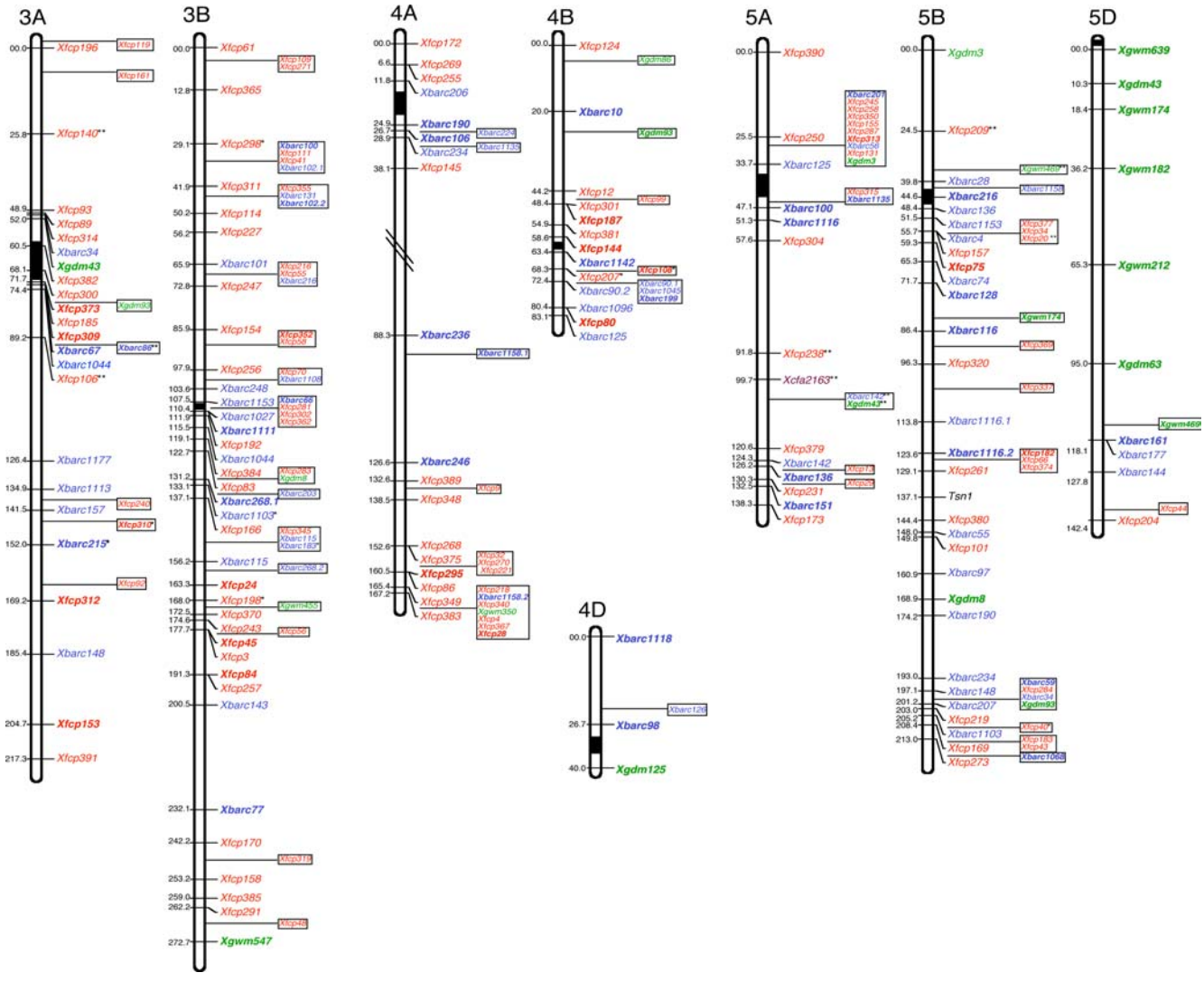

Fig. 2 (Contd.)

66 days among the RI lines. We estimated heritability for this trait to be 94.2%. Composite interval mapping (CIM) indicated a QTL located on the long arm of chromosome 5A for earliness contributed by Grandin, which we designated as *QEet.fcu-5AL* (Table 4, Fig. 4a). This QTL peaked between markers *Xfcp359* and *Xfcp231*, had a LOD = 9.5, and explained 38% of the phenotypic variation. No other QTLs significantly associated with days to heading were identified.

BR34 and Grandin have almost identical average heights of 90 cm and 89 cm, respectively. However, average heights of the RI lines ranged from 67 cm to 122 cm, suggesting that BR34 and Grandin possess different semi-dwarfing genes. Two genomic regions significantly associated with plant height were identified by CIM analysis (Table 4). A QTL with effects for reduced plant height contributed by Grandin was identified on the long arm of chromosome 4B and peaked at marker *Xbarc125*. This QTL, which we designated *QHt.fcu-4BL*, had a LOD = 21.5 and explained 57% of the phenotypic variation for reduced plant height (Fig. 4b). A second QTL associated with effects for reduced plant height contributed by BR34 was identified on chromosome 6A, and we designated this QTL *QHt.fcu-6AS*. CIM indicated that *QHt.fcu-6AS* peaked between markers *Xbarc23* and *Xfcp201*, had an LOD = 4.3, and explained 7% of the phenotypic variation (Fig. 4b).

Discussion

Genome mapping with SSRs and TRAPs

In this investigation, we used two user-friendly PCR-based marker systems to generate a genetic linkage map in an intervarietal HRSW population suitable for QTL detection. Our goal was to exploit the advantages of both TRAPs and SSRs with the premise that TRAPs could be used to generate an abundance of marker loci scattered throughout the genome and SSRs could be used to assign linkage groups to specific chromosomes and draw comparisons with previously published maps. This strategy proved to be efficient and led to the con-

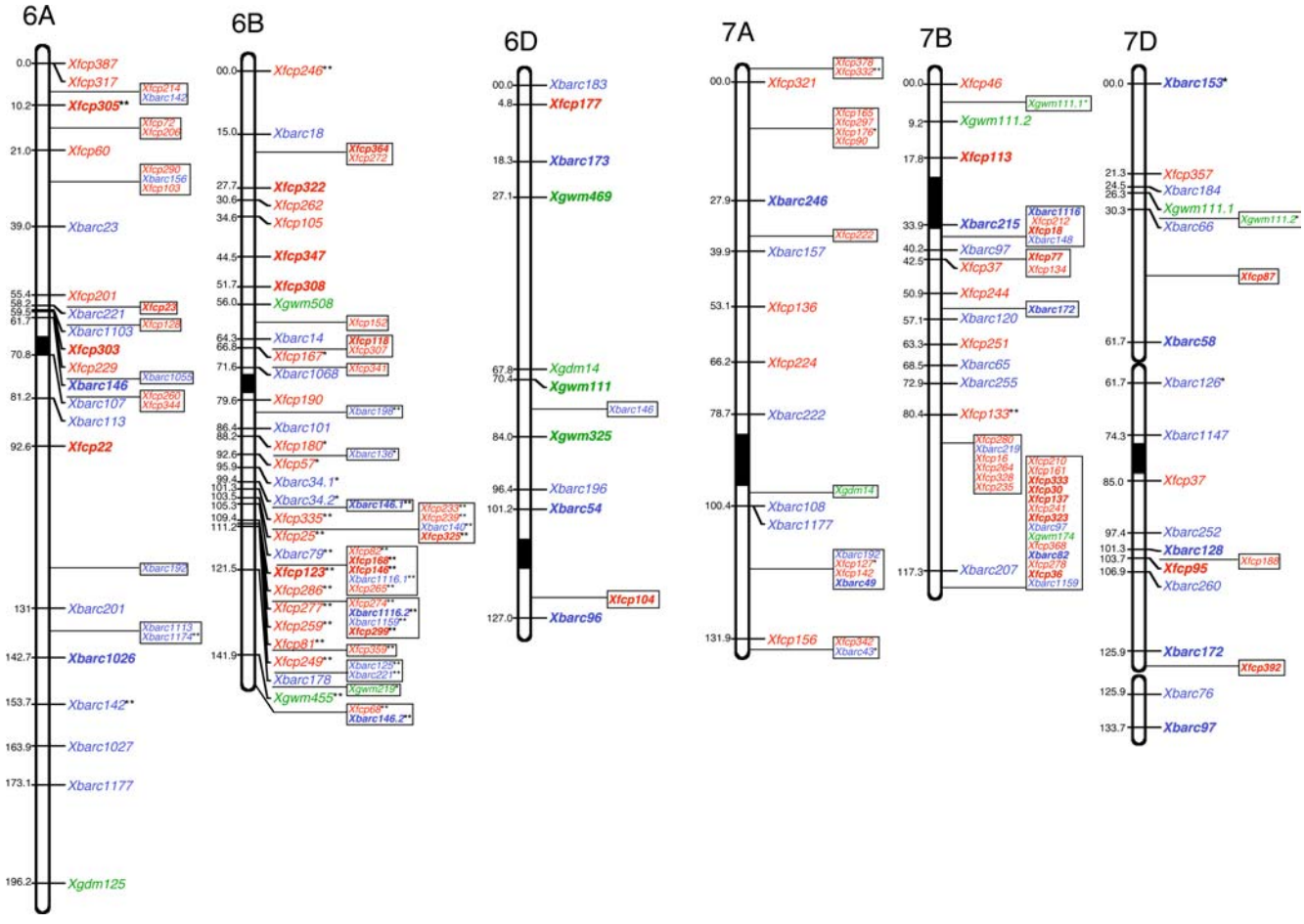

Fig. 2 (Contd.)

struction of a map containing more than 600 loci in a short amount of time.

The TRAP technique is a relatively high-throughput PCR-based marker system, and this is the first report of using TRAPs for the construction of linkage maps in any organism. We found TRAPs to be very efficient compared to other marker systems. First, the TRAP technique generates a number of markers comparable to the AFLP technique. Using a wide cross between *T. turgidum* and *T. dicoccoides*, Elouafi and Nachit (2004) generated 279 polymorphic AFLP fragments from 20 primer combinations. In comparison, we used an intervarietal population to reveal an average of 24 polymorphic markers per reaction. Utilization of TRAP markers in populations derived from wide crosses would presumably yield an even higher frequency of polymorphism.

Second, the TRAP technique does not require extensive pre-PCR processing of templates, as does the AFLP technique. Instead, total genomic DNA is used as template, thereby making TRAPs as user-friendly as SSRs but much more efficient in the number of markers generated. In this study, we mapped 413 TRAP markers in our population in less than 3 weeks, while it took over 1 year to generate 328 SSR markers. Although there is some uncertainty as to the nature of TRAP-amplified fragments, we found that TRAPs are highly repeatable and more effective at assigning linkage groups to chromosomes than SSRs because significantly more loci can be assigned with a single PCR (Fig. 3). These and our unpublished results indicate that TRAPs should be easily transferred and applied to other populations.

TRAPs were not as effective as SSRs for detecting polymorphisms on the D genome. Due to the recent evolution of hexaploid wheat, the D genomes of cultivated wheat are highly homogeneous, making it difficult to obtain adequate marker coverage. The highly polymorphic nature of SSRs makes them ideal for identifying D-genome markers. However, it may be possible to target more TRAP markers to the D genome as well. In preliminary experiments, we have found that using combinations of only random primers instead of a fixed/random primer combination reveals more polymorphisms among closely related genotypes (Chu et al., unpublished). The screening of parents and aneuploid lines with various random primer combinations could be carried out to identify D-genome markers before testing the entire mapping population.

The fixed primers were designed from mapped wheat ESTs, but only a small number of TRAPs mapped to the chromosome harboring the corresponding EST segment. Two reasons may be responsible for this. First, the

Table 3 Chromosome assignment and distribution of markers, length of linkage groups, and marker density of maps generated in the Grandin × BR34 recombinant inbred population

Chromosome	SSRs	TRAPs	Other	Total markers	Anchor markers[a]	Length (cM)	centiMorgans/marker[b]
1A	17	16	0	33	17	166.9	9.8
1B	24	35	1	60	17	124.1	7.3
1D	10	3	0	13	13	163.4	12.6
2A	10	12	0	22	13	123.0	9.5
2B	27	36	0	63	34	197.6	6.0
2D	13	10	0	23	14	148.8	10.6
3A	11	19	0	30	23	217.3	9.4
3B	25	43	0	68	37	272.7	7.4
4A	11	21	0	32	18	167.2	9.3
4B	10	10	0	20	13	83.1	6.4
4D	4	0	0	4	3	40.0	13.3
5A	13	17	0	30	14	138.3	9.9
5B	26	22	1	49	30	213.0	7.1
5D	10	2	0	12	10	142.4	14.2
6A	18	17	0	35	20	196.2	9.8
6B	21	35	0	56	28	141.9	5.1
6D	10	2	0	12	10	127.0	12.7
7A	9	14	0	23	9	131.9	14.6
7B	16	25	0	41	13	117.3	9.0
7D	14	6	0	20	16	133.7	8.4
A genome	89	116	0	205	114	1140.8	10.0
B genome	149	206	2	357	172	1149.7	6.7
D genome	61	23	0	84	66	755.3	11.4
Total	299	345	2	646	352	3045.8	8.67

[a]Markers mapping at an LOD > 3.0
[b]Marker densities were calculated using only markers that mapped at an LOD > 3.0

random primer may amplify fragments from various genomic regions in conjunction with itself. The second reason is presumably due to the low initial annealing temperature in PCR, which allows low annealing specificity and the subsequent amplification of a large number of fragments. Increasing the initial annealing tempera-

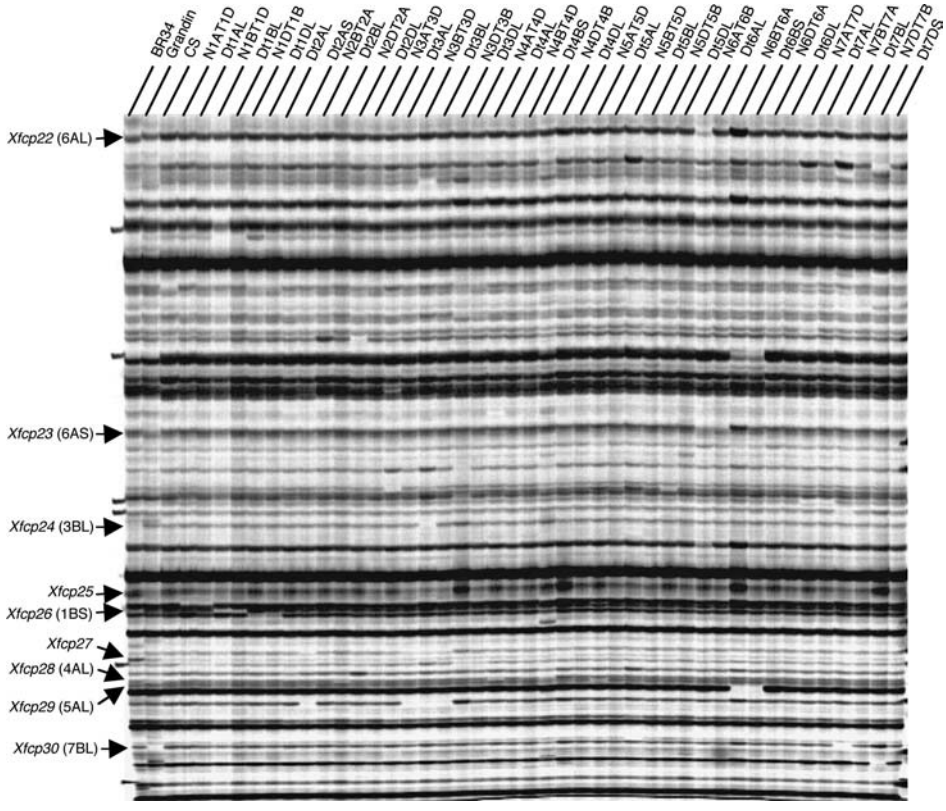

Fig. 3 A portion of a TRAP image generated from the fixed primer W01 and random primer T04 on the CS aneuploids to assign markers to chromosomes or chromosome arms. On the image, seven of nine mapped TRAPs could be assigned to chromosome arms. Designations for the aneuploid lines are listed across the *top*

Table 4 QTLs detected by composite interval mapping for days to heading and reduced plant height in the Grandin × BR34 recombinant inbred population

Trait	Chromosome	Source	QTL	Marker interval	R^2	LOD
DTH[a]	5A	Grandin	*QEet.fcu-5AL*	*Xfcp359-Xfcp231*	0.38	9.5
Plant height	4B	Grandin	*QHt.fcu-4BL*	*Xbarc125*	0.57	21.5
	6A	BR34	*QHt.fcu-6AS*	*Xbarc23-Xfcp201*	0.07	4.3

[a]DTH, Days to heading.

ture would presumably lead to higher specificity and fewer amplified fragments, which may be useful for targeting specific genomic regions. However, the efficiency of the TRAP technique for rapidly generating a large number of markers would be reduced.

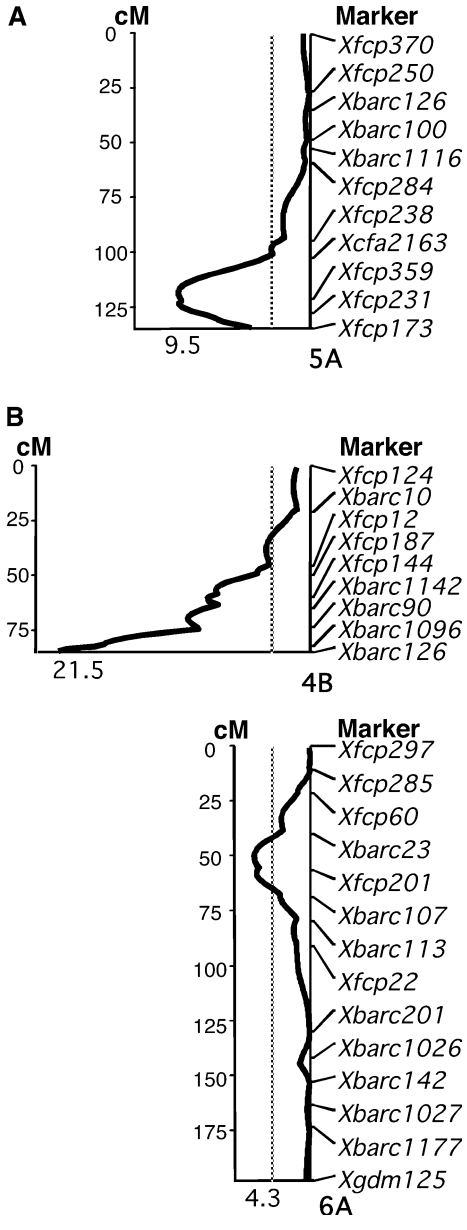

Fig. 4 Interval mapping of QTLs associated with plant height and days to heading in the RI population derived from Grandin × BR34. **a** Interval mapping of chromosome 5A showing a major QTL for days to heading derived from Grandin. **b** Interval mapping of chromosomes 4B and 6A showing the effects of a major QTL (from Grandin) and a minor QTL (from BR34) for reduced plant height

Comparison with previously published maps

The exact genetic size of the hexaploid wheat genome is still unknown, but data from several published maps suggest it is greater than 3,500 cM. The size of the map constructed in the International Triticeae Mapping Initiative (ITMI) population is about 3,700 cM (reviewed by Gupta et al. 1999), and the map generated in the intervarietal cross between Courtot and CS is 3,685 cM in length (Sourdille et al. 2003). Recently, Quarrie et al. (2005) used a doubled haploid population of CS × SQ1 and 567 markers to construct a wheat genetic map covering all 21 chromosomes, with a total length of 3,522 cM. Our map spans 3,045.8 cM of genetic distance and is lacking information for chromosome 3D, which accounts for roughly 170 cM on the ITMI map (Nelson et al. 1995c). Although our map contains over 600 markers, some regions with inadequate coverage and large gaps still exist. The short arms of chromosomes 1A, 2A, 4A, and 5D, and the long arms of 4D, 6D, and 7A contain few markers, or no markers at all. In addition, gaps of significant size exist on 1D, 2B, 2D, 3A, 4A, 5A, 6A, and 7D. The addition of more markers, particularly for A- and D-genome chromosomes will provide more complete genome coverage.

It is difficult to make extensive comparisons of marker orders between our map and previously published maps because the majority of our markers were generated by TRAPs, which have not previously been used to construct wheat genetic maps. However, previously published consensus physical (Sourdille et al. 2004) and genetic (Somers et al. 2004) maps allowed us to draw some comparisons with SSR loci on our map. Of the SSRs that we used for mapping, 71 were in common with those on the maps presented by Somers et al. (2004). Seven of these (*Xbarc24*, *Xbarc90*, *Xbarc91*, *Xbarc95*, *Xbarc101*, *Xbarc140*, and *Xbarc143*) detected loci on chromosomes different from those reported by Somers et al. (2004). *Xbarc95-2D* was assigned to chromosome 2D by NT analysis, and the remaining six markers were each closely linked to other markers that were assigned to chromosomes also by NT analysis. Therefore, we are confident that our chromosome assignments are correct, and it seems that these SSR (and probably others) detect additional loci that have not previously been reported.

The order of common markers along our maps agreed well with those presented by Somers et al. (2004) and Sourdille et al. (2004) with only a few notable exceptions. BARC124 detected loci near the distal ends of group-2 chromosomes on the Somers et al. (2004) and Sourdille et al. (2004) maps, but detected loci much closer to the centromere of chromosomes 2B and 2D in our population. Also, *Xbarc151* mapped distal to *Xcfa2163* on the long arm of 5A in our population, but was located proximal to *Xcfa2163* in the previous maps (Somers et al. 2004; Sourdille et al. 2004). Finally, the region on chromosome 6D involving markers *Xbarc146*, *Xgwm325*, *Xbarc196*, and *Xbarc54* is inverted relative to the order of these markers in Somers et al. (2004) and Sourdille et al. (2004). This apparent inversion may be due to the large gaps flanking the region, and the addition of more markers to 6D should help to resolve this discrepancy.

The absence of chromosome 3D

Although 12 chromosome 3D markers that detected polymorphism between Grandin and BR34 were identified, all RI lines were homozygous for BR34 alleles at these loci. This suggests that all RI lines possess a pair of intact non-recombined 3D chromosomes from BR34. All RI lines of the population were derived from a single F_1 plant. Although rare, monosomics are known to occur spontaneously in nature. It is possible that the Grandin plant used in the cross was monosomic for 3D. We have not determined the frequency of spontaneously occurring monosomics in Grandin, but we reconstructed the cross and reciprocal cross between BR34 and Grandin and evaluated chromosome pairing and constitution in the F_1 to determine if this phenomenon is repeatable. The F_1s had normal chromosome constitution and pairing, suggesting that the loss of Grandin chromosome 3D was specific to the plant used in the original cross.

QTLs for plant height and days to heading

Plant height and days to heading are two important traits for wheat cultivars. A reduction in plant height can improve lodging resistance and partitioning of assimilates to the developing grain. Classical genetic studies indicated that the genetic control of plant height in bread wheat is complex, and most chromosomes harbor factors that can affect it (Law et al. 1973). To date, 21 genes with major effects on plant height have been identified (Worland et al. 1998). The two most common semi-dwarfing genes, *Rht-B1b* and *Rht2-D1b*, are present on the short arms of chromosomes 4B and 4D (McVittie et al. 1978; Börner et al. 1996), respectively, and are both gibberellic acid (GA)-insensitive. Another class of dwarf genes is GA-sensitive, most of which are located on homoeologous groups 2, 7 (Worland et al. 1998), and 5 (Sutka and Kovacs1987). There are also some genes affecting plant height with modulation action (Worland et al. 1998). In addition to *Rht-B1b* and *Rht2-D1b*, Cadalen et al. (1998) identified QTLs associated with reduced plant height on chromosomes 4BS, 7AL, and 7BL in the Courtot/CS population. Börner et al. (2002) identified QTLs on chromosomes 3B, 4A, 5D, and 6A that affected plant height in the ITMI population.

Both BR34 and Grandin are semi-dwarf genotypes, but they possess different dwarfing genes. Markers underlying *QHt.fcu-4BL*, a major QTL with effects for reduced plant height derived from Grandin, were verified by aneuploid analysis to be present on the long arm of chromosome 4B. A QTL for reduced plant height on the long arm of chromosome 4B has not previously been reported. The minor QTL*QHt.fcu-6AS*, derived from BR34, may be the same as the QTL identified on chromosome 6AS in the ITMI population (Börner et al. 2002).

Chromosome 5A carries two major genes, *Vrn-A1* (Law 1966) and *Q*(Miller and Reader 1982, Faris and Gill 2002), for vernalization requirement and ear morphology, respectively. Kato et al. (1999) analyzed a population derived from a CS-*Cappelle-Desprez 5A* substitution line and CS and found two QTLs on 5A associated with ear emergence time. One QTL was in the vicinity of *Vrn-A1*, while the other was near the known location of the *Q* gene. *QEet.fcu-5AL* is also in the vicinity of the *Q* gene because it is located distal to *Xcfa2163*, which, as Sourdille et al. (2004) showed, maps proximal to the deletion bin containing *Q*. This QTL may be the *Q* gene or a closely linked gene responsible for governing ear emergence time. Although heritability for days to heading was high, only one QTL with earliness effects contributed by Grandin was detected. It is likely that BR34 also possesses a gene(s) for earliness that went undetected, possibly due to a lack of markers in a particular genomic region harboring the QTL. The addition of more markers to fill gaps in the map may lead to the identification of a BR34-derived QTL for earliness in this population.

Conclusions

In the investigation reported here, we showed that combining the different PCR-based marker technologies, SSRs and TRAPs, provided an efficient and robust method of generating genetic linkage maps in wheat. SSR markers were useful for assigning linkage groups to chromosomes, and they allowed some comparisons to be made with previously published maps. TRAP markers were very efficient for rapidly generating a large number of markers scattered across the genome, which allowed linkage groups to be joined and many gaps to be filled. TRAPs also showed the same ability as SSRs to assign linkage groups to chromosomes. TRAP markers, like AFLPs, are mostly dominant and, therefore, are more

useful in fixed populations such as RI lines or doubled haploids.

Both marker technologies were efficient in revealing polymorphism between the parents, which are both HRSW cultivars. Mapping in an intervarietal context is of great value for detecting QTLs associated with agronomically important traits and molecular-based breeding strategies. Our mapping population was developed from two very important wheat cultivars. Grandin is a NDSU HRSW variety that has good quality traits and was widely grown within the past decade, while BR34 is an excellent source of resistance to multiple diseases. We tested the utility of our map for detecting QTLs associated with agronomically important traits using plant height and days to heading as examples. The results indicate that the map will be useful for the identification of QTLs associated with other agronomically important traits segregating in this population, such as resistance to tan spot, Stagonospora nodorum blotch, powdery mildew, Fusarium head blight, and leaf rust.

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