Characterization of a partial wheat–Thinopyrum intermedium amphiploid and its reaction to fungal diseases of wheat

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Partial amphiploids between wheat (Triticum aestivum L.) and Thinopyrum species play an important role in the transfer and use of traits from alien species. A wheat-Thinopyrum intermediate partial amphiploid, TAI8335, and its alien parent were characterized by a combination of genomic in situ hybridization (GISH) and cytological observations. Evidence from GISH indicated that the donor parent Th. intermedium possessed seven pairs of S, seven J and 21 J chromosomes. Mitotic observation showed that the majority of TAI8335 plants had 56 chromosomes, but a few had 54 to 55, in some cases with two to three additional telochromosomes. The chromosomes in most pollen mother cells of plants with 2n = 56 formed 28 bivalents, averaging 27.12 in 223 cells, suggesting a basic cytological stability. Sequential GISH patterns using genomic Pseudoroegneria spicata and genomic Th. intermedium DNA as probes revealed that TAI8335 had fourteen chromosomes derived from Th. intermedium and its alien genome consisted of one pair of S-, three pairs of J- and one pair of J-genome chromosomes as well as two translocated chromosome pairs, one being a Robertsonian translocation and another an intercalary translocation, both of which involved J and S genome. Two of the telochromosomes in the aneuploid plants originated from the J genome and one from wheat. Disease screening demonstrated this line was highly resistant to leaf rust, stem rust, stripe rust and powdery mildew. This study showed that the partial amphiploid TAI8335 appears to serve as a novel source for the transfer of resistance genes for multiple fungal pathogens to wheat.

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The ongoing improvement of wheat cultivars is dependent on a continuous supply of genetic variability. Thinopyrum intermedium (Host) Barkworth & D.R. Dewey [syn. Agropyron intermedium (Host) Beauvoir and Elytrigia intermedia (Host) Nevski] (2n = 6x = 42) carries many useful agronomic traits and constitutes a tertiary gene pool for wheat improvement. Th. intermedium is a segmental autoallo-hexaploid, and the determination of its genomic composition has been of interest for a considerable time. However, due to the impact of pairing regulation gene(s) and chromosome pairing complexities as well as the high levels of polymorphism in Giemsa banded patterns within the species, no definite conclusions have been made based on traditional cytogenetic techniques, such as meiotic chromosome pairing and C-banding analysis (Chen et al. 1998).

The transfer of alien genes into wheat (Triticum aestivum L., 2n = 6x = 42, ABD) via wide hybridization makes possible to increase the resistance to biotic and abiotic stress and improvement of quality. In wheat breeding programs, the production of amphiploids or partial amphiploids between wheat and relative species is an important intermediate step for such a gene transfer, because they allow the reliable analysis of the effects of alien genes in the genetic background of wheat and their fertility allows gene transfer even when an F1 hybrid is almost completely sterile (Jiang et al. 1994; Ellneskog-Stam and Merker 2002). The wild relatives, Th. intermedium (2n = 6x = 42, JJ’S), has a wealth of genetic variation for the improvement of resistance to fungal and viral diseases in wheat (Li et al. 2008; Li and Wang 2009). To date, several wheat–Th. intermedium amphiploids, Otrastysuskaya (OT), TAF46, Zhongl to Zhong5, 78829, TAI7044 and TE-3, have been obtained (Chang et al. 2003; Chen 2005; Fedak and Han 2005; Yang et al. 2006). Some of them have been widely used for attempted introgressions of useful traits into wheat, including resistance to viral diseases (Larkin et al. 1995; Friebe et al. 1996; Chen et al. 2003; Li et al. 2008; Li and Wang 2009). However, partial amphiploid still do not cover the entire genome of Th. intermedium, due to the complexity of the genomic composition of the alien parent. The exploitation of a new wheat–Th. intermedium partial amphiploid type still remains necessary for understanding the genetic relationships of the S and J or J genomes of Thinopyrum with those of wheat, and for incorporating disease-resistance
genes from *Th. intermedium* into wheat. TAI8335 is a *Th. intermedium*-derived partial amphiploid that was recently developed in our laboratory. In this study, we attempted to determine the chromosome composition and genomic origins of the alien chromosomes of TAI8335 by genomic *in situ* hybridization (GISH) and to evaluate its potential as a novel source for resistance to fungal pathogens.

**MATERIAL AND METHODS**

**Plant material**

The TAI8335, analyzed in this study, is a BC₁F₂-derived partial wheat–*Th. intermedium* amphiploid line, which was produced by crossing *T. aestivum* cv. Jinchun 5 as the female parent with a *Th. intermedium* cv. Jinchun 5 as the female parent with a *Th. intermedium* accession of unknown origin. The hybrid was backcrossed to another wheat cv., Jinmai 33, and the resulting plants from the BC₁F₂ population that were fertile and intermediate in morphology between wheat and *Th. intermedium* parents were selfed for six generations.

Other plant material included the wheat parents and the *Th. intermedium* accession with the genomic formula E₁E₁St (WANG and ZHANG 1996) or JJS (CHEN et al. 1998) used as a donor, the latter provided by Prof. S. H. Li of the Shanxi Academy of Agricultural Sciences, China. Several well known *Th. intermedium*-derived partial amphiploids were also included in the present disease evaluation: Otrast-syuskaya (OT), TAF46 (CHEN et al. 1999), and Zhong 2, Zhong 4 and 78829, which were derived from the same *Th. intermedium* accession (ZHANG et al. 1996) but had different alien chromosome composition (ZHANG et al. 1996; CHEN et al. 2003; HAN et al. 2004). Also included were TAI7044, which possesses the 6 S + 8 J *Thinopyrum* chromosomes (CHANG et al. 2003), and wheat cultivars Mingxian 169, Jingshuang 16 and Wichita as susceptible controls. The investigation of the chromosome constitution of the wild parent was carried out in a population of eight generations produced from the donor plant by open-pollinating. Total genomic DNA from the wheat cultivar Chinese Spring (CS) (2n = 6x = 42, ABD), *Th. intermedium* and *Pseudoroegneria spicata* (Pursh) Löve (2n = 2x = 14, S) was used as probes or blockers for GISH analyses.

**Chromosome preparation**

Root tips collected from the seedlings were immersed in ice water for about 24 h and fixed in ethanol:acetic acid (3:1) for about one week, then stained using the conventional feulgen method for chromosome counting. For GISH, the roots were stained in 1% acetocarmine for a few minutes and squashed in 45% acetic acid. Cover slips were removed after freezing on dry ice, and the preparations were then dehydrated in ethanol for 5 min prior to *in situ* hybridization.

For meiotic chromosome preparations, anthers from the emerging spikes containing pollen mother cells (PMCs) at MI were fixed in ethanol:acetic acid (3:1) for one day, transferred to 70% ethanol, and kept at 4°C in a refrigerator for about two weeks. Anthers were then stained in 1% acetocarmine and squashed in 45% acetic acid.

**Probe labeling and genomic *in situ* hybridization (GISH)**

Total genomic DNA was extracted from fresh young leaves of *Th. intermedium* and *Ps. spicata* using a DNeasy Plant Mini Kit and following the manufacturer’s instructions (Qiagen Inc., Valencia, CA, USA). The procedure for GISH was as described in ZHANG et al. (2001), with some modifications. Approximately 1 mg of genomic DNA from *Th. intermedium* or *Ps. spicata* was labeled with fluorescein-12-dUTP (FITC detected by yellow-green fluorescence) (Enzo Life Sciences Inc, Farmingdale, NY, USA) in a 50 μl reaction mixture using nick translation. Slides were dehydrated in a series of 70%, 90% and 100% ethanol, and slide-bound chromosomal DNA was denatured in 100 μl of 70% formamide in 2 × saline sodium citrate (SSC) at 80°C for 2 min. The hybridization solution contained 50% deionized formamide, 20 × SSC, 10% dextran sulfate, 0.3 mg ml⁻¹ of sheared salmon testes DNA, and 60 ng of labeled genomic DNA, plus an excess amount of unlabeled genomic DNA of CS, which was sheared to 600–700 bp to block cross-hybridization of probed DNA to wheat chromosomes. The probe-to-blocker ratio was between 1:110 and 1:120. For the wild parent *Th. intermedium*, the genomic DNA from *Ps. spicata* was used as a probe and no blocking DNA was added to the hybridization mixture. The hybridization solutions were denatured by boiling and chilling on ice for about 7 min. Thirty μl of denatured hybridization mixture were applied to each slide and covered with a 20 × 20 mm plastic cover slip. Hybridization was conducted in a humid chamber overnight at 37°C. After hybridization, slides were washed in 2 × SSC at 42°C for 10 min, 50% formamide in 2 × SSC at 42°C for 10 min (equivalent to 82% stringency), 2 × SSC at 42°C for 10 min, and 1 × PBS at room temperature for 5 min. The biotin-labeled probes were detected with a FITC-conjugated ant-biotin antibody (Vector). Chromosomes were counterstained with propidium iodide (PI) and fluoresced red. Slides after GISH were examined by using an epifluorescent microscope, and fluorescent images captured using a SPOT 2.1 charge-coupled device (CCD) camera (Diagnostic Instruments) attached to an epifluorescence Zeiss Axiosplan 2 microscope.

**Disease resistance evaluation**

The partial amphiploid line TAI8335 and its parents, as well as other known *Thinopyrum*-derived partial amphiploids and susceptible checks (Table 3), were screened for...
their reactions to leaf rust and stem rust at Kansas State University (KSU), and stripe rust and powdery mildew evaluations were performed in China.

Screening for rust reactions
Six plants of each line tested were inoculated at the two-leaf seedling stage with seven isolates, including MCDL, PRTUS 25 and PRTUS 35 of _Puccinia triticina_ (for virulence/avirulence formulae see Long et al. 2000), TPMK, RKQQ and QTHJ of _P. graminis_ f. sp. _tritici_ (Pgt) (for virulence/avirulence formulae; Y. Jin and L. Wanschura 2009, Cereal Disease Laboratory) and PST-100 (virulent on Lehmi, Heines VII, Produra, Yanhill, Stephens, Lee, Fielder, Express, Yr8-AVS, Yr9-AVS, Clements and Compair) of _P. striiformis_ f. sp. _tritici_ (Pst), which caused widespread stripe rust epidemics in the US from 2000 to 2005 (Chen and Penman 2006).

Urediospores for each race suspended in Soltrol-170 mineral oil (Chevron-Phillips chemical company) were atomized onto the plants. For leaf rust and stem rust tests, inoculated seedlings and adult plants were incubated in a dew chamber for 18 h at 18°C. Plants were then placed in a greenhouse at 19–21°C with supplemental sodium vapor lighting. For the stripe rust test, inoculated seedlings, inoculated seedlings and adult plants were kept in a dark dew chamber for 24 h at 12 ± 2°C. After inoculation, plants were kept in growth chambers that were set at 16°C (day) and 14°C (night) with a 16-h photoperiod. The infection types (ITs) of leaf rust and stem rust were scored 10–12 days after inoculation. For stripe rust, the IT scoring was done 20 days after inoculation. The rust reaction was rated at seedling and adult stages using the 0–4 scale as illustrated in Roelfs et al. (1992) and McIntosh et al. (1995).

Screening for stripe rust and powdery mildew reaction
Testing for resistance to stripe rust and powdery mildew was conducted, respectively, at the University of Electronic Science and Technology of China and the Shanxi Institute of Crop Genetics, China. Wheat cultivars Mingxian 169 and Jingshuang 16 were used as susceptible checks for the stripe rust and powdery mildew tests, respectively. Three pathotypes (CYR29, CYR31 and CYR32) of _Pst_ were used for screening the stripe rust response. CYR32 had all the virulence factors of CYR31, and was virulent on all differential cultivars, except Zhong 4, _Triticum spelta album_ (Yr5), and Moro (Yr10 and YrMor) (Wan et al. 2004). One-leaf-stage plants were sprayed with distilled water containing 0.05% Tween-20 and brushed with spores of stripe rust races increased on Mingxian 169 plants. Following inoculation, plants was incubated at 10°C and 70% RH in the dark for 24 h. Then, epidemics were induced at 16/11°C day/night with a 14/10 h light/dark photoperiod at 70% RH. ITs of seedlings were scored 14 days after inoculation, using the scale described by McIntosh et al. (1995) with some modifications: 0 = no visible infection; 1 = necrotic/chlorotic flecks, without sporulation; 2 = intermediate sporulation, necrotic/ chlorotic stripes; 3 = abundant large sporulation, necrotic/chlorotic stripes; 4 = abundant large sporulation, without chlorosis. For the seedling test with powdery mildew, three _B. graminis_ f. sp. _tritici_ (Bgt) isolates (E09, E20 and E21) (for virulence/avirulence formulae see Hua et al. 2009) were used. The conidia were dusted onto one-leaf stage plants. Sporulation was promoted at 17°C with a 14/10 h light/dark photoperiod and 60% relative humidity (RH). Host reactions were recorded 7 to 10 days after inoculation, when the susceptible checks were heavily infected. A 0 to 4 IT scale (Sheng 1988) was used to describe host responses to infection. Scores of 0–2 were regarded as resistant and 3–4 as susceptible.

RESULTS

**Genome composition of alien parent Th. intermedium**

The mitotic preparations of the _Th. intermedium_ plant crossed with common wheat were subjected to GISH analysis in this study to determine the genomic composition of the alien parent. Using the S genomic DNA from _Ps. spicata_ as a probe and without a blocker, the 42 chromosomes all fluoresced greenish-yellow either over their entire length or at their centromeric and/or terminal region(s) (Fig. 1). Among them, seven small chromosome pairs were labeled bright greenish-yellow uniformly all along their length, while the remaining fluoresced mostly red, indicating that _Th. intermedium_ had an entire S genome set. Of the remaining 28 chromosomes, seven were hybridized with the probe at the

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**Fig. 1.** GISH pattern of _Th. intermedium_ probed with genomic _Pseudoroegneria spicata_ DNA, which contained seven pairs of S, seven J and 21 J chromosomes.
Table 1. Chromosome number and genomic constitution in 23 plants of TAI8335.

| Plant no. | No. of wheat chromosomes | No. of Th. intermedium chromosomes | S-J translocation | Plant | No. of cells scored | 2n | 28II (%) | I | II | III | IV | Ring | Rod | Total |
|-----------|--------------------------|-----------------------------------|------------------|-------|---------------------|----|--------|----|----|----|----|------|-----|------|
| 2         | 42                       | 2                                 | 6                | 2    | 4                   | 17 | 73.91  |    |    |    |    |      |     |      |
| 55 + 2 telo | 41                     | 2                                 | 6                | 2 + 2 telo | 4   | 1   | 4.35 |
| 55        | 41                       | 2                                 | 6                | 2    | 4                   | 1  | 4.35  |    |    |    |    |      |     |      |
| 54 + 3 telo | 40 + 1 telo               | 2                                 | 6                | 2 + 2 telo | 4   | 1   | 4.35 |
| 54 + 2 telo | 40                     | 2                                 | 6                | 2 + 2 telo | 4   | 1   | 4.35 |
| 54        | 40                       | 2                                 | 6                | 2    | 4                   | 2  | 8.70  |    |    |    |    |      |     |      |

Note: telo-telocentric chromosome.

Table 2. Chromosome pairing at metaphase I in five 56-chromosome plants of TAI8335.

| Plant no. | No. of cells scored | 2n | 28II (%) | I | II | III | IV | Ring | Rod | Total |
|-----------|---------------------|----|----------|---|----|-----|----|------|-----|------|
| 2         | 42                  | 71.43 | 0.81 | 24.16 | 3.08 | 27.24 | 0.14 | 0.07 |
| 4         | 48                  | 75.00 | 1.13 | 24.12 | 2.84 | 26.96 | 0.13 | 0.15 |
| 7         | 46                  | 76.09 | 1.11 | 23.86 | 3.36 | 27.22 | 0.15 |      |
| 13        | 42                  | 73.81 | 0.90 | 23.85 | 3.17 | 27.02 | 0.10 | 0.19 |
| 18        | 45                  | 73.33 | 0.93 | 24.51 | 2.43 | 27.16 | 0.13 | 0.09 |
| Mean      |                     | 73.93 | 0.98 | 24.12 | 2.98 | 27.12 | 0.13 | 0.10 |
Thinopyrum intermedium-derived partial amphiploids Zhong 2, Zhong 4, TA17044, TAF46, OT and 78829 were also tested with the above rust races and powdery mildew isolates. The testing for powdery mildew showed all these known partial amphiploids were highly susceptible to all powdery mildew isolates (Table 3). In the rust screening, reactions of Zhong 2, Zhong 4 and 78829 were immune or highly resistant to all races of leaf, stem and stripe rust. OT was resistant to leaf rust and stem rust and PST100, but susceptible to CYR32 of stripe rust, which is the most widely virulent pathotype in China and virulent to Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr22, Yr23, Yr27, YrHVII, YrHK, YrCle, YrA, YrCV1, YrCV2, YrCV3, YrG, YrSD, YrSO (Wan et al. 2004). TAF46 was immune or resistant to all other rust races tested, but susceptible to Pgt race TPMK, a predominant race in all areas of the United States during the 1990s with the virulence to Sr5, Sr7b, Sr8a, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr36 and SrTmp (Olivera et al. 2007). TA17044 was immune to leaf and Pst race PST100, but susceptible to stem rust and CYR32 (Table 3).

**DISCUSSION**

Knowledge of the chromosome constitution of a donor parent is essential for elucidating the genome origin of...
Table 3. Seedling reactions of Th. intermedium, partial amphiploids and wheat cultivars to various races of three rusts and powdery mildew.

| Line          | $2n =$ | Genomic formula | Leaf rust | Stem rust | Stripe rust | Powdery mildew |
|---------------|--------|-----------------|-----------|-----------|-------------|----------------|
| Th. intermedium | 42     | SJp             | 0         | 0         | 0           | 0              |
| TAI8335       | 56     | $W^b + S + J^a + J$ | 0         | 0         | 0, 0, 0     | 0              |
| TAI7044       | 56     | $W + S + J$     | 0, 0      | 3         | 3, 3        | 0, 0           |
| Zhong 2       | 56     | $W + S + J^a + J$ | 1         | 0, 0      | 0, 0, 1     | 1, 3           |
| Zhong 4       | 56     | $W + S + J^a$   | 0, 0      | 0, 0      | 1           | 0              |
| 78829         | 56     | $W + S$         | 0, 0      | 1         | 0           | 0              |
| TAF46         | 56     | $W + S + J$     | 0, 0      | 2, 3, 1   | 0, 0, 1     | 1, 1           |
| OT            | 56     | $W + S + J$     | 0         | 2         | 1           | 2              |
| Jinchun 5     | 42     | ABDc            | 4         | 3         | 3, 4        | 3              |
| Jinnai 33     | 42     | ABD             | 3         | 3         | 4           | 4              |
| Wichita       | 42     | ABD             | 4         | 4         | 4           | 4              |
| Mingxian 169  | 42     | ABD             | NT        | NT        | NT          | NT             |
| Jingshuang 16 | 42     | ABD             | NT        | NT        | NT          | NT             |

aSeedling infection type (iT) for reactions to leaf rust, stem rust, stripe rust and powdery mildew, follow the 0 to 4 scale described by McIntosh et al. (1995) and Roelfs et al 1992, and Sheng (1988), respectively. The seedling iTs are 0 = no visible symptoms; 0 = hypersensitive flecks or necrotic areas without sporulation; 1 = necrotic and chlorotic areas with restricted sporulation; 2 = moderate sporulation with necrosis and chlorosis; 3 = sporulation with chlorosis; 4 = abundant sporulation without chlorosis; $+/- = uredinia somewhat larger/smaller than normal for the iT. Scores of 0–2 are classified as resistant and 3–4 as susceptible reactions.

bwheat chromosomes
cwheat genome.
dnT: not tested with the corresponding pathotype.
alien chromosomes in intergeneric hybrids and their derivatives. *Thinopyrum intermedium* is a segmental autoallo-hexaploid. Identification of the exact genomic composition of this species has been difficult with traditional cytogenetic techniques, due to chromosome pairing complexity and a high level of polymorphism in chromosome variation. GISH has provided powerful tools for the direct characterization of the chromosome composition of polyploid plant genomes at the DNA level. Using this method, CHEN et al. (1998) found that *Th. intermedium* had 13–14 S, 6–11 J, and 17–21 J chromosomes in different accessions. The alien parent in this study had an unknown origin and its genome was composed of 14 S, 7 J and 21 J chromosomes, as revealed by probing with the genomic DNA of *Ps. spicata*, which shares the common S genome with *Ps. strigosa* (YANG et al. 2006). This variation in chromosome composition of different *Th. intermedium* accessions might be attributed to the outcrossing nature of this species.

In previous reports, the chromosomes of the J genome had FITC signals only or mainly in the centromeric areas when blocked with J/E genomic DNA from *Th. bessarabicum* and *Th. elongatum* (CHEN et al. 1998; CHEN 2005), while in our study there were signals both around the centromere and at the telomere(s) in the absence of blocking DNA (Fig. 1). This difference in GISH banding patterns of J chromosomes seems attributable to the polymorphism of S genome chromatin (repetitive sequences of S genome) at the terminal regions present among the different accesses and/or the absence of blocking DNA. Being an outpollinated perennial species, the chromosome constitution of *Th. intermedium* varies greatly among and within accesses (XU and CONNER 1994). In our experiment, seven single J-genome chromosomes and eight pairs plus five single J-genome chromosomes were observed in most plantlets (Fig. 1), a difference with the alien parent of the Zhong series and 78829, which had three pairs of J and 11 pairs of J chromosomes (TANG et al. 2000).

Evaluation of the cytogenetic stability of intergeneric hybrids is of theoretical and practical significance. *Thinopyrum*-derived partial amphidiploids normally have regular meiosis with high frequencies of bivalent and low multivalent formation (FEDAK et al. 2000). Of the 223 cells with 2n = 56 analyzed here, about 74% of the cells formed the expected 28 bivalents at MI, and only 0.98 unpaired chromosomes occurred per cell. The frequency of univalents was much lower than that reported for TAF46 (2.55), but a little higher than Otrastayuskaya 38 (0.74), both stable partial amphidiploids derived from *Th. intermedium* (FEDAK et al. 2000). This indicated that the present amphiploid had a basic stability in cytology. Meanwhile, this relatively regular meiotic behavior was reflected in the common composition of alien chromosomes among plants and a vigorous growth habit with high fertility. Consequently, the genome of TAI8335 should be largely balanced in terms of homoeologous chromosomes (FEDAK and HAN 2005).

Partial amphiploids play an important role in transferring useful traits present in alien species. It is essential, therefore, to know the exact genomic composition of the added alien chromosomes in the partial amphiploids. GISH, especially using an S genomic DNA probe, provides a powerful diagnostic cytogenetic tool for determining the genomic origin of alien chromosomes in wheat-*Thinopyrum* hybrids, which permits distinction of S, J, and J genomes (CHEN et al. 1998; CHEN 2005). The present GISH pattern revealed that TAI8335 contained a synthetic alien genome composed of two S-genome, six J-genome, two J-genome, and four translocated chromosomes, including one Robertsonian translocation pair and one intercalary translocation pair that were both derived from the J and S genomes (Fig. 2). TAI8335 had a different chromosome composition from those of previously described partial amphiploids Zhong 1 to Zhong 5 (CHEN et al. 2003), TAF46 (CHEN et al. 1999), 78829 (ZHIANG et al. 1996), OT (FEDAK et al. 2000), TAI7045 (CHANG et al. 2001) and TAI7044 (CHANG et al. 2003). Thus, TAI8335 is a novel wheat-*Th. intermedium* partial amphiploid.

Different studies have indicated that the maximum number of J-genome chromosomes ranges from 6 to 11 in different accessions of *Th. intermedium*, suggesting that the J genome may be incomplete within the *Th. intermedium* species (CHEN et al. 1998). *Th. intermedium*-derived partial amphiploids mostly had J-genome chromosomes and interchanges involving J genome in their alien genome. Among them, Zhong 1 to Zhong 5 and TAI7045 all contained four intact J-genome chromosomes (CHEN et al. 2003; CHANG et al. 2001). The present study showed that TAI8335 had six intact chromosomes from the J genome (Fig. 2), whereas only a total of seven J chromosomes were present in its alien parent (Fig. 1). This may be attributed to the preferential transmission of some chromosomes of the J genome to progeny in the crosses of wheat with *Th. intermedium*. Additionally, chromosomes with translocations involving different *Th. intermedium* genomes or between *Th. intermedium* and wheat genomes were reported in several known partial amphiploids, and recent studies have shown that these translocations usually involved S- and J-genome chromosomes (CHEN 2005; FEDAK and HAN 2005). The present GISH results showed that in TAI8335, the genetic exchange of chromosomes from different genomes, either in Robertsonian or intercalary translocation, occurred only between the S- and J-genome chromosomes of *Th. intermedium* (Fig. 2), demonstrating that in this accession, the relationship between S and J genome appears to be closer than with the J genome.

As an important perennial Triticeae species, *Th. intermedium* has frequently been used in wheat improvement
as a donor of various disease resistance genes, in particular for those which to a large extent are lacking in wheat, such as wheat streak mosaic virus (WSMV), its vector the wheat curl mite (WCM) (Aceria tosichella Keifer), barley yellow dwarf virus (BYDV) and Fusarium head blight (FRIEBE et al. 1996; CHEN 2005; FEDAK and HAN 2005). To date, one gene conferring resistance to WSMV, Wsm1 (FRIEBE et al. 1991), and at least two genes specifying resistance to BYDV, Bdv2 (ZHANG et al. 1999) and Bdv3 (OHM et al. 2005), have been identified in Thinopyrum intermedium. These genes were located on the arm of the J- or J-genome chromosome of this species by C-banding, GISH and/or molecular marker analyses (ZHANG et al. 1999; FRIEBE et al. 2009; KONG et al. 2009).

Resistance to rust also was transferred from Thinopyrum intermedium to wheat. FRIEBE et al. (1992) reported that the leaf rust resistance gene Lr38 was located in the distal half of the long arm of the chromosome 7Ai-2, a chromosome from the J genome (TANG et al. 2000), whereas the genes for resistance to stem rust and stripe rust were located either in the short arm or in the proximal region of the long arm of this chromosome. The stem rust resistance gene Sr44 was localized on the short arm of chromosome 7Ai-1(J) (FRIEBE et al. 1996). More recently, two novel powdery mildew resistance genes derived from Thinopyrum intermedium, Pm40 and Pm43, have been characterized and introgressed into wheat (HE et al. 2009; LIO et al. 2009). He et al. (2009) pointed out that Pm43 originated from a J genome chromosome of a wheat-Th. intermedium partial amphiploid. Based on these results, the genes for resistance to WSMV, BYDV, rusts, and powdery mildew are not located on chromosomes of the S-genome but on those of the J- or J-genomes. In the present study, not only six J- and two J-genome chromosomes, but also four translocated chromosomes involving the J genome were identified in TAI8335, suggesting that the J- or J-genome chromosomes appear to be associated with resistance to leaf, stem and stripe rust and powdery mildew in TAI8335. The present data will aid in the transfer of resistance genes for the rusts and powdery mildew from Thinopyrum intermedium into wheat. An attempt has been made to isolate the resistant addition and substitution lines. Progenies from the crosses of TAI8335 with wheat are being screened for rust and powdery mildew resistance and the presence of Thinopyrum intermedium chromosomes.

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