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Genomic in situ hybridization (GISH) analyses of *Thinopyrum intermedium*, its partial amphiploid Zhong 5, and disease-resistant derivatives in wheat

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Abstract Genomic in situ hybridization (GISH) to root-tip cells at mitotic metaphase, using genomic DNA probes from *Thinopyrum intermedium* and *Pseudoroegneria strigosa*, was used to examine the genomic constitution of *Th. intermedium*, the 56-chromosome partial amphiploid to wheat called Zhong 5 and disease-resistant derivatives of Zhong 5, in a wheat background. Evidence from GISH indicated that *Th. intermedium* contained seven pairs of St, seven J^S and 21 J chromosomes; three pairs of *Th. intermedium* chromosomes with satellites in their short arms belonging to the St, J, J genomes and homoeologous groups 1, 1, and 5 respectively. GISH results using different materials and different probes showed that seven pairs of added *Th. intermedium* chromosomes in Zhong 5 included three pairs of St chromosomes, two pairs of J^S chromosomes and two pairs of St-J^S reciprocal tanslocation chromosomes. A pair of chromosomes, which substituted a pair of wheat chromosomes in Yi 4212 and in HG 295 and was added to 21 pairs of wheat chromosomes in the disomic additions Z1, Z2 and Z6, conferred BYDV-resistance and was identical to a pair of St-J^S tanslocation chromosomes (StJ^S) in Zhong 5. The StJ^S chromosome had a special GISH signal pattern and could be easily distinguished from other added chromosomes in Zhong 5; it has not yet been possible to locate the BYDV-resistant gene(s) of this translocated chromosome either in the St chromosome portion belonging to homoeologous group 2 or in the J^S chromosome portion whose homoeologous group relationship is still uncertain. Among 22 chromosome pairs in disomic addition line Z3, the added chromosome pair had satellites and belonged to the St genome and homoeologous group 1. Disomic addition line Z4 carried a pair of added chromosomes which was composed of a group-7 J^S chromosome translocated with a wheat chromosome; this chromosome was different to 7 Ai-1, but was identical to 7 Ai-2. The leaf rust and stem rust resistance genes were located in the distal region of the long arm, whereas the stripe rust resistance gene(s) was located in the short arm or in the proximal region of the long arm of 7 Ai-2. A pair of J^S-wheat translocation chromosomes, which originated from the WJ^S chromosomes in Z4, was added to the disomic addition line Z5; the added chromosomes of Z5 carried leaf and stem rust resistance but not stripe rust resistance; Z5 is a potentially useful source for rust resistance genes in wheat breeding and for cloning these novel rust-resistant genes. GISH analysis using the St genome as a probe has proved advantageous in identifying alien *Th. intermedium* in wheat.

Key words *Thinopyrum intermedium* · Zhong 5 · Addition, substitution and translocation lines · Disease resistance · GISH · Genomic constitution

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Introduction

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey [=*Elytrigia intermedium* (Host) Nevski=*Agropyron intermedium* (Host) Beauvoir] is a perennial auto-allo-hexaploid species (2n=6*x*=42, previously designated with genomes $E_1E_1E_2E_2XX$) that is a potentially useful source of a number of genes for wheat improvement, including barley yellow dwarf virus (BYDV) resistance (Cauderon et al. 1973; Sharma et al. 1984, 1989; Shukle et al. 1987; Brettell et al. 1988; Xin et al. 1988; Banks et al. 1993; Larkin et al. 1995), rust resistance (Wienhues 1966, 1973; Knott 1968, 1989; Cauderon et al. 1973; Friebe et al. 1992 b, 1993; Banks et al. 1993; Larkin et

al. 1995), wheat streak mosaic virus (WSMV) and wheat curl mite resistances (McKinney and Sando 1951; Lay et al. 1971; Wells et al. 1973; Sharm et al. 1984; Stoddard et al. 1987; Chen et al. 1998a, c), common bunt and powdery mildew resistances (Sinigovets 1973, 1976; Franke et al. 1992), low-temperature and drought tolerance (Fedak 1985; Schulz-Schaeffer and Haller 1987), salt tolerance (Dewey 1960, 1984; McGuire and Dvorak 1981; Littlejohn 1988), high protein, an increased number of florets per spike, and a perennial habit (Pienaar 1990). Because of its high crossability with common wheat, a number of useful genes have been transferred from this species to common wheat, which has led to the development of many useful wheat gemplasms and cultivars, including partial amphiploids and addition, substitution, and translocation lines (Gupta 1972; Cauderon et al. 1973; Sharma and Gill 1983; Pienaar, 1990).

Cauderon (1966) and Cauderon et al. (1973) developed a partial amphiploid, designated TAF46 (2n=8*x*=56), containing seven chromosome pairs from *Th. intermedium* added to the full chromosome complement of *Triticum aestivum* (2n=42, AABBDD). TAF46 was used to produce six *T. aestivum–Th. intermedium* disomic chromosome addition lines named L1, L2, L3, L4, L5 and L7 respectively. The homoeologous relationships and genomic origins of all the added *Th. intermedium* chromosomes have been analysed using morphological traits, isozyme and protein markers, karyotype and C-banding, and in situ hybridization (Figueiras et al. 1986; Forster et al. 1987; Friebe et al. 1992a).

Zhong 5, a *T. aestivum–Th. intermedium* partial amphiploid (2n=56) produced by Chinese researchers (Chi et al. 1979; Li and Sun 1981; Sun 1981), contains seven chromosome pairs from *Th. intermedium* added to the full chromosome complement of *T. aestivum* and has resistance to BYDV (Xin et al. 1988), as well as leaf, stem, and stripe rusts (Banks et al. 1993). From crosses between common wheat and Zhong 5, Larkin et al. (1995) developed a series of disomic addition lines (2n=44), named Z1, Z2, Z3, Z4, Z5 and Z6 respectively, which have resistances to BYDV and (or) rusts. Some inferences have been made concerning the homoeologous relationships of the added *Th. intermedium* chromosomes in these lines (except Z5) on the basis of morphological traits, isozyme and protein markers, and restriction fragment length polymorphisms (RFLPs). From crosses between common wheat and Zhong 5, we obtained two BYDV-resistant lines (2n=42) named Yi 4212 and HG 295 respectively; biochemical and cytological analyses indicated that a pair of *Th. intermedium* chromosomes substituted a pair of wheat chromosomes (Nie et al. 1994; Ai et al. 1997). In the present paper, genomic in situ hybridization (GISH) was used to characterize the genomic origins of the added *Th. intermedium* chromosomes in Zhong 5 and in its disease-resistant derivatives.

Materials and methods

Plant materials

(1) Zhong 5: a *T. aestivum–Th. intermedium* partial amphiploid (2n=56) originated from the Heilongjiang Academy of Agricultural Sciences, China (Chi et al. 1979; Li and Sun 1981; Sun 1981). (2) Yi 4212 and HG 295: two disomic substitution lines (2n=42), selected from crosses between common wheat and Zhong 5, have resistance to BYDV (Nie et al. 1994; Ai et al. 1997). (3) Z1, Z2, Z3, Z4, Z5 and Z6: six disomic addition lines (2n=44), selected from crosses between common wheat and Zhong 5. Z1, Z2 and Z6, contain a pair of added *Th. intermedium* chromosomes belonging to homoeologous group 2, and are resistant to BYDV but susceptible to rusts; Z3 has neither BYDV nor rust resistances, and the added *Th. intermedium* chromosomes are homoeologous to group 1; Z4 contains a pair of added *Th. intermedium* chromosomes homoeologous to group 7, and is resistant to leaf, stem and stripe rusts but susceptible to BYDV; Z5 is resistant to leaf and stem rusts but susceptible to stripe rust and BYDV, and its homoeology remains unknown (Larkin et al. 1995). All the wheat parents in (1), (2), and (3) were susceptible to the above-mentioned wheat diseases. (4) Total genomic DNA from *T. aestivum* cv "Chinese Spring", *Th. intermedium*, *Th. elongatum* (Host) D.R. Dewey (2n=14, EE), and *Pseudoroegneria strigosa* (M. Bieb) A. Love (2n=14, StSt) was used as probes or blockers for GISH.

Genomic in situ hybridization (GISH)

Total genomic DNA was isolated from the plant materials by the method of Saghai-Maroof et al. (1984) and labelled with biotin-16-dUTP by the Nick Translation Kit (Boehringer Mannheim Company, Germany). The details and protocols for slide preparation and GISH were as described by Mukai and Gill (1991) and Friebe et al. (1993) with minor modifications. About 10 µl of hybridization mixture was applied to each slide. The hybridization mixture contained 12.5 ng of probe DNA, 500 ng of sheared blocking DNA, 10 µg of sheared salmon sperm DNA, 50% formamide, 2×SSC, and 10% dextran sulphate; 50 µl of rabbit anti-biotin antibody (1:100 dilution) and 50 µl of fluorochrome, FITC-conjugated sheep anti-rabbit antibody (1:100 dilution) were used to detect and visualize labelled chromosomes; 8 µl anti-fade solution containing 1 µg/ml of propidium iodide (PI) was added to counterstain unlabelled chromosomes. All major experimental reagents came from Boehringer Mannheim, Germany. Fluorescence was viewed using an Olympus microscope equipped with a fluorescence attachment (the excitation wavelength for FITC and PI was 450–490 nm). The labelled chromosomes were greenish-yellow (or yellow) in color (the merged color of excited FITC and PI), whereas the unlabelled chromosomes were red in color (the fluorescence color of excited PI). GISH patterns were photographed with Kodak 400 films.

Results

GISH analysis of *Th. intermedium*

Based on the GISH pattern of *Th. intermedium* mitotic metaphase chromosomes using *Ps. strigosa* genomic DNA (St genome) as a probe and *Th. elongatum* genomic DNA (E genome, which is closely related to the J genome from *Thinopyrum bessarabicum*) as a blocker, the *Th. intermedium* chromosomes can be organised into three groups, as follows: (A) seven pairs of small chromosomes were labelled bright greenish-yellow uniformly along all their length, while the remainder fluoresced

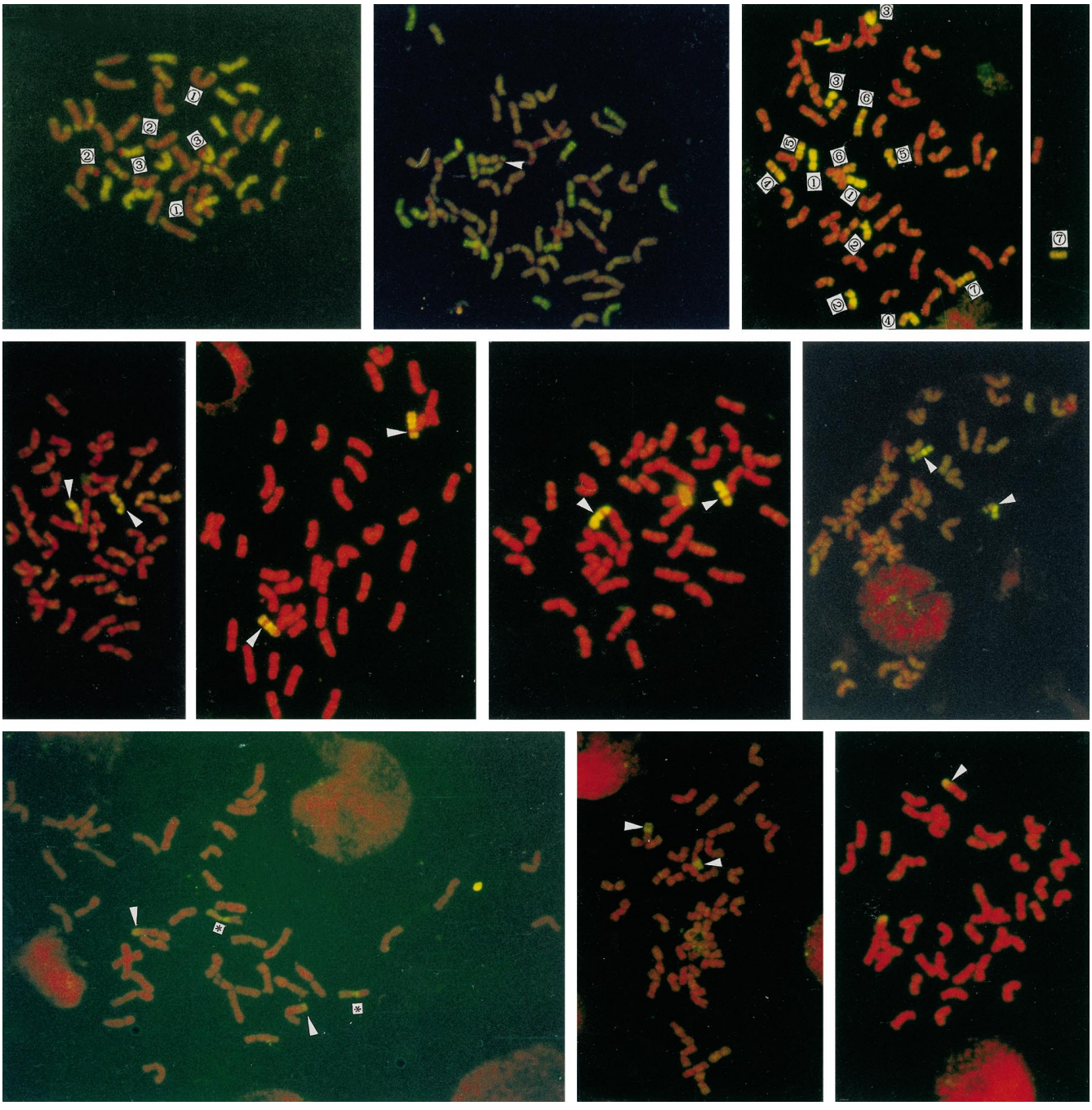

Fig. 1a–j GISH patterns of root-tip cells at mitotic metaphase. **a**, **c**, **e**, **f**, **g**, **h**, and **j** were probed with *Ps. strigosa* genomic DNA, and were blocked with *Th. elongatum* and "Chinese Spring" genomic DNA; **b**, **d**, and **i** were probed with genomic DNA of *Th. intermedium*, and were blocked with genomic DNA of "Chinese Spring". **a** GISH pattern of *Th. intermedium*, which contained seven pairs of St, seven J^S and 21 J chromosomes; ①, ②, and ③ indicate SAT1, SAT2, and SAT3 respectively. **b** GISH pattern of Zhong 5, seven pairs of intact *Th. intermedium* chromosomes were added to Zhong 5, *arrows* indicate a pair of satellited *Th. intermedium* chromosomes. **c** GISH pattern of Zhong 5, seven pairs of added *Th. intermedium* chromosomes in Zhong 5 included three pairs of St chromosomes, two pairs of J^S chromosomes and two pairs of St-J^S reciprocal tanslocation chromosomes. ①, ②, and ③ represent three pairs of intact St chromosomes St1, St2, and St3; ④, ⑤ indicate two pairs of St-J^S reciprocal translocation chromosomes J^SSt, StJ^S; ⑥, ⑦ represent two pairs of intact J^S chromosomes J^S1, J^S2. **d** GISH pattern of Yi 4212, *arrows* indicate a pair of *Th. intermedium* chromosomes which substitute a pair of wheat chromosomes. **e** GISH pattern of Yi 4212, *arrows* indicate that the *Th. intermedium* chromosomes in Yi 4212 were derived from StJ^S chromosomes in Zhong 5. **f** GISH pattern of Z2, *arrows* indicate that a pair of added *Th. intermedium* chromosomes in Z2 were derived from StJ^S chromosomes in Zhong 5. **g** GISH pattern of Z3, a pair of added chromosomes in Z3 were derived from St2 in Zhong 5; *arrows* indicate that the satellite chromosomes belong to the St genome. **h** GISH pattern of Z4, a pair of added J^S chromosomes were translocated with a pair of wheat chromosomes in Z4. *Arrows* indicate a pair of WJ^S chromosomes; *asterisks* indicate a pair of J^SW chromosomes. **i** GISH pattern of Z5, *arrows* indicate a pair of added wheat–*Th. intermedium* translocation chromosomes in Z5. **j** GISH pattern of Z5, the added wheat–*Th. intermedium* translocation chromosomes in Z5 were derived from WJ^S chromosomes in Z4; *arrows* indicate the WJ^S chromosomes

mostly red, indicating that *Th. intermedium* had an St genome set, the other chromosomes comprising two sets of E genomes (Fig. 1a); (B) seven chromosomes of the 28 E-type chromosomes were light greenish-yellow in centromeric and terminal regions, although the signals around the centromeres were a little stronger than that at the telomeres; (C) 21 chromosomes of the 28 E-type chromosomes showed red fluorescence over most of their length except for the terminal regions which were light greenish-yellow (Fig. 1a).

Chen et al. (1998b) proposed the symbol J^S to represent the E (or J) chromosomes with St repeated sequences and GISH signals around the centromeric regions, and the symbol J to represent the E (or J) chromosomes with GISH signals at the terminal regions. From this point on we will adopt Chen's nomenclature, and assign chromosomes in group (B) to the J^S genome, and chromosomes in group (C) to the J genome. Thus, the genome constitution of *Th. intermedium* can be formulated as seven pairs of St plus 7 J^S+21 J chromosomes. Moreover, we can identify three pairs of chromosomes with satellites in their short arms. The longest satellited chromosome had a slight greenish-yellow label at the telomere of the long arm and at the inner border of the satellite in the short arm, and belonged to the J genome. The next-longest satellited chromosome also belonged to the J genome; however, the slight greenish-yellow flourescence in the terminal region of the short arm was close to the outer border of the satellite, and the signal at the terminal region of the long arm in this chromosome was weaker than that in the longest satellited chromosome (Fig. 1a). We named the two satellited J chromosomes SAT1 and SAT2 respectively. The shortest satellited chromosome was entirely labelled with St genomic DNA and belonged to the St genome, and we named it SAT3 (Fig. 1a). Thus, it is easy to distinguish the three satellited chromosomes by GISH analysis using genomic DNA of *Ps. strigosa* as a probe. One surprising aspect of this GISH analysis is that all the satellited regions in these chromosomes (including SAT3) had no hybridization signal with the St probe. The satellites regions, which are rich in the tandem repeated sequence of rDNA and show bright-red PI fluorescence, are easy to distinguish from other unlabelled dark-red regions.

GISH analysis of Zhong 5

GISH, using *Th. intermedium* total genomic DNA (J, J^S and St genomes) as a probe and "Chinese Spring" total genomic DNA (A, B, and D genomes) as a blocker, showed that, among the 56 chromosomes in Zhong 5 root tip-cells at mitotic metaphase, there were seven pairs of chromosomes entirely presenting strong greenish-yellow hybridization signals (Fig. 1b). This indicated that Zhong 5 had seven pairs of intact *Th. intermedium* chromosomes, and carried no wheat–*Th. intermedium* translocation chromosomes. Among the seven pairs of labelled chromosomes, one had a pair of satellites in their short arms (arrows in Fig. 1b). Sometimes, these satellites can not be observed if the mitotic metaphase is not ideal. When Zhong 5 was probed with the *Ps. strigosa* genome and was blocked with *Th. elongatum* and "Chinese Spring" genomes, the seven pairs of added *Th. intermedium* chromosomes can still be clearly distinguished from the 42 wheat chromosomes. However, different chromosome pairs showed great differences in signal strength and distribution:

(1) Three pairs were labelled bright-yellow uniformly along all their length, indicating there were three pairs of intact *Th. intermedium* chromosomes belonging to the St genome. We designated the longest chromosome St1, the next longest one with a small and not very clear satellite St2, and the shortest one St3 (Fig. 1c).
(2) Two pairs of chromosomes showed light-yellow signals in centromeric and telomeric regions, indicating that there were two pairs of intact J^S chromosomes. We named the longest J^S chromosome as J^S1 (it was also the longest of all the added chromosomes); the shorter one was named J^S2 (Fig. 1c).
(3) In the remaining two pairs of chromosomes, the two shorter chromosomes were labelled bright-yellow along most of their length, although the signals at the terminal regions were a little weaker. However, the middle region of the long arm was unlabelled and showed red, which separated the short arm and centromeric region (with a strong signal) from the telomeric region of the long arm (with a weak signal). The unlabelled region equals about 30% of the length of the long arm if the chromosome is in a good state of extension; sometimes, this chromosome can be mistaken for an intact St chromosome if it is too condensed. In the GISH pattern of the two longer chromosomes, only the distal 60% of the long arm was labelled bright-yellow, the other part of the chromosome was mostly red except for the centromere and the terminal region of the short arm, which where light-yellow. There was also a narrow unlabelled region between the distal 60% of the bright-yellow long arm and the light-yellow centromeric region (④, ⑤ in Fig. 1c). This chromosome is easily mistaken for a wheat–*Th. intermedium* translocation chromosome if the weak signals around the centromere and at the terminal region of the short arm disappear because of excessive washing or a high blocker-to-probe ratio. The GISH pattern of *Th. intermedium* showed that no chromosomes had the same hybridization signals as these two pairs of chromosomes (Fig. 1a); moreover, the GISH pattern of Zhong 5 using *Th. intermedium* as a probe indicated that no translocation was observed between wheat chromosomes and the seven pairs of added chromosomes (Fig. 1b). All these observations indicated that a St chromosome and a J^S chromosome had exchanged their distal long arms, resulting in the characteristic hybridization signal pattern on these two pairs of chromosomes in Zhong 5. We named the shorter chromosome StJ^S, indicating that it contained a large part of the St chromosome with a strong signal, and a smaller part from the J^S chromosome with a weak

GISH signal in the terminal region. The longer one was designated $J^{S}St$, indicating that it contained a large part of the J^{S} chromosome and a St chromosome segment (Fig. 1c). In conclusion, the genome composition of added *Th. intermedium* in Zhong 5 included three pairs of St chromosomes, two pairs of J^{S} chromosomes, and two pairs of St–J^{S} reciprocal translocation chromosomes. Regardless of the translocation, 8 of 14 St and 6 of 7 J^{S} chromosomes in *Th. intermedium* were represented in Zhong 5, indicating that Zhong 5 has a synthetic genome set derived from *Th. intermedium.*

GISH analyses of Yi 4212, HG 295, Z1, Z2, and Z6

Yi 4212 gave the same GISH results as HG 295 in analyses using either *Th. intermedium* or *Ps. strigosa* genomic DNA as a probe. When mitotic chromosomes of Yi 4212 and HG 295 (both 2n=42) were probed with *Th. intermedium* genomic DNA and were blocked with "Chinese Spring" total genomic DNA, a pair of chromosomes was entirely labelled with strong greenish-yellow signals (Fig. 1d). This indicated that a pair of *Th. intermedium* chromosomes substituted for a pair of wheat chromosomes, and no wheat–*Th. intermedium* translocation was observed in Yi 4212 and HG295. In GISH analysis using *Ps. strigosa* DNA as a probe and *Th. elongatum* and "Chinese Spring" DNA as a blocker, a pair of chromosomes was labelled bright-yellow along most of their length, except for 30% in the middle regions of the long arms which were red. The signals at the terminal regions were a little weaker (Fig. 1e). This chromosome showed the same hybridization signal as the StJ^{S} chromosome in Zhong 5 (Fig. 1c). GISH analyses, using *Th. intermedium* and *Ps. strigosa* DNA as a probe respectively, showed that in disomic addition lines Z1, Z2 and Z6, each had a pair of added *Th. intermedium* chromosomes which derived from the StJ^{S} chromosomes in Zhong 5 (Fig. 1f). All these results indicated that Yi 4212, HG 295, Z1, Z2 and Z6 carried a pair of StJ^{S} chromosomes. This chromosome pair was homoeologous to group 2 and had high resistance to BYDV (Nie et al. 1994; Larkin et al. 1995; Ai et al. 1997). GISH analysis using the St genome as a probe not only confirmed that the BYDV-resistant *Th. intermedium* chromosomes in Yi 4212, HG 295, Z1, Z2 and Z6 were identical, but also showed that they involved a St–J^{S} translocation chromosome which contained a large part of a St chromosome and the partial long arm of a J^{S} chromosome.

GISH analysis of Z3

GISH patterns using either *Th. intermedium* or St genomic DNA as a probe showed that among the 44 chromosomes in Z3 root-tip cells at mitotic metaphase, a pair of chromosomes was entirely labelled greenish-yellow (Fig. 1g), indicating that Z3 carried a pair of *Th. intermedium* chromosomes belonging to the St genome. A pair of small satellites can be observed at the end of the short arms if the mitotic metaphase is ideal (Fig. 1g). The added *Th. intermedium* in Z3 belonged to homoeologous group 1 (Larkin et al. 1995). The results above showed that only one pair of St chromosomes had satellites in Zhong 5 and in *Th. intermedium* (Fig. 1a, c). These observations indicated that the St chromosomes with small satellites in *Th. intermedium* (SAT3), Zhong 5 (St2) and Z3 were identical, and belonged to homoeologous group 1. One unexpected and unexplained observation is that the satellite regions of this pair of St chromosomes were bright-red in *Th. intermedium*, but were labelled greenish-yellow in Z3 in GISH analysis using the St genome as a probe.

GISH analyses of Z4 and Z5

GISH patterns in Z4 root-tip cells at mitotic metaphase using the *Th. intermedium* genome as a probe showed that there were two chromosome pairs involving a reciprocal translocation between a wheat and a *Th. intermedium* chromosome. The distal 55% of the long arms of the longer chromosome pair and except for the distal 60% of the long arms of the shorter chromosome pair, both were labelled greenish yellow, suggesting that the original added *Th. intermedium* chromosome pair was translocated with a wheat chromosome pair (data not shown). In GISH analysis using *Ps. strigosa* DNA as probe and *Th. elongatum* and "Chinese Spring" DNA as a blocker, the longer chromosome pair had a slight yellow label only at the terminal region of the long arm, while the shorter chromosome pair showed a slight yellow label at the terminal region of the short arm and around the centromere (Fig. 1h). This indicated that the added *Th. intermedium* chromosome in Z4 belonged to the J^{S} genome. We named the shorter translocation chromosome $J^{S}W$, and the longer translocation chromosome WJ^{S} (Fig. 1h).

GISH patterns using the *Th. intermedium* genome as a probe showed that among the 44 chromosomes in Z5, one pair of chromosomes was labelled greenish-yellow on the distal 55% long arms, indicating that a pair of wheat–*Th. intermedium* translocation chromosomes was added to Z5 (Fig. 1i). GISH, using *Ps. strigosa* DNA as a probe and *Th. elongatum* and "Chinese Spring" DNA as a blocker, showed a pair of chromosomes having a slight yellow label only at the terminal region of the long arm (Fig. 1j). This indicated that the added translocation chromosome in Z5 was a wheat-J^{S} translocation chromosome.

Z4 contains a pair of added *Th. intermedium* chromosomes belonging to homoeologous group 7, and is resistant to leaf, stem and stripe rusts; Z5 is resistant to leaf and stem rusts but susceptible to stripe rust, and its homoeology after RFLPs and isozyme analyses remains unknown (Larkin et al. 1995). Banks et al. (1993) reported that the rust resistance of Zhong 5 was conferred by a single pair of *Th. intermedium* chromosomes. Thus, we can conclude that the rust resistance in Z4 and Z5 came

from the same group-7 *Th. intermedium* chromosome. Both Z4 and Z5 are resistant to stem and leaf rust. However, unlike Z4, Z5 did not exhibit stripe rest resistance although both originated from the same cross combination and have the same wheat background. Larkin et al. (1995) inferred that Z5 had a deleted Z4 *Th. intermedium* chromosome, and that the deleted segment included stripe rust, the endopeptidase locus, the peroxidase locus, and the psr129 locus. GISH results confirmed this speculation. Moreover, because Z4 and Z5 had the same origin and the added wheat–J^S translocation chromosome in Z5 and the WJ^S chromosome in Z4 had a similar length and signal pattern, we can conclude that the translocation chromosomes in Z5 were derived from the WJ^S chromosome in Z4. The J^S segment in the WJ^S chromosome carries both leaf and stem rusts, whereas the J^S segment in the J^SW chromosome carries stripe rust, the endopeptidase locus, the peroxidase locus, and the psr129 locus. This also explains why the homoeology of the added chromosomes in Z5 was difficult to determine.

Discussion

Th. intermedium (2n=42) is a segmental autoallo-hexaploid (Dewey 1962, 1984). Its genome designation varies between authors, but there is general agreement that the genomic composition of *Th. intermedium* consists of two very similar genomes plus a third genome of unknown origin. The two similar genomes have been designated as E1 and E2, or E and J, or J1 and J2, which are closely related to the E-genome of *Th. elongatum* (2n=14) or the J genome of *Th. bessarabicum* (2n=14). The uncertain genome has been variously designated as X, N, P, or S (Dewey 1962, 1984; Dvorak 1981; Lǒve 1986; Pienaar 1990). Recent data suggests that the X genome may be closely related to the St (sometimes, designated as S genome) genome in *Pseudoregnaria stipifolia* and *Ps. strigosa* (Liu and Wang 1993; Zhang et al. 1996a, b; Chen et al. 1998a, b). Chen et al. (1998b) demonstrated that different *Th. intermedium* accessions had 13–14 St chromosomes, 6–11 J^S chromosomes, and 17–21 J chromosomes. The *Th. intermedium* accession originating from China had 14 St chromosomes, seven J^S chromosomes, and 21 J chromosomes; our research confirmed these results (Fig. 1a). The difference is that the seven J^S chromosomes showed light signals both around the centromeres and at the telomeres in our experiment, while showing signals only around the centromeres in Chen's results (Chen et al. 1998b). *Th. intermedium* is an open-pollinated perennial species; its genome constitution varies greatly between different populations and even between different plantlets. This also was confirmed by C-banding (Friebe, personal communication). In our experiment, three pairs of J^S and 11 pairs of J chromosomes were also observed in some plantlets (data not show).

Th. intermedium has three pairs of satellite chromosomes including two pairs of J^S and one pair of St chromosomes (Fig. 1a, c, g). In TAF46 and its derived addition lines produced by Cauderon (1966) and Cauderon et al. (1973), the addition line L3 contains a pair of *Th. intermedium* chromosomes which carry two minor satellites in the short arms and belong to homoeologous group 1, while addition line L5 contains a pair of *Th. intermedium* chromosomes which carry two major satellites in the short arms and belong to homoeologous group 5 (Forster et al. 1987; Friebe et al. 1992a). GISH analysis using the St genome as a probe indicated that the *Th. intermedium* chromosome in L3 was SAT2 and the *Th. intermedium* chromosome in L5 was SAT1 (data not shown). Thus, among three pairs of satellited chromosomes in *Th. intermedium*, the longest chromosome, SAT1, which has slight signals at the telomere of the long arm and at the inner border of the satellite in the short arm (GISH pattern using St as a probe), belongs to the J genome and is homoeologous to group 5. The next longest chromosome, SAT2, with slight signals at the end of the long arm and at the outer border of satellite in the short arm, belongs to the J genome and is homoeologous to group 1. The shortest chromosome, SAT3, which is entirely probed with the St genome, belongs to the St genome and is homoeologous to group 1. GISH analysis using *Ps. strigosa* DNA as a probe can easily distinguish the three pairs of satellite chromosomes in *Th. intermedium*. Generally, the SAT1 and SAT2 satellites can be observed in karyotype analysis (Forster et al. 1987; Friebe et al. 1992a); however, the SAT3 satellites are often suppressed and can not be easily observed.

Partial amphiploids play an important role in transferring and utilizing useful alien traits. It is essential therefore to know the exact genomic composition of the added alien chromosomes in the amphiploids. Zhong 5 is a useful wheat–*Th. intermedium* amphiploid for wheat improvement. The genomic composition of the added *Th. intermedium* varies between authors. Combining C-banding and GISH using *Th. intermedium* as probe, Ma et al. (1998) concluded there were six pairs of intact *Th. intermedium* chromosomes and a pair of wheat–*Th. intermedium* translocation chromosomes in Zhong 5. By means of in situ hybridization using a rye-derived 350-bp repeated sequence as a probe, Xin et al. (1988) concluded that there were at least four pairs of X-genome (=St-genome) chromosomes and no wheat–*Th. intermedium* translocation chromosomes; he also confirmed the presence of E (=J or J^S) chromosomes in Zhong 5 by Southern hybridization using a probe that was isolated from *Th. elongatum* (2n=14). From the GISH results using *Ps. strigosa* DNA as a probe, Zhang et al. (1996b) concluded that Zhong 5 had five pairs of St chromosomes, one pair of St-E (E=J or J^S) Robertsonian translocation chromosomes, and one pair of St-E (E=J or J^S) intercalary translocation chromosomes; using the same genomic probe, Chen et al. (1998b) concluded that the seven pairs of added *Th. intermedium* chromosomes in Zhong 5 included two pairs of St chromosomes, two pairs of J^S chromosomes, and three pairs of St-J^S translocation chromosomes. Using the *Th. intermedium* and St

genomes as probes in GISH and combining the GISH results of *Th. intermedium*, Zhong 5 and its derivatives in wheat, we propose that the seven pairs of *Th. intermedium* chromosomes in Zhong 5 include three pairs of St chromosomes, two pairs of J^S chromosomes, and two pairs of St-J^S reciprocal translocation chromosomes (Fig. 1c). We believe the conclusions of this current paper are correct and can explain the different conclusions of previous authors as follows:

(1) In GISH using the St genome as a probe, the signal strength may be different in different regions of a St chromosome. Sometimes, the weak signals in some regions of St chromosomes may disappear because of excessive washing or a high blocker to probe ratio. Thus, a St chromosome can be misinterpreted as a St-JS translocation chromosome.
(2) GISH analysis using the St genome as a probe indicated that JS chromosomes have weak greenish-yellow signals at the telomeres and around the centromeres. This leads to falsely regard a J S chromosome as a St-E (E=J or JS) intercalary translocation chromosome. Moreover, the signals at the telomeres are close to the signal around the centromere if the chromosome is not well extended; combining the factor in (4) below, we usually regard a JS chromosome as a St chromosome.
(3) The StJS chromosome in Zhong 5 is easily misinterpreted as a St chromosome, especially when the chromosome is not in a good state of extension, whereas the JSSt chromosome can be easily misinterpreted as a wheat–Th. intermedium translocation chromosome. However, we can clarify these problems if we compare the GISH pattern of Th. intermedium, Zhong 5 and its derivatives in wheat using the St and Th. intermedium genomes as probes.

Another possible reason is as follow: (4) in GISH using the St genome as a probe, E (=J or JS) and A, B, D genomes as blockers, the Th. intermedium chromosomes, even including the J or JS chromosomes in wheat–Th. intermedium germplasms, can be clearly distinguished from wheat chromosomes. Under these GISH conditions it is easy to mistake the J or JS chromosomes as St. chromosomes. In contrast, with GISH using the E (=J or JS) genome as a probe, and St and A, B, D genomes as blockers, it is difficult to distinguish the J or JS chromosomes from wheat chromosomes and there is very little contrast between the Th. intermedium and wheat chromosomes, because both J (or JS) and wheat chromosomes are labelled with signals. Other researchers observed the same results (Zhang et al. 1996b; Chen et al. 1998c). This is most likely due to the fact that the St genome is less closely related to the A, B, and especially the D, genomes in wheat, than they are to the J or E genomes (Dvorak 1980; Pienaar 1990; Zhang et al. 1996b; Chen et al. 1998c). This would explain why the St genomic probe provides a better contrast between the Th. intermedium and wheat chromosomes than does the Th. intermedium (containing the J, JS and St genomes) genomic probe. The same applies to the genomic composition analysis of Th. intermedium. It is easy to clearly discriminate the 14 St chromosomes from the 28 E chromosomes if we use the St genome as a probe and the E genome as a blocker. In contrast, it is not so easy to distinguish the 28 E chromosomes from 14 St chromosomes if we use the E genome as a probe and the St genome as a blocker. This explains why previous researchers preferred to use the St genome as probe and the E genome as a blocker in the genomic composition analysis of Th. intermedium (Zhang et al. 1996a, b; Chen et al. 1998a, b, c). By GISH using St as a probe, not only can we identify the alien Th. intermedium chromosomes in wheat–Th. intermedium germplasms, but also assign the Th. intermedium chromosomes to St, JS or J genomes. This type of GISH has the advantage of chromosome banding, and makes it especially useful to identify the alien Th. intermedium chromosomes in wheat. In the present paper, the seven pairs of added Th. intermedium in Zhong 5 can be easily distinguished from each other (Fig. 1c).

The added J^S chromosome in Z4 belongs to homoeologous group 7, and is resistant to leaf, stem and stripe rusts but susceptible to BYDV, indicating that this chromosome is different from the 7Ai-1 in L1 (Forster et al. 1987; Friebe et al. 1992a), which is resistant to BYDV (Brettell et al. 1988). Friebe (1992a) described another chromosome belonging to homoeologous group 7, and named it 7Ai-2. This chromosome carries genes conferring resistance to leaf rust in the distal region of the long arm, and has genes conferring resistance to stem rust and stripe rust in the short arm or in the proximal region of the long arm; karyotype and C-banding analysis indicated that 7Ai-2 belonged to the E (=J or J^S) genome (Friebe et al. 1992a, b, 1993). Because there are only two pairs of E (=J or J^S) chromosomes belonging to homoeologous group 7 in *Th. intermedium*, and that the J^S chromosomes in Z4 and 7Ai-2 display similarity in disease resistance, we can conclude that the J^S chromosome in Z4 is identical to 7Ai-2. The third pair of chromosomes homoeologous to group 7 belong to the St genome, and we named it 7Ai-3. Combing our results with those of other authors, we confirm that the genes conferring resistance to leaf rust and stem rust are located in the distal region of the long arm, whereas the gene(s) conferring resistance to stripe rust is located in the short arm or in the proximal region of the long arm of 7Ai-2. The J^S-wheat translocation chromosomes in Z4 and Z5 are potentially useful sources for rust resistance genes in wheat breeding and for cloning these novel rust-resistant genes.

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