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ORIGINAL ARTICLE

Introduction of *Thinopyrum intermedium* ssp. *trichophorum* chromosomes to wheat by trigeneric hybridization involving *Triticum*, *Secale* and *Thinopyrum* genera

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Abstract

Main conclusion **Fluorescence in situ hybridization and molecular markers have confirmed that several chromosomes from *Thinopyrum intermedium* ssp. *trichophorum* have been added to a wheat background, which originated from a cross between a wheat–*Thinopyrum* partial amphiploid and triticale. The lines displayed blue grains and resistance to wheat stripe rust.**

Thinopyrum intermedium has been used as a valuable resource for improving the disease resistance and yield potential of wheat. With the aim to transfer novel genetic variation from *Th. intermedium* species for sustainable wheat breeding, a new trigeneric hybrid was produced by crossing an octoploid wheat–*Th. intermedium* ssp. *trichophorum* partial amphiploid with hexaploid triticale. Fluorescence in situ hybridization (FISH) revealed that *Thinopyrum* chromosomes were transmitted preferably and the number of rye chromosomes tended to decrease gradually in the selfed derivatives of the trigeneric hybrids. Four stable wheat–*Th. intermedium* chromosome substitution, addition and translocation lines were selected, and a $2J^S$ addition line, two substitution lines of $4J^S$(4B) and 4J(4B), and a small 4J.4B translocation line were identified by FISH and molecular markers. It was revealed that the gene(s) responsible for blue grains may located on the FL0.60–1.00 of long arm of *Th. intermedium*-derived 4J chromosome. Disease resistance screenings indicated that chromosomes $4J^S$ and $2J^S$ appear to enhance the resistance to stripe rust in the adult plant stage. The new germplasm with *Th. intermedium* introgression shows promise for utilization of *Thinopyrum* chromosome segments in future wheat improvement.

Introduction

The success of current and future wheat improvement programs is dependent on a continuous supply of genetic variability, which will principally be sourced from the related species. The perennial species, hexaploid wheatgrass, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, is widely distributed in Europe (particularly in the Mediterranean region), western Asia, and northern Africa (Dewey 1984). The relative ease of crossability between wheat and *Th. intermedium* has enabled the development of wheat–*Th. intermedium* partial amphiploids (Cauderon et al. 1973; Chen et al. 1999; Fedak et al. 2000; Bao et al. 2014). Derived from these amphiploids, a number of wheat–*Thinopyrum* chromosome addition, substitution and translocation lines have proved to be a valuable resource for enhancing the disease resistance and yield potential of wheat (Han et al. 2003; Chen 2005; Li and Wang 2009; Wang and Wang 2016). The disease resistance genes, such as *Pm40*, *Pm43*, *Yr50, Lr38* and *Sr44*,

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were transferred from *Th. intermedium* into common wheat (Friebe et al. 1993, 1996; Luo et al. 2009; He et al. 2009; Liu et al. 2013). Genomic in situ hybridization has revealed the complex genomic composition of *Th. intermedium* which includes three genomes named J, J^S, and St, of which the St genome shows a high degree of similarity to that of *Pseudoroegneria,* while the J and J^S genomes appear to be related to the modified *Th. elongatum/Th. bessarabicum* genomes (Chen et al. 1998; Chen 2005; Mahelka et al. 2011, 2013). However, the complete set of chromosomes across the seven linkage groups and three St, J^S, J genomes still have not been transferred to a wheat background. The great geographical and climatic diversity of their native distribution, combined with open pollination, has resulted in high variability and numerous chromosomal rearrangements among *Th. intermedium* sub-species, which has attracted the studies into further wheat–*Th. intermedium* hybridization (Wang 2011; Kantarski et al. 2017).

Thinopyrum intermedium ssp. *trichophorum* displayed a distinctly enriched chromosomal heterochromatin constitution which differs from that of *Th. intermedium* ssp. *intermedium* (Xu and Conner 1994). A partial amphiploid between wheat–*Th. intermedium* ssp. *trichophorum* contained unique seed storage proteins and also displayed novel disease resistance in wheat (Yang et al. 2006). Recently, we isolated a disomic chromosome substitution involving chromosome 1St#2 carrying stripe rust resistance (Hu et al. 2011) and specific gliadin and high-molecular weight glutenin subunits (HMW-GS) genes (Li et al. 2013), as well as 7St addition lines (Song et al. 2013). In addition, two double disomic substitutions were developed (Li et al. 2015). Therefore, continuous developing novel wheat–*Th. intermedium* ssp. *trichophorum* introgression lines may provide effective resistance against new disease pathotypes (Xu et al. 2009).

Trigeneric hybridizations involving the genera *Triticum*, *Aegilops, Secale*, *Dasypyrum*, *Psathyrostachys*, *Thinopyrum*, *Agropyron*, and *Hordeum* are commonly used as bridges to transfer genes from some wild species to wheat (Fedak and Armstrong 1981; Jauhar 1992; Li and Dong 1993; Li et al. 2006; Kang et al. 2012, 2016). The objective of the present study was to transfer and characterize *Th. intermedium* ssp. *trichophorum* chromosomes to wheat using trigeneric hybridization involving a cross between an octoploid wheat–*Thinopyrum* partial amphiploid and hexaploid triticale. New wheat–*Th. intermedium* derivative lines displaying the blue grain character and resistance to stripe rust were identified by sequential FISH and molecular markers.

Materials and methods

Plant materials

The cross between an octoploid ($2n = 8x = 56$) wheat–*Th. intermedium* ssp. *trichophorum* partial amphiploid TE1508 (Song et al. 2013) as the female with hexaploid ($2n = 6x = 42$) triticale Currency as the male parent (Shu et al. 2000) was used to transfer *Thinopyrum* chromosomes to wheat. Lines X24C-14, X24C10, X24C5 and X24C1-1 were developed from the F_6 progeny. *Th. intermedium* PI440028 (StJ^SJ genomes, $2n = 6x = 42$) was obtained from the National Small Grains Collection at Aberdeen, Idaho, USA. Wheat–*Th. intermedium* addition lines L series from TAF46 and Z series from Zhong5 were obtained from Drs Bernd Friebe, Kansas State University, USA, and Zhijian Chang, Shanxi Academy of Agricultural Sciences, China, respectively.

Fluorescence in situ hybridization (FISH)

Seedling root tips of trigeneric hybrids derived lines and their parents were collected and treated with nitrous oxide followed by enzyme digestion, using the procedure of Han et al. (2006). The synthesized oligonucleotide probes Oligo-pSc119.2, Oligo-pTa535, Oligo-pTa71 and Oligo-$(GAA)_7$ were used for identifying the wheat chromosomes according to the description of Tang et al. (2014). Probe Oligo-pSt122 is specific for terminal regions of *Th. intermedium* chromosomes (Li et al. 2016). Oligonucleotide probes were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China). The synthetic oligonucleotides were either 5′ end-labeled with 6-carboxyfluorescein (6-FAM) for green or 6-carboxytetramethylrhodamine (Tamra) for red signals. The protocol of non-denaturing FISH (ND-FISH) by the synthesized probes was described by Fu et al. (2015). After the oligo-based FISH, the sequential FISH with the long terminal repeat (LTR) pDb12H sequence can clearly distinguish the J^S chromosomes in *Th. intermedium* (Liu et al. 2009). This LTR sequence was labeled with Alexa Fluor 488-5-dUTP (Invitrogen) according to Han et al. (2006). Photomicrographs of FISH chromosomes were taken with an Olympus BX-51 microscope equipped with a DP-70 CCD camera.

Molecular marker analysis

DNA was extracted from young leaves of Chinese Spring (CS), *Th. intermedium*, TE1508-1, lines X24C10, X2C14, X24C5, and X24C1-1. PCR-based Landmark Unique Gene (PLUG) primers were designed from rice genomic DNA sequences specific for the syntenic regions of the

corresponding linkage group(s) of wheat genomes, which may amplify Triticeae genome fragments (Ishikawa et al. 2009). Polymerase chain reaction (PCR) was performed in an Icycler Thermal Cycler (Bio-Rad Laboratories, Emeryville, CA) in a 25 μl reaction, containing 10 mmol Tris–HCl (pH 8.3), 2.5 mmol $MgCl_2$, 200 μmol of each dNTP, 100 ng template DNA, 0.2 U Taq polymerase (Takara, Japan) and 400 nmol of each primer. The cycling parameters were 94 °C for 3 min for denaturation, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The amplified products were restriction enzyme digested and electrophoresis was conducted as described by Hu et al. (2012). The PLUG markers without chromosomal physical location in Ishikawa et al. (2009) were searched using the wheat genome database IWGSC WGA v0.4 from https://urgi.versailles.inra.fr/blast/, and confirmed by PCR.

Rust resistance tests

Agronomic traits under field conditions were studied at the Xindu Experimental Station, Chengdu, China during the 2013–2015 wheat growing seasons. Stripe rust (*P. striiformis* f. sp. *Tritici*, Pst) races, CYR32, CYR33 and v26 (Liu et al. 2010), were provided by the Crop Protection Institute, Sichuan Academy of Agricultural Sciences, China. All materials were tested for resistance to mixed isolates in the field. Infection type (IT) was scored 14–15 days after inoculation when rust was fully developed on the susceptible check line SY95-71 (Hu et al. 2011). The IT was recorded based on the 0–4 scale according to Bariana and McIntosh (1993).

Results

FISH karyotype analysis

Cytological analysis of the wheat–*Th. intermedium* ssp. *trichophorum* partial amphiploid TE1508 revealed two distinct variants of chromosome numbers, 56 and 54, the genotypes of which were subsequently named TE1508-1 and TE1508-2, respectively. The mitotic metaphase chromosomes of TE508-1 and TE1508-2 were hybridized with probes Oligo-pSc119.2, Oligo-pTa535, pDb12H, Oligo-$(GAA)_7$ and Oligo-pSt122 by sequential multi-color-FISH (Fig. 1). The FISH hybridization signals of the probes Oligo-pSc119.2 and Oligo-pTa535 could easily identify the wheat chromosomes based on the standard FISH karyotype of wheat chromosomes described by Tang et al. (2014). Both TE1508-1 and TE1508-2 contained only 40 wheat chromosomes with the absence of chromosome 4B (Fig. 1a, c). The difference between TE1508-1 and TE1508-2 was in the number of *Th. intermedium* chromosomes. Line TE1508-1 contained eight pairs of *Thinopyrum* chromosomes and TE1508-2 had seven pairs of *Th. intermedium* chromosomes. The sequential FISH with LTR probe pDb12H on TE1508-1 revealed that three pairs of chromosomes belonged to the J^S group, and were named J^S-1 to J^S-3. In comparison, TE1508-2 had two pairs of J^S genome chromosomes but lacked J^S-3 chromosomes (Fig. 1c, d). We have previously developed *Th. intermedium* chromosome 1St in line AS1677 (Hu et al. 2011), and identified the 6St and 7St chromosomes from the lines X482 (Li et al. 2015) and X484 (Song et al. 2013), respectively. The remaining two pairs of J-chromosomes were named J-1 and J-2 (Fig. 1). The FISH karyotype of eight pairs of *Th. intermedium* chromosomes of TE1508 is shown in Fig. 1e.

The FISH probes Oligo-pSc119.2, Oligo-pTa535 and Oligo-1162 were used to identify the wheat and rye chromosomes in this triticale (Fu et al. 2015). As shown in Fig. 2a, the clear FISH patterns of individual rye chromosomes 1R to 7R can be distinguished from the 28 wheat chromosomes in Currency. The FISH patterns of rye chromosomes were different from the *Thinopyrum* chromosomes in TE1508. Therefore, the FISH probes enable the identification of the eight pairs of individual *Th. intermedium* chromosomes and seven pairs of rye chromosomes in a wheat background.

Transmission of *Thinopyrum* and rye chromosomes

The Oligo-based ND-FISH was able to identify the *Thinopyrum* and rye chromosomes in the trigeneric hybrids and their derived progenies. The chromosome compositions of the F_4 to F_6 progenies by FISH are shown in Table 1. Over 80% of F_4 plants had five to 10 *Th. intermedium* chromosomes and one to six rye chromosomes including the telocentrics (Fig. 2b). In the F_5, 50 plants contained two to six *Th. intermedium* chromosomes and zero to three rye chromosomes (Fig. 2c), and 42 plants had no rye chromosomes. In the F_6, there were one to seven *Th. intermedium* chromosomes, but no rye chromosomes were detected (Fig. 2d), except two plants with a 1BL.1RS translocation. Thus, it seems that *Th. intermedium* chromosomes are more preferentially transmitted than rye chromosomes in the latter generations of selfed progenies. Among the different groups of *Thinopyrum* chromosomes, the J^S chromosomes exhibited higher transmission rates than the J and St chromosomes. A total 36.8 and 34.3% of F_4 to F_6 plants contained at least one J^S-2 chromosome and a 4BS.J^S-3L translocation chromosome, respectively. Only 7.8 and 6.5% plants have the J-1 and J-2 chromosomes, respectively. Other wheat–*Th. intermedium* translocation chromosomes were also observed in about 1.2% of the plants.

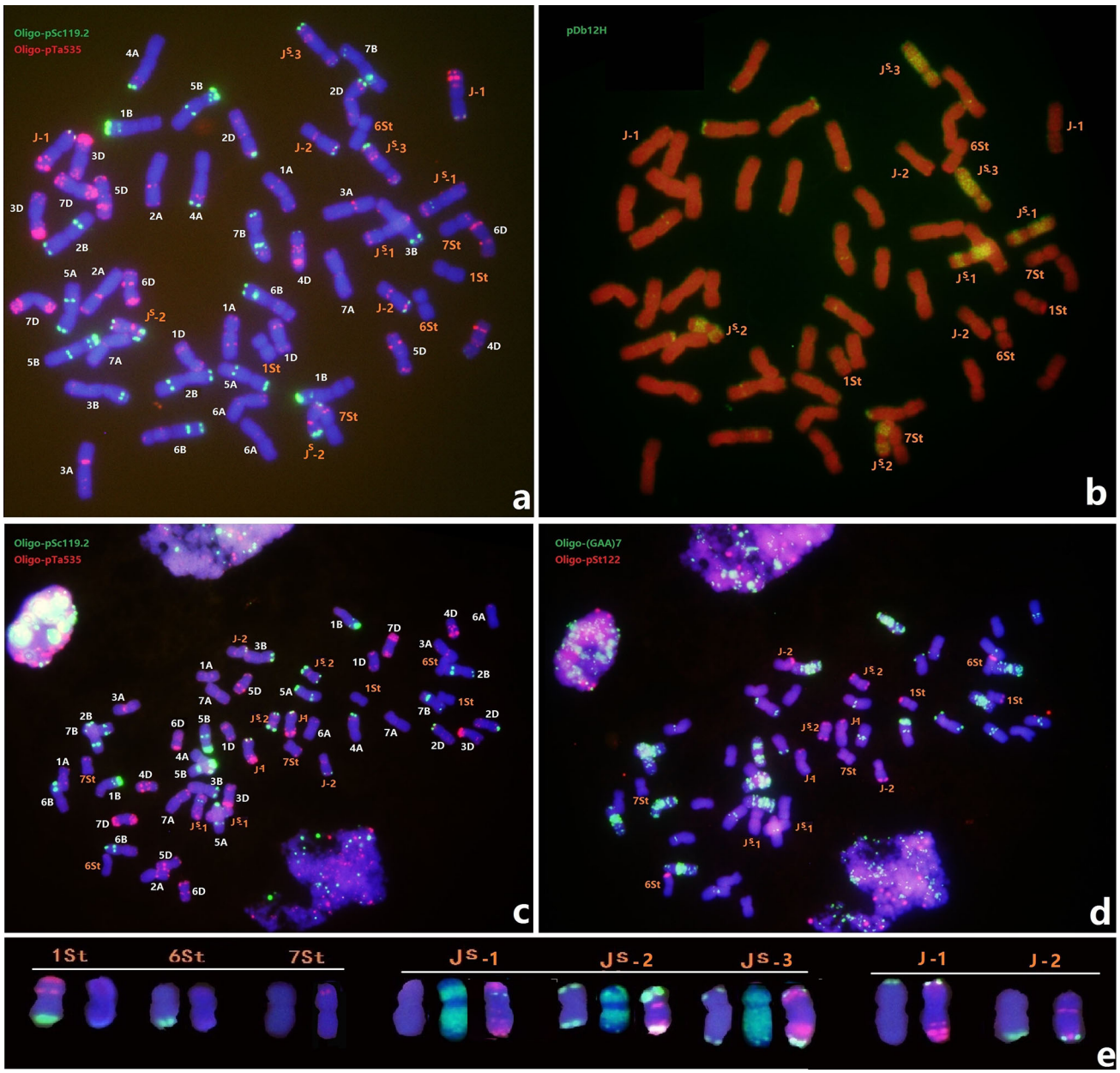

Fig. 1 Sequential FISH patterns of wheat–*Th. intermedium* partial amphiploid TE1508-1 (**a**, **b**), TE1508-2 (**c**, **d**) and karyotype of *Th. intermedium* chromosomes in TE1508 (**e**). The probes for FISH (**a**, **c**) were Oligo-pSc119.2 (*green*) + Oligo-pTa535 (*red*), while the probes for sequential FISH (**b**) were pDb12H (*green*), and FISH (**d**) were Oligo-$(GAA)_7$ (*green*) + Oligo-pSt122 (*red*), respectively. The karyotype (**e**) of *Th. intermedium* chromosomes was shown by the probes at *left* with Oligo-pSt122 (*green*) + Oligo-pTa71 (*red*) and *right* with Oligo-pSc119.2 (*green*) + Oligo-pTa535 (*red*), while in the *middle* with pDb12H (*green*) added for J^S chromosomes, respectively

Chromosome identification of stable wheat-trigeneric derived lines

Sequential multi-color ND-FISH with probes Oligo-pSc119.2, Oligo-pTa535, and Oligo-$(GAA)_7$ was conducted to characterize the cytologically stable lines X24C5, X24C10, X24C14 and X24C1-1 (Fig. 3). The chromosome number of X24C14 was 44, while X24C5, X24C10, and X24C1-1 all had 42 chromosomes. As shown in Fig. 3a, it was found that X24C14 includes all the 42 wheat chromosomes and two alien chromosomes. Probes Oligo-pSc119.2 and Oligo-pTa535 showed a pair of chromosomes with faint Oligo-pTa535 hybridization signals at the telomeric region of long arm, and faint hybridization signals of Oligo-pSc119.2 in the interstitial region of the short arm in X24C14 (Fig. 3a). The FISH hybridization pattern

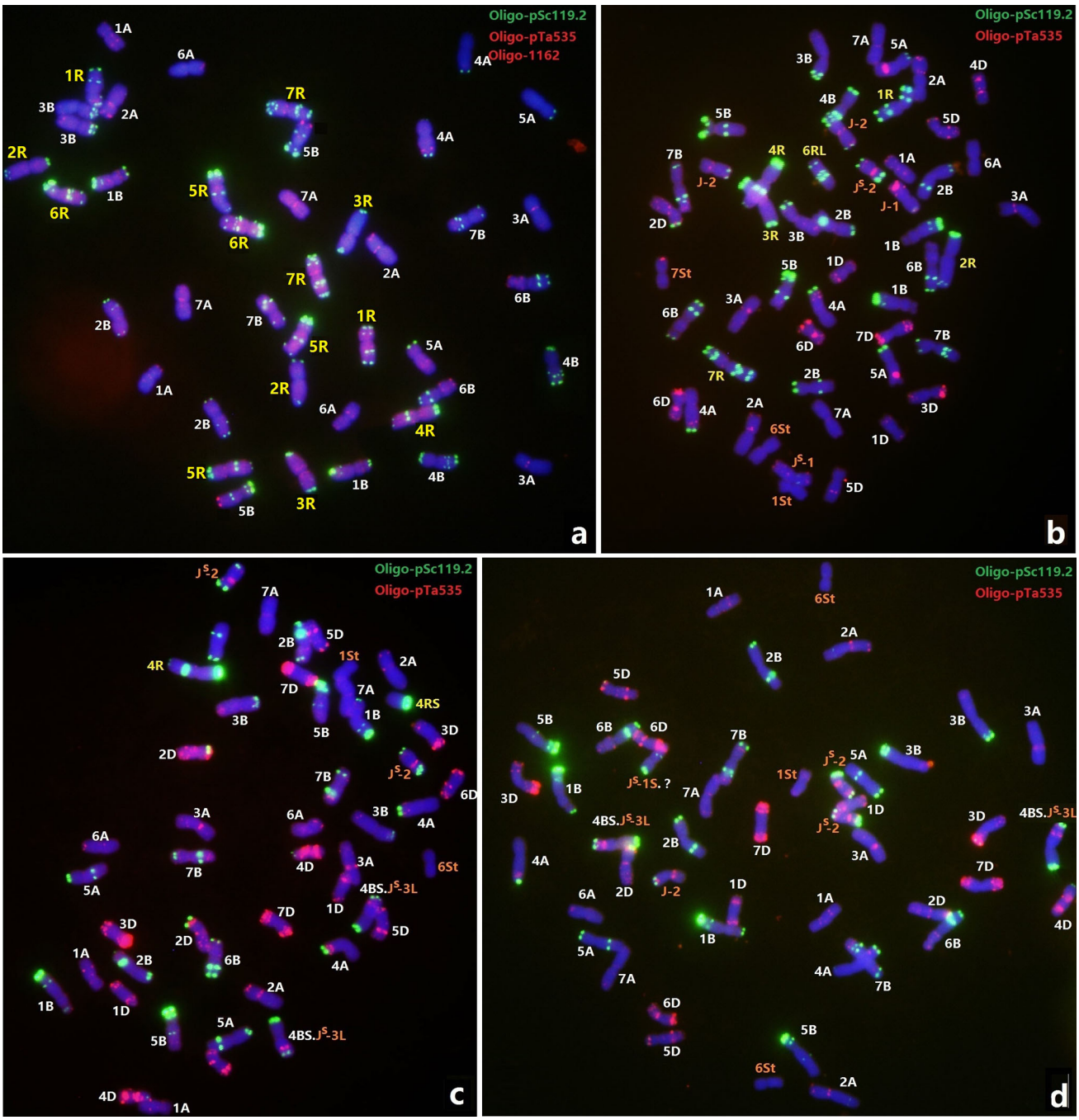

Fig. 2 FISH of Currency triticale (**a**), and the plants of F_4 (**b**), F_5 (**c**) and F_6 (**d**) progenies. The probes Oligo-pSc119.2 (*green*) + Oligo-pTa535 (*red*) were used in FISH (**a**–**d**), and rye chromosomes (**a**) were visualized by probe Oligo-1162 (*red*)

by pDb12H revealed that the added chromosomes belonged to the J^S genome, and were identical to *Th. intermedium* chromosome J^S-1 (Fig. 1e). Therefore, it was identified that the line X24C14 was a chromosome J^S-1 addition line.

Similarly, FISH with probes Oligo-pSc119.2 and Oligo-pTa535 showed that both X24C5 and X24C10 lack the 4B chromosomes of wheat (Fig. 3c, d). The comparison of the introduced alien chromosomes in X24C10 and X24C5 with TE1508 (Fig. 1e) revealed that lines X24C10 and X24C5 were J^S-2(4B) and J-1(4B) substitution lines, respectively.

As shown in Fig. 3e, FISH with probes Oligo-pSc119.2 and Oligo-pTa535 revealed that line X24C1-1 contained a pair of translocated chromosomes. Based on sequential FISH with Oligo-$(GAA)_7$ (Fig. 3f), it was found that the translocated chromosome involved the entire wheat arm 4BS and the distal portion of the long arm of the J-2

Table 1 Number of *Thinopyrum* and rye chromosomes in F_4 to F_6 generations of the cross between TE1508 (wheat–*Th. intermedium* partial amphiploid) and triticale cv. currency

Lines	No. of plants	No. of chromosomes	No. of R-chromosomes	No. of St-chromosomes	No. of J^S chromosomes	No. of J chromosomes
TE1508	5	54–56	0	6	4–6	4
Currency	5	42	14	0	0	0
F_4	56	38–52 (45.8)	0–8 (0.8)	1–6 (2.3)	1–6 (2.8)	1–5 (1.5)
F_5	50	39–48 (43.7)	0–3 (0.5)	0–4 (1.5)	1–5 (3)	0–4 (0.8)
F_6	29	40–44 (42.3)	0–1 (0.1)	1–2 (0.5)	1–4 (4)	0–2 (0.7)

The range of chromosome numbers is given first and the average number is in brackets

chromosome from Fraction Length (FL) 0.60 to the long arm telomere.

Molecular markers analysis

The PLUG markers were useful for developing alien chromosome-specific markers of *Secale*, *Dasypyrum* and *Thinopyrum* chromatin and to assign the alien chromosome to specific homoeologous linkage groups (Hu et al. 2011; Lei et al. 2012; Li et al. 2013). The chromosomal locations of most of the PLUG markers were based on the PCR amplifications as described in Ishikawa et al. (2009), and blasted to the wheat genome database "The IWGSC WGA v0.4" (Tables 2, 3). A total of 500 PLUG markers were used to test the CS, *Th. intermedium* and TE1508. A total of 285 markers gave rise to *Th. intermedium*-specific amplification identical to those in TE1508 after *Taq*I, *Hae*III or *Hap*II digestion of PCR products. A total of 31 pairs of TNAC primers from wheat homoeologous group 2 (Supplementary Table 1) generated the identical bands from *Th. intermedium* to those from the disomic addition line X24C14. These results suggested that the *Th. intermedium* chromosome in X24C14 belonged to homoeologous group 2. After combining these PCR results with the FISH patterns, we concluded that the X24C14 was a J^S-1 addition line, and the J^S-1 was chromosome $2J^S$.

The PLUG markers of wheat homoeologous group 4 (Table 2) also amplified the specific bands with DNA from both X24C5 and X24C10 (Fig. 4), indicating that they both contained *Th. intermedium* chromosomes which belong to group 4. A total of 38 of 42 markers from X24C5 produced *Th. intermedium*-specific polymorphic bands, while 26 markers amplified *Th. intermedium*-specific polymorphic bands from X24C10. Only six markers give identical *Th. intermedium*-specific bands for lines X24C5 and X24C10, indicating that these two group 4 *Th. intermedium* chromosomes were genetically different. In combination with the FISH results, it was indicated that line X24C5 was a $4J^S$(4B) substitution, while line X24C10 was a 4J(4B) substitution. The PLUG markers indicated that chromosome $4J^S$ was more closely related to wheat group 4 chromosomes than was chromosome 4J.

The 4J polymorphic TNAC markers from line X24C10 were further used to amplify in line X24C1-1 (Table 2). Of the 13 4J polymorphic markers on arms 4BL and 4DL, seven markers mapped to the segment distal to FL0.6 and had identical amplification for X24C10 and X24C1-1, while six proximal markers showed no amplification in X24C1-1. Therefore, TNAC markers also confirmed the breakpoints in chromosome 4JL of X24C1-1, which is consistent with the FISH results (Fig. 5).

Plant and grain characters

A set of agronomic traits were measured on each of the ten plants of Currency triticale, TE1508, and the stable wheat–*Th. intermedium* lines grown under field conditions (Table 3). Line X24C10 had significantly reduced plant height and an increase in the numbers of spikes, which possibly implies that the 4J chromosome carries strong dwarfing and tillering gene(s) expressed in the wheat background. The 1000-kernel weight was increased in X24C1-1 and thus probably contributed to a favorable effect on grain yield relative to controls in the 4JL lines.

Grain color of TE1508 displayed a light green shade, those of the triticale line Currency were red, while the wheat–*Th. intermedium* lines X24C10 and X24C1-1 were blue grained (Fig. 6). Based on the karyotypic comparison among the blue-grained lines, it was found that TE1508 and X24C10 contained chromosome 4J. Line X24C1-1 carrying the 4B-4J translocation also showed blue grain. Thus, we conclude that the blue grain is encoded by gene(s) located on the FL0.6–1.00 long arm of chromosome 4J (Fig. 5).

When challenged with CYR32, CYR33 and v26 races of stripe rust, the parents Currency and TE1508 showed resistance to stripe rust at adult plant stages. Lines

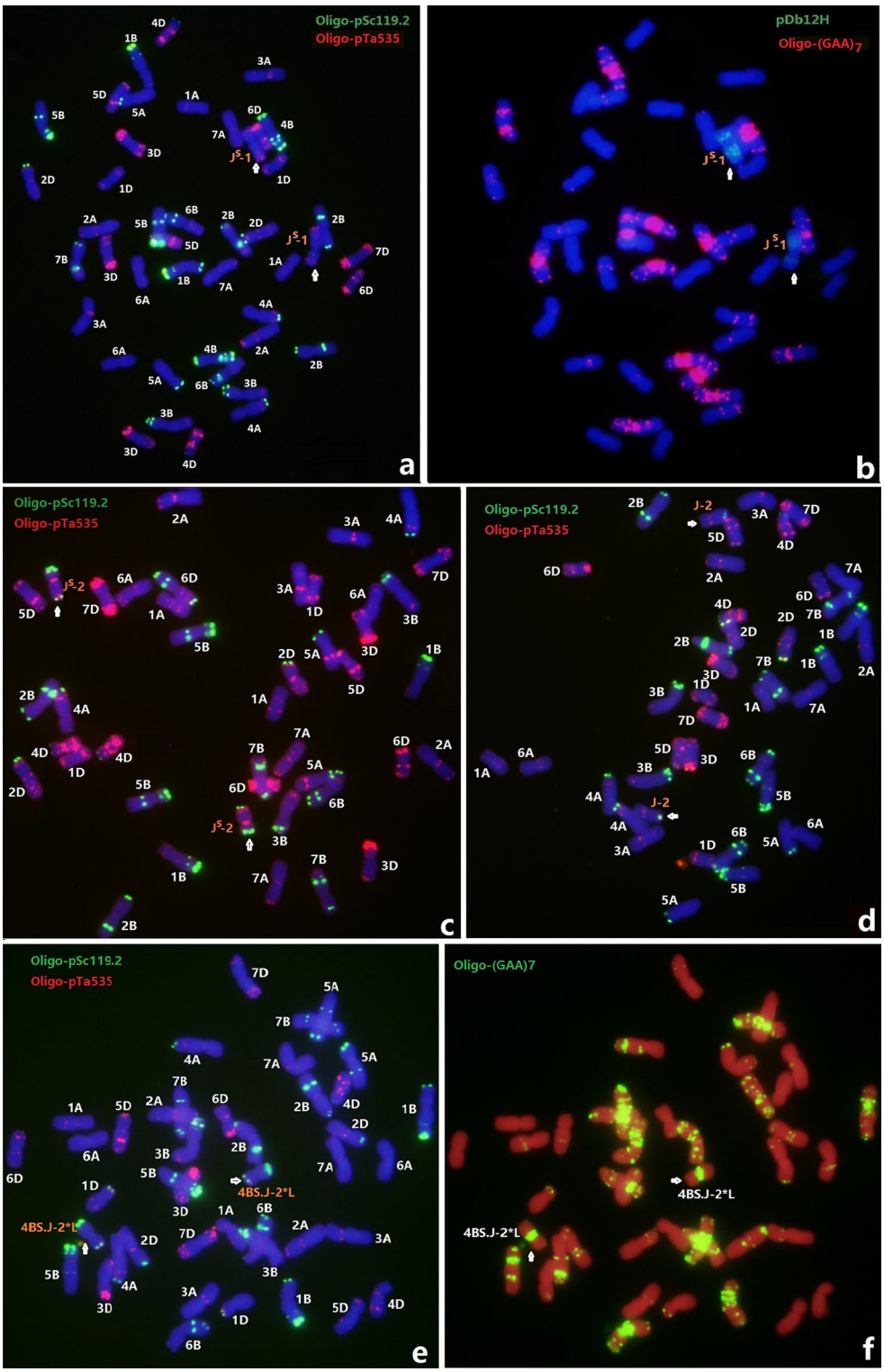

Fig. 3 FISH patterns of wheat–*Th. intermedium*-derived lines X24C14 (**a**, **b**), X24C5 (**c**), X24C10 (**d**), and X24C1-1(**e**, **f**). The probes Oligo-pSc119.2 (*green*) + Oligo-pTa535 (*red*) were used in FISH (**a**, **c**, **d**, **e**), and the probes Oligo-$(GAA)_7$ and pDb12H were used in sequential FISH (**b**, **f**). The *arrows* indicated the *Thinopyrum* chromosomes

X24C14, X24C10 and X24C5 were resistant, while X24C1-1 was susceptible. This indicates that the *Th. intermedium* chromosomes $2J^S$, 4J, and $4J^S$ carry a gene(s) for stripe rust resistance. The wheat–*Th. intermedium* derivatives might be useful resource in wheat breeding programs.

Table 2 The PLUG markers of homoeologous group 4 on X24C10 and X24C5

No.	Primer name	Primer sequences (5′–3′)	Wheat bin map	Restriction enzyme	Length of X24C10 bands/bp	Length of X24C5 bands/bp
1	TNAC1464	F: GGATGCCCTTACAAAGAGGTC R: CGCAGACAGAAGTTAGCCAAG	4AL12-0.43-0.66 4BS1-0.84-1.00 4DS2-0.82-1.00	*Hpa*II	1200	–
2	TNAC1463	F: CGTCTTTATCAAACCCTGCAA R: GTTCACCGAGTTCATCCAGAA	4AL12-0.43-0.66 4BS1-0.84-1.00 4DS2-0.82-1.00	*Hae*III	700	720
3	TNAC1663	F: CAGATAGACCGGGTGGAATTT R: CGAGGTCTACGTCTTCGAGTC	4AL12-0.43-0.66 4BS1-0.84-1.00 4DS2-0.82-1.00	*Taq*I	580	200/490
4	TNAC1656	F: TTCCATGAGGAACTTGTCGAG R: CCGGTCTCACGTCAGCTATT	4AL12-0.43-0.66 4BS1-0.84-1.00 4DS2-0.82-1.00	*Taq*I	800	–
5	TNAC1510	F: GCGTCTGTCTTCATCTTCTGG R: CAAGTGTGCACATGACTGCTT	4AL12-0.43-0.66 4BS8-0.57-0.84 4DS3-0.67-0.82	*Hae*III	800	–
6	TNAC1428	F: GCGTTGATCCTCAGAGAAGAG R: TGAGAAGTCCCATGCAAATCT	4AS1-0.20-0.63 C-4BL14-0.18 4DL9-0.31-0.56	*Taq*I	900	–
7	TNAC1408	F: CAGGAAGTTGGTACCATTGTGA R: CTTGCAGCCTCCTATTGATTC	4AS3-0.76-1.00 4BL5-0.86-0.95 4DL11-0.61-0.71	*Taq*I	600[a]	600
8	TNAC1403	F: CCTCCTCCATTGCGAGATAAC R: GTAGTAACGCTGAAGGGTTCG	4AS3-0.76-1.00 4BL5-0.86-0.95 4DL12-0.71-0.86	*Hae*III	500	–
9	TNAC1398	F: CAAGGCAGGTGCTGATATTGT R: ACCCAGGGTTGACTGACATAA	4AS3-0.76-1.00 4BL5-0.86-0.95 4DL12-0.71-0.86	*Taq*I	1200[a]	1400
10	TNAC1391	F: GTACACGACCACCGAGGAAG R: TGCATCTTTCGACCCTCATAA	5AL23-0.87-1.00 4BL10-0.95-1.00 4DL14-0.86-1.00	*Taq*I	900[a]	–
11	TNAC1406	F: CCAAGAGTTCTGCACGTTGAT R: GTCAGCTTTCCTGTGTGGAAG	**4BL-0.5-0.86**	*Taq*I	800[a]	–
12	TNAC1407	F: GAAGTGATCAGCGCACTCTTT R: AGTTTCCTGATGAACCAACCA	**4AS-0.76-1.00** **4DL-0.5-0.75**	*Taq*I	300[a]	800
13	TNAC1413	F: AGAAATCATCTACATCGGTCCTG R: GATCCATCAGTGGTTCCTCAA	4A**S-0.76-1.00** 4D**L-0.71-0.86**	*Taq*I	320[a]	–
14	TNAC1416	F: CGGTTTCTGCTTTCATTACCA R: GAGTTGCAGCATTAGCTGGAT	4A**S-0.5-0.76** 4D**L-0.71-0.86**	*Taq*I	800[a]	750
15	TNAC1422	F: CTTGGCAAGAACATGCTGAA R: TCTCAAATGATTCCACCGAAG	4B**L-C-0.49**	*Taq*I	350	–
16	TNAC1424	F: GAGGCCCTCATCATCGTTAC R: ATGGGCCCTTTATATTTCAGC	4B**L-C-0.49**	*Taq*I	1100	900
17	TNAC1427	F: AGGCCTGATATGCTTCATTGT R: ATGGTGATGGAGCGGTTC	4B**L-C-0.49** 4D**L-C-0.49**	*Taq*I	300	200/300/360
18	TNAC1443	F: TTCTATCATGCCAATCCCATC R: GAGCATCTGAACTGCTTGAGG	4B**L-C-0.49**	*Taq*I	500	–
19	TNAC1451	F: TTCCAAAGATTGTGAGCATCC R: GCTTGACATACTCGTCCGTTG	4A**L-0.43-0.66** 4D**S-0.62-1.00**	*Hae*III	400	–

Table 2 continued

No.	Primer name	Primer sequences (5′–3′)	Wheat bin map	Restriction enzyme	Length of X24C10 bands/bp	Length of X24C5 bands/bp
20	TNAC1660	F: TGCAACTGCAAGTTATGACCA	4B**S-0.84-1.00**	*Taq*I	600	420
		R: ACATCCAGTTGCCATCACAAT				
21	TNAC1462	F: AGGACCAGTACATTCTGCACAC	4A**L-0.67-1.00**	*Hae*III	320	200
		R: CGAGAGCTTCAGGCTGTCTTA	4B**S-0.84-1.00**			
22	TNAC1667	F: ACTGAAGCATCCGCACCTC	4A**L-0.67-1.00**	*Taq*I	450	–
		R: CCAGACTTCGATGGGCTTAG	4D**S-0.62-1.00**			
23	TNAC1670	F: GGCATTACTTCGACATCCAGA	4B**S-0.84-1.00**	*Hae*III	400	–
		R: ATCCATGCTTCGTTTGACATC				
24	TNAC1671	F: GAGCTGCTGGAGATCATAACG	4A**L-0.67-1.00**	*Taq*I	700	600/650
		R: CGAAAGCAGGCTGTTGTAGTA	**4BS-0.84-1.00**			
25	TNAC1466	F: CAACTTGGTGCCTCTCAACTC	4A**L-0.67-1.00**	*Taq*I	400/520	–
		R: TATAATGGGTCGGCCATCTTC	4D**S-0.62-1.00**			
26	TNAC1467	F: GCCGTCTTGTTCATTGGTTCT	4B**S-0.67-1.00**	*Hae*III	280	–
		R: GCACTAGCGCCATCGATATT	4D**S-0.62-1.00**			
27	TNAC1469	F: ATTCCAGCAGCCTCATTACCT	4A**L-0.67-1.00**	*Hae*III	800	680
		R: GTCCAACCCAGACACGTCTTA	4D**S-0.62-1.00**			
28	TNAC1471	F: AGGCATGAGCATAGGAACTGA	4B**S-0.84-1.00**	*Taq*I	400	–
		R: ACACTGGAGATGGATCTGTCG	4D**S-0.62-1.00**			
29	TNAC1476	F: TTTGGAAGCATTTCTCGTCTC	4B**S-0.84-1.00**	*Taq*I	800	–
		R: ATTCATGTTCAACCAGGCAAA	4D**S-0.62-1.00**			
30	TNAC1861	F: TGAGTTGGTGGACTCCTATGC	4B**S-0.50-0.83**	*Hae*III	800	750/930
		R: AGGAACTTCTCTCCGTTGACC				
31	TNAC1507	F: CAACCTTAGCAAGGACAATCG	4A**L-0.43-0.66**	*Hae*III	500	–
		R: CCTTCATCCTCGTCGAGTTCT	4D**S-0.62-1.00**			
32	TNAC1508	F: GCTTCGCCTCCTCGATCA	4B**S-0.50-0.83**	*Taq*I	400/500	–
		R: TTGCTGTTTCAGCTGTTCTTG	4D**S-0.62-1.00**			
33	TNAC1511	F: ATTATGGTTCCGTTGGTGGAT	4B**S-0.50-0.83**	*Taq*I	400	–
		R: TTTCATCACTTCACCGAGTCC	4D**S-0.62-1.00**			
34	TNAC1594	F: CCTCCAGAACAAGACCCAGAT	4D**S-C-0.49**	*Taq*I	200	–
		R: GCAATGACTCCCTCGAACAT	4D**S-C-0.49**			
35	TNAC1596	F: CCGATTTCCTGGGTGTTAAAT	4B**S-C-0.49**	*Hae*III	600	200/480
		R: TATTCGTGTTGCAGCATGTTC	4D**S-C-0.49**			
36	TNAC1522	F: CTCCCATTGCTTGTGTTGATT	4A**L-0.43-0.66**	*Hae*III	–	710
		R: AAGCTCATCGCTGAGATCAAG	4B**S-C-0.49**			
37	TNAC1597	F: TCCAAGGTCGACATCTCCTTC	4A**L-0.43-0.66**	*Taq*I	–	680
		R: ACTCAGCAGCGTTTATGATGG	4B**S-C-0.49**			
38	TNAC1425	F:CAGCTTTGCCTGTCTCTGAAC	4A**S-0.5-0.76**	*Taq*I	700	700
		R: CAAAGTCGCTTCAAATCTCCA	4D**L-0.71-0.86**			
39	TNAC1410	F: CCCACATGTCGTTCATCATC	4A**S-0.5-0.76**	*Taq*I	350	350/450
		R: CTCCACGGCTTCCACATC	4D**L-0.71-0.86**			
40	TNAC1417	F: TTTGTCAATCTCTGTGCTTGGT	4A**S-0.5-0.76**	*Taq*I	–	600
		R: AAACAGTATGATCCCGACACG	4D**L-C-0.49**			
41	TNAC1419	F: CGAGCAACTGTTCAAGGAGAC	4A**S-0.5-0.76**	*Hae*III	1200/1100	1200
		R: TGAAGCAGGACTTGTGGTAGG	4D**L-C-0.49**			
42	TNAC1444	F: ACATCTGGTGCTGATGCTTCT	**4AL1-0.84-1.00**	*Hae*III	–	880
		R: GTCTGGAGCAGCCATATGAAA	**4DS2-0.82-1.00**			

The physical location marked in bold was referred to the Blast results to database of IGWS v4.0

[a] The amplification also on X24C1-1

Table 3 Comparison of agronomical traits and stripe rust resistance of wheat–*Th. intermedium* derivatives

Genotype	Plant height (cm)	Length of spike (cm)	No. of spikelet	No. of spikes	1000-kernel weight (g)	IT for Pst
Currency	102.5 ± 3.0	18.5 ± 0.4	29.6 ± 2.0	13.7 ± 2.0	41.0 ± 2.0	2
TE1508	109.0 ± 4.6	10.0 ± 0.2	20.8 ± 2.0	14.0 ± 3.0	21.8 ± 0.8	0
X24C14 $2J^S$ addition	93.0 ± 2.8	8.6 ± 0.6	20.0 ± 1.5	6.0 ± 1.5	17.2 ± 1.6	2
X24C10 4J(4B) substitution	72.5 ± 2.4	9.8 ± 0.3	19.5 ± 1.6	13.0 ± 2.0	38.4 ± 0.9	0
X24C5 $4J^S$(4B) substitution	77.5 ± 2.0	8.0 ± 0.2	22.0 ± 1.3	3.2 ± 2.0	27.2 ± 2.0	1
X24C1-1 4J-4B translocation	83.5 ± 3.3	8.3 ± 0.2	24.5 ± 2.2	7.0 ± 1.5	46.7 ± 1.2	4

The numbers for the agronomic traits were the mean and standard deviation. The IT for Pst means the infection type of *P. striiformis* f. sp. *tritici* races

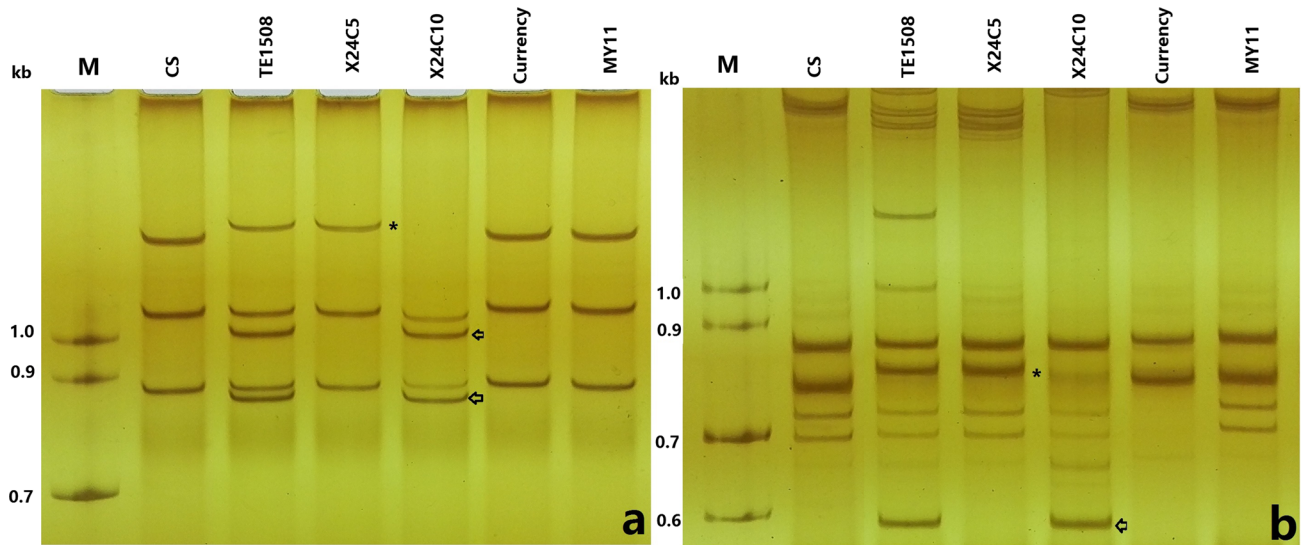

Fig. 4 PCR profiling of markers TNAC1424/*Taq*I (**a**) and TNAC1596/*Hae*III (**b**). *M* indicates molecular marker, *stars* and *arrows* show the specific bands for X24C5 and X24C10, respectively

Discussion

Crossing different amphiploids is an effective and rapid method for producing trigeneric hybrids, which offers the possibility of transferring several alien characters concurrently to cultivated wheat (Orellana et al. 1989).In the present study, we produced trigeneric hybrids between TE1508 and the triticale cultivar Currency. The results indicated that *Th. intermedium* chromosomes remained in the progenies, whereas rye chromosomes were lost quickly during selfing of the populations. Hence, we can conclude that the *Th. intermedium* genomes are probably more closely related to wheat genomes than is the rye genome to wheat. Moreover, the different transmission rates among J^S, J and St-chromosomes in the offspring of trigeneric hybrids also possibly indicate that the J^S-chromosomes may be more closely related to wheat than the other two genomes, which is also supported by the molecular-based evidence (Table 2). Due to distinctly different genetic divergences among the alien genomes and the wheat A, B, D genomes, the transmission rates of the different alien chromosomes led to variations in chromosome numbers in the progenies of trigeneric hybrids (Kosina and Heslop-Harrison 1996). The chromosome rearrangements and intergenomic translocations of wheat and those of alien origin have been previously observed in different trigeneric amphiploid hybrids (Kang et al. 2012, 2016). We also produced translocation lines between J^S and J chromosome and wheat chromosomes in the trigeneric hybrids progenies described here. The lines carry only a small number of alien genes into wheat, which may be of some use to breeders for wheat improvement.

Chromosome painting has been used to identify alien chromosomes, including chromosome number and characterizing structural aberrations in wheat-alien hybrids (Jiang and Gill 1993). Considering that the inter- and intragenomic chromosomal rearrangements also occur commonly in *Th. intermedium* and the wheat–*Th. intermedium* derivatives (Chen et al. 1999; Yang et al. 2006; Deng et al. 2013; Li et al. 2015), identification of individual chromosomes of the complex *Th. intermedium* was considered also

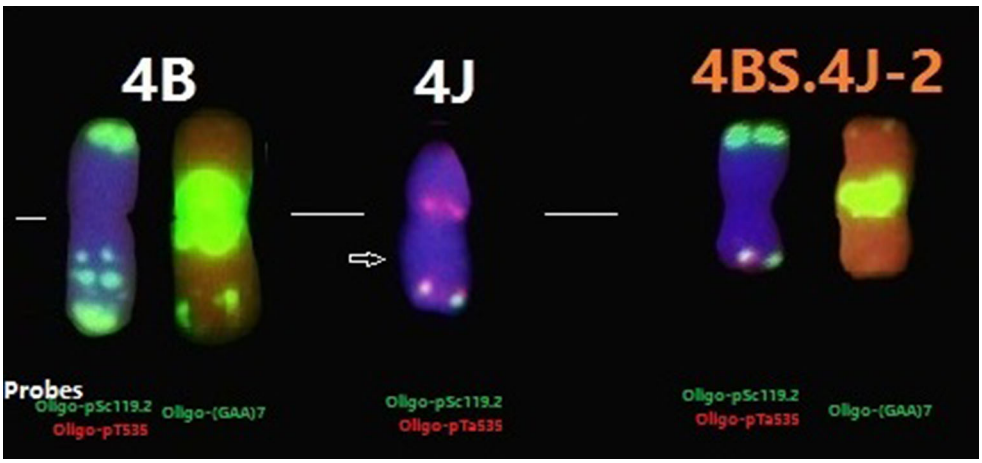

Fig. 5 FISH patterns of chromosomes 4B, 4J and 4J.4B translocation for blue grain. The chromosomes 4B from Currency, 4J from X24C10, and 4J.4B location from X24C1-1. The probes for the chromosomes to the *left* were Oligo-pSc119.2 (*green*) + Oligo-pTa535 (*red*), and probe for the chromosomes to the *right* was Oligo-$(GAA)_7$ (*green*). *Arrow* shows the putative position for the translocation breakpoint

crucial. The genomic in situ hybridization (GISH) technique has been employed to characterize genomes and chromosomes in polyploid *Thinopyrum* species (Chen et al. 1998; Tang et al. 2000; Mahelka et al. 2011). The multicolor GISH (mcGISH) approach has also been sequentially performed to identify the *Thinopyrum* chromosomes in wheat background (Han et al. 2003; Kruppa and Molnár-Láng 2016). The complete hybridization signals of the St genome chromosomes were recently widely accepted by researchers, since they are stable in different *Th. intermedium* accessions. We recently isolated a genome-specific long terminal repeat (LTR) sequence and were able to discriminate between the J^S and J genomes by FISH (Liu et al. 2009; Tang et al. 2011). We further carried out analysis of 11 globally distributed *Th. intermedium* accessions by FISH using the probe pDb12H, and found that the number of J^S-chromosomes was constant 14 chromosomes. We thus concluded that the FISH hybridization signals of pDb12H were specific to the J^S-chromosome of *Th. intermedium*. ND-FISH using the probes Oligo-pSc119.2 and Oligo-pTa535 can enable construction of the karyotype of several *Th. intermedium* chromosomes in the wheat background (Fig. 1e). We recently generated a terminal repetitive probe Oligo-pSt122, which can also assist in distinguishing individual *Th. intermedium* chromosomes in the wheat background (Li et al. 2016). In the present study, the wheat–*Th. intermedium* partial amphiploid TE1508 was identified as having unique *Th. intermedium* ssp. *trichophorum* J^S- and J-chromosomes, which can be used to compare the different origins of *Th. intermedium* chromosomes transferred to wheat.

The chromosomes from *Thinopyrum* species have been successfully introduced into wheat to develop blue-grained germplasm (Li et al. 1986; Zeven 1991; Zhang et al. 2002). The disomic substitution lines consisting of a pair of wheat 4D chromosomes replaced by a pair of *Th. ponticum* 4Ag chromosomes revealed that a gene conferring blue grain is located on this *Thinopyrum* chromosome (Li et al. 1986). Zheng et al. (2006) physically mapped the blue grain gene to the region of FL0.71–0.80 on the long arm of chromosome 4Ag. A blue-grained gene in *Th. bessarabicum* was located on chromosome 4J (Zhang et al. 2002), and was recently located to the region between the centromere and FL0.52 on long arm of chromosome 4J (Shen et al. 2013). Whelan (1989) first reported the grain of *Th. intermedium* ssp. *trichophorum* (formerly named *Agropyron trichophorum*) displayed the blue seeds color. In the present study, we found that the wheat–*Th. intermedium* partial amphiploid TE1508 exhibited blue grains (Fig. 6). Line X24C10 contained a *Th. intermedium* J-chromosome substituted for a pair of 4B chromosomes and displayed the blue grain phenotype (Fig. 3c). Molecular marker analysis in the present study also revealed that the J-chromosome responsible for blue grain belonged to linkage group 4. Moreover, we located the blue grain on the long arm of 4J based on the translocation of 4BS.4JL, and further located on the terminal region of 4JL at the FL0.60–1.00 based on the small segment translocation in X24C1-1 by FISH (Fig. 5).

Thinopyrum intermedium homoeologous group 4 chromosomes have been known to possess novel sources of resistance to both eyespot (*Tapesia yallundae*) and WSMV (Wheat Streak Mosaic Virus) for a long time. Chromosome 4Ai#2 carrying the *Wsm1* gene was further designated as $4J^S$-chromosome by St-based GISH (Li et al. 2004). Other gene(s) for resistance to WSMV were also identified on the long arm of chromosome 4Ai#3 (J-chromosome) from *Th. intermedium* (Friebe et al. 1996; Li et al. 2005). We

Fig. 6 The spikes and seeds of wheat–*Th. intermedium* lines X24C14, X24C10, X24C5, X24C1-1, and their parents TE1508 and currency

observed that the addition line L4 with a 4Ai#1 chromosome (St-genome) was susceptible to stripe rust. In the present study, we determined that the substitution line X24C5 contained the $4J^S$ chromosomes originally from *Th. intermedium* ssp. *trichophorum*. We observed that the terminal region of the original short arm of $4J^S$ of X24C5 contained the strong signals with Oligo-pSc119.2 and Oligo-pSt122, which were absent in $4J^S$-St chromosomes of line X482 previously described by Li et al. (2016) (Supplementary Fig. 1). The distribution of pDb12H hybridization signals confirmed that the $4J^S$-St chromosome had been involved in a translocation event resulting in loss of about 40% of $4J^S$ short arm, which was substituted by a 4St-chromosome, as described by Li et al. (2015). In the field-based stripe rust resistance study, line X482 showed high susceptibility to stripe rust, while X24C5 showed high levels of resistance at the adult plant stage. We, therefore, assume that it is possible that the stripe rust resistance gene in $4J^S$ may be located in the distal region of its short arm at about FL0.60–1.00. Furthermore, we found that the number of polymorphic bands associated with chromosome $4J^S$ when compared to the wheat 4A and 4D wheat chromosomes was fewer than lines carrying the 4J chromosome. Therefore, chromosome $4J^S$ may represent a promising candidate for future homoeologous recombination studies with 4A or 4D.

Three disomic chromosome addition lines, Z1, Z2, and Z6 derived from Zhong 5 and containing chromosome 2Ai#2 from *Th. intermedium*, also consistently showed high levels of resistance to barley yellow dwarf virus

(BYDV) (Xin et al. 1988; Larkin et al. 1995). Chromosome 2Ai#2 is an St-J^S translocation in which majority contains St chromatin and the partial long arm of a J^S chromosome (Tang et al. 2000; Chen et al. 2003). The BYDV resistance gene may locate on the St-segment of 2Ai#2 (Wang et al. 2010). The breakpoint of St-J^S of chromosome 2Ai#2 of Z2 was possibly at the FL0.40 of long arm based on the hybridization with probe pDb12H (Supplementary Fig. 2). The present study identified the *Th. intermedium* $2J^S$ chromosome in X24C14 and characterized that it was a complete J^S chromosome according to the FISH and molecular marker analyses. The FISH patterns with the terminal repetitive probe Oligo-pSt122 showed that the 2Ai#2 chromosome has two distinct hybridization signals in both terminal regions (Supplementary Fig. 2); however, the Oligo-pSt122 did not have any hybridization signal in $2J^S$ of X24C14. Therefore, we have convincingly shown that the chromosomes of the different *Th. intermedium*-derived germplasm can be distinguished by FISH. We found that line X24C14 was resistant to stripe rust, and line Z2 was also responsible for novel rust resistance (Tang et al. 2000). This material is, therefore, considered to be essential for the future studies for transferring novel rust genes from linkage group 2 of *Th. intermedium* into wheat (Hou et al. 2016).

Author contribution statement ZY and GL designed the experiments. JL, BL, HW and ZY performed the experiments, TL and EY analysis the data, ZY and GL wrote the paper.

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