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Cytological and molecular characterization of *Thinopyrum bessarabicum* chromosomes and structural rearrangements introgressed in wheat

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Abstract *Thinopyrum bessarabicum* is an important genetic resource for wheat improvement by chromosome engineering. However, the present low-resolution karyotype limits identification of its chromosomes. Oligonucleotide probes to identify tandem repeats provide an efficient way to produce high-resolution karyotypes in many species. In this study, putative tandem repeats were identified using unassembled sequence reads of *Th. bessarabicum*, and 306 repeat clusters were identified. Among them, 17 had conserved motifs that varied in size from 71 to 856 bp and occupied 0.01% to 1.30% of the genome. Thirty-nine oligonucleotides from 17 clusters were developed, and 21 from 8 clusters produced clear and stable signals in *Th. bessarabicum* chromosomes. Five tandem repeat clusters were distributed only at the telomeric or subtelomeric regions, and the BSCL242 probe produced signals only on chromosome 7JL. The other three were mainly in intercalary and centromeric regions with a few weak signals in telomeric regions. A new multiplex oligonucleotide probe (ONPM#7) containing 13 oligonucloetides distinguished all wheat and *Th. bessarabicum* chromosomes after one round of FISH. The high-resolution karyotype of *Th. bessarabicum* in corresponding with the seven homoeologous group chromosomes of wheat has been developed. Three spontaneous translocations and one isochromosome among *Th. bessarabicum* chromosomes introgressed into wheat thus have been characterized in combined with molecular marker analysis. The ONPM#7 probe and molecular markers provide powerful tools for engineering transfer of chromosomal segments from *Th. bessarabicum* to wheat.

Keywords Fluorescence in situ hybridization · Structural chromosome variation · Chromosome engineering

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Introduction

Repetitive DNA sequences are abundant in eukaryotic plant genomes (Lapitan 1992; Mehrotra and Goyal 2014). There are two basic types of repetitive sequences: namely, interspersed repeats and tandem repeats (Jurka et al. 2007) that have roles in genome evolution and are frequently used for taxonomic and phylogenetic studies (Mehrotra and Goyal 2014). Tandem repeats associated with important chromosomal landmarks, such as centromeres, telomeres, and subtelomeric and other heterochromatic regions, have been widely studied during the last few decades (Heniko et al. 2001; Jiang and Gill 2006; Albert et al. 2010). Chromosome banding and fluorescence in situ

hybridization (FISH) were successively established and widely used for chromosome identification based on heterochromatin banding (Gill et al. 1991; Jiang et al. 1994; Kato et al. 2004; Jiang and Gill 2006; Komuro et al. 2013). More recently, the identification and application of synthesized oligonucleotides as probes greatly simplified the FISH procedure and extended the use of FISH in both chromosome research and engineering (Cuadrado and Jouve 2010; Wang 2013; Tang et al. 2014; Han et al. 2015; Du et al. 2017, 2018; Zhu et al. 2017; Zhang et al. 2019).

Thinopyrum bessarabicum ($2n = 2x = 14$, JJ) is an important genetic resource for wheat improvement due to its salt tolerance, yield-related genes, and blue grain (King et al. 1996, 1997a, b; Qi et al. 2010; Hassani et al. 2010; Shen et al. 2013; Pu et al. 2015). Wheat-*Th. bessarabicum* translocations have been reported in several publications (King et al. 1997a, b; Hassani et al. 2010; Qi et al. 2010; Shen et al. 2013; Pu et al. 2015; Patokar et al. 2016; Grewal et al. 2018). Karyotyping is essential for wheat-*Th. bessarabicum* chromosome engineering. Previous karyotypes of *Th. bessarabicum* were mainly based on the chromosome length and arm ratio (Wang 1985; Hsiao et al. 1986), C-banding (William and Mujeeb-Kazi 1993; Qi et al. 2010; Mirzaghaderi et al. 2010), and FISH using plasmid clones of tandem repeats as probes (Wang 2013; Grewal et al. 2018). Du et al. (2017) reported a karyotype developed from oligopainting where all chromosomes were distinguished but at low resolution; hence, chromosome identification was still difficult. With the development of next-generation sequencing technologies, it became easier to discover tandem repeats from sequencing data based on bioinformatics tools such as Tandem Repeats Finder (TRF) (Benson 1999) and Tandem Repeat Analyzer (TAREAN) (Novák et al. 2017). Here, we report progress in identifying tandem repeats using TAREAN and unassembled sequenced reads from *Th. bessarabicum*. Genomic and physical organization of tandem repeats in *Th. bessarabicum* chromosomes and efficient oligonucleotide probes were identified, which enabled the development of higher-resolution karyotypes of both wheat and *Th. bessarabicum*. All chromosomes could be differentiated, and chromosomes involved in translocations between *Th. bessarabicum* chromosomes were identified and validated with molecular markers.

Materials and methods

Plant materials

A Chinese Spring (CS)-*Th. bessarabicum* amphidiploid and CS-*Th. bessarabicum* disomic addition line DA3J originally came from Dr. Mujeeb-Kazi, CIMMYT, Mexico. *Th. bessarabicum* (PI 531711) was introduced from the USA by Sichuan Agricultural University. All materials used in the study are listed in Table 1.

Methods

Analysis of tandem repeats

The TAREAN program under a Galaxy web-based environment (http://www.repeatexplorer.org) (Novák et al. 2017) was used to obtain tandem repeats from 419,430 Illumina reads (paired-end 2 × 125 bp) of *Th. bessarabicum* DNA (select queue: basic and fast, modify parameters: default). Seventeen putative kinds of satellites were compared with the nr database from NCBI by BLAST (program selection: megablast, https://blast.ncbi.nlm.nih.gov/).

Development of oligonucleotides from putative Th. bessarabicum satellite DNA

To verify the distribution of satellite DNA in *Th. bessarabicum* chromosomes, 1~5 single-strand oligonucleotides (30–50 nt) were designed from each putative satellite cluster by OLIGO 7 (Rychlik 2007) and modified by 6-carboxyfuorescein (FAM) at General Biosystems (Chuzhou, Anhui Province)

Development of 5S ribosomal DNA oligonucleotides from grasses

5S ribosomal DNA (5S rDNA) sequences were downloaded from Rfam (http://rfam.xfam.org/). Multiple sequence alignment among *Oryza sativa*, *Brachypodium distachyon*, *Secale cereale*, *Hordeum vulgare*, *Triticum aestivum*, and *Triticum urartu* identified a highly conserved 116-bp sequence on the 120-bp coding sequence of 5S rDNA. This sequence was used to develop two oligonucleotides (36 nt) by OLIGO 7 (Rychlik 2007), and these were modified by 6-carboxytetramethylrhodamine (TAMRA) at General Biosystems.

Table 1 Materials used in this study

Accession	Chromosome number	Chromosome constitution[a]	Zygosity	Source
Chinese Spring	42	AABBDD	Homo	China
CS-*Th. bessarabicum* amphidiploid	42 + 14 = 56	AABBDDJJ	Homo	Mujeeb-Kazi, CIMMYT
Th. bessarabicum	14	JJ	Homo	USA via Sichuan Agricultural University
18YLS001	42	DS1J(1B)	Homo	Tan (2011)
18YLS320-20	42 + 1 = 43	MA2J	Hetero	This study
18YLS320-45	42 + 1 = 43	MA3J	Hetero	This study
18YLS208	42 + 2 = 44	DA3JS·4JL	Homo	Zhuang et al. (2003); Shen (2011)
18YLS218	42 + 2 = 44	DA3J	Homo	William and Mujeeb-Kazi (1995)
18YLS319	42 + 2 = 44	DA4J	Homo	Dawadundup (2009); Pu et al. (2015); Shen et al. (2013)
18YLS207-8	42 + 1 = 43	MA4JS	Hetero	Shen et al. (2013)
18CJY001	42 + 1 = 43	MA4JL·4JL	Hetero	Shen et al. (2013)
18YLS006	42	DS5J(5A)	Homo	Dawadundup (2009); Yang et al. (2018)
18YLS007	42	DS6J(6A)	Homo	Qian (2007); Liu (2015)
18YLS214	44	DA6JS·2JL	Homo	Shen (2011)
18YLS215-15	39 + 2 = 41	M5B/MS5J(5A)/MS7J(7D)	Hetero	This study

Homo homozygous, *Hetero* heterozygous

[a] For chromosome descriptions: *D* disomic, *M* monosomic, *A* chromosome addition, *S* chromosome substitution

Oligonucleotide FISH and karyotyping

The procedure of chromosome preparation based on the pioneering work of Doležel et al. (1992) and Kato (1999) is described in Huang et al. (2018). Oligonucleotide sequences used for FISH are listed in Table 2.

Three FISH karyotypes with similar patterns were developed previously and used for identification of *Th. bessarabicum* chromosomes (Wang 2013; Du et al. 2017; Grewal et al. 2018). Oligonucleotide probes and multiplexes were developed for identification of both wheat and *Th. bessarabicum* chromosomes (Du et al. 2017). Based on previous reports, a modified oligonucleotide probe multiplex (ONPM#4-1) was formed to identify all chromosomes of *Th. bessarabicum* based on unique signals, satellites, and arm ratios. This multiplex contained seven TAMRA-modified oligonucleotide probes (pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, AFA-4, and pSc119.2) and produced a karyotype similar to that in Du et al. (2017) and Grewal et al. (2018). The karyotype was then used for physical mapping of new oligonucleotides developed in this study.

FISH procedure is described in Huang et al. (2018), and sequential genomic in situ hybridization (GISH) procedure is described in Pu et al. (2015). After FISH chromosomes were visualized with an Olympus BX60 microscope (Olympus, Japan), images were captured with a SPOT Cooled Color Digital (CCD) (Olympus DP72) camera, and image analysis was conducted using Photoshop (v6.0) (Adobe, USA).

Molecular marker analysis

Based on sequences of conserved orthologous genes in Triticeae species and intron polymorphisms among the three orthologs of common wheat, we developed intron-targeted (IT) markers as described in Wang et al. (2017) and Zhang et al. (2017). The IWGSC RefSeq v1.0 annotation and the IWGSC RefSeq v1.0 assembly were downloaded from URGI (https://wheat-urgi.versailles.inra.fr/). We firstly chose sets of genes from chromosomes 2D, 3D, and 7D to calculate exon-exon junction sizes in genomic sequences of homoeologous chromosome groups 2, 3, and 7 by BLASTN (*e* value > 1*e*−5). Intron sizes of corresponding genes were then

Table 2 Oligonucleotide probes used in this study

Probe	Sequence and 5′-end modification	Reference
pAs1-1	TAMRA-5′-GGATGCACTTCGTGTACAAAACGGACAATCTCTTTCAA AGTATCAGGATTTCATCC-3′	Wang (2013); Du et al. (2017)
pAs1-3	TAMRA-5′-TTTTTGTGTGTTCAAAATGCACCATTCAAAGCCACATC ATCATTTTTCAATCCTTT-3′	Wang (2013); Du et al. (2017)
pAs1-4	TAMRA-5′-CTGACTTCATTTGTTATTTTTCATGCATTTACTAATTA TTTTTAGCTATAAGACCC-3′	Wang (2013); Du et al. (2017)
pAs1-6	TAMRA-5′-CATCATTTCATCCACATAGCATGTGCAAGAAA GTTGAGAGGGTTACGGCAAAAACT-3′	Wang (2013); Du et al. (2017)
AFA-3	TAMRA-5′-AAGTATCAGGGTTTCGGACGGAAACTCATCTATTACAA AGGGATT-3′	Du et al. (2017)
AFA-4	TAMRA-5′-CAGTTTTTAAAACATATTTGAACTCCTGACTTTTTGTGTGTT-3′	Du et al. (2017)
pSc119.2.-1	TAMRA-5′-GGCCAGAATCGGCCAAAACTACGAGTGCTGAT GACCGACACGTAAACGCACCCCGGGGT-3′	Wang (2013); Du et al. (2017)
(GAA)10	FAM-5′-GAAGAAGAAGAAGAAGAAGAAGAAGAA-3′	Cuadrado and Jouve (2010); Du et al. (2017)
5S-1	TAMRA-5′-TCATACCAGCACTAAAGCACCGGATCCCATCAGAAC-3′	This study
5S-2	TAMRA-5′-GCGTGCTTGGGCGAGAGTAGTACTAGGATGGGTGAC-3′	This study
BSCL1-1	FAM-5′-ACTAGAGAAGAAACGGGGAACAAACTGATGAAAGAATTAT-3′	This study
BSCL1-2	FAM-5′-TTCACTCAGAACTAACAAGAATGATAACGTGCTCCGA-3′	This study
BSCL5-1	FAM-5′-TAGGTGTTGGAAAGGTATCGGGGAATTGAGATGA-3′	This study
BSCL5-2	FAM-5′-TTTTATGGGCCAACTCATCCGTATGGTCTGCAGAAATAT-3′	This study
BSCL75-1	FAM-5′-ATTCTGGCCCGTTTCGTGGACTATTACTCACTGTTTT-3′	This study
BSCL75-2	FAM-5′-CGAAGTGATTTCCATGATTGTTGAACCCCGAG-3′	This study
BSCL135-1	FAM-5′-GCCTCGACTCGCGTTACCCTAAGATAGACAG-3′	This study
BSCL135-2	FAM-5′-GTGGTGTCACGTGCTCGTCTCGGCGTCCGT-3′	This study
BSCL135-1	TAMRA-5′-GCCTCGACTCGCGTTACCCTAAGATAGACAG-3′	This study
BSCL135-2	TAMRA-5′-GTGGTGTCACGTGCTCGTCTCGGCGTCCGT-3′	This study
BSCL158-1	FAM-5′-TGCACACTAGAACGGTCATAACTTTGTCATTCGGAGTC-3′	This study
BSCL158-2	FAM-5′-AGTACCCTCAAATTCAGCGTTAAGCAGGACTTTT-3′	This study
BSCL158-3	FAM-5′-TTCAAGTAAATCCAGCGCCTATAAGCC-3′	This study
BSCL158-4	FAM-5′-TGGCATGGTGTAACTTATGTGTCTG-3′	This study
BSCL158-5	FAM-5′-CATTGCAGAGAAAAGCGTCACGGGA-3′	This study
BSCL184-1	FAM-5′-TAGCTCTATAACCTAGTACAAATAATTTTACA-3′	This study
BSCL184-2	FAM-5′-ATAGCCTAGTTCAAATAATTTTACACTAGAGTTGA-3′	This study
BSCL156-1	FAM-5′-CCAAACCCACAGCAAGATCCCCTCCGTGTAGACCCGA-3′	This study
BSCL156-2	FAM-5′-TGCTGCCTCTTCAGACATGCCACCACACCTTACG-3′	This study
BSCL156-3	FAM-5′-TTGACCACGAGATCTAGCCCTTTGACTTTCCCC-3′	This study
BSCL242-1	FAM-5′-GTTGCTCAAACTAGAAAATGGACAGATATGGT-3′	This study
BSCL242-2	FAM-5′-TTTGAGGGAATTATATGATGTTATCTTGTTCTCCATG-3′	This study
BSCL242-3	FAM-5′-TCTAAATATTGATCTAGGCCAAGTGGTGCAT-3′	This study

calculated and compared. Homologue alleles in A, B, and D chromosomes whose intron sizes differed by at least 10% were chosen for designing new markers. The primers were designed from exons flanking targeted introns by the use of the online software Primer 3 (version 4.0, http://bioinfo.ut.ee/primer3-0.4.0/). All primers were synthesized by the General Biosystems.

PCR was conducted in a 10 μL system, and the PCR mixture contained 1.5 μL (ca. 25 ng/μL) template DNA, 0.2 μL (10 μM) of each primer, 3 μL of ddH_2O, and 5 μL Master Mix (TSINGKE Biological

Technology, Beijing). Amplifications were conducted for 3 min at 94 °C, followed by 33 cycles of 30 s at 94 °C, 45 s at 49–56 °C depending on specific primers, 1 min 10 s at 72 °C, and a final step of 10 min at 72 °C. PCR products were separated in 8% polyacrylamide gels and visualized with an automatic gel imager JS-680B (Peiqing Science and Technology, Shanghai) after silver staining as described in Pu et al. (2015). Fragment sizes of PCR amplicons were determined by the gel image analysis (SensiAnsys 1.0.3, Peiqing Science and Technology).

Results

Development and physical mapping of tandem repeat oligonucleotides in *Th. bessarabicum*

Following Wang (2013), Du et al. (2017), and Grewal et al. (2018), ONPM#4-1 was developed and produced a basic karyotype that distinguished all chromosomes of *Th. bessarabicum* based on the unique signals, satellites, and arm ratios after one round of FISH (Fig. 1). The basic karyotype was then used for physical mapping of new oligonucleotides developed in this study. Among 39 oligonucleotides developed from 17 putative satellite clusters, 21 from 8 clusters produced clear signals on 1–7 pairs of *Th. bessarabicum* chromosomes. The 8 putative satellites proved to be abundant tandem repeats (Fig. 1, Fig. S1, Table S1).

The new putative satellites were aligned with the known repetitive sequences in the NCBI using BLASTN and then chromosomally located in *Th. bessarabicum* by FAM-modified oligonucleotide FISH. The first four satellite clusters were mapped based on the basic karyotype produced by ONPM#4-1, and all chromosomes could be recognized (Fig. 1).

- BSCL1 was similar to the subtelomeric repeat Spelt52.2 from *Aegilops speltoides* (GenBank: AY117401.1) (Fig. 1b) and was the most abundant putative satellite covering 1.300% of the genome according to TAREAN calculations. It was located in the terminal or subterminal regions of chromosomes 1JS, 4JL, 5JS, 5JL, 6JS, and 7JL.
- BSCL135 was similar to the repetitive sequence pTa-713 in *Triticum aestivum* (GenBank: KC290900.1), occupying 0.110% of the genome and producing the strongest and most widely distributed signals on all chromosomes of *Th. bessarabicum* (Fig. 1f). This oligo is very useful for karyotype analysis.
- BSCL184 (Fig. 1j) and BSCL242 (Fig. 1n) were identified as new types of tandem repeats; BSCL242 produced signals in the subterminal region of 7JL, indicating chromosome specificity, whereas BSCL184 produced signals in the intercalary regions of 4JL and subterminal regions of 7JS and 7JL.

Two 5S rDNA–derived oligonucleotides (TAMRA-modified 5S-1 and 5S-2) were located in the subterminal region of 1JS (Fig. 2a, b).

BSCL135 produced unique signals on all seven chromosomes and made it easier to distinguish all *Th. bessarabicum* chromosomes. Together with TAMRA-modified 5S-1 and 5S-2, TAMRA-modified BSCL135-1 and BSCL135-2 were included in ONPM#4-1 to constitute ONPM#4-2. This was used to locate and map four other (Fig. S1).

- BSCL5 was similar to the repeat sequence clone pLrPstI-4 from *Leymus racemosus* (GenBank: AB016973.1); it occupied 1.200% of the genome and was located in the terminal or subterminal regions of 1JS, 1JL, 2JS, 3JS, and 6JL (Fig. S1b).
- BSCL75 was similar to satellite DNA p25208-193 from *Secale vavilovii* (GenBank: AJ517270.4). It covered 0.380% of the genome and was located in the terminal or subterminal regions of 1JS, 1JL, 2JS, 3JS, 3JL, 4JS, 4JL, 5JS, 6JS, 6JL, and 7JL (Fig. S1f).
- BSCL156 was similar to part of the sequence in BAC LKR001 (GenBank: GU182251.1) from *Triticum turgidum*; it occupied 0.065% of the genome and was located in the intercalary regions of 1JL and the subterminal region of 6JS (Fig. S1j).
- BSCL158 was similar to the XbaI tandem repeat region in BAC 126/C20 (GenBank: FJ904940.1) from *Secale cereale*. It occupied 0.060% of the genome and was located at the terminal or subterminal regions of 3JL, 6JS (occasionally), and 6JL (Fig. S1n).

The physical organization of each tandem repeat cluster is shown in Fig. 2a and b and Fig. S2.

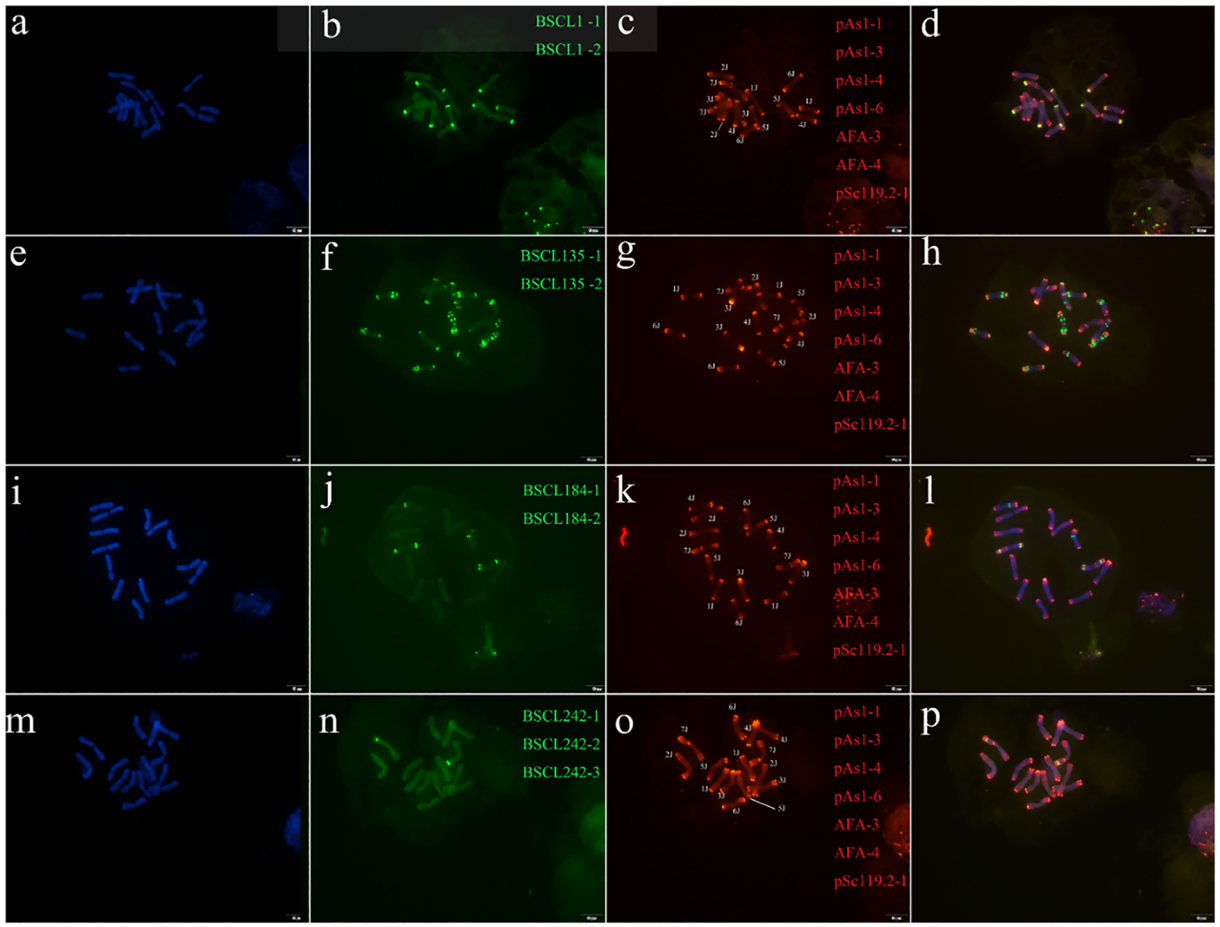


Fig. 1 Physical mapping of tandem repeat oligonucleotides BSCL1 (**a**–**d**), BSCL135 (**e**–**h**), BSCL184 (**i**–**l**), and BSCL242 (**m**–**p**) in *Th. bessarabicum* chromosomes. Blue, chromosomes counterstained with DAPI; green, probe modified with FAM; red, probe modified with TAMRA

Validation of the homoeologous grouping of *Th. bessarabicum* chromosomes

ONPM#7 was developed to confirm the identities of all *Th. bessarabicum* chromosomes in karyotypes produced by ONPM#4-1 and ONPM#4-2 and to develop a new oligonucleotide multiplex probe for identification of both wheat and *Th. bessarabicum* chromosomes. It included TAMRA-modified pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, AFA-4, pSc119.2-1, 5S-1, and 5S-2; FAM modified (GAA)10; and BSCL135-1, BSCL135-2, and BSCL242-1. Separate karyotypes were developed for CS, *Th. bessarabicum*, and CS-*Th. bessarabicum* amphiploid (Figs. 2 and 3). All *Th. bessarabicum* chromosomes showed differing signal combinations on most arms except 2JS and 3JS and corresponded in *Th. bessarabicum* and CS-*Th. bessarabicum* amphiploid. ONPM#7 produced stable signals in different preparations of *Th. bessarabicum* chromosomes following sequential GISH (Fig. S3). The ability to distinguish all *Th. bessarabicum* chromosomes was vastly improved.

For validation of the homoeologous identities of *Th. bessarabicum* chromosomes in different backgrounds, seven *Th. bessarabicum* chromosome introgressions in CS were characterized after ONPM#7 FISH, sequential GISH, and molecular marker analysis. Disomic substitutions of chromosomes 1J, 4J, 5J, and 6J were previously validated using molecular markers (Tan 2011; Shen et al. 2013; Li 2014; Pu et al. 2015; Liu 2015; Yang et al. 2018). The unique patterns of 1J, 5J, and 6J in disomic substitution lines DS1J(1B), DS5J(5A), and DS6J(6A) and that of 4J in the disomic addition line DA4J corresponded to those identified in *Th. bessarabicum* and CS-*Th. bessarabicum*

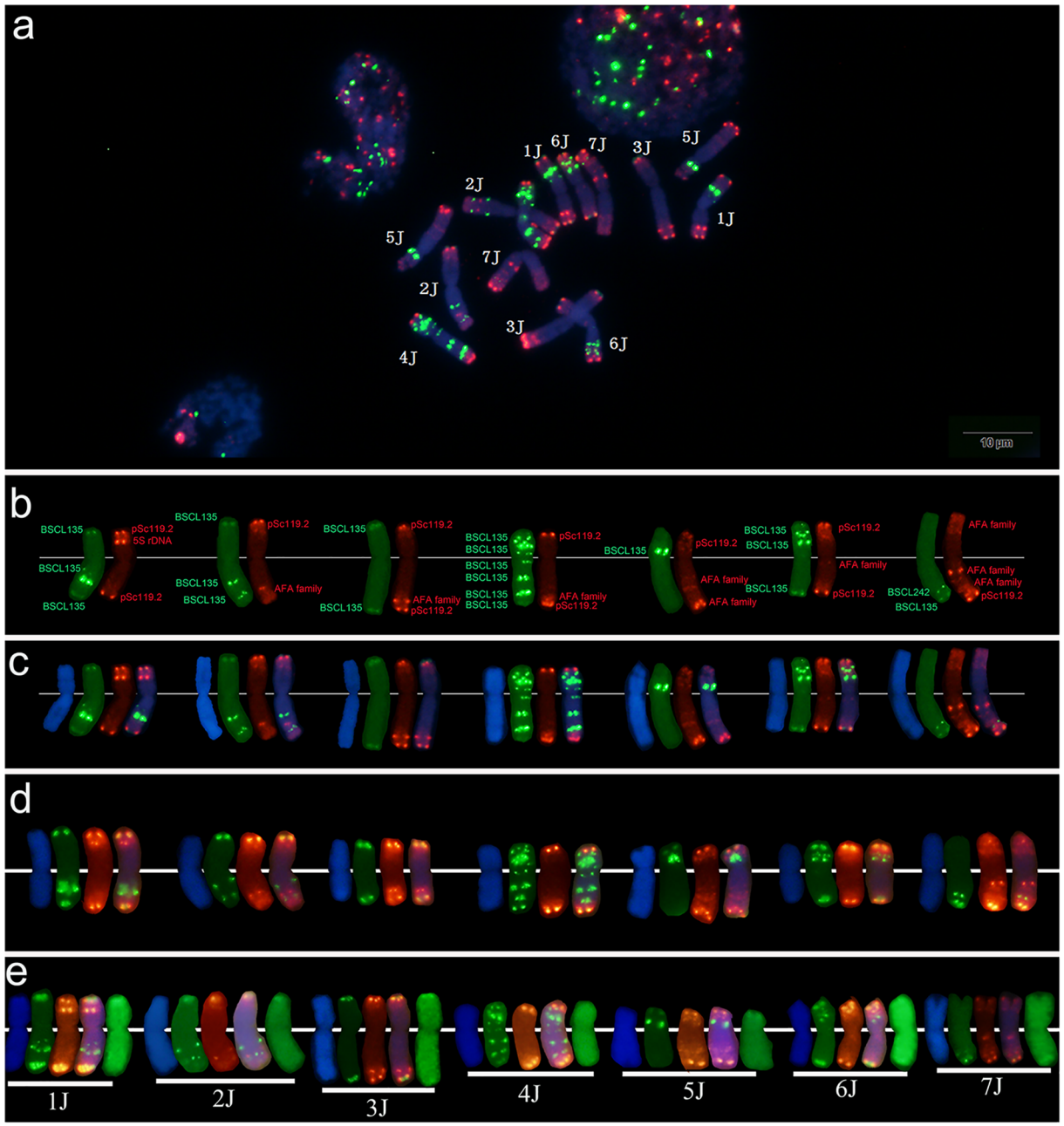


Fig. 2 Chromosome painting of *Th. bessarabicum* using ONPM#7 FISH and sequential GISH (**a**, **b**) and consensus karyotypes of *Th. bessarabicum* (**c**), CS-*T. bessarabicum* amphiploid (**d**), and seven CS-*Th. bessarabicum* alien chromosome lines (**e**). **a** Chromosome painting with ONPM#7. **b** Physical map of tandem repeats pSc119.2, 5S rDNA, AFA family, BSCL135, and BSCL242. **c** Karyotype from *Th. bessarabicum* accession. **d** Karyotype from CS-*T. bessarabicum* amphiploid. **e** Karyotype from seven CS-*T. bessarabicum* alien chromosome lines after ONPM#7 FISH and sequential GISH. DS1J(1B), MA2J, MA3J, DA4J, DS5J(5A), DS6J(6A), and M5B/MS5J(5A)/MS7J(7D). Blue, counterstaining with DAPI; green, probe modified with FAM and Fluorescein-12-dUTP; red, probe modified with TAMRA

amphiploid. To validate the homoeology of the other three chromosomes (2J, 3J, and 7J), three CS-*Th. bessarabicum* introgressions, including the monosomic addition lines MA2J and MA3J and the multiple monosomic addition and substitution line M5B/MS5J(5A)/MS7J(7D), were identified among F_2 plants from CS × CS-

Th. bessarabicum amphiploid (Fig. 2, Figs. S3~S9). To confirm their homoeologies, 52 *Th. bessarabicum*-specific IT markers were developed based on orthologous genes in group 2, 3, and 7 wheat chromosomes. Among them, 21 were from group 2 and located on chromosome 2J, 17 were from group 3 and located on 3J, and 14 were from group 7 and located on 7J (Table S2), thus proving that the three chromosomes belonged to homoeologous groups 2 (Fig. 4), 3 (Fig. 5), and 7.

The karyotypes of CS, CS-*Th. bessarabicum* amphiploid, and seven CS-*Th. bessarabicum* derived lines were compared (Figs. 2 and 3, Figs. S3~S9). All *Th. bessarabicum* chromosomes were identifiable in different backgrounds, with distinguishing signal combinations observed for most chromosome arms except 2JS and 3JS (Fig. 2c–e). Descriptions are as follows:

- Chromosome 1J: one weak and one strong green signal were produced in the middle of the long arm by BSCL135, and the unique signal of 5S rDNA (red) was located in the subterminal region of the short arm.
- Chromosome 2J: a combination of four signals (green-red-green-red) was located on the long arm, and a weak green signal was at the terminal region of the short arm.
- Chromosome 3J: two separate but adjacent red signals located at the subterminal and terminal regions of the long arm differentiated this chromosome.
- Chromosome 4J possessed the most abundant green signals in both arms, making it clearly distinguishable from the other chromosomes.
- Chromosome 5J had a secondary constriction in the subterminal region and a green signal in the centromeric region of the short arm, as well as having the largest, distinguishable arm ratio.
- Chromosome 6J had rich green signals on the short arm and two weak red signals in the centromeric region on the long arm.
- Chromosome 7J: the subterminal region at the terminal of the long arm had unique green signals from BSCL242, but not on the short arm; a strong red signal was present in the centromeric region.

Spontaneous translocations and an isochromosomes involving *Th. bessarabicum* chromosomes

Based on the unique patterns of *Th. bessarabicum* chromosomes after ONPM#7 FISH, three translocations and one isochromosome involving *Th. bessarabicum* chromosomes were identified: T6JS·2JL, T3JS·4JL, T4JS·3JL, and i4JL·4JL.

T6JS·2JL was a Robertsonian translocation with unique signals of 6JS and 2JL in comparison with the intact chromosomes (Fig. 4c, Fig. S10). Among 21

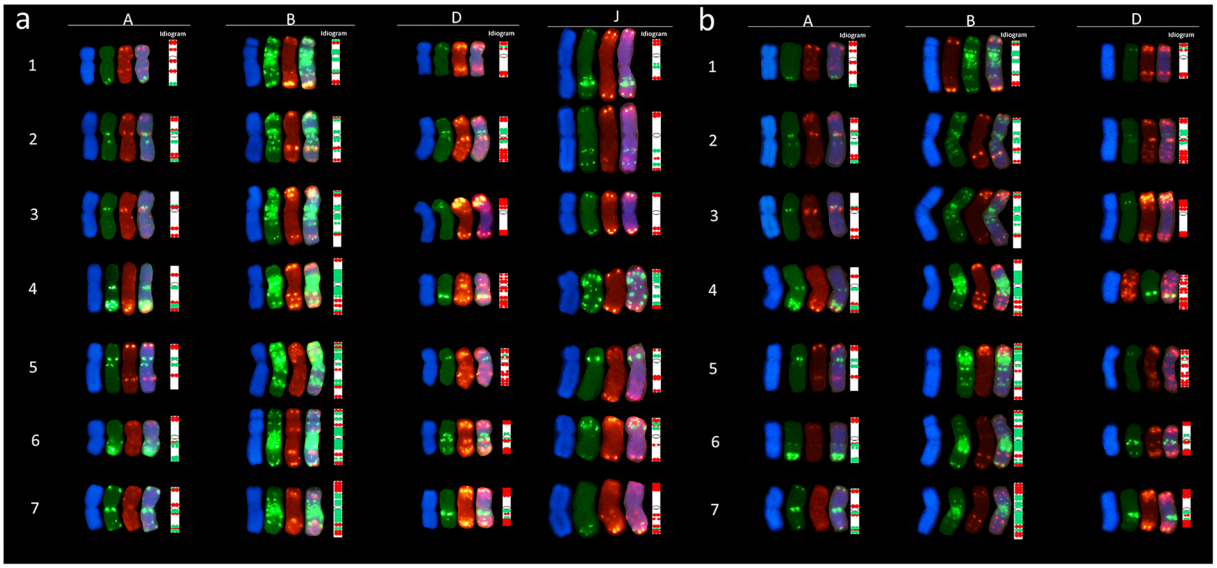


Fig. 3 Karyotypes of CS-*Th. bessarabicum* amphiploid (**a**) and CS (**b**) after ONPM#7 painting. Colors, same as Fig. 2. The alien chromosomes were further validated after sequential GISH in Fig. S3

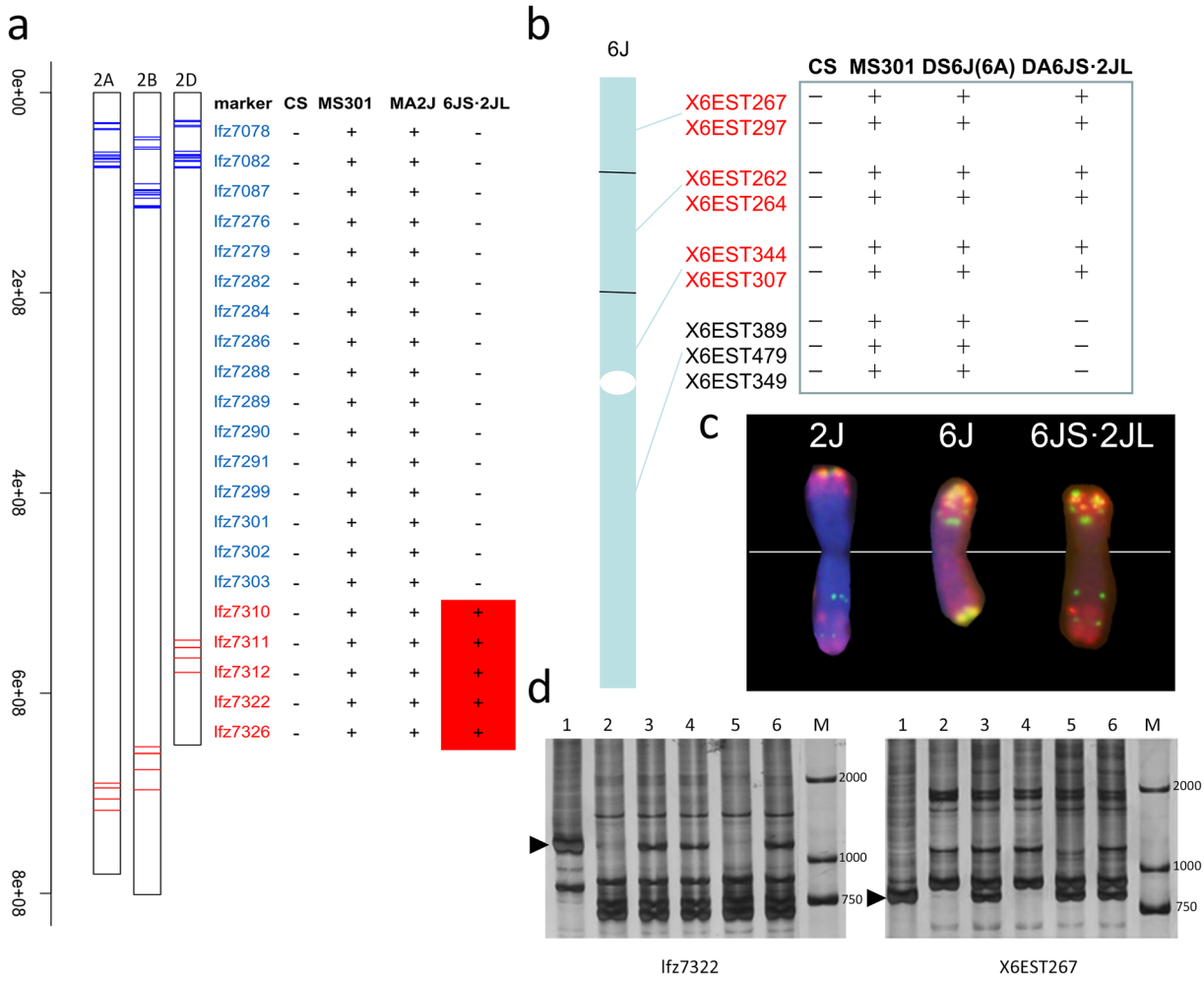


Fig. 4 Homoeologous chromosome determination of *Th. bessarabicum* chromosome 2J using intron-targeted (IT) markers and characterization of spontaneous translocation T6JS·2JL using ONPM#7 FISH. Plus sign indicates the presence of specific *Th. bessarabicum* loci; minus sign indicates the absence of specific loci. Blue indicates markers were in silico-mapped on the short arm and red on the long arm of homoeologous group 2 chromosomes. CS, Chinese Spring; MS301, CS-*Th. bessarabicum* amphiploid. **a** Markers of chromosome 2J. **b** Markers of chromosome 6J. **c** Chromosome painting of chromosome 2J, 6J, and 6JS·2JL using ONPM#7. **d** PCR analysis of two specific markers lfz7322 and X6EST267. 1, *Th. bessarabicum*; 2, CS; 3, MS301; 4, MA2J; 5, DS6J(6A); 6, DA6JS·2JL; the arrows indicate the specific bands. The alien chromosomes were further validated after sequential GISH in Fig. S10

newly developed markers for 2J, only five that mapped to 2L chromosomes were specifically amplified, and specific bands for all 16 markers representing group 2L chromosomes failed to amplify (Fig. 4a). Among nine markers specific for 6J reported by Liu (2015), only six 6JS-specific markers amplified and the other three 6JL-specific markers failed to amplify in the DA6JS·2JL line (Fig. 4b).

Marker analysis of the line 18YLS208 (DAJ7) developed by Zhuang et al. (2003) identified the introduced translocation chromosome as T3JS·4JL (Shen 2011). The present karyotyping confirmed the identity of this chromosome by its unique signals for 3JS and 4JL in comparison with the intact chromosomes (Fig. 5, Fig. S11). Five 4JL-specific markers from Pu et al. (2015) confirmed the identity of 4JL; however, marker *X4est221* was absent (Fig. 5). All the eight markers on 3S chromosomes amplified specific bands confirming the translocation chromosome as T3JS·4JL; none of the nine 3L markers was amplified.

The 3J chromosome in DA3J reported by William and Mujeeb-Kazi (1995) was identified as T4JS·3JL by

both karyotyping and molecular marker analysis (Fig. 5, Fig. S12). This corresponded with a SNP analysis of this chromosome reported by Grewal et al. (2018).

The isochromosome i4JL·4JL reported by Shen et al. (2013) was clearly validated with the unique signals produced by ONPM#7 FISH (Fig. 5).

Fig. 5 Homoeologous chromosome determination of *Th. bessarabicum* chromosome 3J using intron-targeted (IT) markers and characterization of spontaneous translocations T3JS·4JL and 3JS·4JL using ONPM#7 FISH. Plus sign indicates the presence of specific loci of *Th. bessarabicum*; minus sign indicates the absence of specific loci. Blue and red indicate markers that were in silico-mapped on the short arm and long arm, respectively, of homoeologous group 3 chromosomes. CS, Chinese Spring; MS301, CS-*Th. bessarabicum* amphiploid. The alien chromosomes were further validated after sequential GISH in Figs. S11 and S12

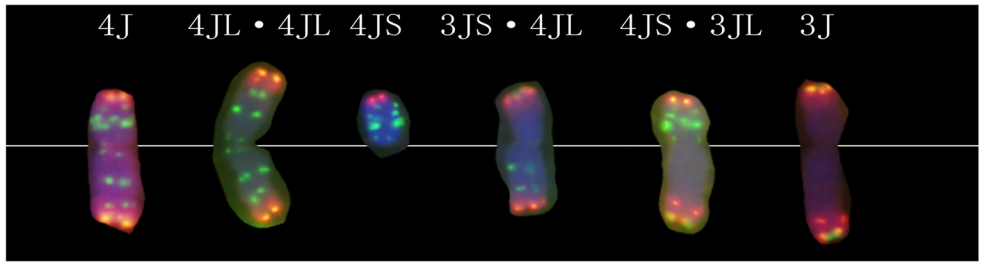
Discussion

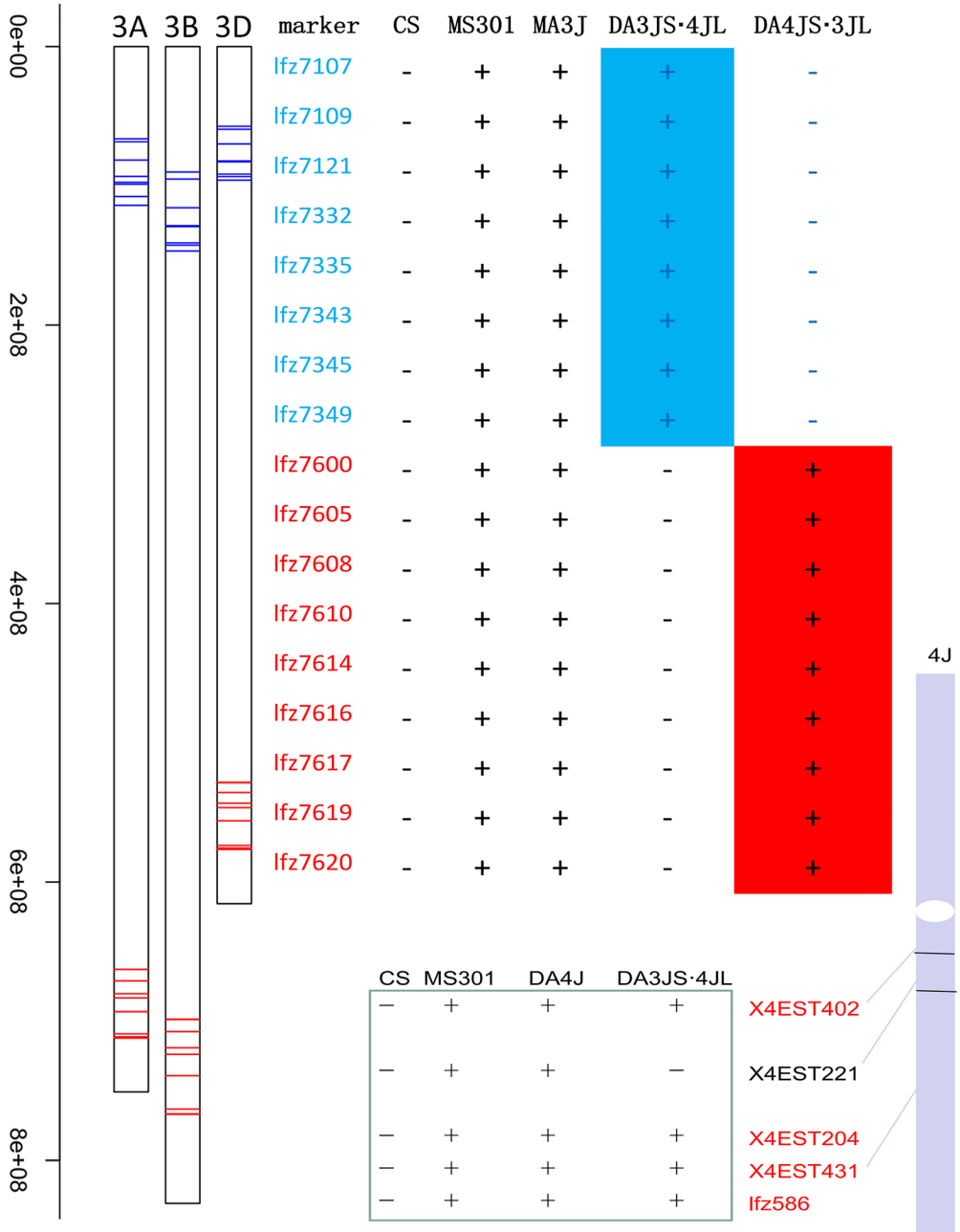
Karyotyping of chromosomes in wheat and its relatives has largely depended on tandem repeat clones pSc119 and pAs1 (Mukai et al. 1993; Cuadrado et al. 1997; Pedersen and Langridge 1997; Ribeiro-Carvalho et al. 2001; Schneider et al. 2003). Various FISH-positive sequences based on plasmid libraries of DNase I–digested genomic DNA were subsequently used to identify more landmarks on wheat chromosomes (Zhang et al. 2004; Komuro et al. 2013). However, BAC clones generally contain large insertions with multiple repeated sequences, some of which label entire wheat chromosomes or whole genomes and conceal landmark signals. It is thus laborious and time-consuming to discover useful tandem repeats in wheat and its wild relatives. Next-generation sequencing and availability of computational pipelines such as RepeatExplorer (Novák et al. 2013) and TAREAN (Novák et al. 2017) enabled graph-based sequence clustering and facilitated identification of groups of reads representing repetitive elements. In combination with TAREAN, we identified 8 tandem repeat clusters by oligonucleotide-based FISH. Two repeats enabled construction of an improved karyotype of *Th. bessarabicum* when combined with previous oligonucleotide probes. The chromosome-specific oligonucleotide BSCL242 producing signal only in the subterminal region of 7JL is one example. This method of developing oligos is more efficient than the previous identification of tandem repeats using K-mer analysis (Du et al. 2017), where only terminal repeats and no intercalary repeats were identified.

Physical mapping of 13 oligonucleotides located five repeats to different positions on *Th. bessarabicum* chromosomes. The pSc119.2 repeat was identified at the terminal regions of one or both ends of all chromosomes, and AFA family repeats were mainly at the telomeric or subtelomeric regions of most chromosomes. However, most strong signals of BSCL135, the most abundant new tandem repeat, were distributed in the intercalary regions of five chromosomes with five chromosomes having weak signals at one or both ends. The other two repeats, 5S rDNA and BSCL242, were present only at the subtelomeric regions of 1JS and 7JL. These results indicated that J genome chromosomes were quite different from those of wheat and rye, where most strong signals produced by pSc119.2 and pAs1 were B genome- and D genome-specific, respectively. BSCL135 appears to be the most abundant repeat thus far reported in *Th. bessarabicum* (Wang 2013; Du et al. 2017; Patokar et al. 2016; Grewal et al. 2018). Five A genome, four B genome, and three D genome chromosomes also contained clear BSCL135 signals in the intercalary regions; thus, BSCL135 could represent a new kind of tandem repeat widely occurring in the Triticeae. This finding parallels a previous report of a plasmid clone consisting of BSCL135-like repeats called pTa-713 in wheat (Komuro et al. 2013); pTa-713 also produced signals in chromosomes of tetraploid *Thinopyrum elongatum* (Li et al. 2018).

GISH is often used to detect alien chromosome introgressions in wheat. However, it is often difficult to detect translocations within alien chromosomes. In previous reports, we identified translocations between *Th. bessarabicum* chromosomes based on molecular markers (Qian 2007; Shen 2011), but it was difficult to confirm such conclusions with GISH and FISH because of limited cytological markers. The present ONPM#7 complex probe produced differentiating signals in most *Th. bessarabicum* chromosome arms and allowed clear cytological identification of three translocations and one isochromosome involving *Th. bessarabicum* chromosomes.

In this study, reciprocal translocations between chromosomes 3J and 4J were identified in Chinese (T3JS·4JL) and CIMMYT (4JS·3JL) wheat lines. Another Robertsonian 6JS·2JL translocation was very similar to a recently reported 6ES-2EL translocation in a wheat-*Th. elongatum* introgression line (Gaál et al. 2018). These results indicate that reciprocal translocations occur not only between wheat and alien


4J
4JL • 4JL
4JS
3JS • 4JL
4JS • 3JL
3J


0e+00
2e+08
4e+08
6e+08
8e+08
3A
3B
3D
marker
CS
MS301
MA3J
DA3JS·4JL
DA4JS·3JL
lfz7107 - + + + -
lfz7109 - + + + -
lfz7121 - + + + -
lfz7332 - + + + -
lfz7335 - + + + -
lfz7343 - + + + -
lfz7345 - + + + -
lfz7349 - + + + -
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lfz7605 - + + - +
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lfz7610 - + + - +
lfz7614 - + + - +
lfz7616 - + + - +
lfz7617 - + + - +
lfz7619 - + + - +
lfz7620 - + + - +
4J
CS MS301 DA4J DA3JS·4JL
– + + + X4EST402
– + + – X4EST221
– + + + X4EST204
– + + + X4EST431
– + + + lfz586

chromosomes but also between alien chromosomes in various wide crosses. Thus, the possibility of chromosome variations should be given more attention in studies of alien introgressions in wheat. High-resolution karyotypes combined with high-density molecular markers will facilitate the identification of such chromosomal variants. The present ONPM#7 probe provides a powerful tool for maintaining chromosome integrity in wide crosses involving wheat and *Th. bessarabicum*.

Conclusions

Identification and physical mapping of tandem repeats revealed that the J genome of *Th. bessarabicum* contains abundant sequences of BSCL135, a new kind of tandem repeat that can be used in Triticeae species to supplement pSc119.2 and pAs1. The complex probe ONPM#7 produced higher-resolution karyotypes of both wheat and *Th. bessarabicum* and enabled the identification of three reciprocal translocations and an isochromosome involving *Th. bessarabicum* chromosomes.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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