Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks

Xiuqiang Huang, Friedrich J. Zeller, Sai L.K. Hsam, Gerhard Wenzel, and Volker Mohler

Abstract: Amplified fragment length polymorphism (AFLP) markers with a total of 256 *Eco*RI + ANN – *Mse*I + CNN primer combinations were investigated employing the common wheat cultivar *Triticum aestivum* 'Chinese Spring.' On average, 103 fragments per primer combination were amplified, ranging from a maximum of 226 fragments to a minimum of 18 fragments. The primer combinations E + AAA – M + CNN and E + ATT – M + CNN produced very few distinct fragments. By using 15 randomly chosen *Eco*RI + ANN – *Mse*I + CNN primer combinations, 928 AFLP markers were allocated to wheat chromosomes, of which 131 were assigned to specific chromosome arms. These AFLP markers were locus-specific and randomly distributed on the different chromosomes. In addition, 6 and 41 AFLP markers were simultaneously absent in two nulli-tetrasomics (NTs) of both homoeologous and non-homoeologous groups, respectively, whereas additional fragments were detected in N1BT1A, N5AT5D, and N6BT6A lines.

Key words: aneuploid, chromosome assignment, *Triticum aestivum*.

Résumé : Des marqueurs AFLP obtenus à l'aide de 256 combinaisons d'amorces (*Eco*RI + ANN – *Mse*I + CNN) ont été étudiés chez le cultivar commun de blé *Triticum aestivum* 'Chinese Spring.' En moyenne, 103 fragments ont été amplifiés pour chaque paire d'amorces, ce nombre variant d'aussi peu que 18 jusqu'à 226 fragments. Les combinaisons E + AAA – M + CNN et E + ATT – M + CNN ont produit très peu de fragments distincts. En prenant 15 combinaisons d'amorces *Eco*RI + ANN – *Mse*I + CNN choisies au hasard, 928 marqueurs AFLP ont pu être localisés sur des chromosomes du blé, dont 131 ont été assignés à des bras chromosomiques spécifiques. Ces marqueurs AFLP étaient spécifiques d'un locus et distribués aléatoirement sur les différents chromosomes. De plus, six et 41 marqueurs AFLP étaient simultanément absents chez deux lignées nulli-tétrasomiques (NTs) pour des chromosomes homéologues ou non-homéologues, respectivement, tandis que des fragments additionnels ont été détectés chez les lignées N1BT1A, N5AT5D et N6BT6A.

Mots clés : aneuploïde, localisation chromosomique, *Triticum aestivum*.

[Traduit par la Rédaction]

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important crops in the world. It is an allohexaploid comprising three genomes A, B, and D, each having seven chromosome pairs. The chromosomes of these genomes can be grouped into seven homoeologous groups. In the past decades, many types of aneuploid genetic stocks such as monosomic (Sears 1954), nulli-tetrasomic (NTs, Sears 1966), and ditelosomic lines (DTs, Sears and Sears 1978) have been developed by exploiting the compensating ability of homoeologous chromosomes. Monosomic and telosomic lines have been extensively used to identify chromosomes carrying particular genes and to map them relative to the centromere. Nulli-tetrasomic and ditelosomic lines are widely employed to assign molecular markers such as restriction fragment length polymorphisms (RFLPs, Anderson et al. 1992; Devey and Hart 1993) and microsatellites (Plaschke et al. 1996; Röder et al. 1995) to specific chromosomes and chromosome arms. However, the use of RFLP markers in wheat has been limited because of low levels of detected polymorphism (Chao et al. 1989; Kam-Morgan et al. 1989; Liu et al. 1990). PCR-based microsatellite markers can detect more alleles per marker than RFLP; the percentages of polymorphic microsatellites were about 22% (Ma et al. 1996) and 36% (Röder et al. 1995) in common wheat.

Recently, AFLP was developed (Vos et al. 1995) as a new DNA marker system combining the features of RFLP and PCR while avoiding the disadvantages encountered in these DNA markers. AFLP is a powerful technique to generate large numbers of markers for the construction of high-density genetic maps (Keim et al. 1997; Qi et al. 1998; van Eck et al. 1995), identification of specific genes (Kasuga et

Corresponding Editor: G.J. Scoles.

Received December 9, 1998. Accepted September 30, 1999.

X. Huang,[1] F.J. Zeller, S.L.K. Hsam, G. Wenzel, and V. Mohler.[2] Technische Universität München, Lehrstuhl für Pflanzenbau und Pflanzenzüchtung, Alte Akademie 12, D–85350 Freising-Weihenstephan, Germany.

[1]Present address: Institut für Pflanzengenetik und Kulturpflanzenforschung Corrensstr. 3 D-06446 Gatersleben, Germany.

[2]Author to whom all correspondence should be addressed (e-mail: mohler@weihenstephan.de).

 © 2000 NRC Canada

al. 1997; Schwarz et al. 1999; Thomas et al. 1995) or QTLs (quantitative trait loci) (Nandi et al. 1997; Toojinda et al. 1998), and assessment of genetic variation and diversity (Maughan et al. 1996; Russell et al. 1997).

In wheat, a single primer combination could detect up to 8 times more polymorphism than a polymorphic RFLP probe (Ma and Lapitan 1998). Hence, usage of AFLP markers in common wheat has been in the evaluation of genetic diversity (Barrett and Kidwell 1998; Burkhamer et al. 1998), and identification of qualitative resistance genes (Hartl et al. 1999) and QTLs (Bai et al. 1999; Parker et al. 1998). The objective of the present study was to provide information on the screening of AFLP markers with 256 *Eco*RI + ANN – *Mse*I + CNN primer combinations and to assign AFLP markers to chromosomes or chromosomal arms in wheat by using aneuploid lines.

Materials and methods

Plant material

Nulli-tetrasomic lines of 'Chinese Spring' (except for 2A and 4A) and several ditelosomic lines of 'Chinese Spring' for 1DS, 1DL, 2AS, 4AL, 6DS, and 6DL were originally obtained from the late Dr. E.R. Sears, University of Missouri. The designations of 4A and 4B used here follow the decisions of the Seventh International Wheat Genetics Symposium held in 1988 at Cambridge, U.K. (Miller and Koebner 1988).

DNA isolation

Total genomic DNA was isolated according to the protocol of Saghai-Maroof et al. (1984) with minor modifications. Briefly, 3–5 g of leaf tissue per sample were ground in liquid nitrogen and incubated at 60°C for 30 min with 15 mL of 1.5× CTAB (cetyltrimethlyammonium bromide) extraction buffer (1.5% (w/v) CTAB, 100 mM Tris–HCl pH 8.0, 20 mM EDTA pH 8.0, 1.05 M NaCl, and 1.5% β-mercaptoethanol) in 50-mL polypropylene tubes. After 5 min cooling on ice, 15 mL 24:1 chloroform : isoamyl alcohol was added. Samples were incubated for 30 min by shaking and then centrifuged at 2100 × *g* for 30 min. The aqueous layer was transferred to a new tube and 20 µL RNase A (10 mg/mL) was added. Samples were incubated for 30 min at room temperature. About one volume of cold isopropanol was added to precipitate DNA. After 30 min incubation at 4°C, precipitated DNA was hooked out and placed in a 2-mL reaction tube containing 1 mL of 75% ethanol. After washing twice with 75% ethanol, the second time overnight, the washing solution was removed and the DNA pellet was dried thoroughly and dissolved in TE buffer. DNA samples were stored at –20°C. The DNA was diluted to a concentration of 100 ng/µL before use in AFLP experiments.

AFLP analysis

The AFLP protocol was as developed by Vos et al. (1995) with modifications. One unit *Mse*I and 5 U *Eco*RI were used to digest 0.5 µg genomic DNA,, and simultaneously 5 pmol *Eco*RI adaptor and 50 pmol *Mse*I adaptor were ligated with 1 U T4–DNA ligase (all enzymes New England Biolabs, Beverly, Mass.) in a buffer containing 10 mM Tris–HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 1 mM ATP, and 50 ng/µL bovine serum albumin in a total volume of 11 µL for 3 h at 37°C. The DNA sample was diluted with $TE_{0.1}$ buffer (10 mM Tris–HCl pH 8.0, 0.1 mM EDTA pH 8.0) to a final volume of 200 µL and stored at –20°C. The sequence of the *Eco*RI adaptor was 5′-CTCGTAGACTGCGTACC-3′, 3′-CTGACGCATGGTTAA-5′, and the sequence of the *Mse*I adaptor was 5′-GACGATGAGTCCTGAG-3′, 3′-TACTCAGGACTCAT-5′.

Preselective amplification of target sequences was performed with *Eco*RI and *Mse*I adaptor-homologous primers, each possessing one additional nucleotide at the 3′ primer end. Polymerase chain reactions were set up with 4 µL diluted restriction–ligation DNA, 2.5 pmol *Eco*RI + A primer, 2.5 pmol *Mse*I + C primer, 0.4 U Taq DNA polymerase (Qiagen GmbH, Hilden, Germany), 0.2 mM of each dNTP (Amersham–Pharmacia Biotech, Uppsala, Sweden) and 1× Qiagen PCR buffer in a total volume of 20 µL. The PCR reaction was performed in a PE 9600 thermal cycler programmed for 20 cycles of 94°C (1 s), 56°C (30 s), 72°C (2 min). To verify successful amplification, 10 µL of the PCR mixture was electrophoresed on a 1.5% agarose gel in 1× TAE buffer stained with 0.5 µg/mL ethidium bromide: a smear of amplified target fragments was visible in the range 100–1500 bp. The remaining 10 µL were diluted 20-fold by adding 190 µL $TE_{0.1}$ buffer, and stored at –20°C.

Selective amplification was achieved with *Eco*RI + ANN and *Mse*I + CNN primers. Only *Eco*RI primers were labeled using either 5-carboxy-fluorescein (5-FAM), or 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxy-fluorescein (JOE), or N,N,N′,N′-tetramethyl-6-carboxyrhodamin (TAMRA). Polymerase chain reactions were carried out using 3 µL diluted pre-amplified DNA, 1 pmol labeled *Eco*RI + ANN primer, 5 pmol unlabeled *Mse*I + CNN primer, 0.4 U Taq DNA polymerase, 0.2 mM each of dNTP and 1× Qiagen PCR buffer in a total volume of 20 µL. For amplification, the following cycle profile was used: one cycle of 30 s at 94°C, 30 s at 65°C, 2 min at 72°C, followed by 8 cycles in which annealing temperature was subsequently lowered 1°C per cycle, and finally 23 cycles of 1 s at 94°C, 30 s at 56°C, 2 min at 72°C. Then, 0.5 µL of 5-FAM-labeled PCR products, 0.6 µL of JOE-labeled PCR products and 0.9 µL of TAMRA-labeled PCR products were pooled. The mixture was made up with 0.15 µL of 6-carboxy-X-rhodamin (ROX)-labeled internal length standard GeneScan–500 ROX (PE–Applied Biosystems) and 0.85 µL formamide dye (98% formamide, 0.005% dextran blue), denatured for 3 min at 90°C and chilled on ice.

Electrophoresis of 36 samples was carried out using 5% denaturing polyacrylamide gels (Long Ranger™, FMC Bioproducts, Rockland, Maine) in 1× TBE electrophoresis buffer (89 mM Tris–base, 89 mM boric acid, 2.0 mM EDTA pH 8.3) on an ABI Prism™ 377 DNA sequencer (PE–Applied Biosystems) at 2500 V for 4 h. For raw data collection, the ABI PRISM™ v. 1.1 collection software was used. AFLP fragments were analysed using GENESCAN™ v. 2.0.2 analysis software (PE–Applied Biosystems) as described in the user's manuals.

For screening of AFLP markers in wheat 256 *Eco*RI + ANN – *Mse*I + CNN primer combinations were applied (Table 1). For chromosomal location of AFLP markers in wheat, the following 15 *Eco*RI + ANN – *Mse*I + CNN primer combinations were used: E + ACA – M + CTA, E + ACA – M + CAC, E + ACA – M + CCT, E + ACA – M + CCG, E + ACA – M + CCC, E + ACT – M + CAC, E + ACT or M + CAA, E + ACT – M + CAG, E + AGA – M + CGA, E + AGA – M + CGT, E + AGA – M + CGG, E + AGA – M + CGC, E + AGA – M + CCC, E + AAT – M + CCA, and E + AAT – M + CCG.

Chromosome (arm) assignment

If an AFLP fragment was absent in a particular nullisomic–tetrasomic line and present in all other 20 lines, we assumed its location on the chromosome was in the nullisomic condition. If a complete ditelosomic set was not available and an AFLP fragment was absent in a ditelosomic line, this AFLP marker was localized on the opposite arm of that chromosome.

© 2000 NRC Canada

Table 1. List of the 256 AFLP primer combinations tested and number of AFLP markers in each primer pair scored in 'Chinese Spring.'

*Eco*RI + ANN	*Mse*I + CNN																Maximum	Minimum	Average
	CCA	CCT	CCG	CCC	CTA	CGG	CGT	CAC	CAT	CGC	CGA	CTC	CAG	CTT	CTG	CAA			
AAT (F)	100	99	124	99	77	80	71	27	47	65	39	40	220	18	137	168	220	18	88
ACG (J)	47	46	53	53	61	50	49	41	47	46	25	66	111	53	106	65	111	25	57
ATA (T)	50	73	65	112	65	87	53	29	32	57	51	48	87	26	50	53	112	26	59
ACT (F)	123	138	128	137	103	109	96	144	176	74	119	117	173	89	101	182	182	74	126
AAA (J)	9	6	8	12	6	5	6	9	4	4	3	4	17	7	13	15	17	3	8
ATG (T)	76	97	72	83	66	111	22	23	28	30	28	22	54	28	34	32	111	22	50
ACA (F)	75	170	98	132	172	96	96	161	150	60	32	126	115	89	157	155	172	32	118
ATT (J)	12	5	10	7	4	3	3	7	2	1	3	3	3	4	7	7	12	1	5
AGC (T)	51	130	122	121	157	84	78	140	134	74	85	88	125	81	53	18	157	18	96
AGA (F)	192	121	28	199	177	155	152	211	226	148	162	190	215	133	204	216	226	28	171
AGG (J)	156	130	55	121	137	112	102	146	162	94	96	126	152	149	141	124	162	55	125
ATC (T)	73	97	46	112	113	61	97	25	40	47	60	75	52	41	80	83	113	25	69
AGT (F)	136	164	117	167	142	120	91	175	154	172	163	152	225	149	138	99	225	91	148
AAG (J)	100	144	99	156	157	126	119	129	97	93	75	136	87	157	112	55	157	55	115
ACC (T)	182	138	126	135	148	105	103	63	129	92	101	158	150	142	130	146	182	63	128
AAC (T)	86	114	126	103	105	99	26	93	91	81	24	90	107	46	103	58	126	24	85
Maximum	192	170	128	199	177	155	152	211	226	172	163	190	225	157	204	216	226		
Minimum[a]	47	46	28	53	61	50	22	23	28	30	24	22	52	18	34	18		18[a]	
Average[a]	103	119	90	124	120	100	83	101	108	81	76	102	134	88	110	104			103[a]

Note: F, 5-FAM dye; J, JOE dye; T, TAMRA dye.
[a]Number of markers from E + AAA – M + CNN and E + ATT – M + CNN was not included.

© 2000 NRC Canada

Results and discussion

Abundance of AFLP markers

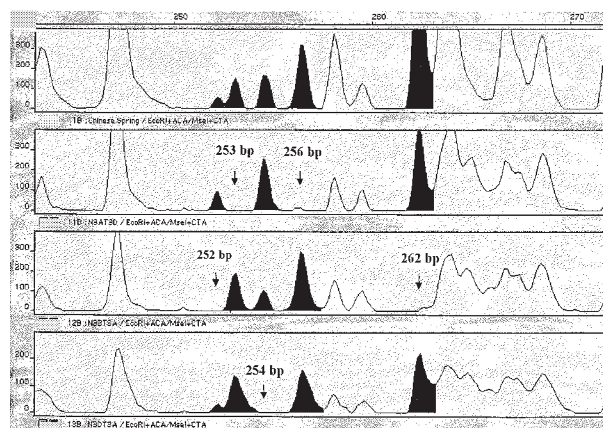
Using 16 *Eco*RI + ANN and 16 *Mse*I + CNN primers, a total of 256 primer combinations (Table 1) were tested with DNA of 'Chinese Spring.' An average of 103 fragments per primer pair was amplified, varying from a maximum of 226 fragments for E + AGA – M + CAT to a minimum of 18 fragments for E + AAT – M + CTT and E + AGC – M + CAA. The number of fragments from E + AAA – M + CNN and E + ATT – M + CNN was not included. Comparison among *Mse*I + CNN primers combined with 16 *Eco*RI + ANN primers showed no obvious difference in the average number of amplified PCR fragments. However, a comparison among *Eco*RI + ANN primers combined with 16 *Mse*I + CNN primers revealed a distinctive difference. The number of fragments from primer combinations E + AGA – M + CNN and E + AGT – M + CNN were on average 171 and 148, respectively, whereas the primer combinations E + AAA – M + CNN and E + ATT – M + CNN produced very few fragments. Similar results were also reported in barley by Schwarz et al. (2000). This indicates that the number of amplified PCR fragments is related directly to *Eco*RI + ANN primer. It appears that two *Eco*RI + ANN primers, E + AAA and E + ATT, due to the few distinct fragments amplified should not be used for AFLP analysis in wheat.

Wheat possesses a large genome with a haploid genome size of 16×10^9 bp (May and Appels 1987). A larger number of DNA fragments could be produced in wheat by using the six-base cutter *Eco*RI in contrast to the eight-base cutter *Sse*8387I (Hartl et al. 1999). The larger number of fragments may generate a larger number of polymorphic markers per primer combination for gene identification. Barrett and Kidwell (1998) reported that the enzyme combination *Pst*I–*Mse*I revealed significantly lower levels of diversity in wheat than the enzyme combination *Eco*RI–*Mse*I. In addition, interpretation of many faint fragments using GENESCAN™ analysis software is comparatively easy relative to scoring weak fragments with conventional radioactive electrophoresis (Ma and Lapitan 1998; Mackill et al. 1996). Moreover, *Eco*RI–*Mse*I is a frequently used enzyme system and is widely applied for AFLP analysis in cereal crops such as rice (Mackill et al. 1996; Nandi et al. 1997) and barley (Qi et al. 1998; Russell et al. 1997). Furthermore, the use of the *Eco*RI–*Mse*I enzyme system in wheat may be efficient to compare AFLP markers among homoeologous groups of other cereal crops. Therefore, information on screening of AFLP markers with 256 *Eco*RI + ANN – *Mse*I + CNN primer combinations in 'Chinese Spring' will be useful for genetic studies in wheat.

Distribution of AFLP marker loci

A total of 928 (39.9%) out of 2328 AFLP markers amplified with 15 randomly chosen *Eco*RI + ANN – *Mse*I + CNN primer combinations could be assigned to individual chromosomes, among which 131 AFLP markers were allocated to specific chromosome arms. The remaining AFLP marker loci that were not assigned to any chromosome may be due to the presence of co-migrating and (or) homoeologous fragments. Five fragments that were obtained with the primer pair *Eco*RI + ACA – *Mse*I + CTA and assigned to group 3 chromosomes are shown in Fig. 1. The fragments 252 bp, 253 bp, and 254 bp (only 1-bp difference in size) were localized on the chromosomes 3B, 3A, and 3D, respectively. These three AFLP markers might be homoeologous alleles of group 3 chromosomes, which needed to be further tested by genetic mapping.

Fig. 1. Chromosomal assignment of AFLP markers, amplified with the primer combination E + ACA – M + CTA and analysed using GENESCAN™ v. 2.0.2 analysis software. The peaks represent fragments, whereas horizontal and vertical scales indicate fragment sizes in bp and relative signal intensity of fluorescence-labelled fragments, repsectively. Arrows indicate missing fragments that were assigned to the respective chromosomes of homoeologous group 3.



All wheat chromosomes were identified by any of the 15 *Eco*RI + ANN – *Mse*I + CNN primer combinations used, indicating that AFLP markers cover the entire wheat genome. The largest number of AFLP marker loci was observed on the B genome with 356 (38.3%), compared to 294 (31.7%) and 278 (30.0%) for genomes A and D, respectively. The results are consistent with those of an earlier study examining microsatellite markers (Plaschke et al. 1996). The homoeologous groups from 1 to 7 were populated by 113 (12.2%), 130 (14.0%), 143 (15.4%), 139 (15.0%), 133 (14.3%), 117 (12.6%), and 153 (16.5%) genome-specific AFLP markers, respectively, indicating that these markers are randomly distributed between the seven chromosome groups. Table 2 shows the primer combination identity of polymorphic fragments with molecular sizes and chromosomal location.

Fragments absent in two or more 'Chinese Spring' NTs and occurrence of additional fragments

Aside from 928 AFLP markers assigned to the different chromosomes, it is noted that 6 and 41 AFLP fragments were absent in two NTs between homoeologous and non-homoeologous chromosomes, respectively. One fragment, E + AGA – M + CGA-267, was missing in four NTs among non-homoeologous chromosomes (Table 2). These fragments could not be assigned to individual chromosomes. In 35 cases, 'Chinese Spring' N5AT5D line was involved, inferring that chromosome 5A has a fewer number of chromosome-specific AFLP markers in comparison with other chromosomes. It appears that a deletion where an AFLP marker was missing in N5AT5D exists in chromosome 5A in

© 2000 NRC Canada

Table 2. Chromosomal location of AFLP markers in wheat and their sizes (bp) using 15 *Eco*RI + ANN – *Mse*I + CNN primer combinations by means of aneuploid lines.

Chrom.	E + AAT – M + CCA	E + AAT – M + CCG	E + ACA – M + CAC	E + ACA – M + CCC	E + ACA – M + CCG
1A	146, 489	448	92, 151, 253		245, 580
1B	130, 326, 480, 465	478, 570	135, 195, 224, 483	119, 370, 402, 635	243, 550
1D	346^{S}, 498^{L}	337^{S}, 405^{L}	255^{L}, 569^{L}	212^{L}, 583^{L}	291^{L}
2A	91^{L}, 283^{L}, 284^{L}, 292^{L}	171^{L}, 226^{L}, 403^{L}	177^{L}, 187^{L}, 453^{L}	65^{L}, 147^{L}, 310^{L}	205^{L}, 206^{L}, 271^{L}, 355^{L}
2B	93, 127, 182, 264, 515	224, $\underline{397}$, 617	140, 556	182, 233, 256, 362, 491	142, 469
2D	180, $\underline{258}$, 335	498	145, 173, 178, 264, 308	135, 194, 294, 389	230, $\underline{381}$
3A	276, 343, 390, 418	98, 154, 247, 250, 517, 520	107, 248, 256, 306, 313, 618	102, 164	98, 139, 450, 583
3B	155, 172, 187	95, 145	$\underline{411}$, 418, $\underline{434}$, 447, 512, 520	149, 167, 228, 640	199, $\underline{214}$, 287, 355, 400, 557
3D	249, 291	430	268, 444	87, 290, 545	370, 414, 492, 523, 524
4A	306^{S}	70^{S}	193^{S}, 263^{S}, 530^{S}	152^{S}, 197^{S}, 448^{S}	297^{S}, $\underline{381}^{S}$
4B	145, 243, 370	197, $\underline{205}$, 210, 562	206, 214, 560	86, 89, 170, 241, 386, 467	$\underline{156}$
4D	83, 125, 240, 272, 323	213, 217	275, 375, 495, 500	374, 406, 446	250, 314
5A	$\underline{190}$, 331	$\underline{205}$, 375, $\underline{397}$	80, $\underline{236}$, 280, $\underline{411}$, $\underline{434}$	250, $\underline{414}$, $\underline{415}$	$\underline{156}$, 401, $\underline{484}$, 548
5B	170, $\underline{258}$	142, 194, 314, 364	203, 210, 217, 220, 288, 291, 515	198, 236, 421	99, 163, $\underline{214}$, 546
5D	176, 185, 427	88, 94	238, 555	66, 567	67, 416, 459
6A	129, 205, 315, 317	265, 323, 346, 630	186, 335, 365, 417, 449, 467, 593	67, 109, 243, 302	367, 487
6B	88, $\underline{190}$, 482	263, 355, 356, 525	120, $\underline{236}$, 354	308, $\underline{414}$, $\underline{415}$, 528	$\underline{484}$
6D	247^{S}, 350^{L}	455^{L}		299^{S}, 405^{S}	
7A	206, 600	325, 439, 545	278, 460	63, 204, 278, 314, 339, 340, 346, 379, 576	240, 515
7B	66, 186, 235, 274, 457	320, 384	79, 152, 163, 182, 282, 292, 338, 442, 443, 485	263, 554	94, 237, 323
7D	76, 119, 351, 366, 369, 387, 560	237, 329, 418	80, 99, 216	455, 540, 613	246, 405, 467

Chrom.	E + ACA – M + CCT	E + ACA – M + CTA	E + ACT – M + CAA	E + ACT – M + CAC	E + ACT – M + CAG
1A	320, 324, 473	284, 379	$\underline{170}$, 273, 277	81, 309, 403	108, 252, 303, 319, 467, 596
1B	165, 225, 303	136, 170, 330	$\underline{170}$, 215, 231, 287, 323, 374, 403, 415	335, 368, 567, 611, 620	98, 151, 244, 431, 498, 640
1D		149^{S}, 357^{S}, 328^{L}	194^{S}, 85^{L}, 288^{L}, 303^{L}, 340^{L}		501^{S}, 84^{L}, 169^{L}
2A	81^{L}, 108^{L}, 189^{L}, 360^{L}, 580^{L}	147^{L}, $\underline{150}^{L}$, 203^{L}, $\underline{262}^{L}$, 288^{L}, 473^{L}	72^{L}, 107^{L}, 240^{L}, 269^{L}, 335^{L}, 582^{L}	259^{L}, 414^{L}	
2B	292, 293, 296, 351, 396, 418, 448, 620	$\underline{346}$, 534	90, 95, $\underline{327}$, 349, 374, 378	226, 363	442
2D	194, 369, $\underline{394}$, 466, 479	127, 241, 275, 315, 295	251, 316, 321	232, 476	307, 340, 507, 560
3A	281	66, 85, 253, 256, 308, 331, 377	161, 245, 293	80, 189, 271, 316, 367	238, 270, 291, 387, 447
3B	$\underline{98}$, 353	$\underline{75}$, $\underline{150}$, 252, $\underline{262}$	414, 576	372, 616	175, 261, 603
3D	249, 280, 474	191, 254, 499	290, 345	89, 105, 110, 122, 268, 280, 356	182, 200, 274, 476, 495, 660
4A	101^{S}, 325^{S}	80^{S}, 88^{S}, 434^{S}	166^{S}, 266^{S}, 602^{S}	75^{S}, 84^{S}, 571^{S}	657^{S}
4B	179, 393	174, 243, 260, 277, 388	172, 198, 214, 312, 380, 390, 454, 507	62, 195, 415, 430, 558	163, $\underline{196}$, 255, $\underline{267}$, 377, 393, $\underline{421}$, 435, 499
4D	252, 442, 450	182, 188, 272	219, 338	61, 291, 610	222, 280, 368, 444, 505
5A	$\underline{98}$, 152, 391, $\underline{411}$	$\underline{75}$, $\underline{115}$, $\underline{346}$	$\underline{327}$, $\underline{362}$, $\underline{369}$	457	$\underline{118}$, $\underline{196}$, $\underline{267}$, $\underline{317}$, $\underline{421}$
5B	274, $\underline{394}$, 500,585	65, 112, 127, 133, 181, 295, 320, 353	67, 133, 232, 294, 301, $\underline{369}$, 449, 478, 575	83, 194, 241, 332, 432, 455	100, $\underline{118}$
5D	129, 160, 199, $\underline{411}$, 436, 530	64, 73, 180, 351, 387, 447, 585	71, 76, 140	257	121, 369, 513, 546
6A	85, 163, 209, 398, 400, 545	240, 286, 410	237, 417	273, 434	103, 276, 428
6B	329, 332, 595	$\underline{115}$, 164, 327, 432	261, $\underline{362}$, 401, 420, 447	97, 225, 398, 500	73, 225, $\underline{317}$
6D	65^{S}, 276^{S}, 229^{L}, 334^{L}	95^{S}, 445^{S}, 457^{L}	300^{L}, 438^{L}		177^{S}, 209^{S}, 243^{S}, 422^{S}

© 2000 NRC Canada

Table 2 (*concluded*).

7A	253, 379, 572	208, 227, 278, 361	173, 257, 280, 628	333, 348, 365, 386	192, 327, 357
7B	122, 152, 155, 245, 330, 358, 530	141, 167, 216	96, 183, 207, $\underline{268}$, 291, 333	329, 337, 536	178, 360, 412
7D	138	103, 158	108, 149, $\underline{268}$, 426	76, 263, 288	
Chrom.	**E + AGA – M + CCC**	**E + AGA – M + CGA**	**E + AGA – M + CGC**	**E + AGA – M + CGG**	**E + AGA – M + CGT**
1A	51, 185, 194	203, 279	341, 360, 465, 553	138	123, 161, 317
1B	184	245, 465	435	58, 333,	451
1D	119^{L}, 197^{L}	277^{S}	77^{L}, 88^{L}	$\underline{246}^{S}$, 232^{L}, 313^{L}, 377^{L}	340^{S}
2A		$\underline{79}^{L}$, $\underline{267}^{L}$, 446^{L}	$\underline{135}^{L}$, 494^{L}, 520^{L}		195^{L}
2B	139	$\underline{161}$, $\underline{229}$, 257, 304, 321, 355, 368, 399, 452	161, 319	401	88, 138, 244, $\underline{263}$, $\underline{420}$
2D	203, 237, 261, 270, 312	489	501, 630	140, 341, 550	156, 287, 306
3A	109, 173	86, 366, 576	104, 242, 359, 417, 442	152, 174, 466, 467	169, 231
3B	62, 117, 161	$\underline{79}$, $\underline{267}$, 385, 455	$\underline{135}$, 241, 389, 518	$\underline{324}$	63, 159, $\underline{258}$, 333, 356, 395
3D		274, 319, 510	236, 372	157, 299, 303	188, 240
4A	199^{S}, 205^{S}, 331^{S}	244^{S}	238^{S}, 412^{S}	318^{S}, 352^{S}	71^{S}, 129^{S}, 315^{S}, 403^{S}
4B	88, 282, 343	92, 215	163	73, 106, 113, $\underline{217}$, 218, 219, 221, 489, 510	165, 367, 462, 582
4D	65, 247	152, 390, 439, 494	225, 445	64, 77, 193, 326	192, 422, 477
5A	75, 118, 149, $\underline{156}$, 202	$\underline{161}$, $\underline{267}$	272, 287	$\underline{151}$, $\underline{217}$, $\underline{324}$, 414, $\underline{431}$, 503	$\underline{179}$, $\underline{258}$, $\underline{263}$, $\underline{362}$, 397, $\underline{420}$, $\underline{522}$
5B	103, 134, 151, 245	64, $\underline{229}$, 264, 317, 406, 544	600	104, 268, 317, 358, 381, 426, $\underline{431}$, 464, 485	210, 243, 332, 438
5D	74, 90, 91, 93, 188, 386	139, 336, 413	226	70, 78, 135, 228	363, 426
6A	368	251, 375, 382, 444	51, 130, 181, 379, 394, 487, 498	420, 472, 501	465
6B	110, 160, 371	219, $\underline{267}$	357	415,	136, $\underline{179}$, $\underline{362}$, $\underline{522}$
6D	307^{L}	72^{S}, 184^{S}	140^{S}, 175^{S}, 280^{S}, 281^{S}	304^{S}, 540^{S}, $\underline{246}^{L}$, 477^{L}, 491^{L}	268^{L}, 312^{L}
7A	60, 124, $\underline{156}$, 304, 310	357, 432	250	$\underline{151}$, 171, 182, 233, 373, 545, 567	74, 325, 402, 424, 528, 536
7B	260, 272, 320	269, 302, 305, 427	188, 293, 313	276, 311, 340, 348, 438	209, 257, 396, 520, 525, 536
7D	85, 444	77	184, 382		625

Note: S, short arm; L, long arm. Underlined markers are absent in two or four NTs and consequently cannot be assigned with confidence to the chromosome group.

corresponding NTs. It is supposed that most of these AFLP markers might be assigned to chromosome 5A. Röder et al. (1995) have also found that the fragment of microsatellite gwm6 was missing after amplification of lines N4BT4A as well as N4DT4A. Anderson et al. (1992) explained the absence of RFLP markers between homoeologous chromosomes as the result of homoeologous recombination.

Fifty-six, 27, and 29 additional fragments were detected in N1BT1A, N5AT5D, and N6BT6A lines, respectively. Also, 31 additional fragments with identical size were present in both N5AT5D and N6BT6A lines. Additional fragments described here were fragments that were present only in one or two CS NT line(s), absent in CS and in other 20 or 19 CS NT lines. Additional fragments independently present in N1BT1A, N5AT5D, and N6BT6A lines may be proposed as the result of mutations that have occurred after the development of three NTs, whereas the occurrence of additional fragments with identical size in N5AT5D and N6BT6A lines may be assumed that the two NTs were developed from one plant of 'Chinese Spring' in which mutations had occurred. Okamoto and Sears (1962) reported a non-homoeologous translocation between chromosomes 6A and 5D which may have been used in the development of the above-mentioned NT lines. On the other hand, caution is needed for using NTs to assign unknown linkage groups to chromosomes in wheat based on AFLP markers because of the inherent anomalies associated with these NTs.

AFLPs as genetic markers in wheat

AFLP technology, unlike that of RFLP, is PCR-based, requires only minimal amounts of starting DNA, and is readily automatable. Unlike RAPDs, AFLP markers have proven to be robust and reliable. And, unlike microsatellite markers and STS (sequence-tagged-satellite) markers, AFLP analysis requires no prior sequence knowledge of the target genome and, therefore, has no associated marker development costs. The present study indicates that the advantages of using AFLP markers are the ability to detect a large number of chromosome-specific AFLP markers, and the high level of polymorphism from comparison of AFLP polymorphism between 'Chinese Spring' and another Chinese landrace (Huang et al. 2000).

© 2000 NRC Canada

The use of three-colour fluorescence allows the achievement of the fast and high throughput desired for examining large numbers of samples (36 lanes × 3 = 108) and the scoring of more data points in a single gel than with conventional radioactive or one-colour-based electrophoresis. The application of an internal-lane size standard ensures superior sizing accuracy by minimizing lane-to-lane or gel-to-gel variation. DNA fragments with size differences as small as one base pair could be differentiated (Fig. 1).

AFLP markers cover all of the wheat genome, hence they should be most applicable for identification of QTLs. Recently, the identification of associations between QTLs for wheat flour colour and AFLP markers has been reported (Parker et al. 1998). Because of the high level of detected polymorphism, AFLP markers are most suitable for evaluation of genetic diversity among wheat cultivars (Barrett and Kidwell 1998; Burkhamer et al. 1998). AFLP analysis will be more informative than other molecular markers and can offer the fastest, most reproducible, and most cost-effective way to generate high density-genetic maps for marker-assisted selection of desirable traits in wheat. The chromosome and arm assignment of AFLP markers provides a valuable tool for anchoring unknown linkage groups to chromosomes from experimental wheat crosses involving 'Chinese Spring.'

Acknowledgements

We wish to thank C. Möller for excellent technical assistance, and G. Schwarz for valuable comments. Financial support by Deutscher Akademischer Austauschdienst (DAAD) is gratefully acknowledged.

References

Anderson, J.A., Ogihara Y., Sorrells, M.E., and Tanksley, S.D. 1992. Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet. **83**: 1035–1043.

Bai, G., Kolb, F.L., Shaner, G., and Domier, L.L. 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology, **89**: 343–348.

Barrett, B.A., and Kidwell, K.K. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. **38**: 1261–1271.

Burkhamer, R.L., Lanning, S.P., Martens, R.J., Martin, J.M., and Talbert, L.E. 1998. Predicting progeny variance from parental divergence in hard red spring wheat. Crop Sci. **38**: 243–248.

Chao, S., Sharp, P.J., Worland, A.J., Warham, E.J., Koebner, R.M.D., and Gale, M.D. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet. **78**: 495–504.

Devey, M.E., and Hart, G.E. 1993. Chromosomal localisation of intergenomic RFLP loci in hexaploid wheat. Genome, **36**: 913–918.

Hartl, L., Mohler, V., Zeller, F.J., Hsam, S.L.K., and Schweizer, G. 1999. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat. Genome, **42**: 322–329.

Kam-Morgan, L.N.W., Gill, B.S., and Muthukrishnan, S. 1989. DNA restriction fragment length polymorphisms: A strategy for genetic mapping of D genome of wheat. Genome, **32**: 724–732.

Kasuga, T., Salimath, S.S., Shi, J., Gijzen, M., Buzzell, R.I., and Bhattacharyya, M.K. 1997. High resolution genetic and physical mapping of molecular markers linked to the *Phytophthora* resistance gene *Rps*1-k in Soybean Mol. Plant–Microbe Interact. **10**: 1035–1044.

Keim, P., Schupp, J.M., Travis, S.E., Clayton, K., Zhu, T., Shi, L.A., Ferreira, A., and Webb, D.M. 1997. A high-density soybean genetic map based upon AFLP markers. Crop Sci. **37**: 537–543.

Liu, Y.G., Mori, N., and Tsunewaki, K. 1990. Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn. J. Genet. **65**: 367–380.

Ma, Z.Q., and Lapitan, N.L.V. 1998. A comparison of amplified and restriction fragment length polymorphism in wheat. Cereal Res. Commun. **26**: 7–13.

Ma, Z.Q., Röder, M., and Sorrells, M.E. 1996. Frequencies and sequence characteristics of di-, tri, and tetra-nucleotide microsatellites in wheat. Genome, **37**: 123–130.

Mackill, D.J., Zhang, Z., Redoña, E.D., and Colowit, P.M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome, **39**: 969–977.

Maughan, P.J., Saghai-Maroof, M.A., Buss, G.R., and Huestis, G.M. 1996. Amplified fragment length polymorphism (AFLP) in soybean: Species diversity, inheritance, and near-isogenic line analysis. Theor. Appl. Genet. **93**: 392–401.

May, C.E., and Appels, R. 1987. The molecular genetics of wheat: Toward an understanding of 16 billion base pairs of DNA. *In* Wheat and wheat improvement. 2nd ed. *Edited by* E.G. Heyne. American Society of Agronomy, Madison, Wis. pp. 165–198.

Miller, T.E., and Koebner, R.M.D. (*Editors)* 1988. Proceedings of the 7th International Wheat Genetics Symposium, held at Cambridge, England, 13–19 July 1988. Agricultural and Food Research Council, Institute of Plant Science Research, Cambridge Laboratory, Trumpington, England.

Nandi, N., Subudhi, P.K., Senadhira, D., Manigbas, N.L., SenMandi, S., and Huang, N. 1997. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol. Gen. Genet. **255**: 1–8.

Okamoto, M., and Sears, E.R. 1962. Chromosomes involved in translocations obtained from haploids of common wheat. Can. J. Genet. Cytol. **4**: 24–30.

Parker, G.D., Chalmers, K.J., Rathjen, A.J., and Langridge, P. 1998. Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **97**: 238–245.

Plaschke, J., Börner, A., Wendehake, K., Ganal, M.W., and Röder, M.S. 1996. The use of wheat aneuploids for the chromosomal assignment of microsatellite loci. Euphytica, **89**: 33–40.

Qi, X., Stam, P., and Lindhout, P. 1998. Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor. Appl. Genet. **96**: 376–384.

Röder, M.S., Plaschke, J., König, S.U., Börner, A., Sorrells, M.S., Tanksley, S.D., and Ganal, M.W. 1995. Abundance, variability and chromosomal location of microsatellite in wheat. Mol. Gen. Genet. **246**: 327–333.

Russell, J.R., Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, W., and Waugh, R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet. **95**: 714–722.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U.S.A. **81**: 8014–8018.

© 2000 NRC Canada

Schwarz, G., Michalek, W., Mohler, V., Wenzel, G., and Jahoor, A. 1999. Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high resolution mapping with AFLP markers. Theor. Appl. Genet. **98**: 521–530.

Schwarz, G., Herz, M., Huang, X.Q., Michalek, W., Jahoor, A., Wenzel, G., and Mohler, V. 2000. Application of fluorescence-based semi-automated AFLP analysis in barley and wheat. Theor. Appl. Genet. (in press).

Sears, E.R. 1954. The aneuploids of common wheat. Mo. Agric. Exp. Stn. Res. Bull. **572**: 1–58.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. *In* Chromosome manipulations and plant genetics. *Edited by* R. Riley and K.R. Lewis. Oliver and Boyd, Edingburgh, Scotland. pp. 29–45.

Sears, E.R., and Sears, L.M.S. 1978. The telocentric chromosomes of common wheat. *In* Proc. 5th Int. Wheat Genet. Symp. 23–28 Feb. 1978. *Edited by* S. Ramanujam. Indian Society of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi. pp. 389–407.

Thomas, C.M., Vos, P., Zabeau, M., Jones, D.A., Norcott, K.A., Chadwick, B.P., and Jones, J.D.G. 1995. Identification of amplified fragment length polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J. **8**: 785–794.

Toojinda, T., Baird, E., Booth, A., Broers, L., Hayes, P., Powell, W., Thomas, W., Vivar, H., and Young, G. 1998. Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: An example of marker-assisted line development. Theor. Appl. Genet. **96**: 123–131.

van Eck, H.J., van der Voort, J.R., Draaistra, J., van Zandvoort, P., van Enckevort, E., Segers, B., Peleman, J., Jacobsen, E., Helder, J., and Bakker, J. 1995. The inheritance and chromosomal location of AFLP markers in non-inbred potato offspring. Mol. Breed. **1**: 397–410.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. **21**: 4407–4414.

© 2000 NRC Canada