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Development of STS and CAPS Markers Specific to Genomes in the Tribe *Triticeae*

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Wild *Triticeae* grasses serve as important gene pools for forages and cereal crops. Knowledge on their genome compositions is pivotal for efficient utilization of this vast gene pool in germplasm enhancement programs. Using DNA sequences of genome-specific RAPD markers, selected primers have been designed to develop sequence tagged site (STS) markers. Genome specificity was lost for a majority of RAPD-to-STS conversions due to the inward extension of primer sequences. However, successful conversion has been achieved for genomes E^b, E^e, St, H, Ns, W, V and Y (an unknown genome in many polyploid *Elymus* species). Several cleaved amplified polymorphic sequence (CAPS) markers were also developed to distinguish the E^b, E^e and R genomes. The identified STS and CAPS markers are useful in suggesting the presence of certain genome(s) in *Triticeae* species and in identifying the alien chromosome or chromosomal segment in wheat addition, substitution, or translocation lines. Use of STS markers has helped to identify wheat addition lines with W- and Y-genome chromosomes derived from hybrids of hexaploid wheat *Triticum aestivum* (AABBDD) and hexaploid *Elymus rectisetus* (StStWWYY). Seven disomic wheat addition lines having different E^b-genome chromosomes are confirmed by the CAPS markers for this genome. This study also provides evidence that barley yellow dwarf virus (BYDV) resistant germplasm lines from Purdue and China are different from those developed in Australia.

Keywords: *Triticeae, genome, RAPD, genome-specific markers, evolution, homology*

INTRODUCTION

Perennial *Triticeae* grasses serve as important gene pools for forages and cereal crops (Dewey, 1984). Knowledge on their genome compositions is pivotal for efficient utilization of this vast gene pool in germplasm enhancement programs. Despite the vast amount of genome research on *Triticeae* species, many of the approximate 350 species have not been scientifically confirmed for their genome compositions (http://herbarium.usu.edu/Triticeae /genmsymb.htm).

A number of genome-specific random amplified polymorphic DNA (RAPD) markers have been identified and sequenced in perennial *Triticeae* species (Wei and Wang, 1995; Zhang et al., 1998; Wang, unpublished). Many species- or genome-specific repetitive sequences have also been reported (Bedbrook et al., 1980; Rayburn and Gill, 1986; Zhang and Dvorak, 1990; Tsujimoto and Gill, 1991; Anamthawat-Jonsson and Heslop-Harrison, 1993; Li et al., 1995). Genome-specific molecular markers are useful in identifying the genome constitution of species in question (Svitashev et al., 1998).

Because RAPD marker is one of many amplified DNA fragments from the polymerase chain reaction (PCR) based on a one single primer 10 bases in length, inexperienced persons may have difficulty using the RAPD technique. Sequence-tagged-site (STS) markers (Tragoonrung et al., 1992) are PCR-based markers generated by a pair of primers (~20 bases long) that are designed according to known DNA sequences. Ideally, only one DNA fragment of a specific length (STS marker) will be amplified from the template DNA containing the target sequence. The STS markers will be more reproducible and specific than the original RAPD marker. On the other hand, the restriction fragment length polymorphism (RFLP) technique requires more genomic DNA and longer time to run an assay. Therefore, PCR-based markers such as STS and cleaved-amplified polymerphic sequence (CAPS; Konieczny and Ausubei, 1993) markers are preferred by most researchers. STS and/or CAPS markers have been developed for identification of species (Li et al., 2002) and chromosomes (Talbert et al., 1994; Blake et al., 1996;

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Erpelding et al., 1996).

In this study, thirty-five sequenced markers presumably specific for various genomes in *Triticeae* were tested for conversion into STS or CAPS markers. The genome symbols are those designated by Wang et al. (1995). The genome specificity and utility of successfully-converted markers were demonstrated using some polyploid *Triticeae* species and wheat-alien addition, substitution, or translocation lines having chromosomes or chromosomal segments of perennial *Triticeae* species.

MATERIALS AND METHODS

Plant materials (Table 1) are raised from seeds and grown in a greenhouse at the USDA-ARS Forage & Range Research Laboratory (FRRL), Logan, Utah. All diploid and several polyploid species having known genomes were used in developing and screening of converted STS markers. Ten BYDV-resistant and -susceptible lines were provided by Dr. Herbert Ohm, Purdue University, Indiana; whereas three lines were provided by Prof. Z.-Y. Xin, Chinese Academy of Agricultural Sciences, Beijing, China. Wheat - *Elymus rectisetus* backcross derivatives were developed at FRRL. These lines and two polyploid *Thinopyrum* species were used for testing the utility of STS and CAPS markers.

RAPD marker sequences published in Li et al. (1995), Wei and Wang (1995), Zhang et al. (1998), and those unpublished by Wang were used for development of STS markers. STS primer pairs (Table 2) were designed for each sequenced genome-specific RAPD marker using program Primers3 (Rozen and Skaletsky, 1997) available online: (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). The selected new primer sites may or may not partially overlap with the original RAPD primer sites. Procedures for DNA extraction, PCR amplification, and visualization of amplification products follow those described by Li et al. (2002) with some modifications. Amount of template DNA in the 25 µl PCR reaction mix was 40 ng for diploid, 80 ng for tetraploid, 120 ng for hexaploid and 200 ng for decaploid species. PCR conditions, mainly the annealing temperature and number of amplification cycles, were tested and optimized for each assay.

If an STS assay failed to produce a target marker for the target genome but produced seemly new genome-specific PCR products, the specific STS products were excised from agarose gel, cloned into pCR2.1 of the TA cloning kit (Invitrogen, USA), and sequenced for a second round of STS development. When necessary, STS products from different genomes were converted to CAPS markers with several restriction endonuclease enzymes. The enzyme-digested PCR product was then separated in a 2% agarose gel containing ethidium bromide in 1ÇTBE.

RESULTS

Based on known sequences of 32 RAPD markers, 33 primer pairs were designed (Table 2). Out of the 33 assays, only eight assays (3, 7, 12, 14, 17, 28, 29, and 30; Table 2) successfully amplified the targeted genome-specific sequences. Five of these are shown in Figures 1 and 2. Both assays 32 (Figure 3) and 33 produced a single band of the target length from genomes **P, E^b**, and **V** (Table 2). From the first round of STS development, some potentially genome-specific markers were identified, cloned and sequenced. Second round STS development successfully produced new STS markers for genomes **E^b, V** (Figure 4), and **H** (Table 2, assays 33 to 36). **E^b**-specific STS marker was also developed in assay 3 (Figure 5a). However, assay 1 amplified one product of the expected length not only from the target genome **E^b** but also from non-target genomes **E^e** and **R** (Figure 5b). To differentiate these three genomes, the F03-1277bp products from **E^b, E^e**, and **R** genomes have been converted to CAPS markers using two restriction enzymes (Table 3, Figure 5c).

Triticum aestivum Ç *Elymus rectisetus* backcross derivatives were tested for the presence of **St-**, **W-**, or **Y-**genome chromosomes using the STS markers. The **St**-genome marker OPD15-**St**498 was not detected in any of the lines tested (Table 4). Lines 0291, 0297, 4319, and 4348 had been tested positive for the **W-**genome marker OPB03-**W**306. The presence of **Y-**genome marker OPB14-**Y**269 was detected in lines 0293, 0294, and 4687. Line 4431 had both OPB03-**W**306 and OPB14-**Y**269 (Table 4). Line 0290 possessed the OPB03-**W**306 marker in a much lower intensity than that in 0291 (Figure 6).

Using the OPF03-1277 marker, Purdue lines P1 to P10 were tested for the presence of **E^b** or **E^e** chromosomes (Figure 7), whereas the three Chinese lines T1 to T3 were tested along with seven wheat addition lines having different **E^b** chromosomes (Figure 8). Those lines with or without BYD resistance yielded the CAPS markers for the **R** genome instead of markers for **E^b** or **E^e**. Each of seven **E^b** chromosomes produced the **E^b**-specific CAPS marker bands using the *Eco*RI restriction endonuclease (Figure 8).

Two polyploid *Thinopyrum* species, *Th.intermedium* and *Th.ponticum*, were assayed using STS and CAPS markers (Table 5). Both species had the **St-**genome STS marker OPB04-**St**341, while only *Th.intermedium* had the **R-**genome

CAPS markers. The use of STS and CAPS markers to detect E^b or E^e in the two species was inconsistent; however, *Th.intermedium* was positive for E^b and *Th.ponticum* was positive for E^e.

Table 1. Plant materials used in various studies to test STS or CAPS markers.

Symbols	Species	ID #	Source	Notes
E^b	***Thinopyrum bessarabicum***	**PI 531710**	**FRRL**	**=J**
E^e	***Th.elongatum***	**PI 531718**	**FRRL**	**=E**
St^{st}	***Pseudoroegneria stipifolia***	**PI 236668**	**FRRL**	**=S**
St^l	***P.libanotica***	**PI 338391**	**FRRL**	**=S**
R	***Secale montanum***	**PI 531829**	**FRRL**	
		PI 531835	**FRRL**	
H	***Hordeum bogdanii***	**PI 499501**	**FRRL**	**perennial**
I	***Hordeum vulgare* var. Walker**	**PI 557000**	**USU**	**annual**
P^c	***Agropyron cristatum***	**PJ-3817**	**FRRL**	
P^m	***A.mongolicum***	**PI 499392**	**FRRL**	
Ns^j	***Psathyrostachys juncea***	**PI 314521**	**FRRL**	
Ns^h	***Ps.huashanica***	**PI 531823**	**FRRL**	
Ns^f	***Ps.fragilis***	**PI 343190**	**FRRL**	
W	***Australopyrum retrofractum***	**PI 531553**	**FRRL**	
V	***Dasypyrum villosum***	**D-2990**	**FRRL**	**annual**
StY	***Elymus longearistatus***	**PI 401282**	**FRRL**	
StWY	***Elymus rectisetus***	**JC1050**	**FRRL**	
JSt(V-J-R)	***Thinopyrum intermedium***	**PI 547315**	**FRRL**	
EEEEE	***Th.ponticum***	**PI 261098**	**FRRL**	
ABD	***Triticum aestivum* var. CS**	**CI*tr* 14108**	**Missouri**	**annual**
P1	***T.aestivum*** lines with	**P107**	**Purdue**	**R to BYDV**
P2	***Th.intermedium*** chromosome	**961341A3-2-2**		**R to BYDV**
P3	or segment	**961341A3-1-2-3**		**R to BYDV**
P4		**98131A1-1-4-9**		**S to BYDV**
P5		**98134G4-1**		**R to BYDV**
P6		**P29 = GP-541**		**R to BYDV**
P7		**169-1**		**R to BYDV**
P8		**632-21**		**R to BYDV**
P9		**177-1**		**S to BYDV**
P10		**69-1**		**S to BYDV**
T1	***T.aestivum*** lines with	**Y920592**	**China**	**R to BYDV**
T2	***Th.intermedium*** chromosome	**Y920592**		**R to BYDV**
T3	segment	**D957-3**		**R to BYDV**
0275, 0290, 0292, 0293, 0294, 0296, 4162, 4431, 4687.	***T.aestivum*** lines with ***Elymus rectisetus*** chromosomes		**FRRL**	**2n = 44**
0291, 4319 4348, 4419 4660, 0297 4183	***T.aestivum*** lines with ***Elymus rectisetus*** chromosomes		**FRRL**	**2n = 42**
$1E^b$ to $7E^b$	***T.aestivum*** lines with a pair of ***Th.bessarabicum*** chromosome		**CIMMYT**	**2n = 44**

Table 2. Primers, GenBank identification numbers of template or marker seguences, target genomes, seguence tagged sites (STS) marker length in bp, polymerase chain reaction (PCR) conditions, and results of PCR with genomes in *Triticeae* (Li et al., 1995; Zhang et al., 1998).

Assay	Primer name	5' ---------- 3'	GenBank ID	Genome	Expected length	Tm	Cycles	Results
1	OPF03F1	TGATCACCTGGTTGATAAGTCA	U43516 [1]	E^b	1277	58	20	E^b, E^e, St, R
	OPF03R1	AAAGTATTTATTCACTCAACCGGATCT				60	20	E^b, E^e, R
2	OPN01F1	GGAATTAAATCACATATGCTGTATGAC	BV679216	E^b	261	60	25	E^b (strong), E^e (weak)
	OPN01R1	CTCACGTTGGTAAGGGAAGA						
3	OPN03F1	TGGTACTCCCCTACCTAAGCA	BV679215	E^e	886	62	30	E^e (900 + 1400 bp)
	OPN03R1	CCCTAGATGTATGCAGGGTCA						E^b (1400 bp)
4	OPD15F	GTGCTGGTGCGGTCATAGA	BV679217	St	498	60	30	St, StY, StWY
	OPD15R	ATCCGTGCTTAGAAAGGTAGCA						
5	OPB04F	GGACTGGAGTTCAGAGCAATC	BV679238	St	341	60	30	St, StY, StWY
	OPB04R	GGACTGGAGTAGCTTTTCAAACA						
6	OPM07F	CGTGACTCAAAGAAATATTGTCAAA	BV679235	Ns	440	58	30	Ns
	OPM07R	CCGTGACTCATGGAAAAGGA						
7	OPB03F2	CCCCTGCCCGATAGATTTTA	BV679211	W	306	60	30	W, StWY
	OPB03R2	CATCCCCCTGGATAAATAAGTG						
8	OPF15F	ACTCCCTGATAAGTGCTTGGA	BV679212	W	575	60	40	W, StWY
	OPF15R	CAGTACTCCCAACCAGCACA						
9	OPB14F1	TCCGCTCTGGGATGTGAC	BV679236	Y	269	55	30	StY, StWY
	OPB14R1	TCCTGAAGGTAAAACTTTCTGTTTTT						
10	C08F2	GGCCACGTGTAGGAATGTG	BV679210	E^b	400	55	30	E^b
	OPC08R	TGGACCGGTGAGATGACAG						
11	C08F3	CAGTCCCCTTCATGTATATCCC	BV679208	V	755	53	40	V
	C08R	TGGACCGGTGAGATGACAG						
12	F15F3	TCCCTGATAAGTGCTTGGAG	BV679209	H	235	55	30	H
	F15R2	GCACAACCCTCATAAGGAGGTA						

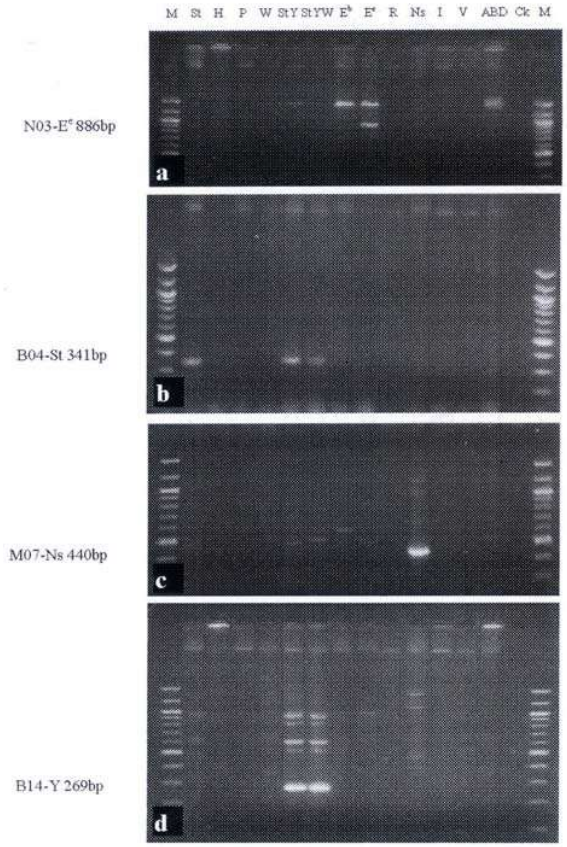

Figure 1. Four sequence-tagged-site (STS) markers for different genomes in the tribe *Triticeae*: (a) N03-**E**^{e}886 amplified at 62ºC for 30 cycles using primers 5'-TGGT ACTCCCCTACCTAAGCA-3' and 5'-CCCTAGATGTA TGCAGGGTCA-3'; (b) B04-**St**341 amplified at 60ºC for 30 cycles using primers 5'-GGACTGGAGTTCAGAG CAATC-3' and 5'-GGACTGGAGTAGCTTTTC AAA-CA-3'; (c) M07-**Ns**440 amplified at 58ºC for 30 cycles using primers 5'-CGTGACTCAAAGAAATATT GTCAAA-3' and 5'-CCGTGACTCATGGAAAAGGA-3'; and (d) B14-**Y**269 amplified at 55ºC for 30 cycles using primers 5'-TCCGCTCTGGGATGTGAC-3' and 5'-TCCTGAAGGTAAAACTTTCTGTTTTT-3'. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. The control (Ck) has all reaction components except the template DNA.

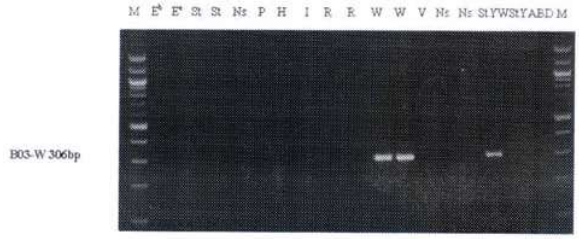

Figure 2. Sequence-tagged-site (STS) marker B03-**W**306 for the **W** genome in the tribe *Triticeae*, amplified at 60ºC for 30 cycles with primers 5'-CCCCTGCCCGATAGATTTTA-3' and 5'-CATCCCCCTGGATAAA TAAGTG-3'. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

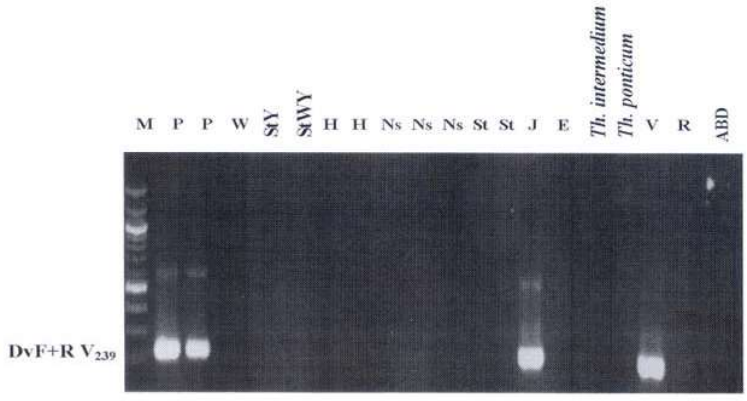

Figure 3. Sequence-tagged-site (STS) marker Dv-**V**239 using primers 5'-GGAACAATTTCGCA CTTACAGCTC-3' and 5'-CCTCGATACCTTTCCAACACCTAC-3' based on the sequence of AF472572 for the **V** genome in the tribe *Triticeae* is also amplified, at 57ºC for 20 cycles, from the **P** genome of *Agropyron cristatum* and *A.mongolicum* as well as the **E^{b} (=J)** genome of *Thinopyrum bessarabicum*. It is amplified neither from other genomes nor from diploid *Th.elongatum*, hexaploid *Th.intermedium*, and decaploid *Th.ponticum*. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

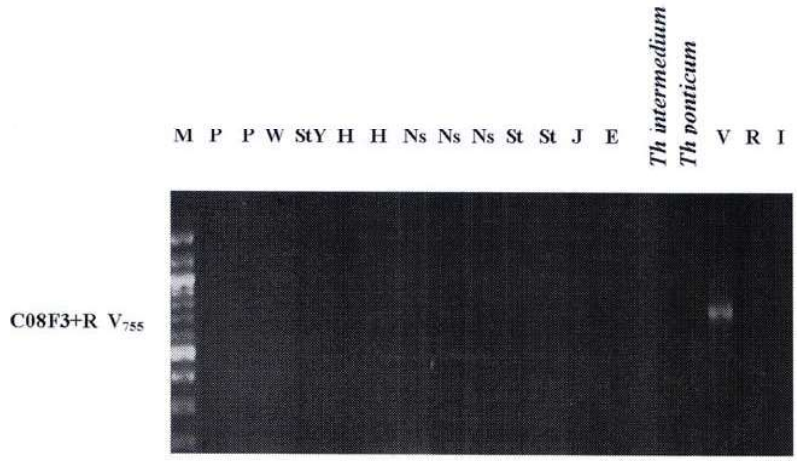

Figure 4. Second-round sequence-tagged-site (STS) marker C08-**V**755 amplified, with primers 5'-CAGTCCCCTTCATGTATATCCC-3' and 5'-TGGACCGGTGAGAT GA-CAG-3' at 53ºC for 40 cycles, only from the **V** genome of *Dasypyrum villosum*. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

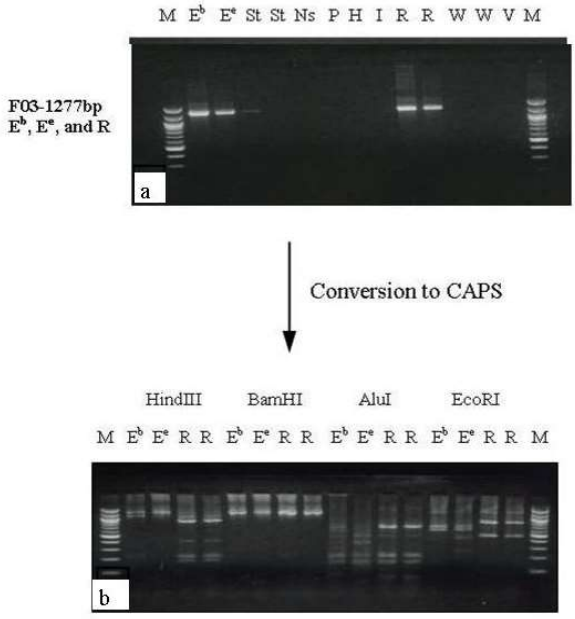

Figure 5. (a) Sequence-tagged-site (STS) marker N01-**E**^{b}261 amplified with primers 5'-GGAATTAA ATCACATATGCTGTATGAC-3' and 5'-CTCACG TTGGTAAGGGAAGA-3' at 60ºC for 25 cycles for the **E**^{b} genome. (b) Primers 5'-TGATCACCTGGTT GATAAGTCA-3' and 5'-AAAGTATTTATTCACT CAACCGGATCT-3' amplified a 1277 bp fragment from **E**^{b}, **E**^{e} and **R** genomes. A faint band could be produced from the **St** genome. (c) The F03-1277 marker was converted to cleaved amplified polymorphic sequence (CAPS) markers using restriction endonucleases. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

Table 3. Cleaved amplified polymorphic sequence (CAPS) markers for **E**^{b}, **E**^{e}, and **R** genomes after restriction digestion by *Eco*RI or *Hin*dIII of the polymerase chain reaction (58º C, 20 cycles) product using primers F03F1 (5'- TGATCACCTGGTTGATAAGTCA-3') and F03R1 (5'-AAAGTATTTATTCACTCAACCGG ATCT-3'). Intense bands are highlighted in bold-face.

Genome	Restriction enzyme	Approximate length of CAPS markers
R	*Eco*RI	**500**, and **800**
	*Hin*dIII	190, 220, 400, **900**, and 1050
E^{b}	*Eco*RI	**600**, and **700**
	*Hin*dIII	220, **1050**, and **1250**
E^{e}	*Eco*RI	250, **450**, **600**, and **700**
	*Hin*dIII	**1250**

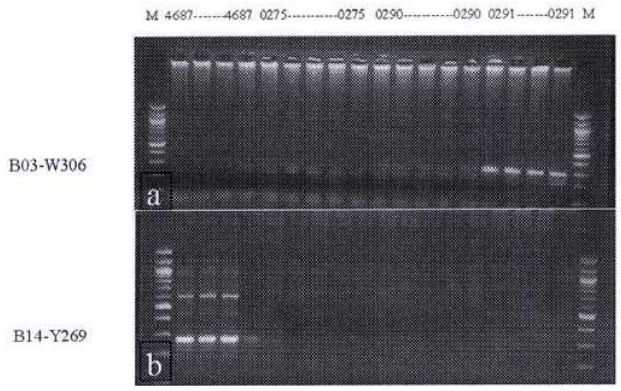

Figure 6. Sequence-tagged-site (STS) markers B03-**W**306 for the **W** genome (a) and B14-**Y**269 for the **Y** genome (b) were used to detect the presence of alien-genome chromosomes in the backcross derivatives of *Triticum aestivum* Ç *Elymus rectisetus* hybrids: 4687 (2n=44, 4 plants), 0275 (2n=44, 5 plants), 0290 (2n=44, 5 plants), and 0291 (2n=42, 4 plants). 0290 and 0291 probably had different **W**-genome chromosomes, whereas 4687 had **Y**-genome chromosomes. Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

Table 4. Results of STS assays on *Triticum aestivum* Ç *Elymus rectisetus* backcross derivatives to detect **St-, W-**, or **Y**-genome chromosomes.

Line ID	Origin	2n	OPB03-W306		OPB14-Y269	OPD15-St498
0275	1026-1-4-1	44	--		--	--
0290	1040-1-3-3	44	+		--	--
0291	1024-4-1-1	42	++		--	--
0292	1034-3-4-2	44	--		--	--
0293	1057-1-2-1	44	--		+	--
0294	1057-3-3-2	44	--		+	--
0296	1045-4-2-1	44	--		--	--
0297	1036-2-2-1	42	++		--	--
4162	1026	44	--		--	--
4183	1026	42	--		--	--
4319	1048	42	++		--	--
4348	1048	42	++		--	--
4419	1048	42	--		--	--
4431	1048	44	++		+	--
4660	1057	42	--		--	--
4687	1057	44	--		+	--

DISCUSSION

Conversion of molecular markers to STS markers is not an easy task. It requires a large number of markers from which suitable primer pairs can be designed. Then PCR conditions need to be optimized to yield discernible results for the target - a specific genome in our case. Even if the designed primer pairs encompass the 5' and 3' end sequences of the original marker, the STS assay may still fail to produce the specific marker from the target genome. We now have STS and CAPS markers for genomes $\mathbf{E}^{b}$, $\mathbf{E}^{e}$, **St**, **H**, **Ns**, **W**, **V**, **Y** and **R** (Tables 2 and 3). By using a combination of assays listed in Table 2, additional genomes may be identified. For example, positive assay 32 or 33 coupled with negative assays 3, 34, and 35 would help identify the **P** genome; and positive assay 26 and negative assay 17 might identify the **I** genome. In view of the low number of available genome-specific STS/CAPS markers, we might have to use both RAPD and STS/CAPS markers in addition to other methods for genome analysis of *Triticeae* species, especially the polyploid species. However, positive results from STS/CAPS assays would first suggest the presence of specific genome(s) in polyploid *Triticeae* species; thus, reducing the number of crosses or procedures in subsequent methods of genome analysis, such as chromosome pairing in hybrids or genomic *in situ* hybridization (GISH).

Triticum aestivum Ç *Elymus rectisetus* hybrids were synthesized and backcrossed to wheat (Liu et al., 1994). Some backcross derivatives have been characterized using RAPD and GISH (Xue and Wang, 1999). Three types of addition lines were identified by RAPD markers: (1) 1048, (2) 1057, and (3) 1026 and 1034. In this study, we further verified that 1048 derivatives had **W**-genome chromosomes and 1057 derivatives had **Y**-genome chromosomes. Derivatives from 1026 and 1034 probably had **St**-genome chromosomes that were

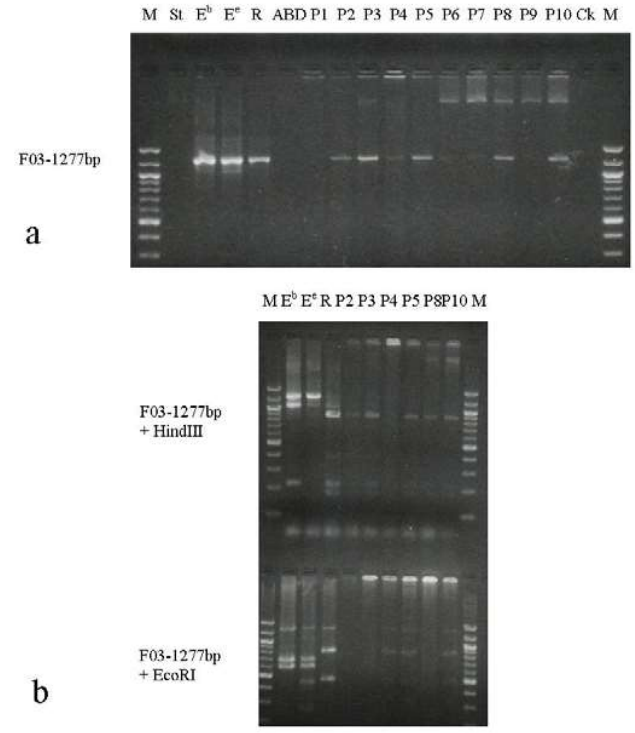

Figure 7. Purdue lines P1 to P10, developed for barley yellow dwarf virus resistance, were assayed for the sequence-tagged-site (STS) F03-1277 fragment (a) and those with the amplified product were assayed for the cleaved amplified polymorphic sequence (CAPS) markers using restriction enzymes *Hin*dIII and *Eco*RI (b). Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. The alien chromosomes in P2, P3, P4, P5, P8, and P10 contained the **R** genome-specific sequence instead of **E**- or **St**-specific sequence.

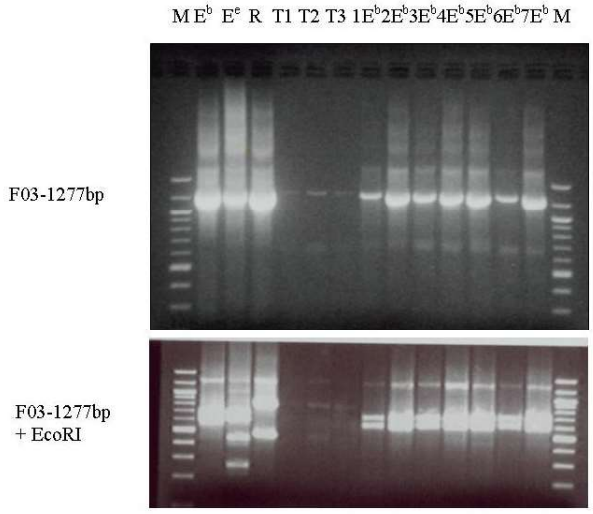

Figure 8. Chinese lines T1 to T3, developed for barley yellow dwarf virus resistance, and seven disomic wheat - *Thinopyrum bessarabicum* addition lines having different $\mathbf{E^b}$ chromosomes, $\mathbf{1E^b}$ to $\mathbf{7E^b}$, were assayed for the sequence-tagged-site (STS) F03-1277 fragment (top) and for the cleaved amplified polymorphic sequence (CAPS) markers using restriction enzyme *Eco*RI (bottom). Bands in lane M are size markers (top to bottom) 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. The alien chromosomes in T1, T2, and T3 contained the **R** genome-specific sequence instead of **E**- or **St**-specific sequence. All seven $\mathbf{E^b}$ chromosomes contain the dispersed sequence U43516 from which the F03-1277 fragment was amplified.

not carrying the OPD15-**St**498 sequence. The fact that this **St**-genome STS marker worked with the whole **St** genome but not with individual **St** chromosomes, suggesting it is a repeated sequence occurring in few of the seven chromosomes, limits its usefulness. Nevertheless, we have identified: (1) 0291 as a wheat substitution lines having a pair of **W**-genome chromosomes, (2) 0290 as a wheat addition line having a different pair of **W**-genome chromosomes, (3) 0293, 0294, and 4687 as wheat addition lines having a pair of **Y**-genome chromosomes, (4) 4431 as a wheat substitution-addition line with a pair of **W**-genome chromosomes and a pair of **Y**-genome chromosomes, and (5) 0297, 4319 and 4348 as wheat substitution lines having **W**-genome chromosomes in addition to probable **St** chromosomes. Lines 1036 and 1048 had been observed to possess 4 to 6 alien chromosomes in GISH studies (Xue and Wang, 1999 and unpublished).

Using F03-1277bp CAPS markers, we confirmed the presence of $\mathbf{E^b}$-specific fragment in all seven chromosomes of the $\mathbf{E^b}$ genome (Figure 8). It has been shown by FISH that this sequence is a dispersed repetitive sequence occurring on all seven $\mathbf{E^b}$ genome chromosomes (Zhang et al., 1998). In contrast, the **V**-genome markers Dv-**V**239 or Dv-**V**205 (assays 32 or 33) developed from AF472572 should be present in six of the seven chromosomes of **V**-genome (Li et al., 1995). In the present study, these markers were also amplified from $\mathbf{E^b}$ and **P** genomes (Table 2), making them less genome-specific than the $\mathbf{E^b}$ CAPS markers.

The most surprising results in this study are the CAPS markers for the **R** genome observed in the Purdue and Chinese lines (Figures 7 and 8). All these lines were also developed from hybrids of wheat and *Th.intermedium* (Sharma et al., 1997, 1999; Crasta et al., 2000; Xin et al., 2001) but believed to be different from those developed in Australia (Banks and Larkin, 1995). The Australian derivatives were developed from the addition line L1 of Cauderon (1966), which has a pair of group-7 **St** chromosome (Hohmann et al., 1996; Wang and Zhang, 1996). *Thinopyrum intermedium* was given the $\mathbf{E^bE^eSt}$ and the $\mathbf{JJ^sSt}$ genome symbol by Liu and Wang (1993) and Chen et al. (1998), respectively. Therefore, the **R**-genome CAPS markers in the Purdue and Chinese lines were speculated as markers for the 1R/1B translocation until Kishii et al. (2005) found that *Th.intermedium* has one **J**, one **St**, and one variant **V** genome. The **J** genome has 11 chromosomes showing fluorescent **St** probe signals at telomeric or subtelomeric sites. The variant **V** genome has nine chromosomes whose centromeric regions were strongly hybridized by **St**-genome probe; thus it is equivalent to the $\mathbf{J^s}$ genome designated by Chen et al. (1998). Because *Th.intermedium* is lacking the two **V**-genome STS markers (Figures 3 and 4) but having the **R**-genome CAPS markers and the variant **V** genome could be weakly hybridized by the **R** genome probe, Kishii et al. (2005) designated the third genome as $\mathbf{(V\text{-}J\text{-}R)^S}$ to suggest that it is a progenitor genome prior to the divergence of these three genomes. The Purdue and Chinese lines appeared to have chromosome or chromosomal segment from this $\mathbf{(V\text{-}J\text{-}R)^S}$ genome rather than from one **St**-genome chromosome as the Australian materials. Because the $\mathbf{J^s}$ genome is also present in *Th.ponticum* (Chen et al., 1998), the $\mathbf{(V\text{-}J\text{-}R)^S}$ genome should be present in this decaploid species. However, *Th.ponticum* did not show the **R**-genome CAPS markers but had only the $\mathbf{E^e}$-specific CAPS marker following the *Hind*III digestion (data not shown). Before a GISH study is carried out to show that the $\mathbf{J^s}$ genome in *Th.ponticum* can be hybridized by the **V** genome probe, the genome symbol for this decaploid species remains **EEEEE** (Table 1).

The present study demonstrated both the usefulness and limitations of STS and CAPS markers for genomes of *Triticeae*. Positive results would always suggest the presence of genome-specific sequences; whereas negative results are not necessarily an indication of their absence due to the very nature of PCR. Furthermore, it is now well known that polyploidization or alloploidy would result in rapid changes in genome organization (Liu et al., 1998a, 1998b; Han et al., 2003). Therefore, use of these genome-specific STS or CAPS markers requires cautions. It is hereby advised that these genome-specific STS or CAPS markers are used as the first-step of genome analysis, followed by GISH and chromosome pairing of appropriate hybrids.

REFERENCES

Banks P.M., Larkin P.J. (1995) Registration of three BYDV-resistant wheat germplasms: TC5, TC6, and TC9. Crop Sci. **35:** 600-601.

Blake T.K., Kadyrzhanova D., Shepard K.W., Islam A.K.M.R., Langridge P.L., McDonald C.L., Erpelding J., Larson S., Blake N.K., Talbert L.E. (1996) STS-PCR markers appropriate for wheat-barley introgression. Theor. Appl. Genet. **93:** 826-832.

Cauderon Y. (1966) Etude cytogenetique de l'evolution du materiel issu de croisement entre *Triticum aestivum* et *Agropyron intermedium*. I. Creation de types d'addition stables. Ann. Amelior Plant (Paris) **16:** 43-70.

Chen Q., Conner R.L., Laroche A., Thomas J.B. (1998) Genome analysis of *Thinopyrum intermedium* and *Th.ponticum* using genomic *in situ* hybridization. Genome **41:** 580-586.

Crasta O.R., Francki M.G., Bucholtz D.B., Sharma H.C., Zhang J., Wang R.-C., Ohm H.W., Anderson J.M. (2000) Identification and characterization of wheat-wheatgrass translocation lines and localization of barley yellow dwarf virus resistance. Genome **43:** 698-706.

Erpelding J.E., Blake N.K., Blake T.K., Talbert L.E. (1996) Transfer of sequence tagged site PCR markers between wheat and barley. Genome **39:** 802-810.

Han F.P., Fedak G., Ouellet T., Liu B. (2003) Rapid genomic changes in interspecific and intergeneric hybrids and allopolyploids of *Triticeae*. Genome **46:** 716-723.

Hohmann U., Badaeva K., Busch W., Friebe B., Gill B.S. (1996) Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome **39:**

336-347.

Kishii M., Wang R.R.C., Tsujimoto H. (2005) GISH analysis revealed new aspect of genomic constitution of *Thinopyrum intermedium*. In: 5th Int. *Triticeae* Symp. Prague, Czech Republic, Book of Abstracts: 21.

Konieczny A., Ausubei F.M. (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4:** 403-410.

Li W.L., Chen P.D., Qi L.L., Liu D.J. (1995) Isolation, characterization and application of a species-specific repeated sequence from *Haynaldia villosa*. Theor. Appl. Genet. **90:** 526-533.

Li X.M., Gardner D.R., Ralphs M.H., Wang R.R.C. (2002) Development of STS and CAPS markers for identification of three tall larkspur (*Delphinium*) species. Genome **45:** 229-235.

Liu B., Vega J.M., Segal G., Abbo S., Rodova M., Feldman M. (1998a) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy non-coding DNA sequences. Genome **41:** 272-277.

Liu B., Vega J.M., Feldman M. (1998b) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. Genome **41:** 535-542.

Liu Z.-W., Wang R.R.C. (1993) Genome analysis of *Elytrigia caespitosa, Lophopyrum nodosum, Pseudoroegneria geniculata* ssp. *scythica*, and *Thinopyrum intermedium*. Genome **36:** 102-111.

Liu Z.-W., Wang R.R.C., Carman J.G. (1994) Hybrids and backcross progenies between wheat (*Triticum aestivum* L.) and apomictic Australian wheatgrass (*Elymus rectisetus* (Nees in Lehm.) A. Lüve and Connor): karyotypic and genomic analyses. Theor. Appl. Genet. **89:** 599-605.

Ozkan H., Levy A.A., Feldman M. (2002) Rapid differentiation of homoeologous chromosomes in newly-formed allopolyploid wheat. Israel J. Plant Sci. **50:** 65-76.

Sharma H.C., Ohm H.W., Perry K.L. (1997) Registration of barley yellow dwarf virus resistant wheat germplasm line P29. Crop Sci. **37:** 1032-1033.

Sharma H.C., Franki M., Crasta O., Gyulai G., Bucholtz D., Ohm H.W., Anderson J., Perry K.L., Patterson P. (1999) Cytological and molecular characterization of wheat lines with *Thinopyrum intermedium* chromosome additions, substitutions and translocations resistant to barley yellow dwarf virus. Cytologia (Tokyo) **64:** 93-100.

Svitashev S., Bryngelssonl T., Li X.-M., Wang R.R.C. (1998) Genome specific repetitive DNA and RAPD markers for genome identification in *Elymus* and *Hordelymus*. Genome **41:** 120-128.

Talbert L.E., Blake N.K., Chee P.W., Blake T.K., Magyar G.M. (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor. Appl. Genet. **87:** 789-794.

Tragoonrung S., Kanazin V., Hayes P.M., Blake T.K. (1992) Sequence-tagged-site-facilitated PCR for barley genome mapping. Theor. Appl. Genet. **84:** 1002-1008.

Wang R.R.C., Zhang X.-Y. (1996) Characterization of the translocated chromosome using fluorescence *in situ* hybridization and random amplified polymorphic DNA on two *Triticum aestivum-Thinopyrum intermedium* translocation lines resistant to wheat streak mosaic or barley yellow dwarf virus. Chrom. Res. **4:** 583-587.

Wang R.R.C., von Bothmer R., Dvorak J., Fedak G., Linde-Laursen I., Muramatsu M. (1995) Genome symbols in the *Triticeae*. In: Proc. of 2nd Int. Triticeae Symp. (Wang R.R.C., Jensen K.B., Jaussi C., eds.), Logan, Utah: 29-34.

Wei J.-Z., Wang R.R.C. (1995) Genome- and species-specific markers and genome relationships of diploid perennial species in *Triticeae* based on RAPD analyses. Genome **38:** 1230-1236.

Xin Z.Y., Zhang Z.Y., Chen X., Lin Z.S., Ma Y.Z., Xu H.J., Banks P.M., Larkin P.J. (2001) Development and characterization of common wheat - *Thinopyrum intermedium* translocation lines with resistance to barley yellow dwarf virus. Euphytica **119:** 161-165.

Xue X., Wang R.R.C. (1999) Detection of *Elymus rectisetus* chromosome segments added to wheat by RAPD and genomic in situ hybridization. Acta Genetica Sinica **5:** 539-545.

Zhang X.-Y., Dong Y.-S., Li P., Wang R.R.C. (1998) Distribution of **E**- and **St**-specific RAPD fragments in few genomes of *Triticeae*. Acta Genetica Sinica **25:** 131-141.