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Precise identification of two wheat–*Thinopyrum intermedium* substitutions reveals the compensation and rearrangement between wheat and *Thinopyrum* chromosomes

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Abstract Two wheat–*Thinopyrum* substitution lines X479 and X482 selected from the progenies of wheat "Mianyang26 (MY26)" × wheat–*Thinopyrum intermedium* ssp. *trichophorum* partial amphiploid were characterized by seed storage protein electrophoresis, genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), and PCR-based molecular markers. Seed storage protein analysis showed that X479 expressed some of *Th. intermedium* ssp. *trichophorum*-specific gliadin and glutenin bands. Chromosome counting and GISH probed by *Pseudoroegneria spicata* genomic DNA indicated that two pairs of *Thinopyrum*-derived chromosomes (St genome and St–J^S translocated chromosomes) substituted for two pairs of wheat chromosomes in both X479 and X482. FISH using pAs1 and pHvG38 as probes showed that chromosomes 1B and 4B, and 4D and 6D were absent in X479 and X482, respectively. Using the newly isolated J^S chromosome-specific repetitive sequence pDb12H as a probe, the FISH signals revealed that the translocation of St–J^S chromosomes in X479 and X482 occurred in repetitive sequence regions of the short arm. The molecular markers based on wheat–rice colinearity confirmed that the chromosome constitutions of X479 and X482 were 1St (1B) + 4St–4J^S (4B) and 4St–J^S (4D) + 6St (6D), respectively. The substitution lines were both fully fertile which suggests that the *Th. intermedium* chromosomes in X479 and X482 substitute well for the corresponding wheat chromosomes. The rust resistance and novel agronomic traits revealed that the substitution lines will be potentially useful for genetic improvement of wheat.

Keywords Fluorescence in situ hybridization · *Thinopyrum intermedium* ssp. *trichophorum* · Substitution lines · Stripe rust resistance

Introduction

As a segmental autoallohexaploid wheatgrass, *Thinopyrum intermedium* consists of two closely related, partially homeologous, genomes and one distinctly diverse genome (Dewey 1984). Efforts to reveal the genomic composition of *Th. intermedium* have been underway for decades and various hypotheses have been proposed (Chen et al. 1998; Mahelka et al. 2011, 2013). The studies indicated that three distinguishable chromosome sets of *Th. intermedium* were recently designated J, J^S, and St genomes. The J genome was

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related to both *Th. elongatum* and *Th. bessarabicum*; however, the J^S genome referred to a modified *Th. elongatum/Th. bessarabicum* genome (Chen et al. 1998; Chen 2005). Since *Th. intermedium* has wide range of adaptation to soil and climate, it is endemic to Central and Southeastern Europe through to Turkey and can also now be found growing wild throughout the western half of the North American continent. This results in the vast genetic diversity in the species of *Th. intermedium* (Wagoner and Schauer 1990). *Th. intermedium* subspecies has been hybridized extensively with wheat and has proved to be a valuable resource for improving wheat disease resistance and yield potential (Li and Wang 2009). Numerous wheat–*Thinopyrum* chromosome addition and substitution lines, as well as partial amphiploids, have been developed, which offer solid genetic resources for determining genetic control of novel genes in the *Th. intermedium* genomes (Chen 2005; Li and Wang 2009). However, extensive genetic diversity exists in the different *Th. intermedium* subspecies, and chromosomal rearrangements have occurred frequently in *Th. intermedium* genomes (Friebe et al. 1992; Xu and Conner 1994; Mahelka et al. 2011). Therefore, it is worthwhile continuously introducing *Th. intermedium* individual chromosomes representing the genetic variation and the novel traits to different wheat background.

Thinopyrum intermedium ssp. *trichophorum,* a pubescent subspecies of wheatgrass (Dewey 1984), displayed more chromosomal heterochromatic bands than chromosomes of *Th. intermedium* ssp. *intermedium* (Xu and Conner 1994) and also appeared to carry novel resistances to several foliar diseases and unique seed storage proteins which may be valuable for wheat improvement (Wills et al. 1998). With the ultimate aim to introduce novel, agronomically important genes from *Th. intermedium* ssp. *trichophorum* to wheat, we developed a wheat–*Th. intermedium* ssp. *trichophorum* partial amphiploid (Yang et al. 2006). The second step for the introgression alien chromosome segments into wheat is to produce addition and/or substitution lines. This was performed by crossing the wheat–*Thinopyrum* partial amphiploid to wheat and reported earlier (Hu et al. 2011; Li et al. 2013a, b; Song et al. 2013).

Here, we report the production and precise characterization of two new, double-disomic *Th. intermedium* chromosome substitution lines for the purpose of (1) analyzing the ability of the *Thinopyrum*-genome chromosomes for compensating the loss of homeologous B- and D-genome chromosomes, (2) mapping of agronomically important genes to a *Th. intermedium* chromosome transferred to wheat, and (3) development of PCR-based markers for identifying the introduced *Th. intermedium* chromosomes.

Materials and methods

Plant materials

Thinopyrum intermedium ssp. *trichophorum* accession PI440125 and *Pseudoroegneria spicata* (St genome, $2n = 2x = 14$) accession PI 232131 were obtained from the USDA National Small Grains Collection at Aberdeen, Idaho. The wheat–*Th. intermedium* ssp. *trichophorum* partial amphiploid, TE-3 (Yang et al. 2006), and wheat line Mianyang 26 (MY26) are maintained at the Triticeae Research Institute, Sichuan Agricultural University, China. Lines X479 and X482 were developed from the progenies of the cross between TE-3 and MY26, and they are deposited at Xindu Experimental Station of University of Electronic Science and Technology of China.

Sequential C-banding and GISH

Seedling root tips were collected, pretreated in water at 0 °C for 24 h, and fixed in ethanol–acetic acid (3:1) for 1 week. Root-tip squashes were stained using the conventional Feulgen method for chromosome counting according to Gill et al. (1991). For GISH analysis, total genomic DNA from *Ps. spicata* was labeled with digoxigenin-11-dUTP by nick translation following the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Sheared genomic DNA of Chinese Spring wheat (CS, genomes ABD, $2n = 42$) was used as blocking DNA. The hybridization mixture was prepared as described by (Mukai et al. 1993). The GISH signal was detected with fluorescein-conjugated antidigoxigenin antibody (Roche Diagnostics, Indianapolis, IN), and the slide was mounted in propidium iodide dissolved in Vectashield® antifade solution (Vector Laboratories, Burlingame, CA). Microphotographs of C-banded and GISH chromosomes were taken with an Olympus BX-51 microscope using a DP-70 CCD camera.

Fluorescence in situ hybridization (FISH)

The probes pAs1, containing a 1-kb DNA fragment isolated from *Aegilops tauschii* in the plasmid pUC8 (Rayburn and Gill 1986), and pHvG38 (Pedersen and Langridge 1997), with GAA repeats were used to identify the B-genome of wheat, were generously provided by Dr. B. Friebe, Wheat Genetic and Genomic Resources Centre, Department of Plant Pathology, Kansas State University, USA. An LTR probe pDbH12 was used to distinguish the J^S genome of *Th. intermedium* as reported by Liu et al. (2009). The probe labeling, hybridization, and detection for FISH were the same as in the GISH protocol.

Seed storage protein electrophoresis

Acid polyacrylamide gel electrophoresis (APAGE) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were used to separate endosperm gliadin proteins and glutenin subunits, respectively. The procedures were described by Yang et al. (2001).

Disease resistance screening

Wheat- and *Thinopyrum*-derived lines X479, X482, and their parents were evaluated in the field during 2010–2013 at Xindu Experimental Station, Sichuan Academy for Agricultural Sciences. The adult-plant resistance to *P. striiformis* f. sp. *tritici* strains CYR31, CYR32, and CYR33 were provided by the Plant Protection Institute, Sichuan Academy of Agricultural Sciences. A subset of 20 plants was selected for these assessments, and their represented parents were also tested. Infection types were evaluated 2–3 weeks after inoculation when uredinia were fully developed. Stripe rust responses were recorded following Ma et al. (1995).

Molecular marker analysis

DNA was extracted from fresh leaves of X479, X482, TE-3, and CS. PCR-based Landmark Unique Gene (PLUG) primers were according to Ishikawa et al. (2009). Polymerase chain reaction (PCR) was performed in an Icycler thermal cycler (Bio-RAD Laboratories, Emeryville, CA) in reaction volumes 25 µl, 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 primer. The cycling parameters were 94 °C for 3 min for denaturing; followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and then, a final extension at 72 °C for 10 min. The amplified products were cloned and sequenced.

Results

Spike and seeds morphology of X479 and X482

Among BC_1F5 progenies from the cross between CS-*Th. intermedium* ssp. *trichophorum* partial amphiploid

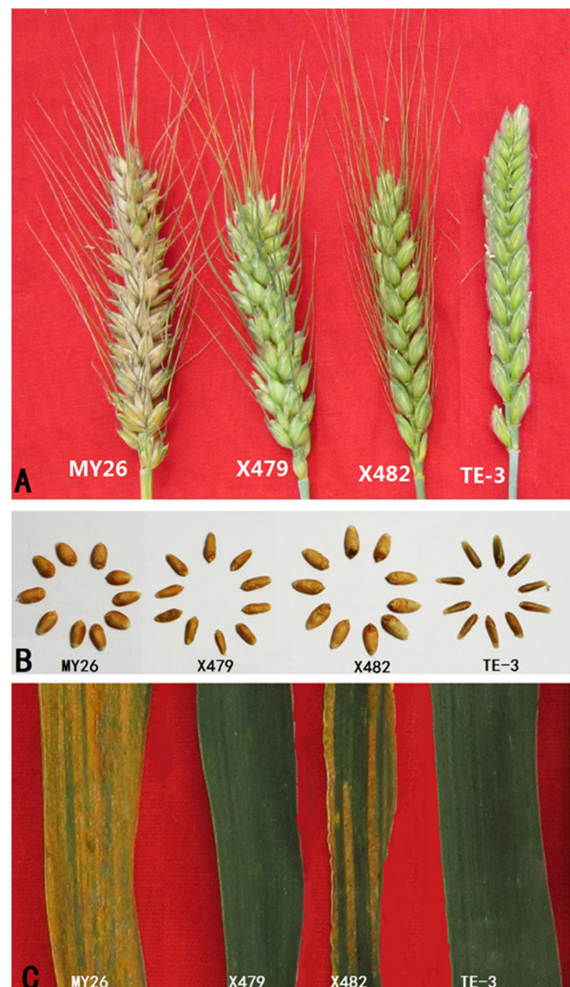

Fig. 1 Adult spikes (**a**), seeds (**b**) and stripe rust resistance (**c**) of MY26, X479, X482, and TE-3

TE-3 and wheat line MY26, each four plants of lines X479 and X482 were selected. As shown in Fig. 1, the spikes morphology of X479 and X482 displayed similar agronomic traits and high seed set to the wheat parent MY26. The lines X479 and X482 had 16–20 spikelets per spike, with shorter spikes than either MY26 or TE-3. The seeds of X479 were relatively thinner and longer than MY26, while the grains of X482 were larger and had a higher 1,000-kernel weight than MY26 (Supplementary Table 1). X479 carried a trait of pubescence on the glumes of spikes, which originated from *Th. intermedium* ssp. *trichophorum* (Yang et al. 2006).

Seed storage protein analysis

The endosperm storage proteins have been considered as useful genetic markers and also utilized on cultivar identification. APAGE produced distinctive bands in the ω, γ, β, and α zones of seed gliadin storage proteins from seeds of CS, TE-3, X479, and X482 plants (Fig. 2a). X479 and X482 displayed clearly different gliadin bands patterns. Within X479 and X482 lines, different seeds produced identical band patterns, indicating their genetic homogeneity at the gliadin loci. The line X479 produced two strong bands in the ω-gliadin zones (arrowed), which are identical to those in the partial amphiploid TE-3. Based on our previous study, the ω-gliadin bands represented the *Th. intermedium* ssp. *trichophorum*-specific bands (Yang et al. 2006).

The high molecular weight glutenin subunit (HMW-GS) composition of X479 and X482 and their parents TE-3 and MY26 were analyzed by SDS–PAGE (Fig. 2b, c). The HMW-GS in TE-3 included *Glu-A1* null, *Glu-B1* subunits 7 + 8, and *Glu-D1* subunits 2 + 12. In MY26, *Glu-A1* subunit 1, *Glu-B1* subunits 7 + 9, and *Glu-D1* subunits 2 + 12 were present. Line X482 showed the HMW-GS of *Glu-B1* subunits 7 + 9 and *Glu-D1* subunits 2 + 12. Line X479 had the *Glu-D1* 2 + 12 subunits, but there were no bands for the *Glu-B1* locus, which is on the long arm of chromosome 1B. It is clear that the *Th. intermedium* group 1 chromosome in X479 contained the *Thinopyrum*-specific HMW-GS and ω-gliadin genes.

GISH and FISH

Root-tip chromosome counts and meiotic observation of PMCs were conducted on 20 plants of lines X479 and X482. We found that all plants had 42 chromosomes and 21 bivalents at meiotic MI, indicating cytological stability of X479 and X482 (Supplementary Fig. 1).

GISH, using total genomic DNA from *Ps. spicata* (St genome) as a probe on mitotic metaphase chromosomes of X479 and X482, was used to determine the introgression of segments of the *Th. intermedium* chromosomes. The St genome-based GISH procedure can distinguish the individual J, J^S, and St chromosomes in *Th. intermedium* (Chen et al.

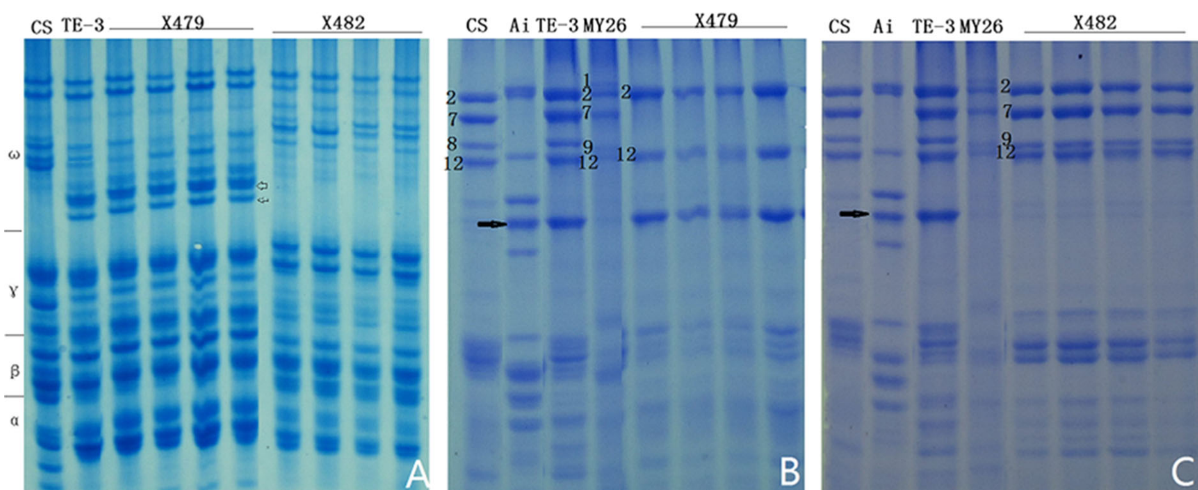

Fig. 2 Gliadin proteins separated by APAGE (**a**) and glutenins separated by SDS–PAGE (**b**, **c**) of X479, X482, and their parents. *Arrows* indicated the *Th. intermedium* ssp. *trichophorum*-specific bands

1998). As indicated in Fig. 3a, e, GISH results show that both X479 and X482 contained two pairs of strongly hybridized chromosomes, of which one pair of chromosomes had signals uniformly along the entire chromosome lengths, indicating that they belonged to *Th. intermedium* St chromosomes. Another pair of chromosomes showed strong GISH signals around the centromeric regions and weaker signals at the telomeric regions, indicating that this pair of chromosomes belonged to the J^S genome, with

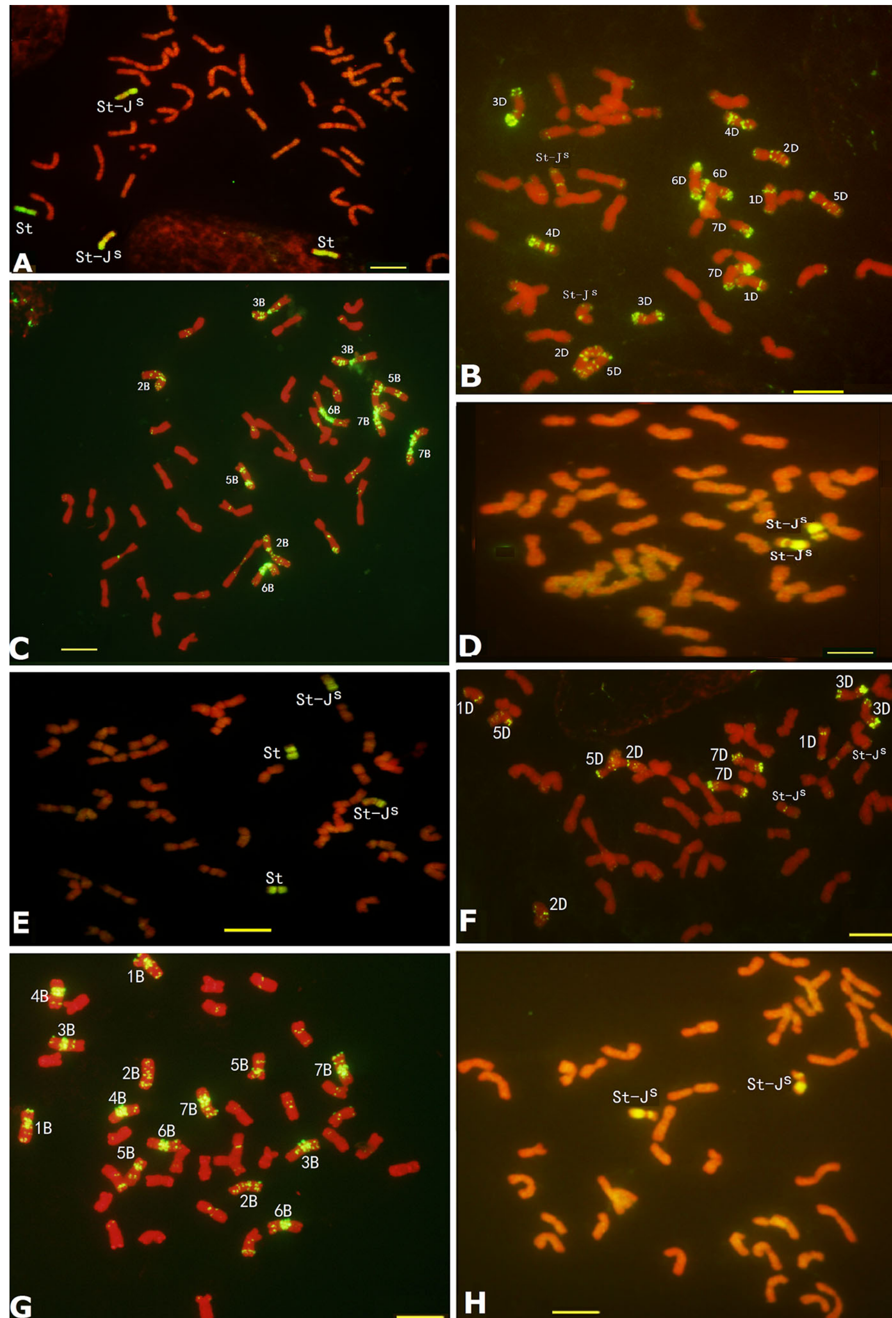

Fig. 3 Genomic and fluorescent in situ hybridization of X479 (**a**–**d**) and X482 (**e**–**h**). The probes used for in situ hybridization were *Ps. spicata* genomic DNA (**a**, **e**), pAs1 (**b**, **f**), pHvG38 (**c**, **g**), and pDb12H (**d**, **h**). *Bar* shows 10 μm

half St genome fragment translocated in the short arms. Therefore, it can be concluded that both lines X479 and X482 contained two St chromosomes and two St–J^S chromosomes.

In order to determine the constitution of wheat chromosomes in X479 and X482, a total of twenty mitotic metaphase chromosomes in X479 and X482 were hybridized by with probes pAs1 and pHvG38 (Fig. 3), respectively. The presence of wheat D-genome chromosomes was demonstrated using probe pAs1 (Rayburn and Gill 1986), while the pHvG38 can be easily detected for the presence of B-genome chromosomes (Pedersen and Langridge 1997). The FISH signals by pAs1 revealed that X479 contained the complete D-genome (Fig. 3b), while X482 had ten D-genome chromosomes and was missing chromosomes 4D and 6D (Fig. 3f). The FISH signals by pHvG38 indicated that chromosomes 1B and 4B were absent in X479 (Fig. 3c), while X482 contained the complete B-genome (Fig. 3g).

FISH using pDb12H as a probe assisted in the identification of the St–J^S chromosomes in X479 and X482 (Fig. 3d, h). A pair of chromosomes in both X479 and X482 displayed identical hybridization signals. The signals covered the most regions of long arm except the centromeric and telomeric regions, and the limited regions in the middle of short arm. Based on the chromosomes distributions of sequential St genome GISH and pDb12H FISH signals outlined by Liu et al. (2009), we considered that the translocation of St–J^S chromosomes in X479 and X482 appeared in the intercalary breakpoint at the short arm of J^S chromosome (Fig. 4).

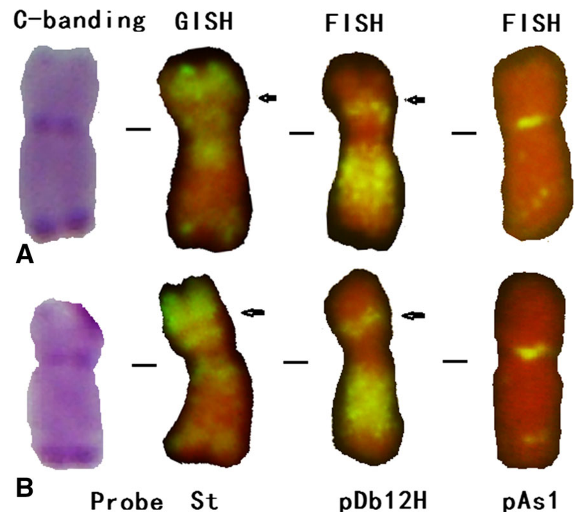

Fig. 4 C-banding and ISH patterns of the 4St–J^S translocation chromosome in X479 (**a**) and X482 (**b**). *Arrow* indicates the putative translocation breakage point

Molecular markers

The PLUG primers were designed based on rice syntenic region, and presumably amplify fragments corresponding to the similar linkage group(s) of wheat genomes (Ishikawa et al. 2009). Our previous studies showed that the PLUG markers were useful for producing *Thinopyrum* chromosome-specific markers (Hu et al. 2012). In total, 145 PLUG markers were tested, including X479, X482, and their parents MY26 and TE-3. Six PLUG markers from homeologous group 1, 6 markers from group 4 and 3 markers from group 6 clearly produced *Th. intermedium*-specific bands in X479 and X482 (Table 1). By using the *Th. intermedium* and *Ps. spicata* (St genome) as control, the results of PLUG primers amplification showed that the *Th. intermedium* chromosomes in line X479 belong to groups 1 and 4, while those of line X482 belong to groups 4 and 6 (Fig. 5).

Chinese Spring nulli-tetrasomic lines were also used to identify the target wheat bands. The amplification of primer TNAC1026 showed the absence of the 1B bands in X479 (Fig. 5a). Similarly, for TNAC1412 (Fig. 5b, c), 4B-specific bands were absent in X479, and 4D bands absent in X482. The amplification of TNAC1743 showed that X482 was missing 6D-specific bands (Fig. 5d). Therefore, we concluded that X479 was a double-disomic 1St (1B), 4St–J^s (4B) substitution line, while X482 was a double-disomic 4St–J^s (4D), 6St (6D) substitution line.

Rust resistances

Lines X479 and X482 and the parental lines TE-3 and MY26 were inoculated with *P. striiformis* f. sp. *tritici* races CYR31, CYR32, and CYR33 at the adult plant stage. TE-3 was immune to these isolates, whereas wheat parent MY26 was highly susceptible. X479 was highly resistant to stripe rust, while X482 was susceptible (Fig. 1c). These results indicated that the stripe rust resistance in X479 was from the chromosome 1St to *Th. intermedium* ssp. *trichophorum*, while the 4St–J^S and 6St may not contain the stripe rust resistance gene(s), or the resistance is not expressed.

Table 1 Amplification of PLUG primers and used in this study

Primers	Homeologous relationship	Primer sequences	Rice homeology ID	Enzymes	*Thinopyrum*-specific bands
TNAC1001	1AS, 1BS, 1DS	F: TTCCCATCTCTTGCCATTAAA R: TTTCCGCTTCCTATGATGCT	LOC_Os05g01240	*Taq*I	1St
TNAC1009	1AS, 1BS, 1DS	F: CGAACGTGACCATCTACATCA R: CATCTGACTTGGTCTTGGCATA	LOC_Os05g06280	*Taq*I	1St
TNAC1010	1AS, 1BS, 1DS	F: GATGCAACTGCAGGAATGAAG R: TCTCTTCTGAAGCGGTCATGT	LOC_Os05g06440	*Taq*I	1St
TNAC1021	1AL, 1BL, 1DL	F: CTCATGCATGCGTTTGTTAAA R: CCAGCTGAAACAAGCATCTTC	LOC_Os05g23430	*Taq*I	1St
TNAC1026	1AL, 1BL, 1DL	F: GGGATAGAACTCTGGGACTTCA R: AGTGCCAGGGCATAATACAGC	LOC_Os05g28200	*Hae*III	1St
TNAC1041	1AL, 1BL, 1DL	F: TCACCACCTCTTTCAGTTGCT R: GCATCAAGGATGAGGAGTCTG	LOC_Os05g42350	*Taq*I	1St
TNAC1412	4AS, 4BL, 4DL	F: CTATGTCCGCAGCCATGAGTA R: CTTCACACCATCCAAGCTTTC	LOC_Os03g12236	*Taq*I	$4J^s$
TNAC1421	4AS, 4BL, 4DL	F: ATCCGCTTCTCCAAGTTCTTC R: GTCCGATCCACTTCTTCAGGT	LOC_Os03g16980	*Taq*I	$4J^s$
TNAC1457	4AL, 4BS, 4DS	F: TTTGATTCCGTACTGCCTGAG R: GCACCATTTGTTCCAGTCAAC	LOC_Os03g45270	*Taq*I	$4J^s$
TNAC1463	4AL, 4BS, 4DS	F: CGTCTTTATCAAACCCTGCAA R: GTTCACCGAGTTCATCCAGAA	LOC_Os03g48930	*Taq*I	$4J^s$
TNAC1468	4AL, 4BS, 4DS	F: GATGATCGCCAAGAAGTTGAG R: CAGAACTCGCTGGAAATGATG	LOC_Os03g50130	*Taq*I	4St
TNAC1510	4AL, 4BS, 4DS	F: GCGTCTGTCTTCATCTTCTGG R: CAAGTGTGCACATGACTGCTT	LOC_Os11g14990	*Taq*I	$4J^s$
TNAC1678	6AS, 6BS, 6DS	F: AAATCTACTCTGCGAGGTTTGC R: TGGGAAGCGCTTACTATTGTG	LOC_Os02g02960	*Taq*I	6St
TNAC1679	6AS, 6BS, 6DS	F: TATTGGCTCAACCAACCATTC R: TTCCAAACCACCCAGTGTGTA	LOC_Os02g03230	*Taq*I	6St
TNAC1743	6AL, 6BL, 6DL	F: CAGTTGATCAGGGCATTCAGT R: TTCCAGTCCTTTGTTTCCACA	LOC_Os02g49360	*Taq*I	6St

Discussion

Thinopyrum intermedium consists of three distinguishable chromosome sets which have been designated as the J, J^S, and St genomes (Chen et al. 1998; Chen 2005). Molecular and cytogenetic evidence has also revealed that remarkable genetic diversity and genomic structural modifications exist across inter-population and intra-population germplasm accessions of *Th. intermedium* (Mahelka et al. 2011, 2013). The study of the variation of agronomical traits is an essential starting point for further utilization of the *Th. intermedium* novel gene(s) for wheat improvement (Li and Wang 2009). In the present study, the *Th. intermedium* ssp. *trichophorum* accession, which displayed a distinctly different chromosomal heterochromatin constitution from the related *Th. intermedium* ssp. *Intermedium,* was used to as donor to produce wheat–*Th. intermedium* substitution lines (Xu and Conner 1994; Yang et al. 2006). The incorporation of *Th. intermedium* chromosomes to wheat enables the effect of individual *Thinopyrum* chromosomes to be determined and clarifies inter-chromosomal homeologies (Hu et al. 2011; Li et al. 2013a, b).

In the present study, we obtained fertile progeny from wheat–*Th. intermedium* ssp. *trichophorum*

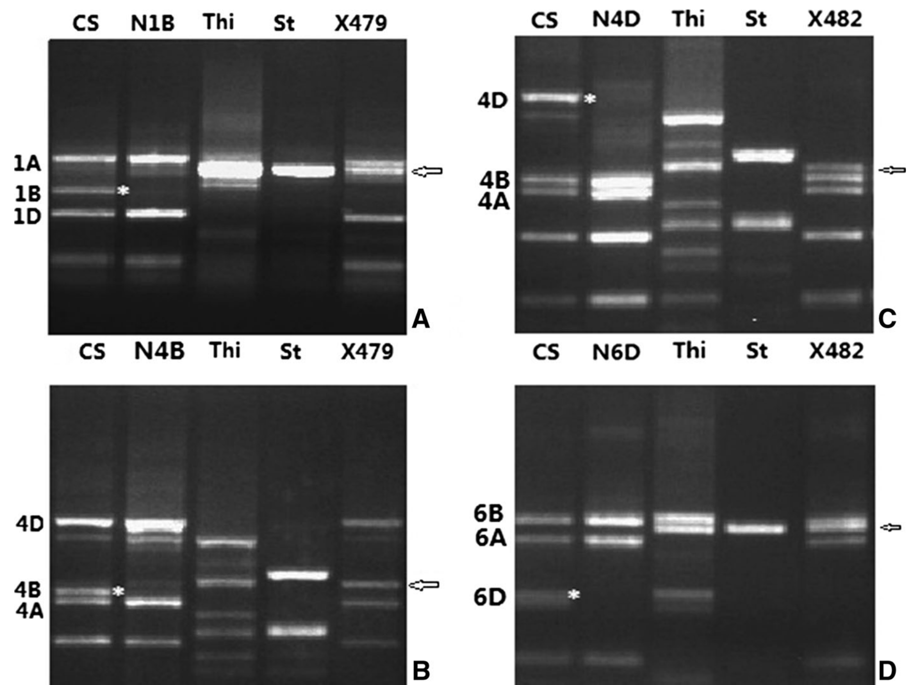

Fig. 5 PCR amplification using PLUG primers TNAC1026 (**a**), TNAC1412 (**b**, **c**), and TNAC1743 (**d**). The arrows indicate the X479- or X482-specific bands identical to that of *Th. intermedium*-derived bands, *star* indicates that the wheat bands absent in X479 or X482. Thi and St refer *Th. intermedium and Ps. spicita,* respectively

double-disomic substitution lines X479 and X482. Based on GISH patterns and C-banding analysis, the *Th. intermedium* chromosomes 1St and $4J^S$–St substituted wheat chromosomes 1B and 4B in X479, while chromosomes $4J^S$–St and 6St substituted wheat chromosomes 4D and 6D in X482. We previous identified a 1St (1D) substitution line (Hu et al. 2011). The results suggested that the groups 1, 4, and 6 *Th. intermedium* chromosomes can be easy to compensate for the loss of corresponding homeologous chromosomes of wheat B- and D-genomes. Wheat-alien chromosome substitution lines are not only of great theoretical interest for elucidating evolutionary relationships, but also of immense practical interest for introducing the rich genetic diversity of donor species for crop improvement (Jiang et al. 1994). In the present study, the C-banding and GISH pattern of chromosome 1St in X479 is identical to our previously identified chromosome 1St#2 in wheat–*Th. intermedium* ssp. *trichophorum* substitution line AS1677 (Hu et al. 2011). The chromosome 1St#2 carried a novel stripe rust resistance gene(s), which when transferred to line X479, conferred resistance to that serious cereal disease. Li et al. (2013a, b) located a *Th. intermedium*-specific HMW-GS, encoded by gene *Glu-1St#2x*, on chromosome 1St#2, which is identical to the additional HMW-GS band scored in X479. However, we also found the *Thinopyrum*-specific ω-gliadin in X479, indicating that chromosome 1St in X479 contained the novel *Th. intermedium*-specific *Gli-1* loci. Meanwhile, the trait for *Th. intermedium* ssp. *Trichophorum*-specific pubescence on the glumes was also observed in X479, suggesting that the gene(s) were located on chromosome 1St of X479.

Recently, Han et al. (2014) reported that four different types of chromosome 6P of *Agropyron cristatum* possessed different desirable genes in wheat–*Ag. cristatum* disomic addition lines. Similar diversity of the 1St chromosomes from different wheat–*Th. intermedium* ssp. *trichophorum* substitution lines may exist across germplasm stocks Friebe et al. (1992) identified a wheat–*Th. intermedium* disomic addition lines L7 (6Ai) originated from TAF 46 (Cauderon et al. 1973), and Chen et al. (1999) further assigned the *Th. intermedium* chromosome in L7 as 6St. The C-banding pattern of the chromosome 6St in X482 appeared clearly different from that the chromosome 6St in L7. We thus have named the group 6 *T. intermedium*-derived chromosome in X482 as "6St#2." We found X482 showed higher grain weight than X479 and wheat parent MY26. It is likely that the chromosome 6St#2 in X482 may confer the important trait of high grain weight useful for wheat breeding.

Due to the highly polyploid nature of the *Th. intermedium* species, the inter- and intra-genomic chromosomal rearrangements also occur commonly in the wheat–*Th. intermedium* partial amphiploids (Chen et al. 1999; Yang et al. 2006; Zeng et al. 2013). The *Th. intermedium* genomic compositions involved different St–J^S genome chromosomes as either Robertsonian, intercalary, or terminal interchanges were found in several partial amphiploids and addition lines (Chen 2005). Tang et al. (2000) identified *Th. intermedium* St–J^S translocated chromosomes in addition lines Z1, Z2, and Z6, which belong to homeologous group 2. In the present study, the GISH–FISH patterns and molecular markers revealed that St–J^S chromosomes in X479 and X482 belonged to homeologous group 4, which differs from the previously reported St–J^S chromosomes in wheat–*Th. intermedium* addition or substitutions (Chen 2005; Li and Wang 2009). Li et al. (2005) reported that *Th. intermedium* J^S chromosomes of homeologous group 4 conferring novel genes for resistance to wheat streak mosaic, wheat curl mite, and eyespot in wheat background. Further disease screening of the 4St–J^S chromosomes in X479 and X482 may discover additional resistance genes. Production of a complete set of *Th. intermedium* substitution and addition lines in a common wheat background will facilitate the study of genomic structures of the introgression of *Thinopyrum* chromosomes. It is possible to reduce the size of alien translocations by inducing recombination between the *Thinopyrum* chromosomes and the corresponding related wheat chromosome(s) after crossing the substitution lines X479 and X482 to wheat using the approaches of marker-assisted chromosome engineering (Niu et al. 2014).

The molecular markers based on comparative genome analysis provided a simple and precise method to target the alien species in a wheat background. The PLUG markers can be useful for alien chromatin identification and assignment of their corresponding linkage groups (Hu et al. 2011; Song et al. 2013; Li et al. 2013a, b). In the present study, the group 1 and group 4 PLUG markers detected polymorphic fragments specific to the *Th. intermedium* chromosome in X479, and the group 4 and group 6 PLUG markers detected for X482 (Fig. 5). The PCR amplification of *Th. intermedium*-specific bands in group 4 of X479 and X482 was identified that the part of short arm was derived from the St genome and the long arm related to J^S genome. These St–J^S chromosomes were not inherited from their parents TE-3 (Yang et al. 2006). Therefore, St–J^S translocation occurred during the processes of cross between wheat and TE-3. Meanwhile, the St–J^S chromosomes can be clearly discriminated by C-banding and FISH by multiple probes such as pDb12H, pHvG38, and pAs1, as well as GISH with St genomic DNA (Fig. 4). From observations of chromosomes after FISH using pDb12H as the probe, it is apparent that the translocation between the J^S and St chromosomes occurred at the short arm. The phenomenon was also commonly observed in the *Aegilops* chromosomes translocation among the species, reported by Molnár et al. (2011). It is suggested that the inter-genomic translocation breakpoints are frequently mapped to repetitive sequences rich chromosomal regions in the allopolyploid species.

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