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RESEARCH ARTICLE

Development and utilization of new sequenced characterized amplified region markers specific for E genome of *Thinopyrum*

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Abstract **Species containing E genome of *Thinopyrum* offered potential to increase the genetic variability and desirable characters for wheat improvement. However, E genome specific marker was rare. The objective of the present report was to develop and identify sequenced characterized amplified region (SCAR) markers that can be used in detecting E chromosome in wheat background for breeding purpose. Total 280 random amplified polymorphic DNA (RAPD) primers were amplified for seeking of E genome specific fragments by using the genomic DNA of *Thinopyrum elongatum* and wheat controls as templates. As a result, six RAPD fragments specific for E genome were found and cloned, and then were converted to SCAR markers. The usability of these markers was validated using a number of E-genome-containing species and wheat as controls. These markers were subsequently located on E chromosomes using specific PCR and fluorescence *in situ* hybridization (FISH). SCAR markers developed in this research could be used in molecular marker assisted selection of wheat breeding with *Thinopyrum* chromatin introgressions.**

Keywords *Thinopyrum*, *Tritrigia*, E genome, SCAR markers, FISH

Introduction

Thinopyrum indtermedium, an auto-allohexaploid, genomic formula is $E^eE^eE^bE^bStSt$ where St genome origins from *Pseduoroengeria*, while E^e and E^b (= J) are closely related to *Th. elongatum* and *Th. bessarabicum* respectively (Chen, 2005). Early cytogenetic data demonstrated the closeness of genome E^e and E^b (Dewey, 1984; Wang and Zhang. 1989). Herein, we designate E genome as the general name of E^e and E^b. The E genome has many outstanding agronomical characters that can be used in wheat breeding, such as salt tolerance (McGuire and Dvorak, 1981; Colmer et al., 2006), waterlogging tolerance (Taeb et al., 1993; McDonald et al., 2001), yellow dwarf resistance and three rust resistances (Sharma and Knott, 1966; Shukle et al., 1987; Sharma et al., 1989; Friebe et al., 1996; Ma et al., 2000; Yang and Ren, 2001; Zhang et al., 2005), Fusarium head blight resistance (Han and Fedak. 2003; Shen et al., 2004; Fu et al., 2012). From the last century, wheat breeders have been transferring the resistance genes from E-genome-containing species to wheat for breeding purpose, and in particularly, a number of wheat-*Thinopyrum* derivative lines have been bred (Sharma and Knott, 1966; Sun, 1981; Li et al., 1985; Sharma et al., 1989; Friebe et al., 1994; Zhang et al., 2005).

Repetitive DNA sequences are proved useful in molecular marker assisted selection (Yang et al., 2006b; Liu et al., 2008). Flavell et al. (1974) pointed out that more than 75% of *Triticeae* genome is repetitive DNA, therefore, different methods including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) have been used in isolating repetitive DNA sequences. RAPD has been commonly used as a simple, low-cost, and time-effective technique, and the SCAR markers converted from RAPD products are very useful in wheat molecular marker assistant breeding (Liu et al., 2008).

Wheat stripe rust and powdery mildew are unusually severe in China. It is urgent to find new resistant resources and to develop new resistant varieties including from wide hybridization between wheat and *Triticeae* species. Recently, we crossed *Thinopyrum intermedium* ssp. *trichophorum* to wheat, and the stripe rust and powdery mildew-immune wheat-*Th. intermedium* ssp. *trichophorum* partial amphi-

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ploids had been produced (Yang et al., 2006a). Further, we crossed the partial amphiploids to wheat and obtained a series of stripe rust and powdery mildew-immune wheat-*Th. intermedium* ssp. *trichophorum* introgressions which can be used as resistant resources in wheat breeding. With aim to produce powerful genome specific molecular markers for fast-identification of wheat-*Thinopyrum* introgressions, we established new SCAR and ISSR markers for St genome (Liu et al., 2007; Hu et al., 2008). It is also necessary to obtain E-genome molecular markers to detect E chromosome in wheat backgrounds. In the present study, we attempt to isolate E-genome specific DNA segments using RAPD technique, and then convert them to effective SCAR-PCR markers. The fluorescence *in situ* hybridization (FISH) was also used to verify the localization of the SCAR markers in wheat-*Thinopyrum* introgression lines.

Material and methods

Plant materials

Materials used in this study and their relevant information including genome composition, accession number, provenance and providers were all listed in Table 1.

DNA extraction and RAPD analysis

Total genomic DNA was prepared from young leaves using SDS protocol (Liu et al., 2006). RAPD amplification was performed in an Icycler thermalcycler (Bio-RAD Laboratories). Total reaction volume is 25 μL containing 10 mmol Tris-HCl (pH 8.3), 2.5 mmol $MgCl_2$, 200 mol of each dNTP, 50 ng template DNA, 0.2 U Taq polymerase (Takara, Japan), and 400 nmol primer. The cycling parameters are 94°C for 3 min to pre-denature; followed by 40 cycles of 94°C for 1 min, 38°C for 1 min, 72°C for 2 min; and then a final extension at 72°C for 10 min.

Cloning and sequencing of the specific RAPD product

The genome-specific RAPD products identified from PCR were excised from 1.0% agarose gels and purified by a gel extraction kit (Qiagen, Valencia, Calif.). The purified products were ligated into the pT7 Blue R-vector using T4 ligase, and then introduced into *Escherichia coli* DH5α, by heat shock transformation. Nucleotide sequencing was performed on a polyacrylamide gel with the ABI prism 377 sequencer (Perkin Elmer) as an automated fluorescent sequencing system. The BLAST program in the GeneBank database was used to search for sequence similarities with DNA (BLASTN).

Specific PCR primer and amplification

Based on the cloned sequence of RAPD products, six pair of specific PCR primers were designed by using software DNAMAN, and synthesized by SBS Biotech, Beijing, China.

Table 1 Materials used in this study

Species	Chromosome number	Genomic composition	Accession No.	Provenance	Provider
Th. elongatum	$2n = 14$	E^eE^e	PI 531719	France	[a]
Th. elongatum	$2n = 14$	E^eE^e	PI 531717	France	[a]
Th. elongatum	$2n = 14$	E^eE^e	PI 531718	Tunisia	[a]
Th. elongatum	$2n = 14$	E^eE^e	PI 153179	Turkey	[a]
Th. bessarabicum	$2n = 14$	E^bE^b (= JJ)	PI 531711	Estonia	[a]
Th. bessarabicum	$2n = 14$	E^bE^b (= JJ)	W6 21890	Ukraine	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 401225	Iran	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 401178	Iran	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 401183	Iran	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 401196	Iran	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 383575	Turkey	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 547332	Turkey	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 547335	Poland	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 547316	Russian Federation	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 547315	Russian Federation	[a]
Th. intermedium	$2n = 42$	$E^eE^eE^bE^b$StSt	PI 547319	Russian Federation	[a]
Octoploid trititrigia	$2n = 56$	AABBDDEE	KD20041	USA	[b]
	$2n = 56$	$40W + 8J + 8J^s$	XY693	China	[c]
	$2n = 56$	AABBDDEE	7430	China	[c]
	$2n = 54$	AABBDD + 2St + 4J + $4J^s$ + 2St-J^s	Zhong2	China	[d]
	$2n = 56$	$40W$ + 4St + 2Js + 2Js-W + 2S-Js + 2St-Js + 2S-J + 2W-Js	Zhong5	China	[d]
	$2n = 56$	$40W$ + 8St + $4J^s$ + 2J + 2J-St	TE	China	[e]
	$2n = 56$	$40W$ + 6St + 8E	7044	China	[f]
	$2n = 56$	AABBDD + 8St + 4E + 2St-E	7045	China	[f]
	$2n = 56$	$40W$ + 2St + 12E + 2St-E	7047	China	[f]
	$2n = 56$	AABBDD + $12J^s$ + 2J	78784	China	[g]

(*Continued*)

Species	Chromosome number	Genomic composition	Accession No.	Provenance	Provider
Th. elongatum	2*n* = 70	- (!)	PI 315352	Former Soviet Union	[a]
Th. elongatum	2*n* = 70	- (!)	PI 308592	Italy	[a]
Th. elongatum	2*n* = 70	- (!)	PI 297871	Argentina	[a]
Th. elongatum	2*n* = 70	- (!)	PI 283164	China	[a]
Th. elongatum	2*n* = 70	- (!)	PI 276399	Germany	[a]
Th. elongatum	2*n* = 70	- (!)	PI 368851	USA	[a]
Th. elongatum	2*n* = 70	- (!)	PI 251443	Turkey	[a]
Th. elongatum	2*n* = 70	- (!)	PI 401008	Turkey	[a]
Th. elongatum	2*n* = 70	- (!)	PI 401009	Turkey	[a]
Th. elongatum	2*n* = 70	- (!)	PI 401010		[a]
Wheat-*Th. elongatum/Th. intermedium* additions	2*n* = 44	AABBDD + one pair 1E	KD20061	USA	[b]
	2*n* = 44	AABBDD + one pair 2E	KD20062	USA	[b]
	2*n* = 44	AABBDD + one pair 3E	KD20063	USA	[b]
	2*n* = 44	AABBDD + one pair 4E	KD20064	USA	[b]
	2*n* = 44	AABBDD + one pair 5E	KD20065	USA	[b]
	2*n* = 44	AABBDD + one pair 6E	KD20066	USA	[b]
	2*n* = 44	AABBDD + one pair 7E	KD20067	USA	[b]
	2*n* = 44	AABBDD + one pair S-J^s	Z1	China	[a]
	2*n* = 44	AABBDD + one pair S-J^s	Z2	China	[a]
	2*n* = 44	AABBDD + one pair S	Z3	China	[a]
	2*n* = 44	AABBDD + one pair *W*-J^s	Z4	China	[a]
	2*n* = 44	AABBDD + one pair *W*-J^s	Z5	China	[a]
	2*n* = 44	AABBDD + one pair S-J^s	Z6	China	[a]
Wheat-*Thinopyrum intermedium* ssp. *trichophorum* introgressions	2*n* = 56	40*W* + 9St + 4J^s + 3J	1908	China	[e1]
	2*n* = 56	40*W* + 8St + 6J^s + 2J	Q156	China	[e1]
	—	—	Q157-Q227	China	[e]
Ps. Spicata	2*n* = 14	StSt	PI 232131	USA	[a]
S. cereale	2*n* = 14	RR	SCND1377	China	[e]
S. africanum	2*n* = 14	R^{a}R^a	CN41211	South Africa	[h]
S. silvestre	2*n* = 14	R^{s}R^s	CN41954	Austria	[h]
D. breviaristatum	2*n* = 28	V^{b}V^{b}V^{b}V^b	KD19791	Morocco	[a]
D. villosum	2*n* = 14	VV	KD19801	Morocco	[i]
H. vulgare	2*n* = 14	HH	CN41966	China	[i]
Ae.comosa	2*n* = 14	MM	PI 317400	Afghanistan	[a]
Ae.umbellulata	2*n* = 14	UU	PI 227339	Iran	[a]
Ae.bicornis	2*n* = 14	S^{b}S^b	CIae 70	Unknown	[a]
Ae. cylindrica	2*n* = 28	CCDD	PI 374320	Yugoslavia	[a]
Ag.cristatum	2*n* = 28	PPPP	PI 578520	Canada	[a]
A.retrofractum	2*n* = 14	WW	PI 547363	Australia	[a]
Eremopyrum	2*n* = 14	FF	PI 227343	Iran	[a]
Ae. tauschii	2*n* = 14	DD	KD20042	China	[e]
T. monococcum	2*n* = 14	AA	PI 427328	Iraq	[a]
T. turgidum	2*n* = 28	AABB	PI 308878	Spain	[a]
T. timopheevii	2*n* = 28	AAGG	PI 297326	Former Soviet Union	[a]
T .teres	2*n* = 28	A^{u}A^{u}DD	KD1988	China	[j]
T. aestivum	2*n* = 42	AABBDD	CS	China	[h]
T. aestivum	2*n* = 42	AABBDD (1RS/1BL)	CN12	China	[i]
T. aestivum	2*n* = 42	AABBDD (1RS/1BL)	CN18	China	[i]
T. aestivum	2*n* = 42	AABBDD (1RS/1BL)	CN17	China	[i]

"-"means not identified; "- (!)" means not identified, but their genome are confirmed to be JJJJJ or JJJJ^{s}J^s or EEEStSt; "W" means W genome, while italic "*W*" means wheat chromosome.

[a] National Plant Germplasm System (NPGS), USDA-ARS, Aberdeen, Idaho USA.
[b] Dr. J. Dvorak, California University, USA.
[c] Aademician Zhenshen Li, Institute of Genetics and Development Biology, Chinese Academy of Sciences.
[d] Research Research Prof. Sancheng Sun, Institute of Crop Genetics, Agricultural Academy of Shanxi Province, China.
[e] The current laboratory.
[e1] The current laboratory, identified in this study.
[f] Research Prof. Zhijian Chan, Institute of Crop Genetics, Agricultural Academy of Shanxi Province, China.
[g]Research Prof. Guanchang Zhong, Shijiazhuang Institute of Agricultural Modernization, Chinese Academy of Sciences.
[h] Missouri botanic garden, USA.
[i] Dr. Zongxiang Tang, Sichuan Agriculture University, China.
[j] Research Prof. Huaren Jiang, Sichuan Agriculture University, China.

The PCR reaction with a 25 μL volume containing 50 ng genomic DNA, 0.2 μmol/L of each primer, 200 μmol/L of each dNTP, 1 × PCR buffer, 2.0 mmol/L $MgCl_2$ and 1U Taq polymerase (Takara, Japan), was performed at 5 min at 94°C; 45 cycles of the following program: 1min at 94°C, 1 min at 60–63°C, and 2 min at 72°C; with a final extension at 72°C for 10min. The PCR products were fractionated on 1% agarose gel.

Fluorescence *in situ* hybridization (FISH)

For FISH analysis, E-genome specific DNA and *Pseduoroengeria spicata* genomic DNA were labeled with digoxigenin-11-dUTP according to the manufacturer's instruction (Roche Diagnostics, Indianapolis, IN). The DNAs were labeled with fluorescence-14-dUTP (Roche Diagnostics) by nick translation. The probes were diluted to a final concentration of 1 μg/mL in the hybridization solution and the hybridization mixture was prepared as described by Mukai et al. (1993). The digoxigenin labeled DNA signals were detected with fluorescein-conjugated antidigoxigenin antibody (Roche Diagnostics). The slides were finally mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, CA) with (0.25 μg/mL) propidium iodide for only FITC detection. Microphotographs of GISH chromosomes were taken with an Olympus BX-51 microscope.

Results

Cloning and sequencing of *Th. elongatum* E genome specific DNA segments

Six random primers, Q10, D14, I4, E11, M4 and H11 were selected from a total of 280 primer sets tested. Using common wheat Chinese Spring, MY11, R57 and R25 as controls, six specific DNA bands about 900 bp, 1000 bp, 700 bp, 400 bp, 1000 bp and 900 bp were amplified from *Th. elongatum*, respectively, but not in wheat controls. These E genome-specific RAPD bands were cloned and sequenced. Their full length were 865 bp, 995 bp, 694 bp, 358 bp, 907 bp and 1033 bp, named $10Q_{865}$ (GenBank accession No.EU331358), $14D_{995}$ (EU331359), $4I_{694}$ (EU331360), $11E_{358}$ (EU331361), $4M_{907}$ (EF566898) and $11H_{1033}$(EU483666), respectively. $10Q_{865}$, $14D_{995}$, $4I_{694}$, $11E_{358}$, $4M_{907}$ and $11H_{1033}$ showed 50.8%, 52%, 45.4%, 45%, 50% and 48.4% GC content, respectively. NCBI BLASTN search showed that $14D_{995}$, $11E_{358}$ and $11H_{1033}$ had no any homology to sequences deposited in NCBI website, indicating they were new sequences. The nucleotide sites 182 to 862 of $10Q_{865}$ had 77% homology with nucleotide sites 80832 to 80152 of *Aegilops tauschii* transposon "Jody" (AY534122S2); The nucleotide sites 4 to 692 of $4I_{694}$ had 77% homology with nucleotide sites 93511 to 94215 of *Triticum aestivum* LTR retrotransposon "Latidu-1p" (DQ537335); The nucleotide sites 373 to 894 of $4M_{907}$ had 86% homology with nucleotide sites 49252 to 48721 of *Triticum monococcum* LTR retrotransposon "Latidu" (AF459639); indicating that $10Q_{865,}$ $4I_{694}$ and $4M_{907}$ were probable transposon or retrotransposon.

Conversion of the RAPD products to specific PCR based markers

According to the nucleotide sequences of $10Q_{865}$, $14D_{995}$, $4I_{694}$, $11E_{358}$, $4M_{907}$ and $11H_{1033}$, six pairs of primers were designed (Table 2). Specific PCR using these six primer pairs showed that corresponding target bands could be detected in all E-genome-containing species including diploid, hexaploid, octoploid and decaploid listed in Table 1, but could not detected in all wheat controls, suggesting that these primer pairs could be used in wheat breeding program for E chromosome detection. The PCR patterns of hexaploid and octoploid *Trititrigia* by using primer pair D14F and D14R were showed in Fig. 1 and Fig. 2, respectively.

To examine whether these markers were unique for E-genome, specific PCR were performed on Triticeae species with different genomes, such as *Secale africanum, S. silvestre, S. cereale* cv. Jingzhou rye, *Aegilops tauschii*, *Dasypyrum breviaristatum*, *Th. intermedium*, *Th. elongatum*,

Table 2 Information about the primers designed in this study

Primer name	Sequence (5′–3′)	Target length(bp)	Annealing temperature
Q10F	TGTGCCCGAATGTAACTGACTA	831	60
Q10R	CTGGACGCTTCCTTGTGCTTC		
D14F	CTTCCCCAAGGCATGTTCCGT	985	62
D14R	CAGAGGAACTAGGGCGGATGA		
I4F	CCGCCTAGTCCACTAATGATT	670	60
I4R	CATCTTCAGCGGTCTTGCAGC		
E11F	GAGTCTCAGGACGAGGTGGAT	347	63
E11R	GCTCCGTCCTCTGGTCACGA		
M4F	GGCGGTTGTCGACATGGAGTT	815	61
M4R	CTGCTCAGAGTTAGTACACGAC		
H11F	CTTCCGCAGTTTGGGCCAGCTG	859	63
H11R	CCGTTGAGGTGGAGGCACCTGC		

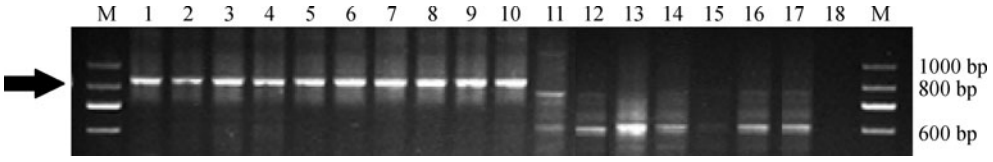

Figure 1 DNA amplification patterns of *Th. elongatum*, *Th. bessarabicum*, Hexaploid Trititrigia and controls with primers D14F and D14R. M: Marker(DL1000); Lanes 1 to 10 are *Th. elongatum* PI 531719, PI 531717, *Th. bessarabicum* PI 531711, W6 21890, *Th. intermedium* PI 401178, PI 401225, PI 383575, PI 547332, PI 547335 and PI 547316, respectively; Lanes 11 to 17 are Chinese Spring, *T. monococcum*, *T. turgidum* subsp. *durum*, *T. timopheevi*, *T. teres* and wheat varieties CN18, CN12, respectively; Lane 18 is blank control. Arrow showed the target PCR bands.

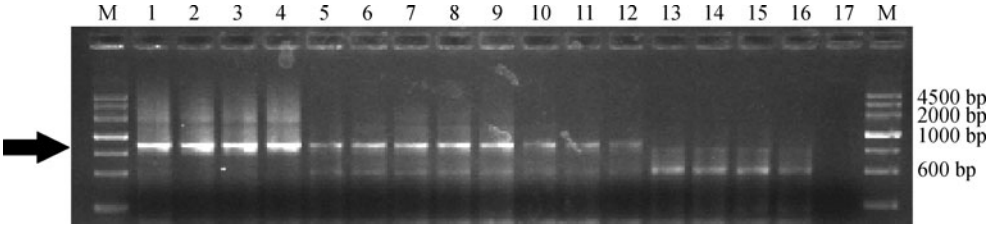

Figure 2 DNA amplification patterns of *Th. elongatum*, Octoploid *Trititrigia* and controls with primers D14F and D14R. M: Marker (DL4500); Lanes 1 to 12 are *Th. elongatum*, Chinese spring- *Th. elongatum* amphiploid, Chinese spring-*Th. bessarabicum* amphiploid, Wheat-*Th. intermedium* ssp. *trichophorum* amphiploid and Octoploid *Trititrigia* 7044, 7045, 7047, 78784, 7430, Zhong2, Zhong5 and XY693, respectively; Lanes 13 to 17 are CS, CN12, CN18 and CN17. Lane 18 is blank control. Arrow showed the target PCR bands.

Ps. spicata, *Ae. bicornis*, *Ae. crassa*, etc, as showed in control column of Table 1. PCR result showed that $10Q_{865}$ existed in *Th. elongatum*, *D. breviaristatum* and *D. villosum* genomes. $4I_{694}$ existed in *Th. elongatum*, *D. breviaristatum*, *D. villosum* and *Australopyrum retrofractum* genomes. $14D_{995}$, $11E_{358}$, $4M_{907}$ and $11H_{1033}$ amplified only in *Th. elongatum*, which indicating they were E-genome unique markers.

Chromosomal location of the specific DNA bands by using PCR and FISH

Attempts were made to assign the six E-genome specific markers to individual *Th. elongatum* chromosome. PCR were performed on a set of Chinese spring-*Th. elongatum* additions using six pair of E-genome specific primers listed in Table 2. All the six primer pairs produced corresponding target bands in seven Chinese Spring-*Th. elongatum* additions, but absent in wheat controls, suggesting that these markers distributed on all the seven E chromosomes. Meanwhile, PCR were also performed on a set of wheat-*Th. intermedium* additions, as a result, only D14F, D14R and E11F, E11R could amplify target DNA bands in wheat-*Th. intermedium* additions (except Z3). The PCR pattern of D14F, D14R in wheat-*Th. elongatum/Th. intermedium* additions was showed in Fig. 3. Because wheat-*Th. intermedium* additions Z1-Z6 contain S-J^s or *W*-J^s chromosome (Z3 contains only S chromosome), D14F, D14R and E11F, E11R could amplify target bands from these lines, indicating that these two pair of primers could not be only used for detecting E chromosome, but also for detecting J^s chromosome, that is to say D14F, D14R and E11F, E11R had broader use than other primer pairs in detecting *Th. intermedium* chromatin in wheat backgrounds.

All six markers developed in this research could amplify wheat-*Th. elongatum* 1E–7E addition, however, it is unknown that whether they could be used to hybridize *Th. elongatum* chromosome or not by FISH. To investigate this, six probes were hybridized to mitotic metaphase chromosomes of Chinese spring-*Th. elongatum* amphiploid,

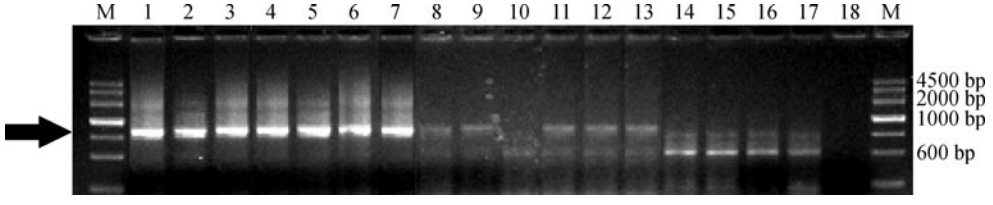

Figure 3 DNA amplification patterns of wheat-*Th. elongatum/Th. intermedium* additions and controls with primers D14F and D14R. M: Marker(DL4500); Lanes 1 to 7 are Chinese spring-*Th. elongatum* additions CSDA1E-CSDA7E; Lanes 8 to 13 are wheat-*Th. intermedium* additions Z1-Z6; Lanes 14 to 17 are CS, CN12, CN18 and CN17, respectively; Lane 18 is blank control. Arrow showed the target PCR bands.

respectively, as a result, only $4I_{694}$ has significant signals on all *Th. elongatum* (Fig. 4A). This suggests that $4I_{694}$ could be used to identify *Th. elongatum* chromosome by FISH in wheat background.

Screening of wheat-*Th. intermedium* ssp. *trichophorum* introgressions using E-genome SCAR markers established

Among the 72 wheat-*Th. intermedium* ssp. *trichophorum* introgression, 1908, Q155, Q156, Q160, Q161, Q168, et al., 27 plants in total amplified the corresponding target bands using all the six primer pairs, indicating that these six molecular markers could detect wheat-*Th. intermedium* ssp. *trichophorum* introgression efficiently, on the other word, these 27 lines contain E chromosome. To verify this, three wheat-*Th. intermedium* ssp. *trichophorum* introgressions, 1908, Q156 and Q168 were randomly selected for genomic in situ hybridization (GISH) analysis. GISH analysis using *Ps. spicata* genomic DNA as probe and with no blocking DNA used on mitotic metaphase chromosomes of 1908, Q156 and Q168 showed that the alien genomic composition of the three introgressions were $9St + 3J^s + 4J$, $8St + 6J^s + 2J$, and $5St + 3J^s + 2J + 3?$ (? = *Th. intermedium* chromosomes not determined), respectively (Fig. 4 B–4D). The GISH result confirmed the PCR result, suggesting that all the six SCAR markers developed in this study wre powerful tools for detecting E chromosome in wheat background.

Discussion

Retrotransposons or transposons have broad evolutionary impact on plant genome due to their prevalence and mobility (Brosius, 1991). A number of retrotransposons of wheat genomes are genome-specific, which provide chance to clone them for evolutional study or germplasm identification. Up to now, many approaches including RAPD, RFLP, AFLP and BAC library scanning were used to clone retrotransposons or transposons. Among them, RAPD is the most rapid and

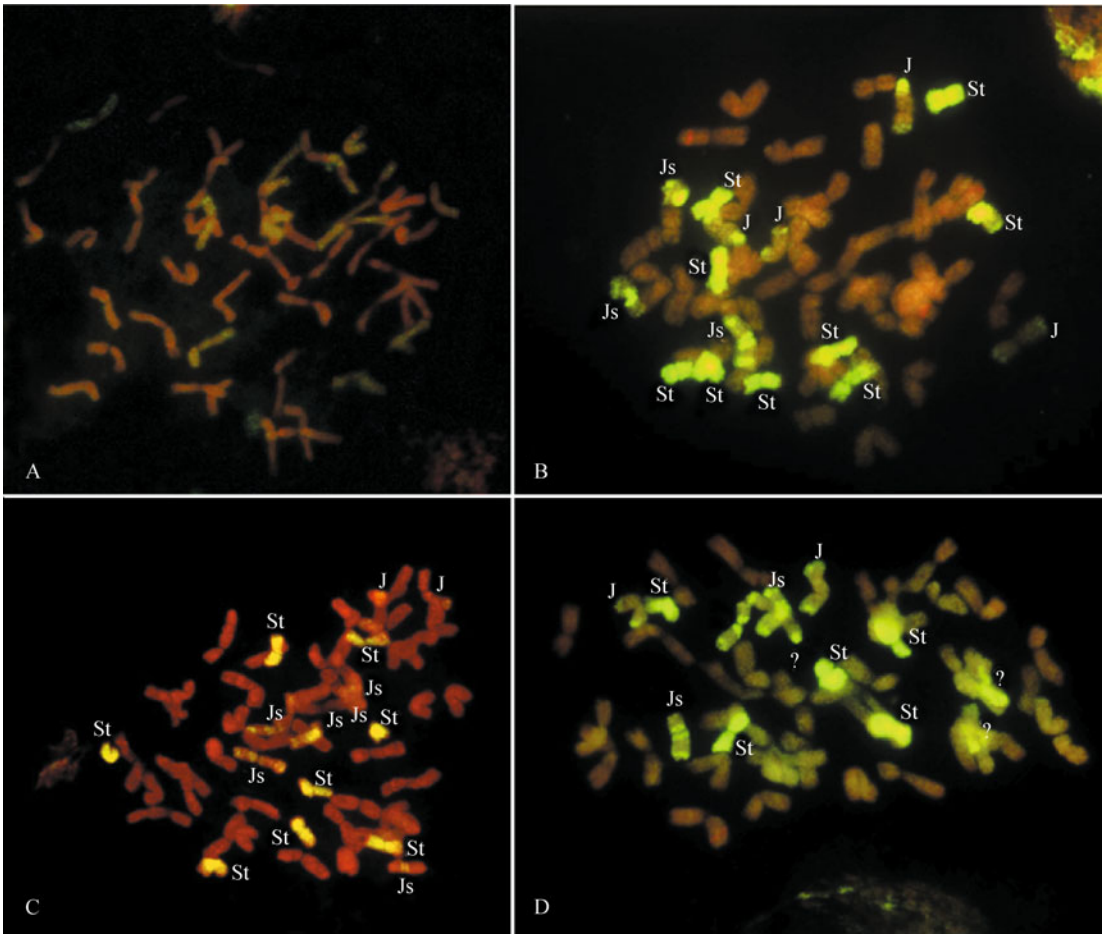

Figure 4 Fluorescence in situ hybridization analysis using $4I_{694}$ (A) and *Ps. spicata* genomic DNA (B-D) as probe and without blocking DNA used on mitotic metaphase chromosomes of Chinese spring-*Th. elongatum* amphiploid (A) and wheat-*Th. intermedium* ssp. *trichophorum* introgressions 1908 (B), Q156 (C) and Q168 (D). ? in Fig. 4D means unknown *Thinopyrum* chromosome due to chromosome overlap or weak hybridization signal.

cheapest approach. Using RAPD technique, Ko et al. (2002) isolated *Ty3*-gypsy retrotransposon from rye, Yang et al. (2006b) cloned *Sabrina*-like retrotransposon from *Dasypyrum* species, Jia et al. (2009) isolated a *Ty1*-copia retrotransposon from *S. africanum*. In this study, we cloned six sequences from *Th. elongatum* using RAPD. The sequences of 14D995, 11E358 and 11H1033 were new, while 10Q865, 4I694 and 4M907 were transposon or retrotransposon. Therefore, 50% sequences cloned from RAPD were transposon or retrotransposon, indicating that RAPD was a good approach for cloning repetitive sequences in wheat and related genomes.

Wide hybridization has contributed to genetic improvement of polyploid wheat (Friebe et al., 1996). Cross amphiploid or partial amphiploid to wheat varieties was commonly used to generate wheat-alien additions, substitutions or translocations. Therefore, a mass of cross offspring need to be screened. Molecular marker assisted selection which is diffusely used in wheat breeding is one of the excellent approach and turns to be an international breeding trend (He et al., 2006). By far, molecular marker has been established in *Secale* (Liu et al., 2006 and 2008), *Dasypyrum* (Yang et al., 2006b) and *Hordeum* (Liu et al., 1996) et al. for breeding purpose. Though *Thinopyrum* species contribute a lot to wheat breeding, the markers established are less that other genera of *Triticeae* because of its complex genome (Liu et al., 2009).

Liu et al. (1998) established RAPD marker of *Agropyron elongatum* 1E and 3E, and You et al. (2002, 2003) obtained E genome specific RAPD and SSR markers. Chen et al. (2007) developed a set of resistance gene analog polymorphism (RGAP) markers for detecting *Th. elongatum* 1E to 7E chromosomes. Li et al. (2007) developed several cleaved amplified polymorphic sequence (CAPS) markers specific to E genome. Recently, Xu et al. (2012) established three SCAR markers, one is specific for chromosome 2E and 3E, the other two distributed on all E chromosomes. All the above markers are established only using *Th. elongatum*, wheat-*Th. elongatum* additions as material, but not be validated by using plentiful of E chromosomes-containing *Thinopyrum* species especially octoploid trititrigia. In the present research, we developed six E-genome SCAR markers which distributed on *Th. elongatum* 1E to 7E chromosomes. The experimental data showed that all the six markers can be used for detecting wheat-*Th. intermedium* ssp. *trichophorum* introgressions and could be used for identifying wheat-*Thinopyrum* hybrids which contain E-genome chromatin. In these six markers, $14D_{995}$ and $11E_{358}$ could be used for detecting both E and J^s chromosome in wheat background. This is the first report that molecular marker could detect both two chromosomes simultaneously. This report represents new tools which can be widely used in wheat breeding for attempting to identify individuals that contain *Thinopyrum* E-genome chromatins.

The ratio of informative markers acquirement is different by using different primers, however, clone genome specific fragment and then convert to SCAR marker is a good approach for marker development. Liu et al. (1998) developed three RAPD markers specific for 1E (marker $OPE\text{-}05_{1300}$ and $OPF\text{-}03_{700}$) and 3E (marker $OPF\text{-}15_{400}$) chromosome from 26 RAPD primers tested, the ratio of informative markers acquirement is 11.5% (3/26). You et al. (2002) screened 100 RAPD primers, as a result, one specific RAPD fragment $OPF3_{1291}$was cloned from *Th. elongatum* and then converted to SCAR marker, the ratio of informative markers acquirement is 1% (1/100). Xu et al. (2012) screened 36 RAPD primers, as a result, two specific RAPD fragment $OPF3_{1407}$ and $LW10_{1487}$ was cloned from *Th. elongatum* and then converted to SCAR marker, the ratio of informative markers acquirement is 5.6% (2/36). In this research, we established six SCAR markers based on the RAPD fragments cloned by screening 280 RAPD primers, the marker acquirement is 2.1% (6/280). It was worth mentioned that primer OPF3 was used without exception in the reports mention above, it seemed that the amplicons were totally different, for we have not detect polymorphic between *Th. elongatum* and Chinese spring, while the rest reports obtain a polymorphic fragment with the length of about 700 bp (Liu et al., 1998), 1291 bp (You et al., 2002) and 1407 bp (Xu et al., 2012), respectively. This might be the disadvantage of RAPD which are not stable among different studies. However, it is no doubt that the conversion of RAPD to SCAR marker is necessary to produce a specific amplification, which can be effectively used to marker assisted selection among different wheat background under different studies.

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Compliance with ethics guidelines

Wenping Gong, Ling Ran, Guangrong Li, Jianping Zhou, Cheng Liu and Zujun Yang declare that they have no conflicts of interest, and this article does not contain any studies with human or animal subjects performed by any of the authors.

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