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

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 translocation 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 translocation chromosomes (StJS) in Zhong 5. The StJS 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 WJS 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

Introduction

*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey [=*Elytrigia intermedium* (Host) Nevski=Agropyron intermedium (Host) Beauvoir] is a perennial autoallo-hexaploid species (2n=6x=42, previously designated with genomes E,E,E,E,E,XX) 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; Friebel 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 (Singovets 1973, 1976; Franke et al. 1992), low-temperature and drought tolerance (Feder 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 germplasms 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=8x=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

Total genomic DNA was isolated from the plant materials by the method of Saghai-Marooof 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 chromosomal DNA, 50 µl 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 fluoresochrome, 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.

#### Genomic in situ hybridization (GISH)

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#### 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