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Molecular cytogenetic characterization of *Thinopyrum intermedium*-derived wheat germplasm specifying resistance to wheat streak mosaic virus

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Abstract *Thinopyrum intermedium* is a promising source of resistance to wheat streak mosaic virus (WSMV), a devastating disease of wheat. Three wheat germplasm lines possessing resistance to WSMV, derived from *Triticum aestivum* × *Th. intermedium* crosses, are analyzed by C-banding and genomic in situ hybridization (GISH) to determine the amount and location of alien chromatin in the transfer lines. Line CI15092 was confirmed as a disomic substitution line in which wheat chromosome 4A was replaced by *Th. intermedium* chromosome 4Ai#2. The other two lines, CI17766 and A29-13-3, carry an identical Robertsonian translocation chromosome in which the complete short arm of chromosome 4Ai#2 was transferred to the long arm of wheat chromosome 4A. Fluorescence in situ hybridization (FISH) using ABD genomic DNA from wheat as a probe and S genomic DNA from *Pseudoroegneria stipifolia* as the blocker, and vice versa, revealed that the entire short arm of the translocation was derived from the short arm of chromosome 4Ai#2 and the breakpoint was located at the centromere. Chromosomal arm ratios (L/S) of 2.12 in CI17766 and 2.15 in A29-13-3 showed that the translocated chromosome is submetacentric. This translocated chromosome is designated as T4AL·4Ai#2S as suggested by Friebe et al. (1991).

Key words Fluorescence in situ hybridization · Translocation · WSMV resistance · *Thinopyrum intermedium* · *Triticum aestivum*

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Introduction

Wheat streak mosaic virus (WSMV) is a damaging viral disease of wheat found in Canada, the United States and many other countries of the world. There is no resistance in wheat but several resistant germplasms from wheat × *Thinopyrum intermedium* hybrids have been developed (Friebe et al. 1991). The wheat germplasm line CI15092, derived from a cross of wheat with *Th. intermedium* (Wells et al. 1973), was identified as a substitution line with immunity to WSMV. The resistance to WSMV exhibited by this line is controlled by a gene(s) located on the short arm of a *Th. intermedium* chromosome, 4Ai#2 (Wang and Liang 1977; Friebe et al. 1991). A translocation line also carrying this resistance was released as CI17766 (Liang et al. 1979). Based on C-banding and in situ hybridization, Friebe et al. (1991) concluded that the translocation involved the short arm of chromosome 4Ai#2 and the long arm of chromosome 4A, which had broken and rejoined at the centromere (i.e., a Robertsonian translocation). These observations contradicted Wang et al. (1980) who, based on chromosome pairing, had concluded that only a part of the short arm of 4Ai#2 had replaced a distal segment of the short arm of chromosome 4A (formerly 4B) in CI17766. Recently, using fluorescence in situ hybridization (FISH) analysis, Wang and Zhang (1996) again reiterate that the translocated chromosome consists of the complete long arm of 4A, the proximal 45% of the short arm of 4A, and the entire short arm of the *Th. intermedium* chromosome 4Ai#2.

However, 4AS is genetically homoeologous to the 4AL and 4DL arms, and its recombination with 4Ai#2S seems highly improbable. To resolve this apparent conflict, genomic in situ hybridization (GISH), C-banding, and phase-contrast microscopy analyses were used in the present study to unequivocally map the physical location of the translocation breakpoint and to estimate the size of the transferred *Th. intermedium* chromosome segment.

Material and methods

Three WSMV-resistant wheat-*Th. intermedium* derivatives were analysed in the present study. Germplasm CI15092 was produced by Wells et al. (1973) and was identified as a 4Ai#2(4D) chromosome substitution line (Friebe et al. 1991). Lines CI17766 and A-29-13-3 are wheat-*Th. intermedium* translocation stocks that were both derived from CI15092. Line CI17766 was derived from the cross of Chinese Spring monosomic for chromosome 5B × CI15092. The resulting F_1 progeny were crossed with the Chinese Spring *ph1* mutant and line CI17766 was selected from the resulting progeny of this second cross and is resistant to all known strains of WSMV (Liang et al. 1979). Line A29-13-3 is a re-selection from CI17766 and exhibits resistance to WSMV at or below 25°C (Wang and Zhang 1996).

To determine the breakpoint and size of the transferred *Th. intermedium* segments and their genomic origin, GISH analysis was used with total genomic DNA probes from the following species: *Triticum aestivum* L. cv Chinese Spring (CS) (2n = 6*x* = 42, genomes ABD); *Thinopyrum elongatum* (Host) Beauv. (2n = 2*x* = 14, genome E); *Thinopyrum bessarabicum* (Savul. and Rayss) A. Love (2n = 2*x* = 14, genome J); and *Pseudoroegneria stipifolia* (Czern ex Nevski) A. Love (2n = 2*x* = 14, genome S).

C-banding was performed using the technique described by Gill et al. (1991). The GISH protocol followed the procedure described by Chen et al. (1995). Chromosome preparations were carried out according to Chen et al. (1996). To clarify the position of the primary constriction, mitotic chromosomes in root-tip cells were photographed in phase contrast before GISH analysis. The length of 20–30 alien or translocated chromosomes were measured after GISH and the mean length of the short and long arms and the total length of the translocated chromosomes in these lines were compared using Student's *t*-test (Steel and Torrie 1980).

The rationale for the notational designations of *Th. intermedium* chromosomes was discussed by Friebe et al. (1991, 1996). Thus, for chromosome '4Ai#2' the Arabic number 4 identifies that it belongs to homoeologous group 4, the letters 'Ai' denote its origin from *Agropyron intermedium* (now called *Th. intermedium*) and '#2' identifies it as the second group-4 *Th. intermedium* chromosome transferred to wheat (Friebe et al. 1991). For T4AL·4Ai#2S, the letter 'T' identifies a translocation chromosome, 4AL = long arm of chromosome 4A of wheat, the symbol '•' indicates a breakpoint at the centromere, and 4Ai#2S = short arm of 4Ai#2 of *Th. intermedium* (Raupp et al. 1995).

Results

Germplasm line CI15092

GISH analysis of CI15092, using genomic DNA of Chinese Spring as a probe and *Ps. stipifolia* S genomic DNA as the blocker, labelled 20 out of the 21 chromosome pairs over their entire lengths (yellow) but one chromosome pair remained unlabelled (appears red because of counterstaining with propidine iodide) (Fig. 1 a). The reverse analysis using S genomic DNA as a probe and wheat genomic DNA as the blocker, labelled one chromosome pair and left the other 20 pairs unlabelled (Fig. 1b). Under the reverse conditions, the alien pair showed strongest labelling around the telomeres and centromeres with a weakly labelled segment at the center of each arm (Fig. 1 b). Measurement of 20 *Th. intermedium* 4Ai#2 chromosomes in ten GISH-labelled cells of CI15092 gave an average length of 6.26 μm (S = 2.88, L = 3.38) and an arm ratio (L/S) of 1.18 (Table 1). The C-banding pattern of 4Ai#2 in CI15092 is distinctive, with a small subtelomeric C-band in the short arm and a proximal C-band and prominent terminal C-band in the long arm (Fig. 2), substituting a chromosome pair 4A of wheat, as previously reported by Friebe et al. (1991).

Germplasm lines CI17766 and A29-13-3

GISH analysis of CI17766 and its re-selection, A29-13-3, using a wheat genomic DNA probe and an S genome DNA blocker (Figs. 1 c, g), together with the reverse GISH analysis (S-genome DNA as a probe and wheat genomic DNA as the blocker) (Figs. 1 d, h), all demonstrated that these lines carry a translocated

Fig. 1a–h GISH on somatic metaphase cells from root tips of substitution line CI15092 and translocation lines CI17766 and A29-13-3 using genomic DNA from Chinese Spring (ABD genomes), *Pseudoroegneria stipifolia* (S genome) and *Th. bessarabicum* (J genome) as probes. Sites of probe hybridization fluoresce *yellow or greenish yellow* with FITC, while non-hybridized sites fluoresce *red* with propidium-iodide counterstain. **a** Line CI15092 shows two red submetacentric *Th. intermedium* 4Ai#2 chromosomes and 40 yellow wheat chromosomes labelled with wheat genomic DNA probe in the presence of an S genomic DNA blocker. **b** The two alien chromosomes 4Ai#2 in line CI15092 strongly hybridized at terminal and centromeric regions when detected with the S genomic DNA probe and the wheat genomic DNA blocker. **c** One pair of submetacentric translocated chromosomes in line CI17766 was detected by a wheat genomic DNA probe and an S genomic DNA blocker. The translocation breakpoint is at the centromere. **d** Line CI17766 displays the two yellow 4Ai#2 chromosome arms carrying a GISH fingerprint detected by an S genomic DNA probe attached onto two red wheat chromosome arms with breakpoints at the centromere. **e** Detection of one or two artificial tertiaries constrictions occurring on the long arm of wheat chromosomes in line CI17766 after GISH with wheat genomic DNA as a probe and S genomic DNA as a blocker. **f** Detection of one pair of submetacentric translocated chromosomes with a breakpoint at the centromere by the J genomic DNA probe in line CI17766. Fourteen smaller wheat chromosomes were also stained *light yellow* by the S genomic DNA. **g** Detection of one pair of a submetacentric translocated chromosomes in line A29-13-3 using wheat genomic DNA as a probe. **h** A cell of A29-13-3 showing strong hybridization signals near the centromere area on short arms of the two translocated chromosomes when S genomic DNA was used as a probe and wheat genomic DNA was used as a blocker

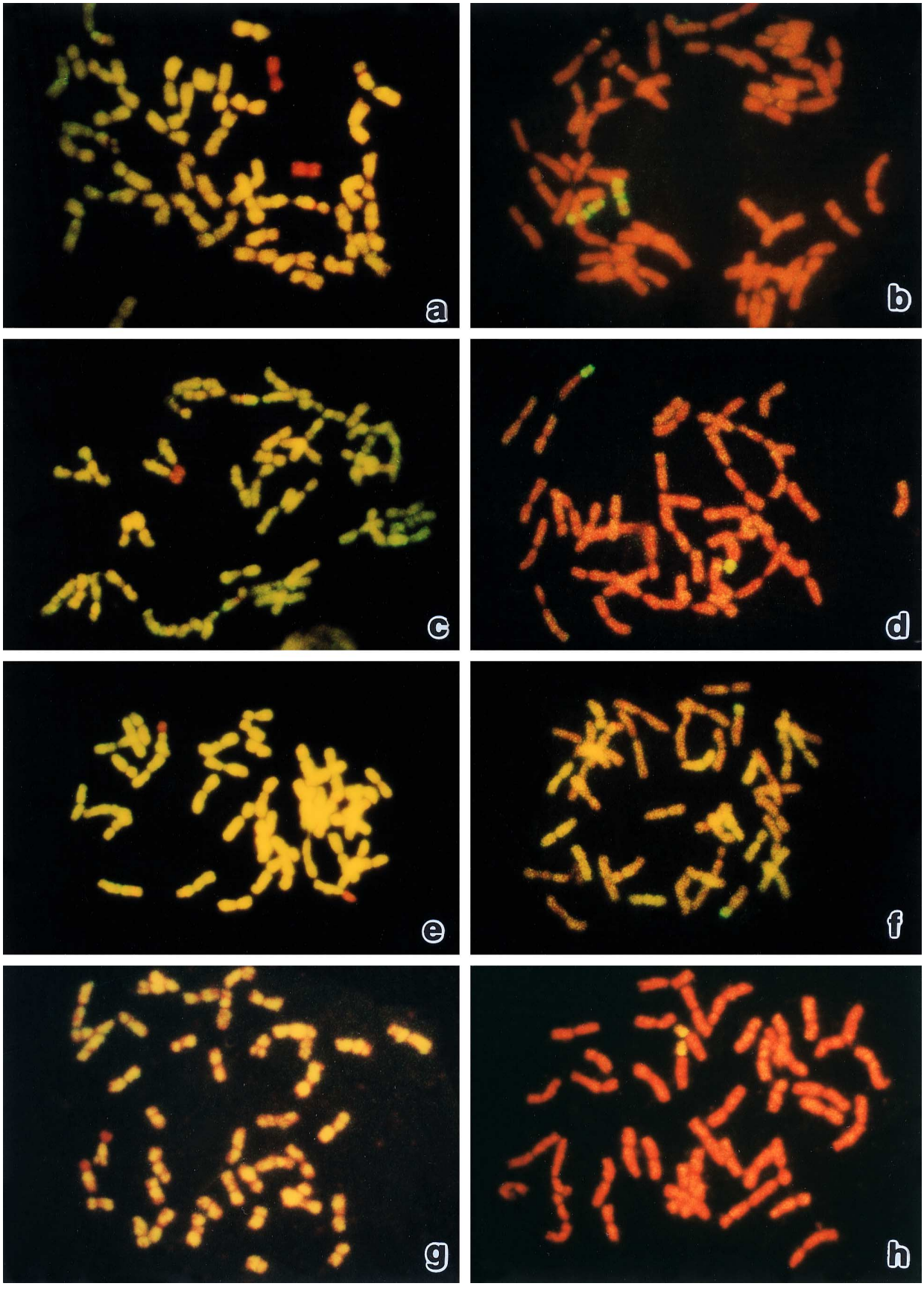
a
b
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Table 1 Length (μm) of different chromosomes in CS, CI15092, CI17766 and A29-13-3. a, within-columns, means followed by the same letter are not significantly different from each other according to Student's *t*-test at $P = 0.01$

Chromosome	Short arm	Long arm	Total	Arm ratio
4A (CS)	3.68 ± 0.43a	6.01 ± 0.70a	9.69 ± 1.08a	1.64b
4Ai#2 (CI15092)	2.88 ± 0.34b	3.38 ± 0.31b	6.26 ± 0.55c	1.18c
4AL·4Ai#2S (CI17766)	2.76 ± 0.33b	5.86 ± 0.79a	8.62 ± 1.07b	2.12a
4AL·4Ai#2S (A29-13-3)	2.95 ± 0.54b	6.33 ± 1.13a	9.27 ± 1.64ab	2.15a

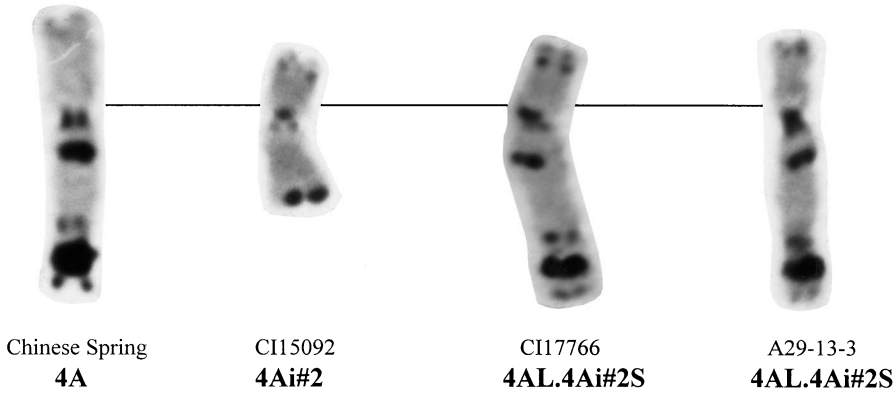

Fig. 2 C-banding patterns of critical chromosomes present in the WSMV-resistant germplasm

chromosome in which a short segment from *Th. intermedium* was joined to a long wheat segment with the breakpoint apparently located at the centromere. This coincidence of the breakpoint and centromere has been the subject of conflicting reports in the literature (Friebe et al. 1991; Wang and Zhang 1996). Unequivocal identification of primary and secondary constrictions is possible if mitotic metaphases are first analysed in phase contrast followed by chromosome banding or GISH analyses (Endo and Gill 1984). Under negative phase contrast, only primary constrictions (centromeres) and secondary constrictions (nucleolar organisers) are clearly visible (Fig. 3 a and c). However, following GISH-staining tertiary constrictions sometimes became evident, especially in those chromosomes that were labelled by the wheat genomic-DNA labelled probe (Figs. 1 e and 3 b). Based on observations of 30 GISH-labelled cells in CI17766 using a wheat genomic DNA probe, 47% of the translocated chromosomes showed a narrow band with no hybridisation signal in the middle of the long arm, thus creating the appearance of a double centromeric chromosome (Fig. 1 e). For this reason, a comparison of chromosomes in phase contrast with GISH (CI1776)- and reverse GISH (A29-13-3)-labelled chromosomes of the same cell was used to clearly demonstrate that the breakpoint in the translocated chromosome pair coincided with the centromere (Fig. 3).

GISH images of CI17766 were also produced using genomic DNA from *Th. bessarabicum* (genome J) and *Th. elongatum* (genome E) as probes in the presence of wheat genomic DNA as a blocker. As with the S genome probe, the J genome probe also detected a pair of translocated chromosomes with a breakpoint at the centromere. The entire short arm was labelled and the long arm was unlabelled (Fig. 1f). In addition to the signal on the short arm of the translocated chromosome, 14 other chromosomes were also weakly labelled light yellow by J genomic DNA (Fig. 1f). Because these chromosomes were smaller than average, they probably belong to the D genome of wheat. Genomic DNA from *Th. elongatum* gave similar results to the S or J genomic DNA probes except that its hybridization signal on the translocated chromosome was weaker. These differences in signal intensities may be a reflection of genomic repetitive DNA sequence homology.

C-banding analysis of CI17766 and A29-13-3 showed identical results with respect to the translocated chromosome (Fig. 2). In each case, the short arm of the translocated pair was identical to the short arm of the alien pair in CI15092 (4Ai#2), while the long arm was identical to the long arm of chromosome 4A of Chinese Spring (Fig. 2). Measurements of chromosome length were also made on comparable preparations of Chinese Spring, CI15092, CI17766 and A29-13-3 (Table 1). The average length of the translocated chromosome in CI17766 is 8.62 μm (S = 2.76; L = 5.86) with an arm ratio (L/S) of 2.12. The average length of the translocated chromosome in line A29-13-3 is 9.27 μm (S = 2.95 μm; L = 6.33 μm) with a arm ratio (L/S) of 2.15. The total length of the translocated chromosome in line A29-13-3 is a little longer than that found in CI17766, but the estimated value of

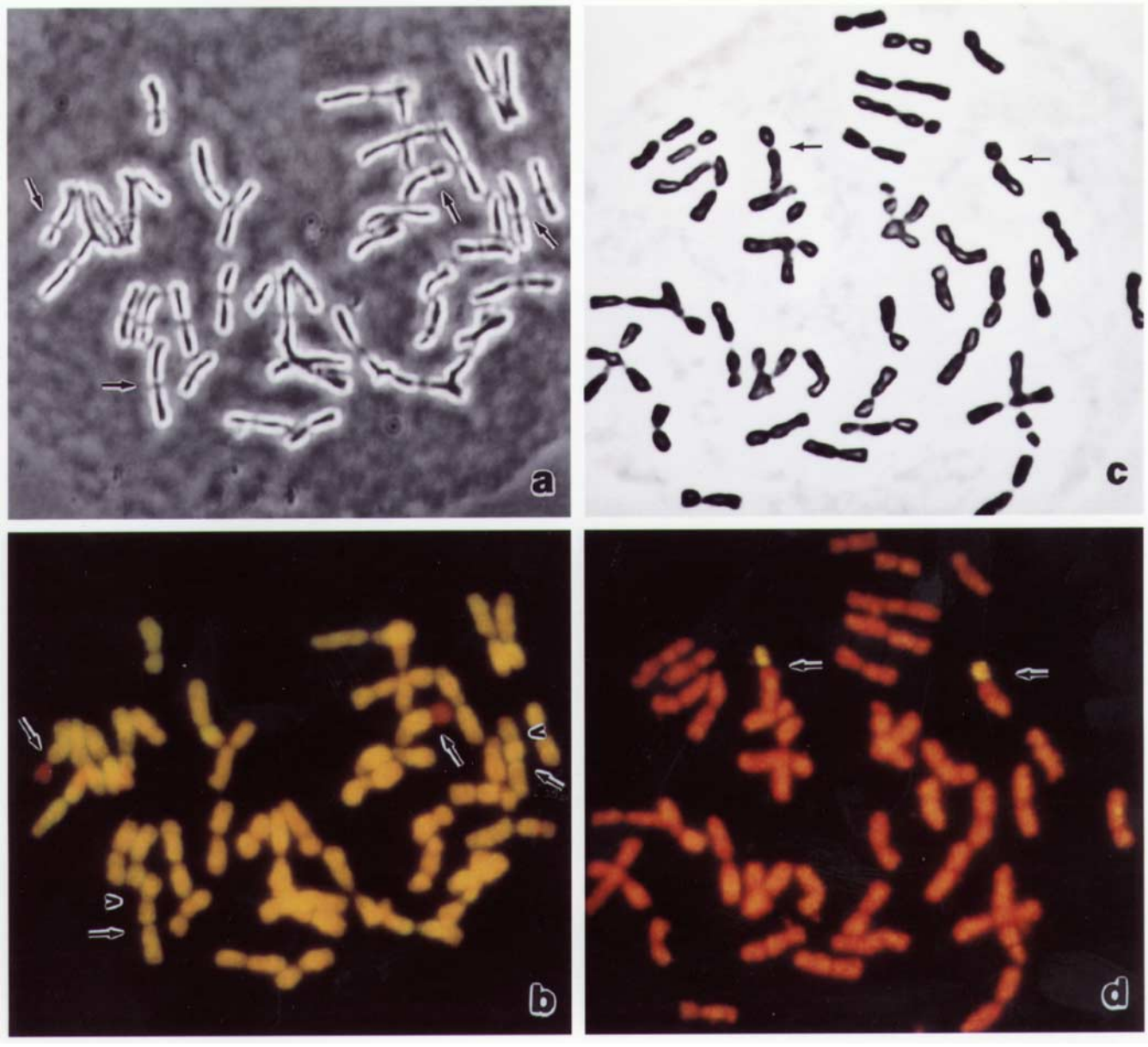

Fig. 3a–d Comparison of metaphase chromosomes under phase contrast and GISH in the same cell of the translocation lines CI17766 (**a** and **b**) and A29-13-3 (**c** and **d**). Centromeres are marked by *arrows*, while *arrow heads* indicated the tertiary constriction that occurred after GISH when wheat genomic DNA is used as the probe. **a** Phase-contrast image of a root-tip cell of CI17766 showing the position of the centromere on the translocated chromosome. **b** Tertiary constrictions (*arrow heads*) that became evident along the long arm of same wheat chromosomes shown in after GISH using a wheat genomic DNA probe and an S genomic DNA blocker. **c** Phase-contrast image of a root-tip cell of A29-13-3 showing the position of the centromere on the translocated chromosome. **d** GISH using an S genomic DNA probe and a wheat DNA blocker for the same cell as in **c**

$t = -1.81$ was not significant ($P = 0.01$; *df* 58). No statistically significant difference was found among the lines CI15092, CI17766, and A29-13-3 for the mean length of the short arm of 4Ai#2. Similarly, no significant differences were found between the long arms of 4A of Chinese Spring and those of the T4AL·4Ai#2S translocated chromosomes of CI17766 ($t = -0.7$; *df* 48). The results for the size and arm ratio of the translocated chromosome in both CI17766 and A29-13-3 strongly support the GISH and C-banding results that the translocated chromosome resulted through breakage at the centromere and re-union in a Robertsonian translocation between the short arm of 4Ai#2 and the long arm of 4A.

Discussion

C-banding and GISH analyses revealed the presence of an identical Robertsonian translocation in lines

CI17766 and A29-13-3. Comparisons of the C-banding pattern and chromosome lengths of the original 4A, 4Ai#2, and the translocated chromosomes T4A·4Ai#2 showed that the translocation consisted of the entire short arm of 4Ai#2 of *Th. intermedium* and the entire long arm of chromosome 4A of wheat joined at the centromere. Furthermore comparison of chromosomes under phase contrast and after GISH revealed that the breakpoints in both CI17766 and A29-13-3 were located at the centromere. The translocated chromosomes in CI17766 and A29-13-3 were both submetacentric with similar arm ratios (L/S) of 2.12 and 2.15, respectively.

Wang and Zhang (1996) reported that the translocated chromosome in A29-13-3 was metacentric with a non-centromeric breakpoint located about the middle of the short arm, whereas previous studies based on C-banding analysis identified the translocated chromosome as sub-metacentric and suggested a centromeric breakpoint with the complete arms of 4AL and 4Ai#2S being present (Friebe et al. 1991). The present data are in agreement with the latter study and identified the translocated chromosome as T4AL·4Ai#2S. It should be noted that in about half of the GISH-labelled cells analysed, the T4AL·4Ai#2S translocated chromosome appeared to have, in addition to the centromere, another constriction-like structure located in the middle of the long arm (Fig. 1 e). This artefact was caused by the lack of hybridization signal in this region, most likely resulting from twisting of the chromosome arms, and was probably the reason for the mis-identification of the translocated chromosome in the study by Wang and Zhang (1996). Lack of a hybridization signal in some chromosome regions is common in GISH experiments (Xu et al. 1994) and was also observed for the long arms of other wheat chromosomes in the present study (Fig. 3 b).

When examined under phase contrast, each chromosome possessed only one primary constriction or centromere. In the few cases where more than one constriction occurred, they were nucleolar organisers (secondary constrictions) on chromosomes 1B or 6B of wheat (Endo and Gill 1984). Although no other constriction-like structures were noted under phase contrast, obvious examples of tertiary constrictions appeared in the same chromosomes following GISH with wheat genomic DNA as the probe. Consequently, determining the location of the centromere from GISH-stained preparations alone may become somewhat subjective. While GISH staining provides unambiguous evidence of translocation breakpoints, prior phase-contrast photography of cells provided a useful adjunct to this method in order to demonstrate convincingly the relationship between the primary constriction or centromere and the translocation breakpoint.

The wheat-*Th. intermedium* translocated chromosome in CI17766 and A29-13-3 was assumed to be derived from induced homoeologous recombination (Liang et al. 1979). However, the wheat chromosome 4A involved in this translocation is structurally rearranged (Naranjo et al. 1988). Chromosome 4A was involved in a large paracentric inversion and a cyclical translocation with chromosomes 5A and 7B. As a result, the physically shorter arm is genetically related to other group-4 long arms and the physically longer arm shows homoeology to group-4 short arms, with the distal segment being homoeologous to group-7 short arms (Mickelson-Young et al. 1995). Thus, the 4Ai#2 short arm can not compensate for the loss of the short arm of chromosome 4A which is homoeologous to the group-4 long arms. Because of the rearranged structure of chromosome 4A, the 4Ai#2S arm probably had no chance for homoeologous pairing and recombination with either arm of chromosome 4A. In the F_1 hybrid of CS mono 5B × CI15092 three univalents were present (Liang et al. 1979), involving chromosomes 5B, 4A, and 4Ai#2. Univalents have a tendency to break at the centromere, followed by fusion of the broken chromosome arms (Sears 1952). Depending on which arms are involved in the fusion, different whole-arm translocations can be produced (Lukaszewski and Gustafson 1983). The centromeric breakpoint in T4AL·4Ai#2S and the blurred homoeology of chromosome 4A suggests that this translocation was most likely produced by centromeric breakage and re-fusion.

In the present GISH study, the 4Ai#2 chromosome showed special GISH patterns with different genomic DNA probes. The S genomic DNA probe hybridized strongly at centromeric and distal regions, but not at the middle of the 4Ai#2 arm. This indicates that chromosome 4Ai#2 is genetically related to the S genome but not directly derived from this genome. The GISH results, using J or E genomic DNA probes, showed that these probes strongly hybridized to the entire length of the 4Ai#2 arms. These results suggest that chromosome 4Ai#2 was most likely derived from the J or E genomes. However, it is interesting to note that the S genomic probe provided better discrimination of the *Th. intermedium* chromatin than did J or E genomic DNA. This is most likely due to the fact that the S genome is less closely related to the ABD genomes than are the J or E genomes. This would explain why the J and E genomic probes hybridized with about 14 small wheat chromosomes which were possibly those of the D genome of wheat. Other molecular and cytogenetic studies have also confirmed these points (Dvorak 1980; Zhang et al. 1996).

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