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STS-PCR markers appropriate for wheat-barley introgression

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Abstract Introgression of chromosomal segments across large taxonomic distances has long been an objective of scientists interested in understanding the relationships between genes and their effect on phenotype. Barley and wheat represent cultivated members of the *Triticeae* with different zones of adaptation, different responses to pathogens, and different end-use characteristics. Introduction of small, well-characterized chromosomal segments among grass relatives presents an opportunity to both better understand how genes perform in novel genomic environments and to learn more about the evolutionary novelties which differentiate related species. Since the distribution of the wheat-barley addition lines, the potential power and value of a comprehensive series of wheat/barley translocation lines has been widely appreciated. A scarcity of easy-to-use markers which unambiguously distinguish barley loci from their wheat homologues has limited the ability of scientists to identify the relatively rare inter-chromosomal recombination events which are the necessary antecedents of these lines. Since the single most critical pathogen affecting U.S. wheat producers is Karnal bunt (*Tilletia indica*) and since barley carries a gene conferring immunity, molecular markers may prove practically and immediately important. In this report we describe a series of 135 barley-specific markers amplified by 115 primer sets developed from sequences from previously mapped restriction fragment length polymorphism (RFLP) markers. These easily distinguish the cognate barley products from their wheat counterparts and should find ready use in the identification of lines which contain wheat/barley translocation events.

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Introduction

The conversion of mapped RFLP and randomly amplified polymorphic DNA (RAPD) markers to their sequence-tagged-site (STS) (Olson et al. 1989) counterparts has proven an effective method of obtaining easy-to-use, reliable markers of genes valuable enough to merit marker-assisted-selection (Williamson et al. 1993; Nieto-Lopez and Blake 1994). Marker conversion has thus far been performed on an 'as-needed' basis, with little or no effort given to genome-wide conversion efforts. Several laboratories have participated in marker conversion efforts in the grasses (Tragoonrung et al. 1992; Sorokin et al. 1994; Ghareyazie et al. 1995, Van Campenhout et al. 1995) with the objective of either demonstrating the value of the technology or marking specific genes of interest.

Between 1974 and 1982 a broad international effort was mounted to make intergeneric gene transfer a reliable, predictable technology. Development of chromosomal addition and substitution lines among wheat relatives (Shepard and Islam 1981; Sethi and Miller 1986) coupled with genomic in-situ hybridization and the isolation of mutants with increased rates of intergenomic chromosomal recombination (Koebner and Shepherd 1985; King et al. 1994a, b; Jiang et al. 1994) provided the basis for the transfer of agronomically important genes from wild relatives into wheat.

These efforts required effective selection for genes providing unique single-gene sources of a modified phenotype, coupled with cytological verification of specific translocations. While enormously useful in transferring genes which confer large phenotypic effects, this sort of approach generally failed to permit the unambiguous identification of lines containing genes which confer less well-defined phenotypic modifications.

Islam and Shepherd (1992) proposed the transfer of small individual segments of barley chromosomes span-

ning the complete barley genome to form a series of wheat-barley recombinant lines. Genome-wide marker conversion and release has been an objective of the Montana State University barley and wheat programs for the past 2 years. In this report we describe 115 polymerase chain reaction (PCR) primer sets that direct the amplification of 135 barley-specific markers, which are appropriate for the tagging of barley chromosomal segments during their introgression into wheat. Clone sequences are available on Graingenes (WWW server: http://wheat.pw.usda.gov/graingenes.html) and through GenBank. Images of primer set amplifications using the wheat/barley addition lines are available on Hordeum (WWW server: http://hordeum.oscs.montana.edu). Small aliquots of all primer sets described in this publication are available from the senior author.

Materials and methods

Plant materials

Mapping markers to chromosomes was performed using DNA isolated from the wheat-barley addition lines (Shepherd and Islam 1981). This work was initiated while the senior author was on leave to the Waite Institute (Glen Osmond. SA), and the DNA used was derived from plants cytologically characterized by Dr. R. Islam and isolated in the laboratory of Dr. P. Langridge.

Source of clones and primer sequences

Most of the clones sequenced in this project were supplied by Dr. A. Kleinhofs. the curator of mapped clones for the North American Barley Genome Mapping Project (NABGMP) (Kleinhofs et al. 1993). Several were generous gifts of Dr. M. Sorrells and had been previously mapped in barley (Shin et al. 1990, Heun et al. 1991, Kanazin et al. 1993 a. b) or oat (O'Donoghue et al. 1995). Two clones were derived from the *T. tauschii* library of Dr. B. Gill (Gill et al. 1991). Double-stranded sequencing of plasmid isolated using alkaline lysis was performed manually using the dideoxy chain termination method with [P^{32}] as label (Sanger et al. 1977). Following proofreading. sequences were deposited in GenBank and in GrainGenes. Approximately 400 bp of sequence were read per clone. Priming sequences were selected using OLIGO™, a software package which permitted selection of primers designed to have optimum annealing temperatures near 50°C and minimal tendency to show internal secondary structure or to form dimers.

All amplifications were done in 25 µl, in 200-µl capped microfuge tubes under 1 drop (approximately 50 µl) of mineral oil. In each reaction mix were included 0.25 units Taq polymerase, 0.2 µ*M* primers, approximately 50 ng DNA, 0.1 m*M* each dNTP, 1.5 m*M* $MgCl_2$ and reaction buffer. Reactions were performed in MJ Research Thermocyclers. Following initial denaturation (94° C, 4 minutes) samples were subjected to 33 cycles of 1 min at 94° C, 1 min at 52°C, 1.2 min at 72°C. followed by a final extension reaction of 5 min at 72°C.

All samples were analyzed in 6% polyacrylamide gels (29:1 ratio of monomer to crosslinker) in 1×TBE (Maniatis et al. 1982). Gels were run for 2.5 h at 210 V, stained with ethidium bromide and photographed using Polaroid type 667 film or using a CCD camera and thermal printer The figure included herein was obtained following gel photography using Polaroid type 55 positive/negative film

The cloning of PCR products, when required, was performed using the TA Cloning Kit (Invitrogen) according to the manufacturers' recommendations. Following isolation of cell lines containing desired inserts, plasmid production and double-stranded sequencing were performed as previously described (Kanazin et al. 1993a).

The 115 primer sets described in this report direct the amplification of one or more barley-specific products which are easily detected using the wheat-barley addition lines. The products described may be detected without modification.

Results

Within Table 1 is contained the simplifying assumption that if a RFLP marker and its PCR derivative lie on the same chromosome then they most likely lie at the same locus. About 20% of our PCR markers have been recombinationally mapped within one or more of the doubled haploid or recombinant inbred populations used by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993; Blake et al. 1993). Thusfar, the assumption of allelism has proven correct. We expect and will welcome revisions of marker locations as colleagues dispel particular aspects of the general assumption of allelism between RFLP markers and their STS-PCR derivatives.

Chromosome 1H (barley chromosome 5)

Eleven STS-PCR markers were found on chromosome 1H. The short arm is particularly well-represented with markers which have been recombinationally mapped. Two primer sets direct amplification of different components of the *Hor-2* multigene family (Kanazin et al. 1993a. b). Both are highly informative and have been determined to cosegregate with the *Hor-2* locus (Kanazin et al. 1993a). The marker *KsuD14* was previously reported in Nieto-Lopez and Blake (1994). and *ABA004* was previously reported in Kleinhofs et al. (1993).

Chromosome 2H (barley chromosome 2)

Out of 24 primer sets which direct amplification of barley-specific products from chromosome 2H, 7 derive from clones mapped by RFLP analysis to other chromosomes. Two (aABG055 and aABC261) derive from RFLP clones which identify chromosome 1H loci, and 2 (aABG004 and aABC323) from RFLP clones which identify chromosome 3H loci. The RFLP identified by clone WG541 mapped to 5H. Clones ABC170 and ABC160 hybridized to segregating fragments which mapped to several chromosomes (Kleinhofs et al. 1993). Seventeen barley-specific PCR products located on chromosome 2H shared chromosomal location with RFLP polymorphisms. Six markers (ABA005, aRRN5s1, aMst510, aMst126, aABG004 and aABC454) have been recombinationally mapped.

Chromosome 3H (barley chromosome 3)

Nineteen STS- PCR markers were found on chromosome 3H. Five derive from clones producing RFLPs which mapped to other chromosomes. Two of these (ABC160 and

Table 1 STS-PCR markers mapped to wheat-barley addition lines listed by chromosome

Primer name	Left primer (5′ → 3′)	Right primer (5′ → 3′)	STS[d]	RFLP[e]
ABA004[c, d]	CCACCAAGCGTGGAGTC	GGGTGGCGTGGGGTG	1H	1H
aABG452[a]	TCTACCTAGCTTCTTTCAAA	AGCTGCCACCACCCCCAGTG	1H	1H
aMST102[a, e]	CCACCATGAAGACCTTCCTC	TCGCAGGATCCTGTACAACG	1H	1H
aMST101[a, e]	CCACCATGAAGACCTTCCTC	ACCTTGCATGGGTTTAGCTG	1H	1H
aABG053[b]	ACTTAAAATAGTCCGCACTG	TAATTGCATGAGTCTGAATC	1H	1H
aABG500[a]	AAGAAGAACCCGGAGAATCT	GCTAGAACTTGACCAATCTC	1H	1H
aABG059[a]	GCCATCACGAGTTTAGTCTT	TTCGGGTTGAGTCTGTTAGC	1H	1H
aABC160[c]	AACAAGGCCGCATGGATGTA	GCCAGCGCTCCGGTCTGGTC	1H	1H
			2H	
			3H	
aD14[c, h]	CGCTTTTACCGAGATTGGTC	CCAAAGAGCATCCATGGTGT	1H	1H, 7H
			4H	
aABG055[c]	CAGGCAAGATTGACGCAGTA	ATCCGCAGCAGATCGAGGAC	1H	1H
			2H	
			7H	
aABC152[b]	TCCGCAAGTACCAGAAGAGC	GACAAGGAAAGCCAATCAAC	1H	1H
			7H	
aABG613[b]	TTATTGCAAAGGAAACAAAA	ACATATTTCCCTTCCCAGTC	2H	2H
ABA005[a, d]	GGTACGAACATGGAGGTACT	CTGTCAGAAATAGGAGGCGG	2H	2H
aABC454[b]	GCGTGCGAGGGGAAGGAGAA	TTCACAGCCGAAACACTTGT	2H	2H
aABG058[b]	AGAACTGGAGGAACGAGATA	ACCCAGGCTTGATTATTAGG	2H	2H
aABG356[a]	TCAACTGAGGTAGAATACTA	CCAACAATAAAGAATCAAAT	2H	2H
aABG153[c]	AACACCTCCTGGCTCTCAG	GCCTCTGCCGCTGGAACTAC	2H	2H
aABG317[c]	AACTCTGGGTGGTTTGTGAA	CATGATGGGTCAAGCTCTGT	2H	2H
aRRN5S1[a, f]	TGGGAAGTCCTCGTGTTGCA	CGCGGTGGGAAAAGTCACAC	2H	2H
aBCD175.1[b]	TGTAAACCAGCCATCCGACA	CATGGAGGATGTCGCTCATC	2H	2H
aMST510[a, i]	GGTCTTTCATGTACCTACC	CGAGCTCCTGTCGAGG	2H	2H
aABC306[c]	ACGGCCTCAGGACCTACTCC	GCATACACATACATTCACAT	2H	2H
aABG716[a]	ATGGCATTAGAAGTCGATCT	CTCCACACCATACTTGAATC	2H	2H
aABG609[a]	ATCAATGGAGATTTGCTTAC	GTGTTTACATGCTTGTCATA	2H	2H, 3H
aMST126[a, j]	GCTGTCCTCACCACCGTCGT	ACTGATGCCGCCACCAAACA	2H	2H
			7H	
aABG602[b]	GCAAAATCCCTCCATACATC	CTTCCTTCTATCGCCTTCTC	2H	2H
			4H	
			6H	
aABC252[b]	AAGCTCACCAAGTCCCAGTC	CACAAGGCTCAAAACATAAC	2H	2H
			4H	
aABC451[a]	CAGTAACCAAAACGACAAAC	TTCAACGAGGCATCAATCTT	2H	2H
			4H	
aABC261[c]	AAAGTCAAGAGTTGCATCAA	AGGAAGCTCAAGAAGGTGAA	2H	1H
			3H	
			4H	
aABG004[c]	CGGGAGGAGGGGAAGAGGAC	AGCACCAAAGCACCTGAACC	2H	3H
			4H	
aABC323[c]	GCCACATCACCCTCTT	TCGTCACAGGTTTCAC	2H	3H
aABC170[c]	ACGGTTGCTGTGGGTGTGAT	AACGATACAATGACCAGAGC	2H	6H
aRRN5S2[a, f]	TGGGAAGTCCTCGTGTTGCA	AGAGGTCGTGGCGGTGCTCG	3H	3H
aCDO474[c]	TTTGGACCGTTCAGAGTTGC	ACGTCCTCTCCAAGGCCAAT	3H	2H, 3H
aABG070[a]	GGACCAAGCAAATATCTCAG	AACACGAGTTTGAATTTTAC	3H	3H
aABG010[a]	TGAGTTCCTTGATAGATACA	CTTAGCATTTGAGTTTGAGA	3H	3H
aABG377[a]	GCTGCTATGAGGAGAGAACC	TGGTATGAAACAGGTGAATA	3H	3H
aABG654[a]	ACAGCGGAGAAAAAGATACA	AAGTTCGGATGGGACAAGAT	3H	3H
aABG57.4[a]	TTATAAGCATAGACTGCGGT	GGTCACCCATCCAGTTCTTG	3H	3H
aABC156.2[a]	TCATCATCCAAGAACGAAGG	GGATATGAAGATTCTGATCT	3H	3H
aABG057 1[c]	CCTTATTCTACACAACAGAT	GGTCACCCATCCAGTTCTTG	3H	3H
aABG57.2[a]	TTATAAGCATAGACTGCGGT	GCACGAGTGAGCTGAGAGTG	3H	3H
aABG57.3[a]	CCTTATTCTACACAACAGAT	GCACGAGTGAGCTGAGAGTG	3H	3H
aABG396.1[a]	GGGTCACAAAGACGGAGGAG	AGGAAACCTATGTAATCATC	3H	3H
aABC156.1[c]	ATGAGGAGGGACACACTGGT	GAGAGGAGGACAAGCAACAC	3H	3H
			6H	
aABG459[c]	CCACGCTCGCTTGCTGACTC	GCCACCACGCTCTCCATTGT	3H	2H
aBCD269[c]	ATTCGGCACGAGCAATGGCT	CCAACACACACCATTACACC	3H	6H
aABC151[c]	GTGCCGTAGATAGGAAGGTG	CATGGTACAAACTCTCAACT	3H	7H
aABG075[c]	AGCATGTACAAGAACAGAAA	CCATTAGTGACACAGGTGAG	3H	7H
aWG464	AGGACTGTGAAGATGCTACT	AGTCCAAATGATGTCACAGG	4H	4H
ABA003[a, d]	GCTGCGCGCTTCAGCT	GACCTCCACGAGTTGC	4H	4H
aCDO475[c]	GACACATTGACCGCATCTTA	CCTTCACCTCGCTCCCTACC	4H	4H
aABG394[b]	GGGTGTTGCTCATGCTCTTT	CGATGTAATAAAAATTAGGT	4H	4H
aBTA002[a]	CCGTGTGGTATTTCTTTCTC	TTGTCCTCGCTGACGAACTC	4H	4H
aABG715[b]	GGGACGACTGTTAATGAGAT	GGGCATGAGAGCAATTCAAG	4H	4H
aABG618[c]	GTTCATCTCAACGGGTCAGT	CTGTGGTCATCAATCCAGTG	4H	4H

Table 1 (Continued)

Primer name	Left primer (5′ → 3′)	Right primer (5′ → 3′)	STS[d]	RFLP[e]
aABG472[a]	GGCGAAGATGTCCTTAGATA	GGGATTTGGAAAAAGTTTAT	4H	4H
aABG054[b]	GATGTCCAACGGTGGCTTGA	GTGCTTGGCGGTCGACCAGT	4H	4H
aABC303[c]	GAGGCTGTTATTATCTTACC	CTTTACAGCGACGAGACCAG	4H	4H
aBARG010[a]	CCAACAAAACAAAACACATA	TGAAGCCCAAGCAGGACATT	4H	4H
aABG466[c]	TGCACGCGCTGTGGCATCTC	CCAAGCATTCCAACCTTAGC	4H	4H
aABC164[c]	CGCACACCTCTCCCTCCTTC	ATGCCCATCTTCTGCAACTC	4H	1H
aABG484[b]	TAAATCACCAACATTGAAAC	TAGTTAGAGAGGGAGAACAA	4H	4H
aABG498[c]	CTGACTACTGGATGGACCAC	TTACTGAAGAAAAACCTGTC	4H	4H
aABC455	GTGGGTTCCTCTTTGTTGAG	CACGCTCCTAAACGCACTCC	4H	7H
aABC468[a]	CCACCAGATAACTCTTCATA	TGAGCAATACAGGGCTGATG	4H	2H
aWG940[c]	GCACACACAAACGACGACGG	GCATTGCAATACAGTGAGTC	4H	3H
aABC168[a]	GTAAGAACAAAACCCAAAAC	GGAGACCCAGCGGAAGGAGA	4H	5H
aABG020.2[b]	TCGCGATGATGTTCTTCAGG	TGGACCCAGAAGGAGAGAGA	4H	6H
aABG712[b]	GCTTCTGGTCTGTGTTCTTG	AAATATGGTTGGTCAAAGTT	5H	5H
aABC717[a]	CAATACGGCAACAAATAACA	CCCCACCAAAATTACCAGTC	5H	5H
ABA001[a, d]	GGGGAGATATCGACCAAAGT	CACGCCCTCGCCAACGCTCTCCA	5H	5H
aABG473.11	TTCCTGGCTACAAATACACC	GGGCAATGTCGCTTTACTGT	5H	5H
aABC302.1[a]	GTGGTAAAGGAGCGGTTGAT	CAGAGTACAGGACAGACAGT	5H	5H
aBG123[c, k]	TCCAAGAAGAGTCAAGTAAA	CAAGCAGTAGGAATAAGAAC	5H	5H
aABC483[c]	GGGCACGACGACACACTCAG	TGCATCACCGACGACAGACC	5H	5H
aPST337[a, g]	ATCCAGTTCTTGTGCACCTG	AGCTACGTGGATCACACCAC	5H	5H
aABC718[c]	CAACAGAAAGCTGCTAGAGC	ATAGGAACGGTGGTGATTTT	5H	5H
aABC155[a]	TCTGTGTGTTATTCCTCTTA	ACGAAAAATCAATACTGGTC	5H	5H
aWG541[c]	AATTCGATCGACACCGTCGGA	TCCAACCAAAGGAACGAAGG	2H	5H
			5H	
aABC302.3[a]	ATAAAGGAGAAGATTGAGTC	ATAAGGAACAGGAACAGAGT	5H	5H
			7H	
aABG473.1[a]	ACTTTGTTCTCCATTATGAG	TGTCGCTTTACTGTATTTAT	5H	5H
aABG391[c]	CATCAAACTCAATGCAAGTG	CGGTGAATTCCGTCATTT	5H	5H
aABC156.5[c]	CATAATGTGGGGTGCCATAC	CAAGGAGGATCAAGTAGTCA	5H	2H, 3H
				7H
aABG397 1[a]	GCAAAACACACTAGATTTTA	ATGCGAGCAGAAACTTAGGA	5H	4H
aABG711[c]	GGGGTACACGAAGAGGTAGA	AATAGAGAAGGGGGAGAGGT	5H	6H
aCDO673[c]	ACGAGCGTCAGTCGGTCACT	GCCATTGGTATTGCGAGAGG	5H	7H
aMST109[a]	CATATCTCATTCTACTTGAG	TGGAACGAGGGAAAAAACAG	6H	6H
aABG378[b]	TTAGTCATAGAATCCCTGTT	AAAATTCGCCTGTGCTGTGT	6H	6H
aABG466[c]	TGCACGCGCTGTGGCATCTC	CCAAGCATTCCAACCTTAGC	4H	6H
			5H	
			6H	
aABG473.1[a]	ACTTTGTTCTCCATTATGAG	TGTCGCTTTACTGTATTTAT	6H	6H
aABG458.1[c]	AGTCTTGCGCATGGTGACAC	CACCAATTGCATCAAAGCTC	6H	6H
aABG379[a]	GCTGAGTTACAGAAAGTTCC	TTTGTAGTTCTGATGGTGTG	6H	6H
aABG065[a]	TGAGTATCTATCGGCGGTTA	GGCGACTCTGATGCTACGAT	6H	3H
aBCD402.1[c]	ACCTTGATCACCTGATCCAC	TGCCACAACGACGTGACGCA	6H	4H
aWG232 1[c]	CCGCTTTCCTCAGTGTTTCA	TTCAACCCCAGGTATGCTGT	6H	4H
aABG064[b]	GGGTATGCATGGACGTGGTC	GGCTGAATCATCCGCTCATA	6H	5H
aABG471[b]	TGGATTTGATGGCGGAGACC	CAAGACTGACAACACAAGAC	6H	5H
aABC310[a]	TCCTGATGGTCCTCTTATGC	ACATAGTTCTCTTCCCAGTA	6H	5H, 7H
aABC305[b]	GACAACGGCCAACAAATCTA	AGCATGTCATTGAACACTTC	6H	7H
aWG669[c]	AAAGGAGGAGCCCAGTGATT	GAAGGAAAGGTTCCCCATCC	6H	7H
aCDO213[c]	GGCAGTTGAATGAGTTTGAG	TAATCACGAGGTCACTAATC	6H	5H
			7H	
aABG701[c]	GAATACCTGTTGCCCAAAGA	CAACTGAATGCCAGCAATAC	7H	7H
aMST107[c, d]	ATGGCCCGCAC(C/G)AAGCAGAC	AGCTGGATGTCCTTGGGCAT	7H	7H
aABG603[a]	AAGTGCAAGCAGGCTCTGAC	GGTGTTGGTGTCGAGCACTC	7H	7H
aWG686.1[b]	TCGCTTTACCACAATTTCAG	GCTGTTCATATAAAAGGAGA	7H	7H
aABC253[b]	GCATGGTGACAGATTTCAAA	AGGGAATGCAGATCTCACAC	7H	7H
aNMST108[c, d]	ATGGCCCGCAC(C/G)AAGCAGAC	GACTTCCT(C/G)GCCGCCTGCAA	7H	7H
aABC465[a]	CACGACAGACGGACCAAATG	GCTACTGGGACAAAATCTCC	7H	7H
aABC152[a]	TCCGCAAGTACCAGAAGAGC	GACAAGGAAAGCCAATCAAC	7H	7H
aABC255[a]	CGAATTCCGAGACATCAAAT	GATCATCGTGGATGGAGTGT	7H	7H
aABG320[b]	AFACGAGTGGACACATGATG	GATCCAACAGCAAGGAAAGA	7H	7H
aWG996[c]	CCTTCACTGACCCCTAAATA	AGCCCAGGTTCTACAACAAC	7H	2H
aABG460[b]	TGGAGGAGAGCGGAAGAGAT	TTGTGTGGTAAAAGTAAAAT	7H	3H
aABG020.11[c]	GCAAAGCGACTATTTTTCAT	TGGACCCAGAAGGAGAGTGC	7H	6H

[a] Gel pattern similar to panel A of Fig. 1
[b] Gel pattern similar to panel B of Fig. 1
[c] Gel pattern similar to panel C of Fig 1
[d] Previously cited in Kleinhofs et al. (1993)
[e] Previously cited in Kanazin et al (1993a)
[f] Previously cited in Kanazin et al (1993b)
[g] Previously cited in Tragoonrung et al. (1992)
[h] Previously cited in Nieto and Blake (1994)
[i] Previously cited in Shin et al. (1990)
[j] Previously cited in Blake et al. (1993)
[k] Cited in Hayes et al. (submitted)

ABC261) lie on chromosome 1H, 1 (ABG459) lies on 2H and 2 (ABG075 and ABC151) lie on 7H. Seven markers (aABG70, aABG377, aWG110, aCDO113, aRrn5s2, aABG396.1, aABC156c) have been recombinationally mapped. Details of this recombinational analysis are presented in a companion paper (Larson et al. 1996).

Chromosome 4H (barley chromosome 4)

Twenty-seven STS-PCR markers were mapped to barley chromosome 4. Thirteen markers derive from clones providing RFLPs which map to alternative chromosomes, 6 from PCR reactions producing multiple mappable bands. Only 3 of the chromosome 4 markers (aBTA002, aBarG10 and aABA003) have been recombinationally located within chromosome 4H, and none of these derived from an RFLP marker developed by the NABGMP.

Chromosome 5H (barley chromosome 7)

We found 19 barley-specific PCR markers which mapped to chromosome 5H. Fourteen derive from clones which identified RFLP markers previously mapped to chromosome 5H, while 5 markers derived from clones which provided RFLPs mapped to other chromosomes. As with the RFLP map, the long arm of 5H is highly populated with markers, while the coverage of the short arm and satellite is sparse. These STS-PCR markers appear to span most of the chromosome, ranging from aABC483 near the end of the satellite to aPst337 near the distal end of the long arm of chromosome 5H (Kleinhofs et al. 1993, Blake et al. 1993). Eight markers have been recombinationally mapped, and allelism with the previously mapped RFLP demonstrated.

Chromosome 6H (barley chromosome 6)

Only 6 of the 18 markers STS-PCR markers assigned to chromosome 6H matched supporting RFLP mapping data. Nine of the 18 derived from clones which provided RFLP markers mapped to other chromosomes. None of the STS-PCR markers on chromosome 6H have been recombinationally mapped.

Chromosome 7H (barley chromosome 1)

Of 18 unique STS-PCR markers mapped to chromosome 7H, 10 derive from clones producing RFLPs also mapping to chromosome 7H. Eight derive from clones producing RFLPs mapped to other chromosomes. Of these, 1 (WG996) mapped to chromosome 2H, ABG460 mapped to 3H, ABG366 mapped to 4H, ABC302 mapped to 5H, ABG20 mapped to 6H and ABC152 produced mappable RFLPs on several chromosomes.

Discussion

In this report we describe and release 115 primer sets which direct the amplification of 135 products from barley which can be unambiguously distinguished from their wheat counterparts by simple gel electrophoresis. Chromosomes 1H, 2H, 3H and 5H appear to contain markers sufficiently well-distributed to identify translocation events in any region of each of these chromosomes. Chromosome 4H contains a gene conferring resistance to Karnal bunt (*Tilletia indica* Mitra), and our efforts to cover this chromosome with definitively mapped markers continues. The short arm of chromosome 6H likewise remains poorly marked, although 12 markers are currently unmapped within this chromosome, and the short arm of chromosome 7H remains unmapped although 10 markers remain unlocated. As recombinational location of these currently unlocated markers is completed a much greater proportion of the genome will likely be effectively marked.

The chromosomes which are well-populated with recombinationally located STS-PCR markers have been more thoroughly studied by our laboratory than those less well-characterized. Many of these markers have been utilized in marker-assisted selection projects including the introgression of genes conferring tolerance to *Diuraphis noxia* (Nieto-Lopez and Blake 1994), grain yield (Larson et al. 1996), seed dormancy (Oberthur et al. 1995), stripe rust resistance (P. Hayes, personal communication), spot blotch resistance and net blotch resistance (B. Stephenson, personal communication).

Multiple primer sets have been developed from several RFLP clones, and occasionally from sequences obtained from sequence analysis of the PCR products of primer sets derived from RFLP clones. Details regarding the derivation of each primer set are available from the senior author and have been explained in GrainGenes.

When we contrast the location of STS products with the RFLP map we find that 85 amplification products map to chromosomes which contain RFLP counterparts. Fifty products map to chromosomes which appear not to contain appropriate RFLP counterparts. No chromosomal pattern of mismatch between RFLP marker and PCR derivative was obvious, with the possible exception of markers on chromosome 6. In this instance, 4 STS-PCR markers derived from clones identifying RFLPs on chromosome 7H, and 3 from clones identifying chromosome 5H RFLPs may indicate a non-random association.

The type of clone used for RFLP mapping and STS production appeared to impact the likelihood that the STS derived from it would map to the predicted chromosome. Of the cDNA-derived ABC primer sets, 46% (18/39) mapped to a chromosome containing a cognate RFLP locus, while 75% (45/60) of the genomic DNA-derived ABG STS markers mapped to a chromosome predicted by RFLP analysis. When we contrast the classes of amplification ("A" and "B" groups vs "C" group) a possible explanation becomes apparent (Fig. 1). Summing over the barley-derived genomic and cDNA-derived STSs, 50/68 (74%) of the primer

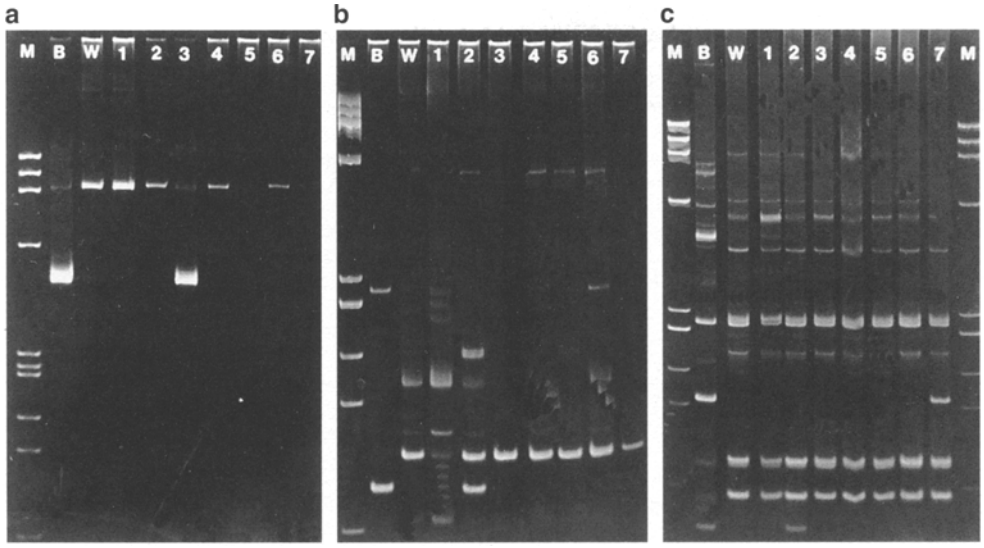

Fig. 1a–c Examples of amplification products described in Table 1. **Panel a** Products of amplification resolved in a 6% acrylamide gel using primer set aABG70 vs 'Betzes' barley (*B*), 'Chinese Spring' wheat (*W*) and the seven wheat/barley addition lines (1–7). Note the nearly complete specificity of the primer set for the barley allele on chromosome 3. **Panel b** Primer set aABC454 vs 'Betzes' barley (*B*), 'Chinese spring' wheat (*W*), and the seven wheat/barley addition lines (1–7). Note that while products are amplified from both barley and wheat DNA, the products are differently sized. **Panel c** Primer set a ABG055 vs 'Betzes' barley (*B*), 'Chinese Spring' wheat (*W*) and the seven wheat/barley addition lines (1–7). Note that while two uniquely-sized barley products are produced, fragments of common size are also produced from barley and wheat. These three general 'types' of amplification were commonly observed. In Table 1 the superscripts by each primer set name refer to the general type of amplification pattern observed. *Hae*III-restricted PhiX 174 DNA is used as molecular weight marker (*M*) in each panel

sets producing a single product resulted in a product which mapped to the chromosome predicted by RFLP analysis. When the primer sets resulting in multiple products were evaluated, only 18/41 products (44%) mapped to chromosomes predicted by RFLP analysis. Nearly 60% (30/51) of the primer sets derived from cDNAs directed the amplification of multiple products, while only about 35% (21/60) of the primer sets derived from barley genomic sequences directed the amplification of multiple products. The cDNA clones used in the NABGMP may overrepresent sequences with homology to dispersed multigene and pseudogene families.

Conversion of markers from RFLP to PCR technology, while conceptually straightforward, can be a tedious process. With this report, we make available a relatively large number of primer sets with products which are mapped to chromosomes.

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