EST-derived SSR markers from defined regions of the wheat genome to identify *Lophopyrum elongatum* specific loci

Daniel J. Mullan, Amanda Platteter, Natasha L. Teakle, Rudi Appels, Timothy D. Colmer, Joseph M. Anderson, and Michael G. Francki

Abstract: *Lophopyrum elongatum*, a close relative of wheat, provides a source of novel genes for wheat improvement. Molecular markers were developed to monitor the introgression of *L. elongatum* chromosome segments into hexaploid wheat. Existing simple sequence repeats (SSRs) derived from genomic libraries were initially screened for detecting *L. elongatum* loci in wheat, but only 6 of the 163 markers tested were successful. To increase detection of *L. elongatum* specific loci, 165 SSRs were identified from wheat expressed sequence tags (ESTs), where their chromosomal positions in wheat were known from deletion bin mapping. Detailed sequence analysis identified 41 SSRs within this group as potentially superior in their ability to detect *L. elongatum* loci. BLASTN alignments were used to position primers within regions of the ESTs that have sequence conservation with at least 1 similar EST from another cereal species. The targeting of primers in this manner enabled 14 *L. elongatum* markers from 41 wheat ESTs to be identified, whereas only 2 from 124 primers designed in random positions flanking SSRs detected *L. elongatum* loci. Addition and ditelosomic lines were used to assign all 22 markers to specific chromosome locations in *L. elongatum.* Nine of these SSR markers were assigned to homoeologous chromosome locations based on their similar position in hexaploid wheat. The remaining markers mapped to other *L. elongatum* chromosomes indicating a degree of chromosome rearrangements, paralogous sequences and (or) sequence variation between the 2 species. The EST-SSR markers were also used to screen other wheatgrass species indicating further chromosome rearrangements and (or) sequence variation between wheatgrass genomes. This study details methodologies for the generation of SSRs for detecting *L. elongatum* loci.

Key words: Lophopyrum elongatum, expressed sequence tags (EST), simple sequence repeat (SSR), EST-SSR, synteny, alien introgression.

Résumé : Le *Lophopyrum elongatum*, un proche parent du blé, constitue un réservoir de gènes d'intérêt pour l'amélioration génétique du blé. Des marqueurs moléculaires ont été développés pour suivre l'introgression de segments chromosomiques du *L. elongatum* chez le blé hexaploïde. Des microsatellites existants provenant de banques génomiques ont d'abord été criblés pour détecter des locus polymorphes chez le *L. elongatum*, mais seulement 6 de 163 marqueurs étaient informatifs. Afin d'accroître le taux de succès chez le *L. elongatum*, 165 microsatellites ont été identifiés au sein d'EST (étiquettes de séquences exprimées) du blé dont la position chromosomique était déjà connue par cartographie de délétants. Des analyses détaillées des séquences ont permis d'identifier 41 microsatellites potentiellement supérieurs pour la détection de locus chez le *L. elongatum*. Des alignements BLASTN ont été employés pour situer les amorces au sein de régions conservées chez au moins un autre EST semblable provenant d'une autre céréale. Un tel ciblage des amorces a permis d'obtenir 14 marqueurs polymorphes chez le *L. elongatum* parmi les 41 EST analysés, alors que seulement 2 des 124 amorces placées au hasard autour du microsatellite auront révélé du polymorphisme à ces locus chez le *L. elongatum*. Des lignées d'addition et ditélosomiques ont été employées pour déterminer

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D.J. Mullan, N.L. Teakle, and T.D. Colmer. School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia.
A. Platteter and J.M. Anderson. USDA-ARS, Department of Agronomy, Purdue University, West Lafayette, IN 47907–2054, USA.
R. Appels. Department of Agriculture and Molecular Plant Breeding Cooperative Research Centre, Locked Bag No. 4, Bentley, WA 6983, Australia.
M.G. Francki.[1] Department of Agriculture and Value Added Wheat Cooperative Research Centre, Locked Bag No. 4, Bentley, WA 6983, Australia.
D.J. Mullan, N.L. Teakle, R. Appels, and M.G. Francki. WA State Agricultural Biotechnology Center, Murdoch University, Perth, WA 6150, Australia.

[1]Corresponding author (e-mail: mfrancki@agric.wa.gov.au).

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l'emplacement des 22 marqueurs. Neuf de ces microsatellites ont été assignés à des locus homéologues par comparaison à la situation chez le blé. Les autres étaient situés à des sites nonhoméologues chez le *L. elongatum*, ce qui indique un degré de réarrangement chromosomique, de séquences paralogues ou de variation de séquence entre ces 2 espèces. Les microsatellites codants (EST-SSR) ont également été utilisés pour examiner d'autres espèces d'agropyres et ces analyses ont suggéré d'autres réarrangements chromosomiques ou variation de séquence entre les génomes d'agropyres. Cette étude décrit des méthodologies pour la production de microsatellites en vue de la détection de locus chez le *L. elongatum*.

Mots clés : Lophopyrum elongatum, étiquettes de gènes exprimés, microsatellites, EST-SSR, synténie, introgression de gènes étrangers.

[Traduit par la Rédaction]

Introduction

Tertiary gene pools from related species are an attractive source of new genetic variation for wheat improvement. *Lophopyrum elongatum* (Host) A. Lőve [syn. *Thinopyrum elongatum* or *Agropyrum elongatum*], a closely related species to bread wheat, is tolerant to many biotic and abiotic stresses. *Lophopyrum elongatum* has been exploited for a wide range of traits including resistance to pests and diseases (Sharma et al. 1989; Shukle et al. 1987; Friebe et al. 1996; Yang and Ren 2001; Shen et al. 2004), and adaptive mechanisms to drought (Roundy 1985), waterlogging (Taeb et al. 1993), and salinity (Omielan et al. 1991; Deal et al. 1999). Exploiting this genetic resource has become an objective within many wheat improvement initiatives and involves the introgression of small portions of *L. elongatum* chromosome segments into wheat. Previously, molecular techniques involving in situ hybridization and molecular markers, such as RFLPs (for review see Fedak 1999), have been used to identify small segments of alien chromatin. With the studies in this paper, the aim to define new markers to increase the efficiency of screening large population sizes for detecting small alien segments required the development of alternative marker systems.

Microsatellite markers or simple sequence repeats (SSRs) have become a valuable tool for genetic studies, as they are able to efficiently screen large population sizes. SSRs have been used in a variety of plant species and in genetic mapping initiatives for cereals including wheat (Bryan et al. 1997; Roder et al. 1998), barley (Ramsay et al. 2000), and rice (Temnykh et al. 2000). Microsatellites are highly reproducible codominant markers that offer the potential for automation, facilitating the rapid screening of plant lines (for review, see Gupta et al. 1999). The process of SSR-marker development from genomic libraries is justified for major crop species, but it is too expensive and labor intensive for related species that lack significant agricultural importance. Studies have explored the possibilities of using genomic SSRs generated from 1 species for use in related species. Rarely, however, has a significant number of SSR markers been transferred across species and used in alien introgression studies (Roder et al. 1995; Peil et al. 1998). An alternative is to use the increasing availability of other sequencing resources from crop species for developing SSR markers in related species.

The increasing availability of DNA-sequence information from public databases provides a valuable resource for SSR-marker development. The International Triticeae EST Cooperative (ITEC), together with other sequencing projects, provided more than 500 000 cereal EST sequences to the public domain. Contributing to the EST initiative is the physical mapping of more than 5000 of these sequences in wheat. Cytogenetic resources have been used to position wheat ESTs into 159 deletion bins, which has resulted in assigning chromosomal locations to more than 16 000 loci across the wheat genome (http://wheat.pw.usda.gov/wEST/binmaps/) (Qi et al. 2002). This mapping information is a useful resource for the development of novel molecular markers. Expressed sequences represent transcriptional regions of the genome and show a high degree of DNA conservation between species. Consequently, utilizing this sequence information for the generation of SSRs may increase the potential of detecting *L. elongatum* loci in wheat. Furthermore, the assignment of ESTs to specific locations on the wheat genome and their potential transferability enables detailed comparative genome analysis between species. The exploitation of existing EST-sequence information offers a new resource for the development of SSR markers suitable for detecting *L. elongatum* loci in wheat.

The main aim of this study was to use mapped wheat ESTs as the basis for developing SSR markers capable of detecting *L. elongatum* loci in wheat. The objectives included (*i*) comparing the ability of genomic-derived wheat SSR markers to detect *L. elongatum* loci, (*ii*) assigning EST-SSR markers to regions of *L. elongatum* chromosomes, and (*iii*) comparing the physical locations of the EST-SSRs on wheat and *L. elongatum* chromosomes.

Materials and methods

Plant materials and DNA extraction

Bread wheat (*Triticum aestivum* L. 'Chinese Spring' (CS); $2n = 6x = 42$; genome AABBDD) and a CS – *Lophopyrum elongatum* amphiploid (Host A. Lőve; $2n = 8x = 56$; genome AABBDDEE) were used to first identify *L. elongatum* loci. Seven wheat – *L. elongatum* addition lines and 12 ditelosomic lines (4ES and 5ES lines were unavailable) were also used to assign loci to *L. elongatum* chromosome arms. The addition lines were checked cytogenetically and contained the correct chromosome complement, except for the 1E addition line, which contained either monosomic or disomic *L. elongatum* chromosomes (McDonald et al. 2001). Additionally, nullitetrasomic lines were selected to confirm the deletion mapping information for some of the ESTs used in this study. The cytogenetic stocks were obtained from J. Dvorák (University of California, Davis, California, USA)

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(Dvorák and Knott 1974). The *Lophopyrum intermedium* allohexaploid (‘Oahe’; $2n = 6x = 42$; genome EEEEXX), and P29, a group 7 wheat – *L. intermedium* disomic substitution line (Sharma et al. 1997), obtained from H. Ohm (Purdue University, Indiana, USA) were also analyzed. DNA from all lines was extracted from leaves following the phenol–chloroform extraction method of Francki et al. (1997).

Genomic SSRs and database mining of mapped ESTs for EST-SSR development

Wheat genomic SSRs were selected from across the wheat genome for detection of *L. elongatum* loci. One hundred thirty-eight *Xgwm* (Roder et al. 1998) and 25 *Xgdm* (Pestova et al. 2000) SSRs were used for this purpose.

As of November 2003, a total of 5651 5′-EST sequences consisting of 16 603 mapped loci in wheat were available from the EST deletion mapping initiative (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). EST sequences from each of the 7 wheat chromosome groups were downloaded in FASTA format for input into SSR detection and analysis software. RepeatMasker software (http://www.repeatmasker.org/) was used to screen the ESTs for simple and compound microsatellites using default settings, with “DNA source: grasses”, and the “only mask simple sequence repeat and low complexity DNA” option selected. EST-SSRs with at least 5 di-, tri-, tetra-, or penta- repeats were selected.

Identification of conserved regions of ESTs and primer design for detecting *L. elongatum* loci

The ESTs containing SSRs were used in BLASTN analysis (version 2.2.6) against EST databases from wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), maize (*Zea mays*), rye (*Secale cereale*), and *Triticum monococcum*. Conserved regions within the ESTs that flanked the SSR motif were identified by sequence alignment with at least 1 other grass species and defined as having greater than 85% identity, or 80% identity with 2 other species over the primer annealing site. BLASTN analysis used “cost to open a gap: 11” and “cost to extend a gap: 1” parameters.

Primer3 and Primer Express software were used to design primers flanking SSRs (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) (Rozen and Skaletsky 2000; Applied Biosystems). Sequence information was provided in FASTA format, with the “target” option used to ensure primers were designed within conserved regions flanking the repeat motif. Default settings were used from Primer3 and Primer Express software, with “optimum primer size: 20 bp” and “optimum temperature: 60 °C”. When using Primer3 software, GC% was restricted from 20% to 80%. Primers were designed to amplify products of size 150–500 bp and were assigned a *Xedm* (X EST derived microsatellite) designation according to the order in which they were identified. The strategy for identifying EST-SSRs and primer design is detailed in Fig. 1.

PCR amplification and allele sizing

Amplification of EST-SSR markers and the identification of *L. elongatum* loci was first optimized using CS and CS – *L. elongatum* amphiploid genomic DNA. Annealing temperatures were optimized for each primer pair, with the thermal cycling conditions outlined in Table 1. Those SSRs able to detect *L. elongatum* amplicons in the amphiploid were screened against DNA from the 7 addition lines and 12 ditelosomic lines to assign markers to *L. elongatum* chromosome arm locations. PCRs contained 1.5 mmol $MgCl_2$/L, 0.2 mmol of each deoxynucleotide/L, 0.3 mmol of each primer/L, 1× PCR buffer (Fischer Biotech, Perth, Australia), 0.5 U *Taq* DNA polymerase (Fischer Biotech, Perth, Australia), and 25 ng template DNA in a 25 μL reaction volume. PCR products were separated on 8% polyacrylamide gels in 0.5× Tris–borate–EDTA using a Protean II xi Cell gel system (BioRad, California, USA). Gels were electrophoresed at constant voltage (90 V) for 16 h, stained with ethidium bromide, and visualized under UV light with a Gel Doc System (BioRad, Milan, Italy).

Results

Identification of genomic and EST-SSRs detecting *L. elongatum* loci in wheat

SSRs derived from genomic libraries and previously reported to be distributed across the wheat genome were initially screened to assess their suitability to detect *L. elongatum* loci in wheat. In a preliminary analysis, 138 *Xgwm* (Roder et al. 1998) and 25 *Xgdm* (Pestova et al. 2000) markers were used to screen genomic DNA from CS and the amphiploid. Only 6 SSRs derived from genomic libraries detected polymorphic *L. elongatum* amplicons using a range of PCR cycling conditions (Table 1), indicating low levels of detectable *L. elongatum* markers (Table 2).

To increase the detection of loci *L. elongatum*, SSR markers were developed from wheat ESTs. Initially, primers were designed that randomly flanked repeat motifs within the ESTs. One hundred twenty-four *Xedm* markers were designed and screened against DNA from CS and the CS – *L. elongatum* amphiploid. Eighty-eight markers amplified CS DNA, but only 2 of these detected *L. elongatum* amplicons, representing less than 3% efficiency in marker identification. The low efficiency when primers were designed randomly flanking repeat motifs supported the need for a more targeted approach to primer design.

The efficiency of loci detection was increased through identifying SSR markers by having primers located within conserved flanking regions of wheat ESTs (see Fig. 1 for a summary of this strategy). An example of primers positioned within conserved regions in an EST is shown in Fig. 2. The multiple sequence alignment of *Xedm*80 shows the forward and reverse primers located within conserved regions flanking the repeat motif. The forward primer has 100% identity with a *T. monococcum* and barley EST, and the reverse primer has 100% identity with *T. monococcum* and 80% barley. Variation in repetitive sequences between members of the Triticeae was observed, as the number of (GCT) repeats is reduced from 8 in wheat to 4 in *T. monococcum*, and none in barley. A total of 41 *Xedm* markers were developed by targeting primers within similar conserved EST regions, and 39 amplified wheat genomic DNA (Table 3). The screening of CS and the CS – *L. elongatum* amphiploid showed that 14 of these *Xedm* markers amplified 17 loci in *L. elongatum* (Table 2) indicating a significant improvement in the efficiency of detecting *L. elongatum* markers in wheat. An ex-

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Fig. 1. Strategy for identifying and designing SSRs from wheat ESTs for amplification of *Lophopyrum elongatum* loci in wheat.

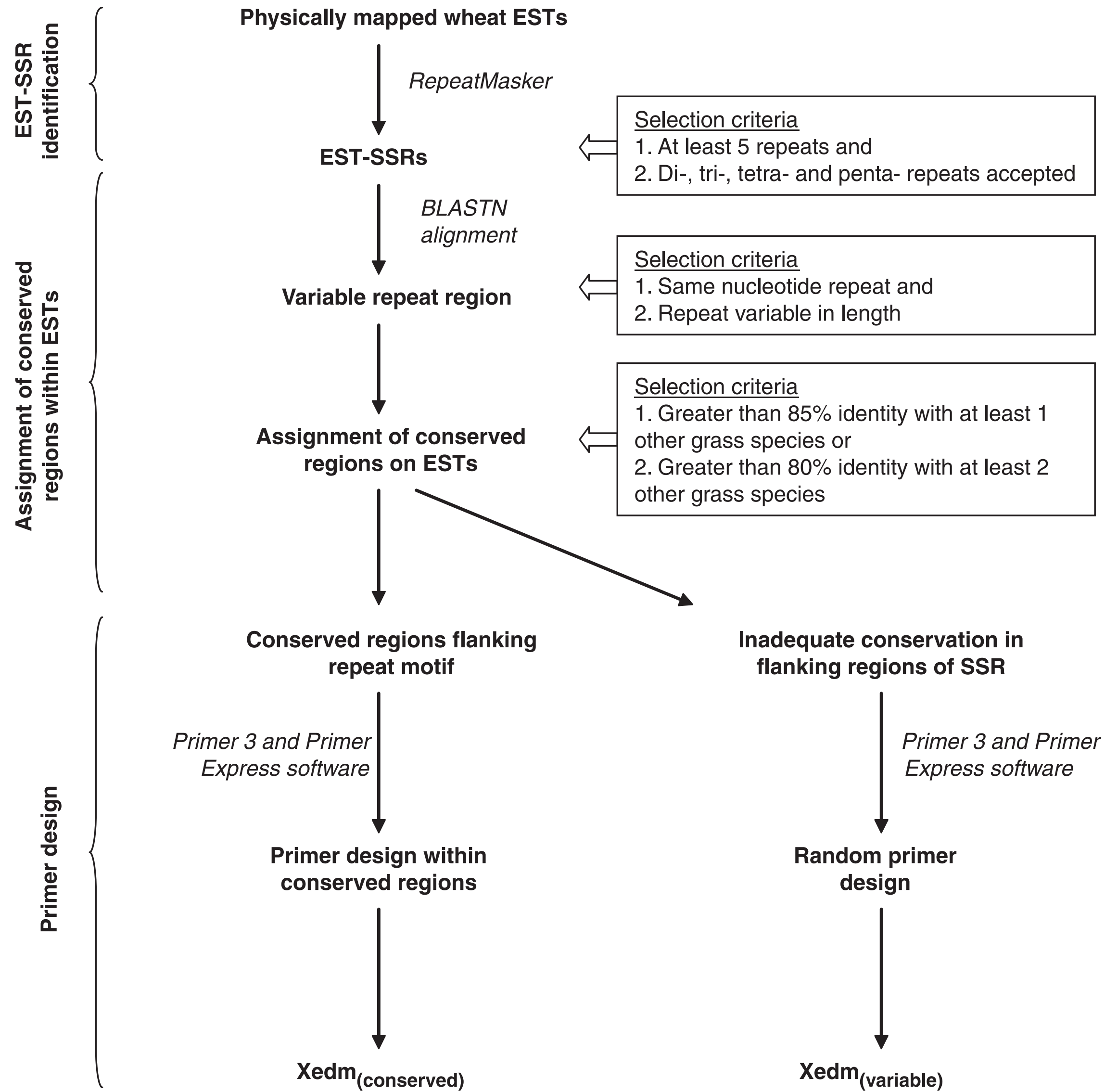

Table 1. PCR conditions used to amplify *Xedm* and *Xgwm* SSRs using genomic DNA from wheat and wheat – *L. elongatum* aneuploid lines.

	Denature 1 cycle		Touchdown				Amplification			Extension 1 cycle
No.	3 min	No. of cycles	30 s	30 s	30 s	No. of cycles	30 s	30 s	30 s	3 min
1	94 °C	—	—	—	—	40	94 °C	50 °C	72 °C	72 °C
2	94 °C	—	—	—	—	40	94 °C	55 °C	72 °C	72 °C
3	94 °C	—	—	—	—	40	94 °C	60 °C	72 °C	72 °C
4	94 °C	10	94 °C	55–47 °C	72 °C	30	94 °C	47 °C	72 °C	72 °C
5	94 °C	10	94 °C	60–50 °C	72 °C	30	94 °C	50 °C	72 °C	72 °C
6	94 °C	10	94 °C	65–55 °C	72 °C	30	94 °C	55 °C	72 °C	72 °C

ample of the amplification of polymorphic loci using *Xedm*80 as a marker is shown in Fig. 3. A 200-bp fragment was amplified by *Xedm*80 in the amphiploid, but not detected in the wheat parent, indicating an *L. elongatum* locus. Further analysis using CS – *L. elongatum* addition and ditelosomic lines localized this marker to the short arm of the *Lophopyrum* group 6 chromosome (Fig. 3).

Assigning SSRs to *L. elongatum* chromosome locations

To provide an informative source of markers, SSRs were

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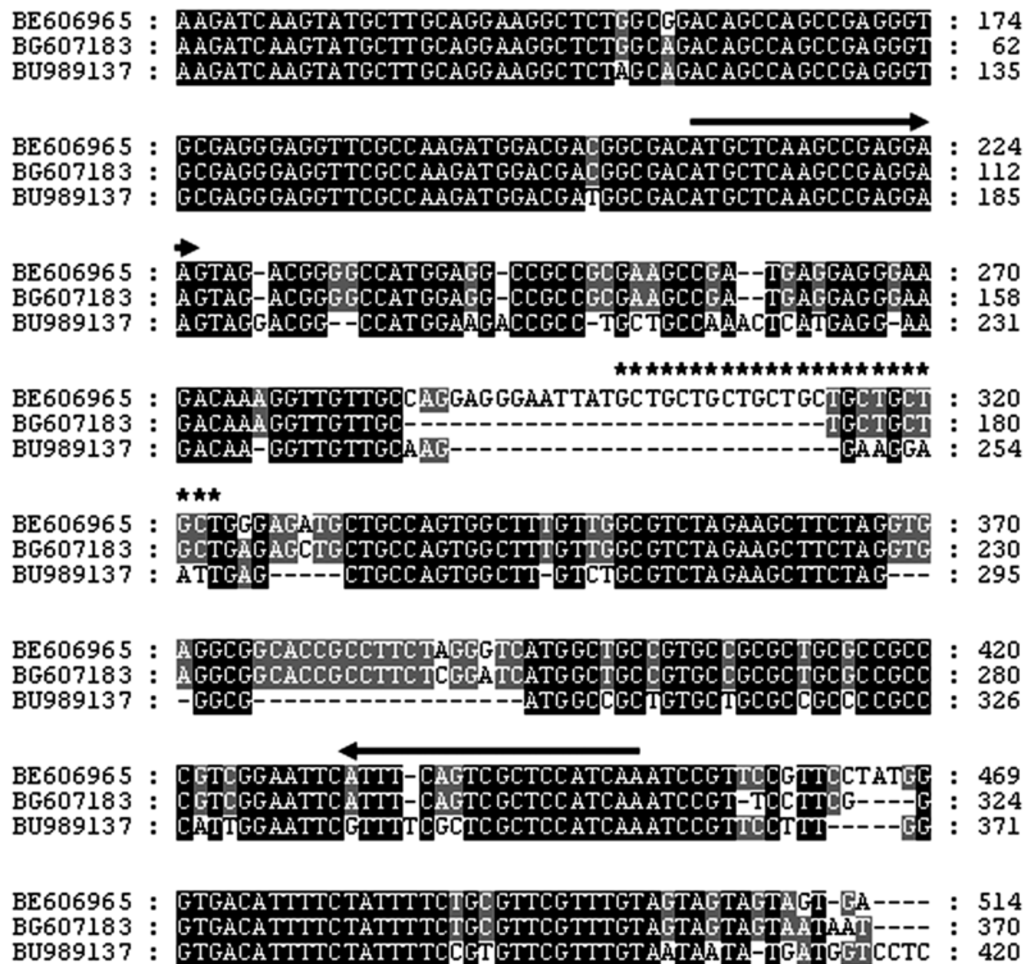
Table 2. Summary of SSR markers detecting *Lophopyrum elongatum* loci in wheat.

	Total no. of markers screened	No. of markers detecting polymorphic *L. elongatum* loci	No. of polymorphic *L. elongatum* loci detected	Percentage detection of polymorphic *L. elongatum* loci
Xgwm and *Xgdm* SSRs	163	6	7	4.3
$Xedm_{(\text{variable})}$ SSRs	86	2	2	2.3
$Xedm_{(\text{conserved})}$ SSRs	39	14	17	44

Note: *Xgwm* and *Xgdm* markers were derived from genomic libraries (Roder et al. 1998; Pestova et al. 2000), $Xedm_{(\text{variable})}$ SSRs were designed with primers randomly positioned in unconserved regions of wheat ESTs, while $Xedm_{(\text{conserved})}$ SSRs were designed with primers within conserved regions of ESTs.

Fig. 2. Multiple sequence alignment of *Xedm*80 (BG606965) with nucleotide sequences from *Triticum monococcum* (BG607183) and barley (BU989137). Arrows above nucleotide sequence indicate forward and reverse primers, and asterisks indicate the repeat motif. Pileup (GCG) software (Genetics Computer Group, Madison, Wisconsin, USA) was used for alignment of sequence with "gap creation penalty: 11" and "gap extension penalty: 1". Prettybox (GCG) software was used for displaying multiple sequence alignment (Genetics Computer Group Inc. 1998, Purdue University, Indiana, USA).



assigned to chromosome locations in *L. elongatum*. The 22 markers (*Xgwm* and *Xedm*) were initially assigned to chromosomes via addition lines, and then the markers assigned chromosome arm locations by screening with ditelosomic lines. The location of all *Xgwm* and *Xedm* markers detecting *L. elongatum* loci in wheat are shown in Fig. 4. A total of 26 loci are distributed across 6 of the 7 chromosomes, with 4 markers (*Xgwm*335, *Xedm*8, *Xedm*28, and *Xedm*96) amplifying loci on more than 1 chromosome. Interestingly, no markers were assigned to *L. elongatum* group 4. Nine of the EST-SSR markers were assigned to more precise *L. elongatum* chromosome locations, based on their position on wheat nonhomoeologous chromosome arms by deletion mapping. Table 3 shows that each of the remaining *Xedm* markers were derived from wheat chromosome groups 1–5 and were located on nonhomoeologous *L. elongatum* chromosomes 2, 3, 5, 6, or 7. For each of these markers nullitetrasomics were used to confirm the wheat chromosomal locations derived from deletion mapping (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). Five of the *Xedm*

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Table 3. Primer design and amplification of *Xedm* and *Xgwm* markers in wheat and wheat – *L. elongatum* aneuploid lines.

SSR ID	GenBank accession No.	No. of loci	Mapped loci	Deletion map location	Repeat	Cycling conditions[a]	Left primer	Right primer	*L. elongatum* chromosome location of polymorphic loci	Designed within conserved regions?[c]
Xedm 8	BE406368	5	4	C-5AS1, 5AS1, 5BS4	(ACC)7	4	GACCCCCACTTCCTGCTC	GGGCAAAACTGTGGCTATTG	2E-S, 3E-L	Y
Xedm 15	BE423792	4	3	C-2AL1, 2AL1, 2DL3	(CAG)7	5	GATGACTCAAGTGGCCAACC	GTTCCTCGGCTTCTTGGAC	—	Y
Xedm 16	BE424364	3	3	7AL21, 7BL10, 7DL2	(CGG)5	5	TCACCTAACAGCACCACGAG	GCCGAGTACCAGCAGTACCA	7E-S	Y
Xedm 17	BE424439	—	—	1[b]	(CAA)5	6	TAGCTGCATCTCTGGTTTGG	CTAGTTGTTGTTGCTGCGAAA	1E	Y
Xedm 24	BE443931	3	3	1AS3, 1BSsat18, 1DS5	(CGA)8	6	GCTTAGCTGATGGCTCGTCT	GCGCACTGTAATGTTGGTTG	—	Y
Xedm 26	BE444101	8	5	5AL10, 5BL16, 5DL5, 7AS5, 7DS4	(GA)11	5	GGGAATCGAACCATTGCTTA	AGGGATCAGCTGAAGCAAGA	—	Y
Xedm 28	BE444731	3	2	4BL1, C-4DL9	(GGAA)6	5	GCTCACTCACGCATCATAGC	GTTGGCGGAATCCTTCTTC	2E-S, 3E-L	Y
Xedm 34	BE489982	3	1	7BS1	(CA)9	5	TTGTTTCTTGGCTGGGTTTC	GATCTGCCAAAGCCTTCATAA	7E-S	Y
Xedm 46	BE498388	4	3	C-2AS5, 2BS1, 2DS5	(GGGAA)5	5	CCGCCGTCCGTCCT	GCAGGAGGCCTCGTTGTAG	5E-L	N
Xedm 51	BE498948	5	3	5AL, 1AS3, 1BSsat19	(GGA)10	5	AGGGCAAGTTCGATCTACGA	CAAGTTCCACGGAACCAACT	—	Y
Xedm 54	BE499184	3	2	2AL, 5BS6	(GA)18	1	CGTCCTTGTTCTTGCTTCAA	TGAGCAGGCCATAAGGAAAC	5E-S	N
Xedm 61	BE500714	3	3	C-1AS1, C-1BS10, C-1DS3	(GAAA)5	6	CATCATAGGCGAGATGGACA	TGGGATGGTGTTGTATGCTG	—	Y
Xedm 74	BE604050	3	2	1AL3, 1DL2	(GGA)5	5	GTAAGTCGACCGAGGAGACG	CCTTCTTGCTTGGCATTCTC	1E-L	Y
Xedm 75	BE604051	3	1	1DL2	(TGG)6	6	TCGGAGATGGAGCTGGAG	CATAACAGTGCCCACACCAC	—	Y
Xedm 80	BE606965	3	1	C-3DS3	(TGC)8	5	ATGCTCAAGCCGAGGAAGTA	TTGATGGAGCGACTGAAATG	6E-S	Y
Xedm 92	BF202718	2	1	1DL2	(CATAT)5	5	ATCCGCGACGACAATGTAAG	CTGGTGTGGATCTTGACGTG	1E-L	Y
Xedm 94	BF292128	5	1	7DS4	(GCC)5	6	CTACTGCTGGCACTGCTGCT	CTGTATTGGCGGCATGATCT	—	Y
Xedm 96	BF292343	4	1	3AS4	(AGC)8	5	TCCAAGACTGTGCCTCAAGA	GCGTTGAACTCCTGTTCCAT	2E-S, 3E-S	Y
Xedm 97	BF293021	8	4	2AL1, 2BL2, 2DL3	(TGTC)5	6	AGCTGGGGAAGTCCTGATG	CTGGACCAGCAAGGAAATGT	—	Y
Xedm 104	BF474264	—	—	2[b]	(TGA)5	6	AAGCTTGGTCGTGGAAGAGA	CGGGACCTGTGCTTCTTATC	—	Y

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Table 3. (*continued*).

SSR ID	GenBank accession No.	No. of loci	Mapped loci	Deletion map location	Repeat	Cycling conditions[a]	Left primer	Right primer	*L. elongatum* chromosome location of polymorphic loci	Designed within conserved regions?[c]
Xedm 105	BF474428	3	2	7AL21, 7BL7	(CGG)5	5	ACCGCCAGGGA GCTCTGC	GATGTCCTTCTG GCCGTACT	7E-L	Y
Xedm 109	BF483478	6	4	3BL7, C-7BS1, 7DS5	(GAA)6	5	ATGAGGCACAA GTGGATGGT	CTCCTGAAGGG AAAAGCTCA	7E-S	Y
Xedm 111	BF483478	6	4	3BL7, C-7BS1, 7DS5	(GAA)6	5	TGAAGATGGAG CAGGAGCTT	TGGATTTCTCCC TCAGGAAA	—	Y
Xedm 120	BG263656	9	4	C-5AL10, C-5BL14	(TCC)6	5	ACCGTCCCTGCC CTCTTC	GGGAACCTTCC AAGATGTGA	—	Y
Xedm 121	BG264057	6	4	C-2AL1, 2BL2, 2DL3	(CGG)9	6	ACCATCGTGAAG ACGAGAGC	CAGATCTGGCAG TCGATGG	—	Y
Xedm 129	BE426728	5	1	6DS2[b]	(GAT)7	4	AAGAAAGGTGC TTCGTCCAA	GTTGCTGCACTC AGCTGTTC	—	Y
Xedm 139	BE494877	3	3	1AS3, 1BSsat18, 1DS5	(CAGG)6	4	GCAGGCAAGAG AGGAGACG	CAGGTTGTGGCC GTAGGT	—	Y
Xedm 140	BE499510	6	2	1AL3, 1BL1	(CGA)6	5	CCTCCGACGAGA TCTGCTAC	GCTTTGGCTTCC GGTACG	6E	Y
Xedm 141	BE398320	2	2	2AS, 2BL	(CTT)6	5	GACTGAGGGGT TGACAAAGC	CTGTCAGCACTT TCTGAGTTCC	—	Y
Xedm 142	BE403860	10	8	2AS5, 2DS5, 4AL12, 4BS1, 4DS2, 7BL7, 7DL2	(CGG)5	5	CGGGGTTCAGG TTCCAC	GGCTCGATCTTG TTGAGGTC	—	Y
Xedm 143	BE442751	8	1	2DS5	(CTTTT)5	6	GCAATATCGGCA TCCTTTTG	ATCCACATTCCT TGCAAACC	—	Y
Xedm 144	BE489333	1	1	2AS5	(CGG)5	5	GTCGGGATCTTG CTGCTG	GCATGTGGCAGA GCATGAC	—	Y
Xedm 147	BF200700	5	3	C-3AL3, C-3BL2, 3D	(GCC)5	4	CCAGGCCAATCA CATCG	GCCTAGGTGCTC AGTTCCAC	—	Y
Xedm 148	BF292124	20	7	3BS1, 4AL4, 4BL1, 4DL13, 6BS, 6DL6	(AGG)4 (AGG)3	4	CCAGACGAGGA GGAGGAG	ATTGACGGTGTC GAGGTAGG	—	Y
Xedm 149	BE498400	4	2	5BL16, 6BL5	(TTCT)8	4	ATCCACGCCAAG CAGAAG	CTGTGGGAAGA AGTGCCTTG	6E-L	Y
Xedm 151	BE405680	3	2	C-6AL4, 6DL6	(CCAT)6	5	ACAGATGGTCCA CCTCCAAG	CCCACGGCTACA AGTGTCC	—	Y
Xedm 153	BE498715	9	2	7DL2	(GGA)7	5	GAGGAGCCCAA GCAGCAG	GTCGTCGTACTC CATCGTCA	—	Y
Xedm 154	BE498933	5	5	5AL12, C-5DL1, 7AS5, 7BS1, 7DS4	(GAA)5	5	GGAGAGGCCAC TGTTTTTGA	GGCACATCACTC CCTTCTTC	—	Y
Xedm 155	BE606843	3	3	7AL18, 7DL2	(GCC)4 (TCC)3	4	CGTCTCCGTCTC CACCAC	GATCTCTGGTGA AGCGTTCC	—	Y
Xedm 158	BE489333	1	1	2AS5	(CGG)5	5	CTACGTCGTCTA CCGCTGCT	CTTGGGCATCAT CGATCTTC	7E	Y

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Table 3. (*concluded*).

SSR ID	GenBank accession No.	No. of loci	Mapped loci	Deletion map location	Repeat	Cycling conditions[a]	Left primer	Right primer	*L. elongatum* chromosome location of polymorphic loci	Designed within conserved regions?[c]
Xedm 159	BE490444	14	7	2BS3, 2DS53	(CCCCAG)4	6	GGCCAATCAAG AAGCACATC	GAAGGAACGGG GTGGTAGTC	—	Y
Xgwm 149	—	—	—	4BL5	(GA)23	2	CATTGTTTTCTG CCTCTAGCC	CTAGCATCGAAC CTGAACAAG	6E-S	—
Xgwm 156	—	—	—	C-5AL-12	(GT)14	3	CCAACCGTGCTA TTAGTCATTC	CAATGCAGGCCC TCCTAAC	7E-S	—
Xgwm 179	—	—	—	5AL	(GT)15	2	AAGTTGAGTTGA TGCGGGAG	CCATGACCAGCA TCCACTC	6E	—
Xgwm 205	—	—	—	5AS3, 5DS	(CT)21	3	CGACCCGGTTCA CTTCAG	AGTCGCCGTTGT ATAGTGCC	5E-S	—
Xgwm 292	—	—	—	C-5DL1	(CT)38	3	TCACCGTGGTCA CCGAC	CCACCGAGCCGA TAATGTAC	6E-S	—
Xgwm 335	—	—	—	5BL6	(GA)14 (GCGT)3	2	CGTACTCCACTC CACACGG	CGGTCCAAGTG CTACCTTTC	5E-L, 7E-L	—

Note: ESTs from which *Xedm* SSRs were designed are identified by GenBank accession number; repeat motif information and wheat deletion mapping information is provided (Sourdille et al. 2004). Primer sequence information and PCR conditions are also detailed for both *Xedm* and *Xgwm* markers. The chromosome positions of markers identifying polymorphic *L. elongatum* loci are indicated, as are those *Xedm* markers that were designed with primers in conserved regions of the wheat ESTs.

[a]Numbers refer to PCR conditions in Table 1.

[b]Deletion mapping information was removed from EST-SQL database after marker development.

[c]Indicates whether primer was designed for a conserved (Y) or variable (N) region.

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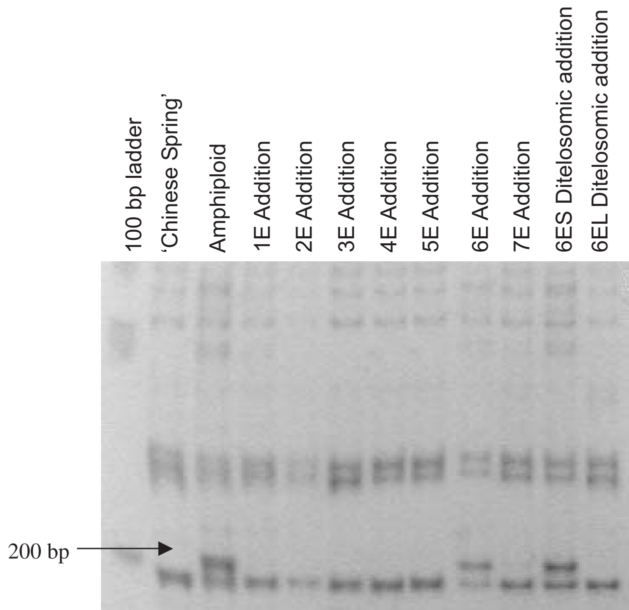
Fig. 3. EST-SSR marker *Xedm80* amplifying loci in CS, CS – *L. elongatum* amphiploid, 7 addition lines, and 2 ditelosomic lines from *L. elongatum* chromosome 6E. Bands were separated on an 8% polyacrylamide gel, stained with ethidium bromide, and visualized under UV light. Band sizes were estimated from the 100-bp ladder included with the 200-bp *L. elongatum* band shown by the arrow.



markers were confirmed to be on the expected wheat chromosome, whereas 2 mapped to other regions. The assignment of SSRs to *L. elongatum* chromosomes enables these *Xedm* markers to be used as more informative markers for monitoring alien chromosome introgression.

Transferability of SSRs to *L. intermedium*

The ability of EST-derived SSR markers to detect loci in related wheatgrass species was assessed through amplification of *Lophopyrum intermedium* genomic DNA. Four *Xedm* markers (*Xedm*16, *Xedm*34, *Xedm*105, and *Xedm*109) positioned on *L. elongatum* chromosome 7E were used to amplify loci in an *L. intermedium* group 7 substitution line containing chromosome 7X 7E (P29). Although sequences were amplified in *L. intermedium* 'Oahe' (original donor of alien group 7 in P29), no marker was able to distinguish *L. intermedium* group 7 from wheat loci in P29 (Fig. 5).

Discussion

The design of SSRs from EST databases is a new ap proach to identifying markers for detecting alien chromatin in wheat. The present study described the development of SSR markers for detection of *L. elongatum* loci. Although genomic-derived SSRs and randomly designed primers flanking SSRs in ESTs were screened, their low efficiency in detecting loci (5% and 2%, respectively) emphasized the importance of using a more targeted approach. Transfer of wheat EST-SSRs to *L. elongatum* was achieved through the design of primers in conserved regions identified by BLASTN searching of related ESTs from other species. The identification of *L. elongatum* loci and their ability to be assigned chromosomal locations makes EST-SSRs attractive markers for the characterization of wheat lines containing *L. elongatum* chromosome segments.

In this study, more than 150 SSR markers developed from wheat genomic libraries (Roder et al. 1998; Pestova et al. 2000) were screened as potential *L. elongatum* markers, but only 6 identified *L. elongatum* amplicons in wheat. The low number of markers detecting in *L. elongatum* loci in wheat is most likely due to the fact that more than 75% of the wheat genome is composed of repetitive DNA (for a recent review, see Appels et al. 2003), which are typically species specific. Therefore, a large proportion of genomic-derived SSRs may have been derived from repetitive elements that were not transferable across species to *L. elongatum*. Previous work has demonstrated that the transferability of genomic SSRs across plant species is unpredictable. For example, studies between wheat and *Hordeum chilense* report that 36% of the *Xgwm* SSR markers tested could be used for analysis of introgression lines (Hernandez et al. 2002), whereas other studies show that transferability is lower within families and species (Brown et al. 1996; Chee et al. 2004). Inability to detect loci across species is most likely due to primers being positioned within regions of the genome that have inconsistent sequence conservation. By ensuring that targeted sequences are highly conserved between 2 species, microsatellite markers derived from ESTs are likely to be transferable across species. The methodologies detailed in this study could be applied to other species, in an attempt to increase SSR availability and for comparative genome analysis, particularly in those species without extensive genomic and DNA-marker resources. For example, recent studies across legume species have shown DNA-sequence conservation of expressed sequences between *Medicago truncatula*, *Glycine max*, and *Arabidopsis thaliana* (Yan et al. 2004) could potentially identify a number of EST-SSR markers for *Lupinus angustifolius* (Francki and Mullan 2004).

ESTs assigned to specific locations on wheat chromosomes by RFLP deletion mapping were deliberately targeted so that *L. elongatum* loci could be assigned to nonhomoeologous chromosomes. A high degree of synteny exists between wheat and many of its relatives (Dvorák 1980; Devos and Gale 1997; Appels et al. 2003). Because *L. elongatum* is also closely related to wheat, synteny may be extended to assign chromosomal locations of EST-SSRs from wheat to *L. elongatum.* Only 9 of the 16 *Xedm* markers could be assigned to homoeologous regions of *L. elongatum* chromosomes. The remaining *Xedm* markers were located to other regions of the *L. elongatum* genome, indicating either a nonsyntenic relationship between some regions of the wheat and *L. elongatum* genome or amplification of paralogous sequences when converting EST-SSRs to a PCR-based system. In this study, *Xedm* markers not assigned to homoeologous *L. elongatum* chromosomes were mapped to their respective wheat chromosomes by nullisomic–tetrasomic analysis (data not shown). The genetically mapped *Xgwm* markers and their positions on nonhomoeologous chromosomes provide further evidence for breaks in synteny between wheat and wheatgrass genomes. Dvorák (1980) reported homoeology

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Fig. 4. Chromosome locations of *Xedm* and *Xgwm* markers amplifying polymorphic loci in *L. elongatum*. Chromosome arm locations (S, short; L, long) were assigned via analysis of addition and ditelosomic lines. Markers were assigned more precise physical locations when *L. elongatum* loci amplified on chromosomes homologous to wheat locations assigned via deletion bin mapping. Markers positioned on nonhomologous *L. elongatum* chromosomes are indicated by shading; for wheat deletion bin locations refer to Table 3. Asterisk indicates SSR marker located on other *L. elongatum* chromosomes.

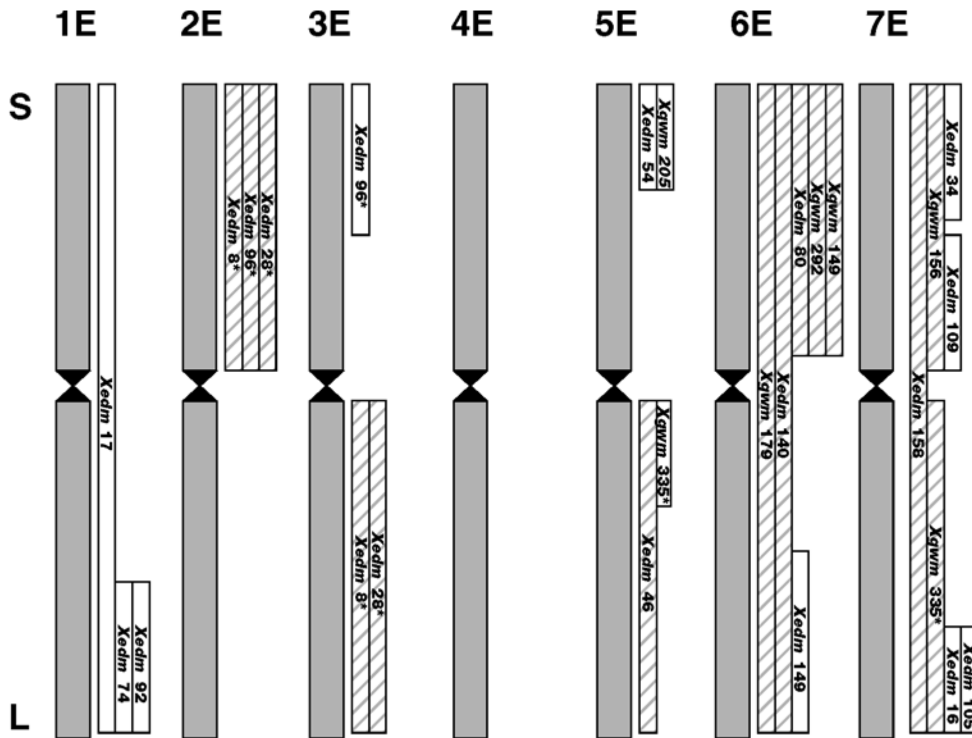
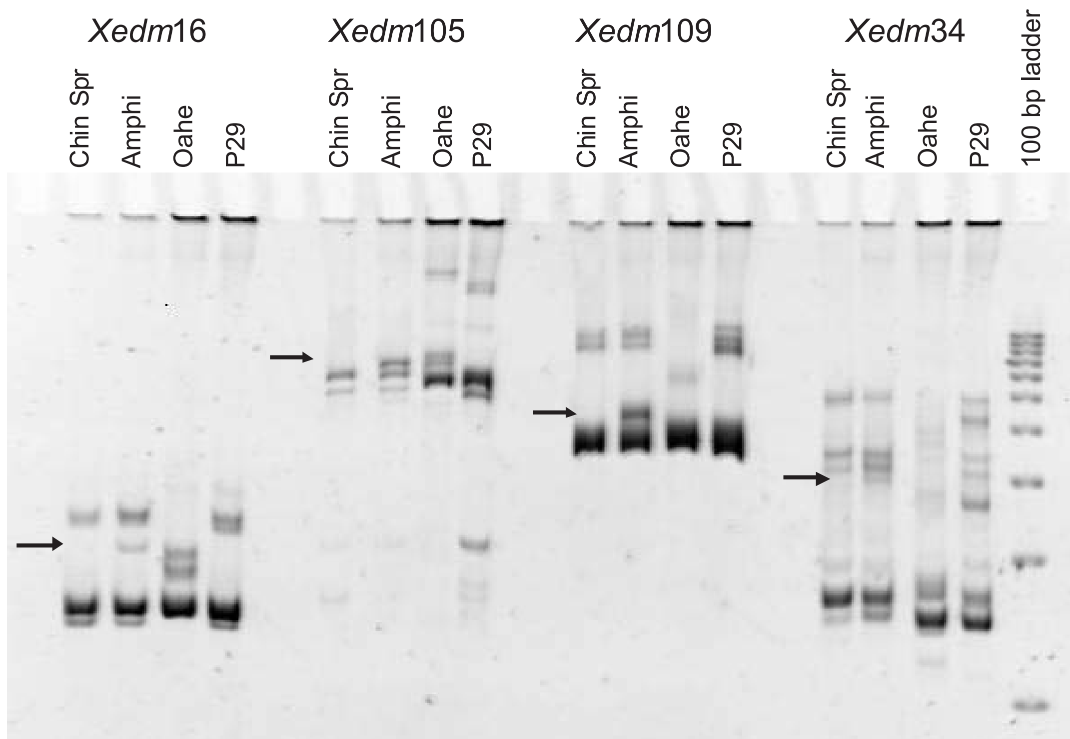


Fig. 5. EST-derived SSR markers, *Xedm*16, *Xedm*105, *Xedm*109, and *Xedm*34, amplifying loci in 'Chinese Spring' (Chin Spr), Chin Spr – *L. elongatum* amphiploid (Amphi), *Lophopyrum intermedium* ('Oahe'), and P29, a group 7 wheat – *L. intermedium* disomic substitution line (Sharma et al. 1997). Bands were separated on an 8% polyacrylamide gel and visualized under UV light after staining with ethidium bromide. Band sizes were estimated from the 100 bp ladder, with the *L. elongatum* loci indicated for each marker by an arrow.



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between wheat and *L. elongatum* chromosomes; but translocations and inversions between the 2 species were also identified.

More recently, studies have shown that chromosomes from some cereals have remained mostly conserved, whereas others have undergone major structural rearrangements during speciation (Gale and Devos 1998). This has been extended to include the A, B, and D genomes of bread wheat and the C genome of its wild relative, *Aegilops markgrafii* (Peil et al. 1998). It is still unknown what level of conservation exists between wheat and wheatgrass genomes, but preliminary results from this study indicate that synteny between wheat and *L. elongatum* may be more complicated than previously thought. Whereas there are indications that breaks in synteny do exist between wheat and wheatgrass genomes, we cannot exclude the possibility that paralogous sequences in different genomes are confounding comparative genome analysis. The assignment of multiple loci to several *L. elongatum* chromosomes provides evidence of these paralogous sequences. Although synteny was useful in localizing some *Xedm* markers to small regions of the *L. elongatum* genome, further localization of nonhomoeologous *Xedm* and *Xgwm* markers to specific regions on *L. elongatum* chromosomes will require the exploitation of translocation lines or genetic mapping information.

The identification of *Xedm* markers that detect *L. elongatum* loci indicates that they may also amplify loci in other related wheatgrass species. However, 4 *Xedm* markers amplifying loci from chromosome 7E in *L. elongatum* were unable to distinguish *L. intermedium* from wheat loci in P29. This line contains a group 7 substitution from the X genome in *L. intermedium* (Sharma et al. 1997; Francki et al. 1997). It is likely that the 7X and 7E chromosomes and their sequences are significantly diverged where EST-SSR markers were unable to detect similar alleles in the homoeologous wheatgrass chromosomes.

ESTs are becoming widely used in the development of new markers in both plants and animals (Eujayl et al. 2002; Mamo et al. 2003; Scott et al. 2000). The exploitation of existing EST databases utilizes a readily available resource that can be used as a platform from which markers in wheatgrass may be identified with minimal time or expense. This approach has provided numerous SSR markers that complement the existing markers used to characterize wheat genotypes with smaller *L. elongatum* chromosome segments. Sequence analysis has enhanced the efficiency of EST-SSR marker development through the identification and targeting of primer design within conserved regions. Accessing ESTs with known physical locations on the wheat genome enabled, in some cases, a more precise assignment of the markers to *L. elongatum* chromosome locations. This information has also provided preliminary information on DNA-sequence diversity and synteny within wheatgrass and between other cereal genomes.

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