Barley allele-specific amplicons useful for identifying wheat-barley recombinant chromosomes

Koji Murai^{1,*}, Shin Taketa², A. K. M. Rafiqul Islam³, and Ken W. Shepherd³

¹Department of Bioscience, Fukui Prefectural University, Matsuoka-cho, Fukui 910-1195, Japan

²Department of Life Sciences, Faculty of Agriculture, Kagawa University, Ikenobe, Kagawa 761-0795, Japan

³Department of Plant Science, Waite Campus, University of Adelaide, Glen Osmond, SA 5064, Australia

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Barley (*Hordeum vulgare* L.) is potentially a new source of genes for wheat (*Triticum aestivum* L.) improvement. Wheat-barley chromosome recombinant lines provide a means for introgressing barley genes to wheat genome by chromosome engineering, and since these are expected to occur only rarely in special cytogenetic stocks, an efficient selection skill is necessary to identify them. To convert RFLP markers to barley allele-specific PCR markers useful for effective production of wheat-barley recombinant lines, 91 primer sets derived from RFLP clones which were previously mapped to the barley chromosomes were examined for PCR amplification using 'Chinese Spring' wheat, 'Betzes' barley and the wheat-barley chromosome addition lines. The polymorphisms were detected by an agarose gel electrophoresis of the PCR products without digestion with restriction enzymes. Out of 81 primer sets producing polymorphisms between the wheat and barley genomes, 26 amplified barley chromosome-specific DNAs which were confirmed to be located on the same chromosome as the RFLP markers by using the wheat-barley chromosome addition lines. These amplified DNAs represent barley allele-specific amplicons, which distinguish barley alleles from their wheat homoeologous counterparts. The present investigation revealed a higher probability for obtaining allele-specific amplicons from genomic DNA-derived RFLP markers than from cDNA-derived ones. The barley allele-specific amplicons developed in this study, namely, four for chromosome 2H, two for 3H, seven for 4H, eight for 5H, one for 6H and four for 7H, are suitable for identifying 'Chinese Spring' wheat- 'Betzes' barley recombinant chromosomes. However, one out of eight barley allele-specific amplicons on chromosome 5H did not detect a unique barley band in a 'New Golden' barley chromosome 5H addition line of 'Shinchunaga' wheat, indicating there may be a need to reconstruct allele-specific amplicons with different barley cultivars.

INTRODUCTION

Introgression of genetic materials, particularly disease resistance genes, from related species such as *Aegilops*, *Agropyron* and *Secale* has been widely used for the improvement of bread wheat (*Triticum aestivum* L.) (reviewed in Islam and Shepherd, 1991; Mujeeb-Kazi, 1993). Barley (*Hordeum vulgare* L.) is a potential new source of genes for wheat improvement, e.g., genes conferring resistance to barley yellow dwarf virus (McGuire and Qualset, 1990), leaf and yellow rust (Islam and Shepherd, 1990), cereal cyst nematode (Islam and Shepherd, 1992),

Russian wheat aphid (Nieto-Lopez and Blake, 1994), and Karnal bunt (Chauhan and Singh, 1994). Recently, Murai et al. (1997a) examined heading characters of wheat-barley chromosome addition lines produced by Islam et al. (1981) and Koba et al. (1997), and found that barley chromosome 5H has genetic effects on accelerating narrow-sense earliness, decreasing vernalization requirement and/or increasing photoperiodic sensitivity. To introduce the barley genes for early heading on chromosome 5H into wheat, we are planning to produce wheat-barley 5H chromosome recombinant lines (Murai et al. 1997b).

To transfer alien gene into wheat, it is preferable if the wheat and alien chromosomes can be induced to pair and recombine at meiosis. However, a major problem for producing wheat-barley recombinant lines is the low pairing

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* Corresponding author. E-mail: murai@fpu.ac.jp

frequency between wheat and barley chromosomes, resulting in a low frequency of recombinants. Islam and Shepherd (1988,1992) reported that barley chromosomes paired with their wheat homoeologues with frequencies ranging from 0.3 to 2.6%. Because of this low frequency of pairing, it is necessary to have an effective selection system to detect the rare wheat-barley recombinants. Islam and Shepherd (1992) used five isozyme markers for selecting wheat-barley recombinants involving chromosomes 3H or 6H. However, isozyme markers are not widely applicable for this purpose because the number of suitably located markers is limited. Murai (1995) developed 22 barley chromosome-specific RAPD (random amplified polymorphic DNA) markers, but their locations within chromosomes are not known.

Several RFLP (restriction fragment length polymorphism) maps have been developed in barley (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993; Langridge et al., 1995; Qi et al., 1996), and these RFLP markers have been used as informative molecular markers in genome analysis. However, RFLP analysis is too time consuming and expensive to apply to the large-scale screening for wheat-barley recombinant lines. Conversion of RFLP markers to STS (sequence tagged site)-PCR (polymerase chain reaction) markers offers several experimental advantages, i.e., they can be applied to relatively small amounts of DNA and can be applied rapidly and cheaply. When RFLP markers are converted to STS-PCR markers, it is crucial to confirm that they occur at the same chromosome location. Erpelding et al. (1996) found that the RFLP and equivalent STS-PCR markers were located on the same chromosomes in barley in 28 cases out of 50. More recently, Mano et al. (1999) identified the chromosome locations of 38 STS-PCR markers and observed that the locations were identical with those of the RFLP markers except in three cases. These results emphasize that re-localization of the STS-PCR markers is necessary after their conversion from an RFLP marker. Furthermore, STS-PCR markers often require a restriction enzyme digestion following amplification to produce a polymorphism (Tragoonrun et al., 1992). For the efficient screening of rare wheat-barley recombinants, it would be more suitable to use direct amplification of barley alleles (allele-specific amplicons) which can be distinguished from their wheat counterparts without restriction enzyme digestion. Blake et al. (1996) reported 135 barley allele-specific amplicons amplified by 115 primer sets developed from sequences of previously mapped RFLP clones. In their procedure, PCR products were electrophoresed in a 6% polyacrylamide gel for 2.5 h. However, the barley allele-specific amplicons would be more convenient for practical use if they could be detected more rapidly by an agarose gel electrophoresis. Here we describe a simplified detection of barley allele-specific amplicons using electrophoresis of the PCR products in a 1.5% agarose gel for 0.5 h at

100 V.

The present experiments were designed (1) to evaluate 91 PCR primer sets derived from RFLP markers to determine which of these produced polymorphisms between 'Chinese Spring' wheat and 'Betzes' barley with the simplified detection system, (2) to localize the barley-specific amplified DNAs by using the wheat-barley chromosome addition lines, and (3) to optimize annealing condition for each primer set. Out of the 91 primer sets, 40 had been examined earlier by Blake et al. (1996) and found to produce barley allele-specific amplicons. These primer sets were re-examined for producing barley allele-specific amplicons with the simplified detection system. The remaining 51 primer sets were newly tested to search for additional barley allele-specific amplicons.

MATERIALS AND METHODS

Plant materials. Polymorphism of PCR products between wheat and barley was detected using 'Chinese Spring' wheat and 'Betzes' barley as reference samples. A set of wheat-barley disomic addition lines possessing each of 'Betzes' chromosomes 2H to 7H in the background of 'Chinese Spring' (Islam et al., 1981) were used to localize the PCR products to the barley chromosomes. Addition line 1H was not available because of problems of sterility when it is added to wheat (Islam et al., 1981). Furthermore, a 'New Golden' barley chromosome 5H addition line of 'Shinchunaga' wheat (Koba et al. 1997) was used for amplification of barley allele-specific amplicons on chromosome 5H. The nomenclature for the barley chromosomes is based on their homeologous relationships with the wheat chromosomes, i.e., barley chromosome 1=7H, 2=2H, 3=3H, 4=4H, 5=1H, 6=6H and 7=5H (Linde-Laursen et al., 1997).

Primer evaluation. A total of 91 primer sets developed from the sequences of previously mapped barley RFLP clones were evaluated (Table 1). The primer sets were developed from several sources: (1) barley genomic DNA clones (ABG and BG; Kleinhofs et al., 1993; Heun et al., 1991); (2) barley cDNA clones (ABC and BCD; Kleinhofs et al., 1993; Heun et al., 1991); (3) wheat genomic DNA clones (WG; Heun et al., 1991); (4) oat cDNA clones (CDO; Heun et al., 1991), and others. Following Larson et al. (1996), the PCR primers/markers were distinguished from RFLP clones/markers by using the small-case letter "a" in front of the marker locus name. Multiple primer sets were developed from several RFLP clones; e.g., both aABG473.1 and aABG473.11 were from RFLP clone ABG473. The sequence data of the primer sets are available on GrainGenes (WWW server: <http://wheat.pw.usda.gov/>). The data sources are Drs. Tom Blake, Andris Kleinhofs, Mark E. Sorrells, Bikram S. Gill, Gottfried Kuenzel, and Andreas Graner. The aBmy1 primer set

Table 1. List of primer sets used in this study, together with the chromosome location of RFLP markers from which the PCR primers were derived

Primer name ^a	RFLP-1 ^b	RFLP-2 ^c	STS ^d	Amplicon ^e	Primer name ^a	RFLP-1 ^b	RFLP-2 ^c	STS ^d	Amplicon ^e
aABG318	2H	2HS	nd	IV	aCDO749	5H	5HS	nd	IV
aABG2.1	2H	2HS	nd	V	aWG530	5H	5HL	nd	IV
aABG358.1	2H	2HS	nd	V	aBG123	2H, 5H	2HL	5H	I(5H)
aABG459	2H	2HS	3H	IV	aABC302.3	5H	5HL	5H, 7H	III
aCDO370	2H	2HS	nd	IV	aWG564.1	5H	5HL	nd	IV
aABG602	2H	nd	2H, 4H, 6H	III	aABC168	5H	5HL	4H	IV
aABG716	2H	nd	2H	III	aABG473.1	5H	5HL	5H, 6H	I
aCDO770	2H	2HS	nd	IV	aABG473.11	5H	5HL	5H	III
aABC468	2H	2HS	4H	IV	aABC717	5H	5HL	5H	I
aABG356	2H	2HS	2H	III	aWG1026.1	4H, 5H	5HL	nd	III(5H)
aWG996	2H	2HL	7H	IV	aABG712	5H	5HL	5H	III
aABG14.1	2H	2HL	nd	II	aABC155	5H	5HL	5H	I
aCDO588.1	2H	2HL	nd	IV	aCDO213	5H	5HL	6H, 7H	IV
aABC153	2H	2HL	nd	I	aABC622	5H	nd	nd	IV
aCDO395	3H	3HS	nd	V	aABG707	5H	5HL	nd	V
aABG471	3H	3HS	6H	IV	aABG463	5H	5HL	nd	IV
aWG178	3H	3HL	nd	IV	aABG314	5H(2)	nd	nd	II
aABG396.1	3H	3HS	3H	III	aABC309	5H	5HL	nd	IV
aABC323	3H	nd	2H	IV	aCDO506	5H	5HL	nd	IV
aBCD828	3H	3HS	nd	IV	aABG378	6H	6HS	6H	III
aABG377	3H	3HL	3H	III	aABG62	nd	6HS	nd	IV
aWG940	3H	3HL	4H	IV	aABG474	6H	6HS	nd	II
aABG4	3H	3HL	2H, 4H	IV	aABG20.1	6H	6HS	nd	IV
aWG110.1	3H	3HL	nd	V	aBCD269	6H	6HL	3H	IV
aABC166	3H	3HL	nd	IV	aABC170	6H(2)	nd	2H	IV
aWG622	4H	4HS	nd	V	aABA301	7H	7HS	nd	V
aABG3.1	4H	4HS	nd	IV	aABG704	7H	7HS	nd	V
aABG484	4H	4HS	4H	III	aABG77	7H	nd	nd	II
aWG232.1	4H	4HS	6H	IV	aABG75	7H	7HS	3H	IV
aWG464	4H	4HL	4H	III	aBCD129	7H	7HS	nd	IV
aCDO541	4H	4HL	nd	V	aCDO545.1	7H	7HS	nd	IV
aWG181	4H	4HL	nd	IV	aCDO475	7H	7HS	4H	IV
aABG472	4H	4HL	4H	I	aABG380	7H	7HS	nd	III
aABG394	4H	4HL	4H	III	aABC158	7H	7HS	nd	IV
aABG618	4H	nd	4H	III	aCDO36	7H	nd	nd	IV
aWG114	4H	4HL	nd	II	aABC465	7H	7HS	7H	III
aABG54	4H	4HL	4H	III	aABC255	7H	7HS	7H	III
aABG397.1	4H	4HL	5H	IV	aABC308	7H	7HS	nd	IV
aABG397.2	4H	4HL	nd	IV	aCDO464	7H	7HL	nd	IV
aABG366	4H	4HL	nd	IV	aWG719	7H	7HS	nd	IV
aCDO63	4H	4HL	nd	IV	aABC254	7H	7HS	nd	IV
aABG601	4H	nd	nd	IV	aABC455	7H	7HS	4H	IV
aBCD402.1	4H(2)	4HL	6H	IV	aABC310	5H, 7H	7HL	6H	I(7H)
aBCD402.11	4H(2)	4HL	nd	IV	aABC305	7H	7HL	6H	IV
aBCD402.12	4H(2)	4HL	nd	IV	aABG461	7H	7HL	nd	V
aBmy1	4H	4HL	nd	I					

^a Primers are listed in the order of the RFLP marker position in each chromosome, i.e., from distal in short arm to distal in long arm, according to the RFLP map of Qi et al. (1996).

The position of aABG62 was determined from the RFLP map of Langridge et al. (1995).

^b Chromosome location of the RFLP markers following Qi et al. (1996). (2) indicates two loci of the RFLP clone were observed in the same chromosome. nd means not determined.

^c Chromosome location of the RFLP markers following Langridge et al. (1995). nd means not determined.

^d Chromosome location of the STS-PCR markers following Blake et al. (1996). nd means not determined.

^e Classification of primer sets revealed by this study (refer to Table 2). Chromosome location of amplicon is indicated by parentheses when the parent RFLP marker is located on two different chromosomes.

was derived from the RFLP marker Bmy1 corresponding to barley β -amylase gene (Kreis et al., 1987), which was located on chromosome 4H RFLP map (Langridge et al.,

1995; Qi et al., 1996).

Plant DNA was extracted by a modified CTAB method described previously (Abo-elwafa et al., 1995) and used as

a template for the PCR assay. Each 20 µl amplification reaction consisted of 10mM Tris-HCl (pH 9.0), 50mM KCl, 0.1% Triton X-100, 1.5mM MgCl₂, 160µ M each of dNTPs, 200nM primer, 0.5U of Taq polymerase (Gibco BRL; Toyobo), and about 50ng of template DNA. Amplification was performed under the following conditions: at 94°C for 3 min; followed by 30 cycles at 45, 50 or 55°C for 1 min, at 72°C for 2 min, and at 94°C for 1 min; followed by at 72°C for 3 min. Amplification products were separated on 1.5% agarose gel for 0.5 h at 100V and visualized with ethidium bromide.

Southern hybridization analysis. Five barley allele-specific amplicons on chromosome 5H, aABC302.3, aABG473.1, aWG1026.1, aABG712 and aABC155, were subjected to Southern analysis to determine the relationship of the PCR products to the corresponding RFLP clones used to design the primer sets. PCR products were transferred to a nylon membrane (Hybond N⁺, Amersham), hybridized with the RFLP clones, and detected using the ECL direct nucleic acid labeling and detection system (Amersham).

RESULTS AND DISCUSSION

Classification of PCR primer sets. Initially all of the 91 primer sets developed from RFLP clones were evaluated by PCR assay at 45°C as the annealing temperature to determine whether they produced polymorphisms between ‘Chinese Spring’ wheat and ‘Betzes’ barley without digestion with restriction enzymes (Table 1). A total of 81 primer sets were found to produce barley-specific PCR products. The chromosome locations of the barley-specific amplified DNAs were determined by analyzing the

wheat-barley chromosome addition lines. The PCR primer sets were classified into five classes (Table 2). Class I and II primer sets amplified DNAs from barley but not from wheat. Class III and IV produced PCR products (including minor amplified DNAs) from both wheat and barley, but the products were different in size. The barley-specific PCR products amplified with Class I and III primers and the RFLP markers from which the PCR primers were developed were found to be located on the same chromosomes. With Class II and IV, RFLP markers had a different chromosome location from the barley-specific PCR products. Class V primer sets gave PCR products with the same size in both wheat and barley, and further treatments such as restriction digestion would have been necessary to produce polymorphisms.

In barley, Erpelding et al. (1996) reported that the chromosome locations of PCR products matched those of the RFLP markers in 28 cases out of 50 (56%). In our data, however, PCR products were localized on the same chromosomes as the RFLP markers in only 26 cases (with Class I and III primer sets) out of 91 (29%). Several factors are thought to have contributed to the disagreement between the chromosome locations of RFLP markers determined by linkage analysis and those of the PCR markers determined by analysis of wheat-barley addition lines. One contributor was due to the lack of polymorphism between wheat and barley, which prevented localization of the PCR products. To overcome this problem, restriction digestion of the PCR products would be expected to increase the frequency of polymorphisms and allow mapping of the PCR markers, as used by Erpelding et al. (1996). Another possible contributor is that the PCR primers amplified sequences from multiple loci on different chromosomes. Alternatively, some PCR prim-

Table 2. Classification of PCR primer sets based on the amplification of wheat and barley DNAs, and number of primers belonging to each class

Class	PCR amplification				Source of RFLP clone ^a				
	CS ^b	Be ^b	CS-Be ^c	Specificity ^d	Barley genomic (ABG,BG)	Barley cDNA (ABC,BCD)	Wheat genomic (WG)	Oat cDNA (CDO)	Others ^e
I	–	+		+	3	4	0	0	1
II	–	+		–	4	0	1	0	0
III	+	+	Poly.	+	13	3	2	0	0
IV	+	+	Poly.	–	13	18	8	11	0
V	+	+	Iden.		5	0	2	2	1
Total					38	25	13	13	2
I + III (%)					42.1	28.0	15.4	0	–

^a RFLP clone source from which the primer sets were designed
^b CS: ‘Chinese Spring’ wheat, Be: ‘Betzes’ barley, +: PCR amplified, –: PCR unamplified
^c Poly.: Polymorphic between CS and Be in major and/or minor PCR products, Iden.: Identical in both major and minor PCR products
^d +: Barley chromosome-specific, –: Unspecific
^e aBmy1 (Class I) and aABA301 (Class V)
^f Percentage of PCR primer sets producing barley allele-specific amplified DNAs

Table 3. Barley allele-specific amplicons developed

Primer ^a Name	Sequence (5' – 3')	Optimal annealing Temp.(°C)	PCR products ^b		Size of amplicon (bp) ^c	Chromo- some
			CS	Be		
aABG602	CTTCCTTCTATCGCCTTCTC GCAAAATCCCTCCATACATC	55	++	+	1080	2H
aABG716	CTCCACACCATACTTGAATC ATGGCATTAGAAGTCGATCT	55	+	+	1140	2H
aABG356	TCAACTGAGGTAGAATACTA CCAACAATAAAGAATCAAAT	55	—*	++	410(510) ^d	2H
<u>aABC153</u>	GCCTCTGCCGCTGGAACCTAC AAACACCTCCTGGCTCTCAG	55	—	+	270	2H
aABG396.1	AGGAAACCTATGTAATCATC GGGTCACAAAGACGGAGGAG	55	+	+	360	3H
aABG377	GTGTTACAGCCACCTATTTTC GCTGCTATGAGGAGAGAACC	55	—*	+	460	3H
aABG484	TAGTTAGAGAGGGAGAACA TAAATCACCAACATTGAAAC	55	—*	+	1030	4H
aWG464	AGTCCAAATGATGTCACAGG AGGACTGTGAAGATGCTACT	55	+	+++	1420	4H
aABG472	GGGATTTGGAAGATTTTAT GGCGAAGATGTCCTTAGATA	50	-	+	870	4H
aABG394	CGATGTAATAAAAATTAGGT GGGTGTTGCTCATGCTCTTT	50	++	+	280	4H
aABG618	CTGTGGTCATCAATCCAGTG GTTTCATCTCAACGGGTCAGT	55	—*	+	560	4H
aABG54	GTGCTTGGCGGTCGACCAGT GATGTCCAACGGTGGCTTGA	55	+++	+++	270	4H
<u>aBmy1</u>	GATGAAGCAAACAAGGTC CACATAACATAAAGGGTAGTAAA	50	—	+	1200	4H
aBG123	TCCAAGAAGAGTCAAGTAAA CAAGCAGTAGGAATAAGAAC	50	—	+	1420	5H
aABC302.3	ATAAGGAACAGGAACAGAGT ATAAAGGAGAAGATTGAGTC	55	++	+	870	5H
aABG473.1	TGTCGCTTTACTGTATTTAT ACTTTGTTCTCCATTATGAG	55	—	+	370	5H
aABG473.11	GGGCAATGTCGCTTTACTGT TTCCTGGCTACAAATACACC	55	—*	+	280	5H
aABC717	CAATACGGCAACAAATAACA CCCCACCAAAATTACCAGTC	55	—	+	730	5H
<u>aWG1026.1</u>	ATGGGATCACCTGGAGGATG TGCTAAACAAGACTTGCTG	55	+	+	950	5H
aABG712	AAATATGGTTGGTCAAAGTT GCTTCTGGTCTGTGTTCTTG	55	+++	+++	460	5H
aABC155	TCTGTGTGTTATTCCTCTTA ACGAAAAATCAATACTGGTC	55	—	+	410	5H
aABG378	TTAGTCATAGAATCCCTGTT AAAATTGCTGCTGTGCTGTGT	55	+	+	230	6H
<u>aABG380</u>	CACGAACAGAAGGAGACTAA AAACCCTACATCATCTTTTT	50	—*	++	400(340) ^d	7H
aABC465	GCTACTGGGACAAATCTCC CACGACAGACGGACCAAATG	55	—*	+	540	7H
aABC255	GATCATCGTGGATGGAGTGT CGAATTCCGAGACATCAAAT	55	—*	+	650	7H
<u>aABC310</u>	TCCTGATGGTCCTCTTATGC ACATAGTTCTCTTCCCAGTA	55	—	+	1520	7H

^a Underlined primer sets indicate novel barley allele-specific amplicons found in current study^b +: single band, ++: two bands, +++: more than three bands, -: not amplified, —*: amplified band(s) detected at 45°C as the annealing temperature^c Size estimated by comparison with known size standards using 1.5% agarose gel electrophoresis^d Visible minor band

ers might have amplified nonhomologous sequences. Finally, structural changes to the added barley chromosome, such as deletion or insertion, might have occurred during introgression of the barley chromosome to wheat background, resulting in failure of PCR products to be amplified.

Class I and III primer sets produced barley-specific PCR products allowing barley alleles to be distinguished from their wheat homoeologous counterparts without restriction digestion. These PCR products can be regarded as barley allele-specific amplicons, and they were found in approximately 42% of the primers derived from genomic DNAs and approximately 28% of those from cDNAs (Table 2). This difference in frequency is probably due to the cDNA-derived RFLP clones often being sequences of multiple loci whereas genomic DNA-derived RFLP clones have unique sequences. The aBmy1 primer directs specific amplification of the barley β -amylase gene locus, indicating that sequences of cloned genes are a good source for developing allele-specific amplicons.

Development of barley allele-specific amplicons.

For Class I and III primer sets, the PCR assay was conducted at annealing temperatures of 50 and 55°C to determine the optimal annealing temperature for barley allele-specific amplicons. Table 3 summarizes the PCR patterns obtained with 'Chinese Spring' and 'Betzes' with optimal annealing temperature and the sizes of barley allele-specific amplicons. In some cases, unspecific (minor) PCR products disappeared when the optimal annealing temperature was used (Fig. 1). Altogether, we developed 26 barley-allele specific amplicons, namely, four for chromosome 2H, two for 3H, seven for 4H, eight for 5H, one for 6H and four for 7H. As shown in Fig. 2, there

were two types of barley allele-specific amplicons. In the first type, the PCR product was obtained from barley DNA but not from wheat DNA (for example, aABG484 amplicon). In the second type, the amplified DNAs differed in their sizes between wheat and barley (for example, aWG464). Amplicons of the latter type are likely to be more informative than the former because they can be used as co-dominant markers.

Blake et al. (1996) detected 135 barley allele-specific amplicons amplified by 115 primer sets. We re-examined 40 of these primers and confirmed that barley allele-specific amplicons could be produced using our simplified detection system. Blake et al. (1996) counted barley-spe-

Fig. 1. Amplification with the primer set aABG618 under different annealing temperature (45 or 55°C), for 'Chinese Spring' wheat (CS), 'Betzes' barley (Be), and wheat-barley 3H and 4H chromosome addition lines (3H and 4H, respectively). *Hae*III-digested ϕ x174 DNA is used as molecular weight marker (M).

Fig. 2. Amplification with the primer set aABG484 (left) and aWG464 (right) for the wheat-barley addition lines (2H-7H), along with 'Chinese Spring' wheat (CS) and 'Betzes' barley (Be). Mixture of *Hind*III-digested λ DNA and *Hae*III-digested ϕ x174 DNA is used as molecular weight marker (M).

Fig. 3. Amplification with the primer set aABC155 (left), aWG1026.1 (middle) and aABG712 (right) for the wheat-barley addition lines (2H-7H), along with 'Chinese Spring' wheat (CS) and 'Betzes' barley (Be), and Southern blots of the PCR products hybridized with corresponding RFLP clones. *Hae*III-digested ϕ x174 DNA is used as molecular weight marker (M).

cific PCR products that were located on a different chromosome to the RFLP markers among the barley allele-specific amplicons. In our study, however, only those barley-specific PCR products which had the same chromosome location as the parent RFLP markers were considered to be barley allele-specific amplicons. The present data were consistent with the results of Blake et al. (1996), except for the location of aABC310 (Table 1). This disagreement might have resulted from the different PCR conditions used in the two studies. Five of the 26 amplicons listed in Table 3, namely, aABC153 on chromosome 2H, aBmy1 on 4H, aWG1026.1 on 5H, aABG380 and aABC310 on 7H, were novel barley allele-specific amplicons.

PCR markers are expected to share the same locus as their parent RFLP makers with the consequence that an RFLP marker and its PCR derivative should lie on the same chromosome. However, Erpelding et al. (1996) found two exceptions in 14 cases where a PCR product located on the same chromosome as the RFLP marker failed to hybridize to its RFLP clone, indicating that non-homologous sequences had been amplified and mapped to the chromosome. Furthermore, Larson et al. (1996) re-

Fig. 4. Linkage map of eight amplicons on chromosome 5H developed from RFLP markers. Map position of amplicons was cited from the RFLP map (Qi et al. 1996) with the assumption that an RFLP marker and the amplicon lie on the same locus. ABA306B and MWG602 are RFLP markers, and the chromosome is oriented with the short arm at the top. Underlined amplicons were confirmed to correspond to their parent RFLP clones by Southern hybridization analysis.

ported that an STS-PCR marker did not coincide with the parent RFLP locus but it did map to the same chromosome. Therefore, linkage analysis of PCR markers or hybridization analysis between RFLP clones and their PCR products should be carried out to determine the location of the derived PCR markers. Five amplicons on chromosome 5H, namely, aABC302.3, aABG473.1, aWG1026.1, aABG712 and aABC155, were analyzed by Southern hybridizations to determine whether the PCR products were homologous to the corresponding RFLP clones used to design the primer sets. The results showed that all of the PCR primer sets tested amplified the sequences homologous to the RFLP clones (Fig. 3), demonstrating that the amplicons occupy the same loci as the parent RFLP markers. Based on the RFLP map of Qi et al. (1996), a linkage map of barley allele-specific amplicons on chromosome 5H is shown in Fig. 4.

To determine whether the barley allele-specific amplicons developed in this study are applicable for other wheat-barley genotypes, the primer sets producing barley allele-specific amplicons on chromosome 5H were examined for PCR amplification in a 'New Golden' barley chromosome 5H addition line of 'Shinchunaga' wheat. One out of eight barley allele-specific amplicons on chromosome 5H, i.e., aABC155, did not amplify in the addition line having 'New Golden' chromosome 5H (Fig. 5). This suggests a need for reconstructing allele-specific amplicons in each barley cultivars used.

Fig. 5. Amplification with the primer sets yielding barley allele-specific amplicons on chromosome 5H, i.e., aABC302.3, aABG473.11, aABG712, and aABC155 (left to right), for the 'Chinese Spring' wheat-'Betzes' barley 5H addition line (Be5H) and 'Shinchunaga' wheat-'New Golden' barley 5H addition line (NG5H). *Hae*III-digested ϕ x174 DNA is used as molecular weight marker (M).

In conclusion, we developed 26 easily detectable barley allele-specific amplicons, five of which were novel barley allele-specific amplicons. Our main objective of developing barley allele-specific amplicons is to screen wheat-barley recombinant chromosomes for homoeologous group 5. Five barley allele-specific amplicons on the chromosome 5H are available for this purpose now. However, we did not get barley allele-specific amplicons located on short arm as well as distal position of long arm. To obtain new amplicons located on these regions, alternative methods such as development of amplicons from barley allele-specific RAPD markers (Murai 1995) should be introduced.

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