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MOLECULAR CYTOGENETIC CHARACTERIZATION OF E^b-GENOME CHROMOSOMES IN *THINOPYRUM BESSARABICUM* DISOMIC ADDITION LINES OF BREAD WHEAT[1]

Ji-Yi Zhang,* Xiao-Mei Li,* Richard R.-C. Wang,[2,]* A. Cortes,† V. Rosas,† and A. Mujeeb-Kazi†

*USDA-ARS, Forage and Range Research Laboratory, 695 North 1100 East, Logan, Utah 84322-6300, U.S.A.; and †International Maize and Wheat Improvement Center (CIMMYT), Londres 40, Apartado Postal 6-641, Delg. Cuauhtemoc 06600, Mexico

Thinopyrum bessarabicum (2n = 2x = 14, E^bE^b = JJ) is recognized for its high tolerance to soil salinity and the scab disease caused by *Fusarium graminearum*. Seven *Triticum aestivum*/*T. bessarabicum* disomic addition lines (2n = 44 = 21″ ABD + 1″ E^b) developed at the International Maize and Wheat Improvement Center were assayed. Genomic *in situ* hybridization (GISH) showed that six of the seven were true disomic alien addition lines, whereas one was a duplication-translocation addition line in which four-fifths of the additional chromosome was an alien chromosome segment and one-fifth was a wheat chromosome segment. Using AFLP fragments amplified from 32 selective primer pairs, 389 fragments were assigned to *T. bessarabicum* chromosomes. The duplication-translocation addition line had no unique AFLP fragments but shared 25 fragments with the $7E^b$ addition line, indicating that the translocation chromosome probably involved the $7E^b$ rather than the $3E^b$, as originally described. The presumed $6E^b$ addition line was determined to have a pair of $4E^b$ chromosomes. Thus, the $3E^b$ and $6E^b$ addition lines are missing. At present, there are 48 AFLP markers for $1E^b$, 67 for $2E^b$, 39 for $4E^b$, 59 for $5E^b$, and 54 for $7E^b$. In addition, two RAPD markers for $1E^b$, two for $2E^b$, six for $4E^b$, one for $5E^b$, and three for $7E^b$ were identified. Sixty-eight AFLP markers were present in the amphidiploid but absent in all tested disomic addition lines, making them putative markers for $3E^b$ and/or $6E^b$. Also, 50 AFLP and six RAPD markers occur in at least five E^b chromosomes. These molecular markers, when used in conjunction with GISH, will be useful in identifying the two missing E^b addition lines and in monitoring the introgression of E^b chromosomal segments into wheat.

Keywords: marker, salt tolerance, scab resistance, translocation, wheatgrass, *Thinopyrum bessarabicum*, addition line.

Introduction

Thinopyrum bessarabicum (2n = 2x = 14, E^bE^b = JJ) is a wild, rhizomatous maritime sand couch grass distributed in Crimea (Dewey 1984). It is recognized for its high tolerance to salinity, i.e., being able to withstand up to 350 mol mL^{-3} of NaCl (Gorham et al. 1985; King et al. 1996, 1997). It also possesses resistance to the Columbia root-knot nematode, *Meloidogyne chitwoodi* (Jensen and Griffin 1994), and tolerance to the scab disease caused by *Fusarium graminearum* (A. Mujeeb-Kazi, personal communication). In addition, *T. bessarabicum* is the probable donor species that contributed the E^b genome to many polyploid wheatgrasses (Liu and Wang 1992, 1993*a*, 1993*b*; Zhang et al. 1996). Many polyploid wheatgrasses, such as *Thinopyrum intermedium* and *Thinopyrum ponticum*, are important forage grasses and valuable germ plasm resources for wheat (*Triticum aestivum*) improvement (Wang and Zhang 1996; Zhang et al. 1996; Q. Chen et al. 1998; Fedak 1999). Understanding the organization of E^b genome and its phylogenetic relationships with other related genomes will greatly facilitate the utilization of these grasses for the introgression of useful genes into wheat.

Alien chromosome addition lines are usually generated to transfer agronomically important gene(s) from wild relatives into cultivated crops. This has been a cost-effective means of fostering germ plasm utilization. In addition, alien chromosome addition lines have also been used for localizing genes for valuable traits on specific chromosomes (Kindiger et al. 1996; Yildirim et al. 1998; Ma et al. 1999), construction of DNA libraries for specific chromosomes following microdissection (Jung et al. 1992), isolation of chromosome-specific DNA sequences (Clarke et al. 1995; Delaney et al. 1995), selective isolation and/or chromosome mapping of cDNAs (Korzun et al. 1996; Li et al. 1996; Biyashev et al. 1997), research on genome composition and chromosome structure (Ananiev et al. 1998; X. Y. Zhang et al. 1998), and assignment of DNA markers to specific chromosomes (Liu et al. 1996; Suen et al. 1997; Gallego et al. 1998; van Heusden et al. 2000). In recent years, disomic or monosomic addition lines have also been used to study nuclear architecture (Abranches et al. 1998) and to clone specific DNA sequences with the help of representation difference analysis (RDA) techniques (Delaney et al. 1995; Z. J. Chen et al. 1998).

In plants, molecular markers such as RFLP, SSR, RAPD, and AFLP are useful for studying genome organization, for understanding evolutionary changes and phylogeny (Sharma et al. 1996; Aggarwal et al. 1999), for assisting in alien chro-

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[2] Author for correspondence; e-mail rrcwang@cc.usu.edu.

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Table 1

Plant Materials Used in This Study and Their Chromosome Constitutions

Identification	Parentage or source	Chromosome constitution	
		Presumed	Actual
CS	Chinese Spring	2n = 42 = 21″ ABD	
Gen.	Genaro-81	2n = 42 = 21″ ABD	
W95001	CS/*Thinopyrum bessarabicum*	2n = 56 = 21″ ABD + 7″ E^b	
W95002	CS/*T. bessarabicum*//Gen.	2n = 44 = 21″ ABD + 1″ ($1E^b$)	
W95003	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($2E^b$)	
W95004	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($3E^b$)	= 21″ ABD + 1″ (4/5 $7E^b$ − 1/5 ?wheat)
W95005	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($4E^b$)	
W95006	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($5E^b$)	
W95007	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($6E^b$)	= 21″ ABD + 1″ ($4E^b$)
W95008	CS/*T. bessarabicum*//2*Gen.	2n = 44 = 21″ ABD + 1″ ($7E^b$)	

mosome (or segment) identification, and for facilitating selection in breeding programs. As demonstrated in numerous studies, AFLP, which combines the reliability of restriction enzyme digestion with stringent PCR condition for primer annealing, is a powerful technique for developing a large number of reproducible markers per assay across a wide variety of organisms (Vos et al. 1995; Powell et al. 1996; Jones et al. 1997; Zhu et al. 1998; Cato et al. 1999; Crouch et al. 1999). The multifluorophore detection system used in some DNA sequencers further increases the capacity and accuracy of AFLP compared with ^{33}P-based analysis.

A set of seven disomic addition lines of wheat with *Thinopyrum bessarabicum* chromosomes was produced at the International Maize and Wheat Improvement Center (CIMMYT). Specific proteins/isozymes and genomic *in situ* hybridization (GISH) were used for detecting the presence of *T. bessarabicum* chromosomes in the advanced backcross derivatives and for tentatively establishing the homoeology of these added chromosomes (William and Mujeeb-Kazi 1995). Molecular markers are still needed to detect the *T. bessarabicum* chromosomes in wheat background and to monitor alien chromosomal segment introgression in wheat improvement efforts.

By combining the molecular marker and GISH technologies, this research was aimed at characterizing the *T. bessarabicum* disomic addition lines for effective utilization of genetic resources in genome analysis and wheat–germ plasm enhancement.

Table 2

Sequence of AFLP Adaptors and Primers

Name	Sequence
EcoR I-adaptor	5′-CTC GTA GAC TGC GTA CC-3′ 3′-CAT CTG ACG CAT GGT TAA-5′
Mse I-adaptor	5′-GAC GAT GAG TCC TGA G-3′ 3′-TA CTC AGG ACT CAT-5′
E00	5′-GAC TGC GTA CCA ATT C-3′
M00	5′-GAT GAG TCC TGA GTA A-3′
EcoR I primer for preamplification	E00 + A
Mse I primer for preamplification	M00 + C
EcoR I primers	E36 = E00 + ACC E37 = E00 + ACG E40 = E00 + AGC E41 = E00 + AGG
Mse I primers	M47 = M00 + CAA M48 = M00 + CAC M49 = M00 + CAG M50 = M00 + CAT M59 = M00 + CTA M60 = M00 + CTC M61 = M00 + CTG M62 = M00 + CTT

Material and Methods

Plants

Seeds of a set of putatively disomic addition lines (2n = 44 = 22″; 21″ ABD + 1″ E^b) were obtained from CIMMYT and planted in a greenhouse with the wheat parental lines (table 1).

DNA Extraction

Young leaves were harvested individually and ground into powder in liquid nitrogen. DNA extraction was performed according to the chloroform-phenol method (Williams et al. 1992), with one additional purification step using chloroform to obtain high-quality DNA. Equal amounts of DNA from three individuals of each disomic addition line that had been confirmed with GISH detection were pooled for RAPD and AFLP assay.

GISH Assay

Root tips from germinating seeds were excised and then immersed in ice water for 24 h before being fixed in ethanol : acetic (3 : 1) fixative. Slides were prepared by squashing in 45% acetic acid after the tips were macerated at 37°C with an enzyme mixture containing 2% cellulase (Serva 16419, 1.3 U mg^{-1}, Feinbiochemica) and 2.5% pectinase (Sigma P4716, 25 U mg^{-1}, Sigma Chemical). Slides were treated with RNase in 2 × SSC and stabilized in 4% freshly prepared depolymerized paraformaldehyde solution in 1 × PBS buffer.

Because of its superior discriminating power between E-genome and ABD-genome chromosomes (Wang and Zhang

1996; Zhang et al. 1996; Q. Chen et al. 1998), genomic DNA from *Pseudoroegneria stipifolia* (St genome) was labeled with biotin-16-dUTP using the BioNick labeling system (Life Technologies). Ninety nanograms of labeled DNA were added as the probe in the hybridization solution with 120–150 times the amount of autoclaved genomic DNA of wheat (*Triticum aestivum* cv. Chinese Spring, ABD genome) and 50 times the amount of autoclaved salmon sperm DNA as the block. Hybridization was accomplished on a thermal cycler (Hybaid Omni Gene) with 75°C as the denaturation temperature for 5 min. The optimal hybridization temperature was 37°C when the solution contained 50% formamide and 2 × SSC. Stringent washing was effected in a solution with 20% formamide and 0.1 × SSC at 42°C. Hybridization signals were amplified and detected using fluorescent isothiocyanate (FITC)-Avidin D and biotinylated goat antiavidin D (A2001 and BA-0300, respectively; Vector Laboratories). The chromosomes were counterstained by 0.02%–0.05% propidium iodide in vector shield mounting medium (Vector Laboratories).

AFLP Analysis

Two parental wheat cultivars (Chinese Spring [CS] and Genaro-81), one artificial CS/*Thinopyrum bessarabicum* amphiploid (2n = 56 = 28″ ABDE^b), and seven cytogenetic stocks with alien E^b chromosomes (table 1) were used to separate E^b chromosomes and assign E^b chromosome-specific AFLP markers. The AFLP procedure used in this study was a combination, with slight modifications, of the protocol of Vos et al. (1995), the AFLP Plant Mapping Protocol (PE Applied Biosystems), and AFLP Analysis System I (Life Technologies). EcoR I (referred to in the following as E) and Mse I (referred to in the following as M) were used for the double digestion of 300 ng of template DNA. Following digestion, 12.5 μL of ligation solution containing adaptors (table 2) and T4 DNA ligase were added. Ligation reaction was performed at 20°C for 2 h, and then transferred to 14°C overnight. The solution was subsequently diluted fivefold in 1 × TE solution. Preamplification using primers containing one selective nucleotide each, namely E00 + A and M00 + C (GIBCO BRL AFLP Preamp primer mix I kit, Life Technologies), was performed in Perkin-Elmer/Cetus 9600 thermocyclers using the following temperature profile: 21 cycles of 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C. Reaction solutions were diluted in 1 : 20 ratio. Selective amplification of 10 μL of reaction solution containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM each dNTP, 1.5 μL of diluted preamplification solution, 2 μM of M-primer, and 0.04 μM of fluorescent-labeled E-Primer, and 1 U of GIBCO BRL Taq DNA Polymerase (Life Technologies) was performed in Perkin-Elmer/Cetus 9700 thermocyclers using the following profile: 18 cycles of 30 s at 94°C in every cycle, 30 s at 65°C that dropped 0.5°C in each cycle until it reached 56°C, and 60 s at 72°C in every cycle; then 23 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C, ending with 5 min extension at 72°C. One microliter of PCR reaction product was mixed with 2 μL of formamide, 0.5 μL of loading dye, and 0.3 μL of Rox 500, the internal lane size standard DNA (PE Applied Biosystem). Following denaturation, 1.2 μL of each sample was loaded on a 6% sequencing polyacrylamide gel (Long Ranger gel, FMC BioProducts, Rockland, Maine). Electrophoresis was performed on an ABI 373 automated sequencer, and data were collected and processed with GeneScan 3.1 (PE Applied Biosystem). The gel images were aligned in 1-bp bins by using the software Genographer (Benham et al. 1999).

RAPD

The decamer oligonucleotides kits were obtained from Operon Technologies. Stoffel fragments of AmpliTaq DNA polymerase, 10 × buffer, and 25 mM $MgCl_2$ were obtained from Perkin-Elmer. The optimized amplification reaction mixture (25 μL) contained 13.3 μL of sterile ddH_2O, 2.5 μL of 10 × buffer, 2 μL of 8 mM dNTP, 2 μL of 10 μM primer, 3 μL of 25mM $MgCl_2$, 0.2 μL (2 U) of Stoffel fragment, and 2 μL of template DNA (2.5 ng μL^{-1}), and was covered by 30 μL of mineral oil. The PCR reaction was performed with the GeneAmp PCR system 9700 for 40 cycles of 93°C for 1 min, 35°C for 1 min, 71°C for 2 min, stored at 4°C. The amplification products were separated in a 2% agarose gels, which contained 0.5 μg of ethidium bromide in 1 × TBE buffer (pH 8.3). DNA fragments were photographed under UV light, and their size was determined by comparison with DNA size markers.

Results

GISH Characterization of E^b Addition Lines

Genomic *in situ* hybridization of the *Triticum aestivum* cv. CS/*Thinopyrum bessarabicum* partial amphidiploid showed that 14 of the 56 chromosomes were yellow or yellowish green, exhibiting *T. bessarabicum* chromosomes, while the remaining 42 were all red or orange red, signifying wheat chromosomes from the combined red fluorescence of propidium iodide and a slight green fluorescence from FITC. This demonstrates that all E^b chromosomes in a wheat background can be visualized through GISH in different colors, varying from yellow to green when St genomic DNA is used as the probe. GISH assays of seven addition lines revealed that this set of addition lines encompassed only six different true disomic addition lines with intact *T. bessarabicum* chromosomes and one translocation-duplication (= tertiary) disomic addition line (fig. 1). W95004 contained two chromosomes of which fourth-fifths of the total length, from the long arm end, was yellowish green, whereas the remaining one-fifth was red (fig. 1*C*). This indicates that the alien chromosomes in this line had been subjected to translocation with an unknown wheat chromosome.

E^b Chromosome Identification Using AFLP Markers

Utilization of 32 AFLP selective primer combinations (table 2) amplified ca. 3545 fragments ranging from 50 to 500 bp, with an average of 111 (14 for E-ACC/M-CAC to 150 for E-ACC/M-CAG) scored AFLP bands per primer pair. E^b-specific AFLP markers were identified as bands present in the partial amphidiploid and one or two specific E^b chromosome addition line(s) but absent in two parental wheat lines and other addition lines. In total, 389 markers specific to E^b chromosomes were identified (table 3; fig. 2), accounting for 10.9% of the amplification products scored. This was lower than the expected (25% [= 14/56] chromatin ratio). Among the markers, 50 occurred in all lines with E^b chromosomes, indicating a

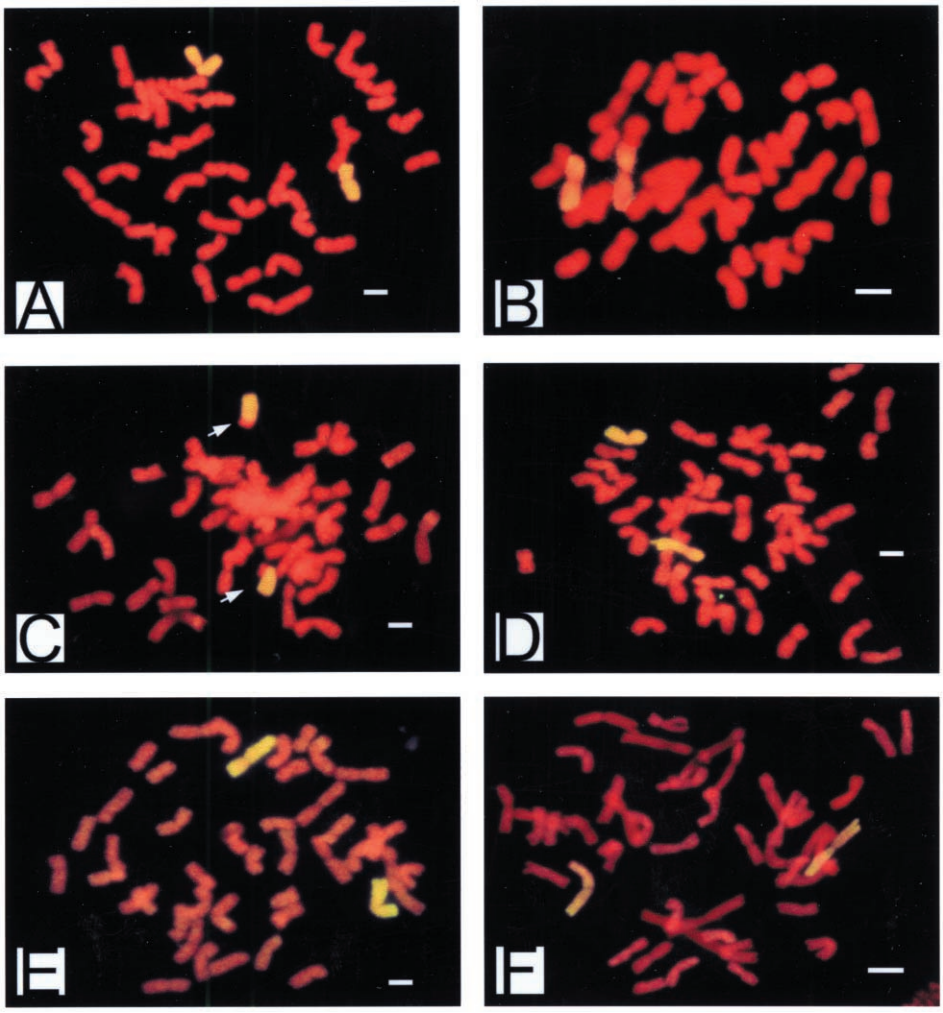

Fig. 1 Genomic *in situ* hybridization of *Triticum aestivum*/*Thinopyrum bessarabicum* disomic addition lines using biotin-labeled genomic DNA of *Pseudoroegneria stipifolia* (St genome) as probe and unlabeled wheat (ABD genomes) DNA as block. Yellowish chromosomes are fluorescent isothiocyanate–detected *Thinopyrum* chromosomes. $1E^b$ of W95002 (*A*), $2E^b$ of W95003 (*B*), $7E^b$ wheat tertiary chromosome of W95004 (*C*), $4E^b$ of W95005 (*D*), $5E^b$ of W95006 (*E*), and $7E^b$ of W95008 (*F*). Wheat chromosomes appear in red or orange due to propidium iodide. Bars = 10 μm.

Table 3

Distribution of Specific AFLP Markers among Cytogenetic Stocks of *Triticum aestivum*/*Thinopyrum bessarabicum* Disomic Addition Lines

Identification	E^b chromosome	Number of AFLP markers
W95001 and W95002	$1E^b$	48
W95001 and W95003	$2E^b$	67
W95001, W95005, and W95007	$4E^b$	39
W95001 and W95006	$5E^b$	59
W95001 and W95008	$7E^b$	54
W95001, W95002, and W95003	$1E^b$ and $2E^b$	1
W95001, W95003, and W95008	$2E^b$ and $7E^b$	1
W95001, W95006, and W95008	$5E^b$ and $7E^b$	2
W95001 and all five lines	All five E^b above	50
W95001 but none of five	$3E^b$ and $6E^b$	68
Total		389

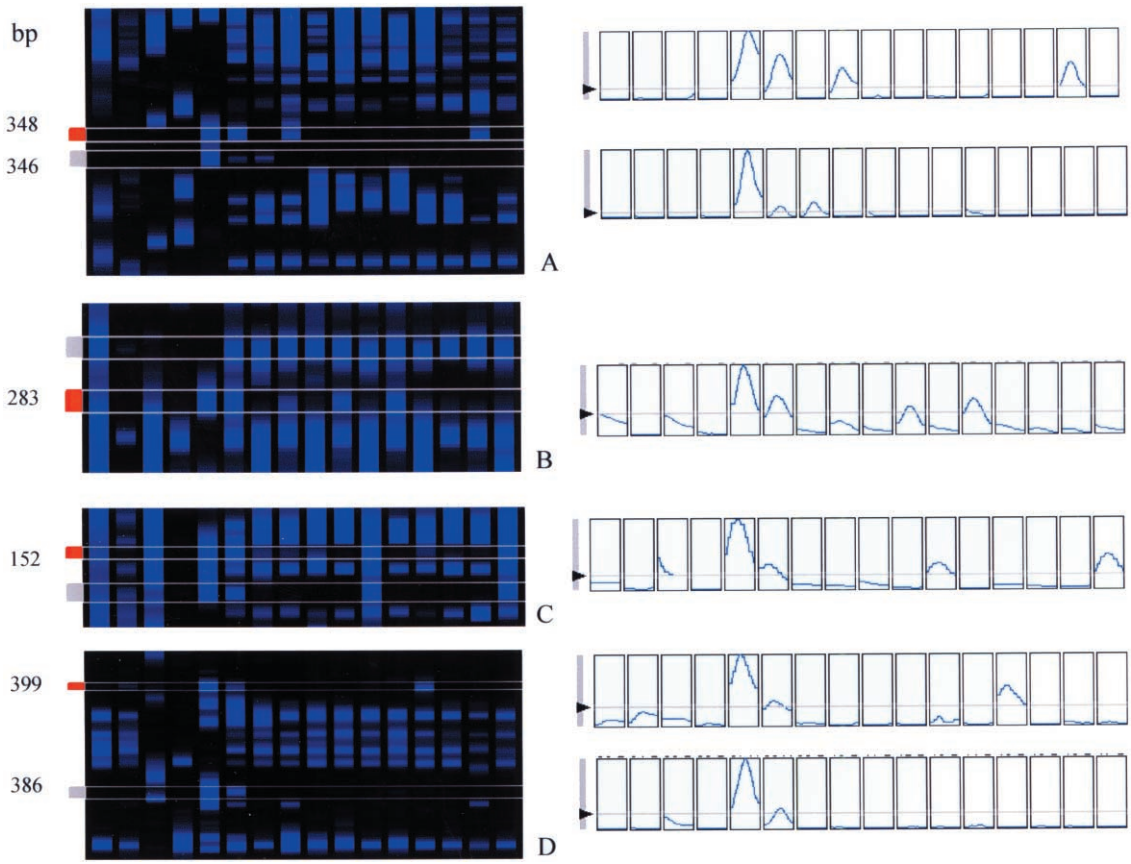

Fig. 2 Genographer-generated gel (left) and thumbnail (right) profiles of AFLP fragments from primer combinations E36M59 (*A*–C) and E36M49 (*D*). Template DNA samples and their genome constitutions are (left to right): wheat cultivar Chinese Spring (ABD), cultivar Genaro-81 (ABD), *Pseudoroegneria stipifolia* (St), *Thinopyrum elongatus* (E^e), *Thinopyrum bessarabicum* (E^b), W95001 (21″ ABD + 7″ E^b), W95002 (21″ ABD + 1″ $1E^b$), W95003 (21″ ABD + 1″ $2E^b$), W95004 (21″ ABD + 1″ $7E^b$-wh), W95005 (21″ ABD + 1″ $4E^b$), W95006 (21″ ABD + 1″ $5E^b$), W95007 (21″ ABD + 1″ $4E^b$), W95008 (21″ ABD + 1″ $7E^b$), W95009 (21″ ABD + 1″ ?E^b-wh), $2E^b$ addition line, and $5E^b$ addition line. The last two are those from King et al. (1996). The thumbnail profiles demonstrate the AFLP markers E36M59.348 (top *A*) for $2E^b$, E36M59.346 (bottom *A*) for $1E^b$, E36M59.283 (*B*) for $4E^b$, E36M59.152 (*C*) for $5E^b$, E36M49.399 (top *D*) for $7E^b$, and E36M49.386 (bottom *D*) for the missing $3E^b$ or $6E^b$. Note the difference in intensity of each marker between the diploid E^b species (lane 5) and the partial amphidiploid (lane 6) due to the relative proportion of the target chromosome in the total genome content, i.e., one out seven in the diploid E^b and one out of 28 in the partial amphidiploid.

wide distribution in the E^b genome. In lines W95002, W95003, and W95006, 48, 67, and 59 specific AFLP markers were identified for the $1E^b$, $2E^b$, and $5E^b$ chromosomes, respectively. W95008 had 29 specific bands and shared 25 additional markers with line W95004. W95004 lacked unique AFLP markers. Thus, the alien chromosome arm in W95004 must have been a part of $7E^b$, which was intact in W95008.

W95005 and W95007 shared 39 AFLP markers, i.e., there was not a single marker unique to either line. That number was significantly higher than that found for markers specific to any two E^b chromosomes, strongly indicating that both lines have the same E^b chromosome. Indeed, both lines had blue endosperm in the seed, a morphological character specific to homoeologous group 4. Therefore, W95005 and W95007 most likely have a pair of $4E^b$ chromosomes. An additional 68 AFLP markers were found in the amphiploid that were absent in all the addition lines. They are potential markers for $3E^b$ and $6E^b$, the two chromosomes not represented among this cytogenetic stocks.

E^b-Specific RAPD Markers

From 52 Operon random primers, few chromosome-specific RAPD markers, ranging from one to six, were identified for each E^b chromosome in the five disomic addition lines (table 4). Six RAPD markers, including the previously reported $OPF03_{1296}$ (Zhang et al. 1996, 1998), were found in the CS/*T. bessarabicum* partial amphidiploid and in all five disomic addition lines.

Discussion

The most difficult and time-consuming process for constructing a set of addition lines can be alien chromosome iden-

Table 4

RAPD Markers Specific to E^b-Genome Chromosomes in Cytogenetic Stocks of *Triticum aestivum/Thinopyrum bessarabicum* Disomic Addition Lines

Identification	E^b chromosome	RAPD markers[a]
W95001 and W95002	$1E^b$	$OPF07_{370}$, $OPBA17_{390}$
W95001 and W95003	$2E^b$	$OPD12_{500}$, $OPP04_{350}$
W95001, W95005, and W95007	$4E^b$	$OPB09_{680}$, $OPAA11_{350}$, $OPAY05_{720}$, $OPAZ04_{1100}$, $OPAZ08_{750}$, $OPAZ13_{680}$
W95001 and W95006	$5E^b$	$OPH11_{500}$
W95001 and W95008	$7E^b$	$OPAZ06_{780}$, $OPAZ17_{610}$, $OPAY08_{720}$
W95001, W95002, and W95006	$1E^b$ and $5E^b$	$OPB16_{700}$
W95001, W95003, and W95006	$2E^b$ and $5E^b$	$OPAY16_{420}$
W95001, W95002, W95003, and W95008	$1E^b$, $2E^b$, and $7E^b$	$OPAY07_{600}$
W95001 and all five lines	All five E^b above	$OPO17_{600}$, $OPZ08_{450}$, $OPE10_{580}$, $OPF03_{1296}$, $OPAY07_{900}$, $OPAZ02_{550}$

[a] Subscript numbers are approximate lengths in base pairs of the RAPD markers, except $OPF03_{1296}$ that has been sequenced (GenBank accession U43516).

tification. Morphological traits, chromosome-banding techniques, and biochemical markers have limitations and low accuracy. GISH is the first step to unequivocally detect the presence of alien chromosomes before molecular techniques can be applied to separate these alien chromosomes into different homoeologous groups. Because the E^b genome is relatively closer to the ABD genomes of wheat, it is better to use the St genomic DNA as a probe in GISH to detect E-genome chromosomes in the wheat background (Wang and Zhang 1996; Zhang et al. 1996; Q. Chen et al. 1998). Using this technique, we determined that the seven lines studied here contained six true disomic addition lines and one tertiary disomic addition line (fig. 1).

Molecular markers such as RFLP, SSR, and radioisotope-based AFLP are not easy nor speedy methods for chromosome identification. The application of multifluorophore and semi-automated AFLP analysis on a DNA sequencer provides an expeditious and accurate method (Schwarz et al. 2000). Results reported in this study also show that AFLP is a powerful technique to detect and identify alien chromosomes. For instance, the selective primer pair E-ACC/M-CTA (=E36M59) alone can amplify one marker (E36M59.346) for $1E^b$, four (E36M59.109, E36M59.191, E36M59.308, and E36M59.348) for $2E^b$, two (E36M59.162 and E36M59.283) for $4E^b$, five (E36M59.149, E36M59.152, E36M59.198, E36M59.260, and E36M59.420) for $5E^b$, two (E36M59.397 and E36M59.425) for $7E^b$, and 13 for all five chromosomes. This is far more efficient than any other reported molecular method.

In a previous study, line W95007 was tentatively identified as the $6E^b$ disomic addition line based on biochemical diagnostics (William and Mujeeb-Kazi 1995). This study showed that W95007 is identical to line W95005, which has the $4E^b$, based on specific AFLP markers and the fact that both have seeds with blue aleurone. Another correction was made to line W95004, previously considered to have $3E^b$, in which the translocation chromosome was evidenced by GISH (fig. 1*C*) and its partial $7E^b$ was revealed by AFLP results (table 3). The seven addition lines currently available contain only five of the seven E^b chromosomes. The $3E^b$ and $6E^b$ addition lines are missing.

The abundance of specific DNA markers can, to some extent, reveal the divergence of the alien genome or individual chromosome from its progenitor. Shan et al. (1999) reported that 22.3% of the AFLP bands are barley chromosome-specific in a wheat background when only five disomic addition lines were studied. This is much higher than the 10.9% reported here for five E^b addition lines and indicates a more divergent relationship between the I genome of *Hordeum vulgare* and ABD of common wheat than between the E^b genome of *Thinopyrum bessarabicum* and ABD. This AFLP assay also detected more chromosome-specific markers in $2E^b$ and $5E^b$ than in other chromosomes.

Repetitive DNA fragments were also revealed by AFLP among the different E^b chromosomes. Of the 389 E^b genome–specific AFLP fragments, 50 were located on all (or at least five) E^b chromosomes. Twenty-five others were amplified in more than one disomic addition line (data not shown). Thus, ca. 19% of AFLP bands occurred in more than one E^b chromosome. This is higher than that found in a study of AFLP on *Allium cepa* chromosomes (van Heusden et al. 2000). More duplicated AFLP fragments were identified from $2E^b$, $5E^b$, and $7E^b$ than from any other E^b chromosomes.

The number of AFLP and RAPD markers assigned to individual E^b chromosome was not evenly distributed, ranging from 39 for $4E^b$ to 67 for $2E^b$. Fifty-nine AFLP markers were identified for $5E^b$, which ranked as the second. For all E^b or at least the five chromosomes presently studied, 50 common markers were found. Additionally, 68 markers were assumed to be located on the two missing chromosomes, $3E^b$ and $6E^b$. Most of the 68 markers can be used to search for these desired addition lines.

The AFLP technique is an efficient DNA fingerprinting tool that can generate a large number of molecular markers. However, most of the AFLP markers are difficult to convert to sequence-tagged-site (STS) markers (Shan et al. 2000). The requirement of sophisticated instruments and expensive reagents also limit its usage. However, the RAPD technique can be used by almost every laboratory and most RAPD markers can be easily converted to STS markers. Therefore, we investigated E^b chromosomes using both AFLP and RAPD techniques and report here both types of markers. These AFLP and RAPD markers are useful in chromosome identification, mapping, and introgression.

In conclusion, using both GISH and molecular markers we

made an accurate identification of the added alien chromosomes in the first incomplete set of addition lines derived from the partial amphidiploid of *Triticum aestivum* × *T. bessarabicum*. We will continue to use these techniques to construct a complete set of seven addition lines in a wheat background other than Chinese Spring.

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