Theor Appl Genet (1993) 86:141–149

Radiation-induced nonhomoeologous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust

B. Friebe[1], J. Jiang[1], B. S. Gill[1], and P. L. Dyck[2]

[1] Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506-5501, USA
[2] Agriculture Canada, Winnipeg, Manitoba R3T 2M9, Canada

Received August 5, 1992; Accepted September 19, 1992
Communicated by K. Tsunewaki

Summary. The *Agropyron intermedium* chromosome 7Ai#2 is the source of the leaf rust resistance gene *Lr38* which was transferred to wheat by irradiation. The chromosomal constitutions of eight radiation-induced rust-resistant wheat-*Agropyron intermedium* derivatives were analyzed by C-banding and genomic in-situ hybridization (GISH). Five lines were identified as wheat-*Ag. intermedium* chromosome translocation lines with the translocation chromosomes T2AS · 2AL-7Ai#2L, T5AL · 5AS-7Ai#2L, T1DS · 1DL-7Ai#2L, T3DL · 3DS-7Ai#2L, and T6DS · 6DL-7Ai#2L. The sizes of the 7Ai#2L segments in mitotic metaphases of these translocations are 2.42 μm, 4.20 μm, 2.55 μm, 2.78 μm, and 4.19 μm, respectively. One line was identified as a wheat-*Ag. intermedium* chromosome addition line. The added *Ag. intermedium* chromosome in this line is different from 7Ai#2. This line has resistance to leaf rust and stem rust. Based on the rust reactions, and the C-banding and GISH results, the remaining two lines do not contain any *Ag. intermedium*-derived chromatin.

Key words: Common wheat – Alien translocations – *Agropyron intermedium* – Leaf rust resistance – In-situ hybridization – C-banding

Introduction

Agropyron intermedium (Host) P. B. [= *Thinopyrum intermedium* (Host) Barkworth and Dewey] is an autoalloploid (2n = 6x = 42, genomically $E_1E_1E_2E_2XX$) wild relative of cultivated bread wheat, *Triticum aestivum* L. em Thell (2n = 6x = 42, genomically AABBDD). *Ag. intermedium* is known to possess genes conferring resistance to wheat streak mosaic virus (McKinney and Sando 1951), barley yellow dwarf virus (Xin et al. 1988), and rusts (Knott 1989), making this species suitable for improving the distance resistance of wheat.

C-banding, and genomic in-situ hybridization (GISH), using biotin-labeled total genomic DNA of the donor species and unlabeled total genomic blocking DNA of the recipient as a probe, are now well established and highly efficient techniques for detecting alien chromatin in wheat (Le et al. 1989; Friebe et al. 1991a; Mukai and Gill 1991).

Recently we reported transfer of a leaf rust resistance gene (*Lr38*), derived from a goup 7 *Ag. intermedium* chromosome designated 7Ai#2, to wheat via a radiation-induced T2AS · 2AL-7Ai#2L wheat-*Ag. intermedium* chromosome translocation (Friebe et al. 1992a). This material was selected by A. Wienhues (1966, 1973) in irradiated progenies of wheat-*Ag. intermedium* chromosome addition line resistant to leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*), stripe rust (*Puccinia striiformis* West f. sp. *tritici*), and stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn.).

In the present study, we describe the chromosomal constitution of additional radiation-induced wheat-*Ag. intermedium* translocation lines by C-banding and GISH analysis. This material may have significance in breeding superior rust-resistant cultivars.

Materials and methods

Plant materials

The material analyzed consisted of eight wheat-*Agropyron intermedium* derivatives, designated T2, T4, T6, T7, T24, T25, T28, and T33. All lines were selected by Dr. A. Wienhues in X- or ^{60}Co-irradiated progenies of a wheat-*Ag. intermedium* chromo-

Correspondence to: B. Friebe

some addition line which was resistant to leaf rust, stripe rust, and stem rust. The parent wheat cultivar 'Heine IV' was susceptible to these fungi. The production and pedigrees of these lines were described in detail previously (Wienhues 1966, 1973). All lines were kindly provided by Dr. Nitzche, Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany, and were obtained from Dr. F. J. Zeller, Institut für Pflanzenbau und Pflanzenzüchtung, Technische Universität München-Weihenstephan, Germany.

Cytogenic analysis

For chromosome identification the C-banding protocol described by Gill et al. (1991) was used. For GISH analysis, the protocols of Le et al. (1989) and Mukai and Gill (1991) were followed with minor modifications. Genomic DNA of *Ag. intermedium* was isolated and labeled with biotin-11-UTP by nick translation. Hybridization was carried out in 10 µl per slide of a mixture containing 5–10 ng of labeled *Ag. intermedium* genomic DNA, 0.5–1 µg of sheared wheat genomic DNA as blocker, 5–10 µg of sheared salmon sperm DNA, 50% formamide, 2 × SSC, and 10% dextran sulfate. Post-hybridization wash, and signal detection with streptavidin horseradish peroxidase and diaminobenzidine tetrahydrochloride, were as described by Mukai and Gill (1991). For fluorescence detection (FISH), rabbit anti-biotin antibody (Enzo Diagnostics) was applied to chromosome preparations after the post-hybridization wash. Following incubation at 37 °C for 30 min slides were washed three times in 1 × PBS at room temperature (5 min each), treated with FITC-conjugated goat anti-rabbit antibody (Enzo Diagnostics), and incubated at 37 °C for 30 min. The slides were again washed three times with 1 × PBS at room temperature (5 min each). A thin layer of antifade solution (Johnson et al. 1981) containing 1 µg/ml of propidium iodide was then added for counterstaining and overlaid with a cover slip. The FITC and propidium iodide were excited by light at 450–490 nm wavelength using an Olympus reflected light fluorescence attachment.

The *Ag. intermedium* segments were identified by their bright yellow fluorescein fluorescence, whereas wheat chromosomes showed a red propidium iodide fluorescence. If detection was with streptavidin horseradish peroxidase and diaminobenzidine tetrahydrochloride, the *Ag. intermedium* chromatin had brown labeling, whereas chromosomes of wheat appeared blue due to Giemsa counterstaining. *Ag. intermedium* chromatin was detected in metaphase cells as well as in interphase nuclei.

Meiotic chromosome pairing was analyzed in aceto-carmine-stained pollen mother cells (PMCs).

Measurements were carried out on 20 C-banded chromosomes and on 20 chromosomes after GISH do determine the translocation breakpoints. Positions of breakpoints were calculated as a fraction of the total chromosome arm length from the centromere (fraction length). Photographs of C-banded chromosomes were taken with a Zeiss Photomicroscope III using Kodak Imagelink HQ microfilm 1461. For in-situ hybridization, photographs were taken with an Olympus BH-2 photomicroscope using either Kodak technical Pan film 2415 or Kodak Ektar 1000 color print film.

Resistance analysis

The *Ag. intermedium*-derived lines were tested with race 1 (122-54) and race 30 (176-58) of *P. recondita tritici* and races C17, C10, 69-MD-193A and 85AF10-1 of *P. graminis tritici* at the seedling stage. A mixture of leaf rust isolates was also used. The infection types produced were classified 12–14 days after inoculation using the system described by Stakman et al. (1962).

Results

In-situ hybridization analysis

FISH patterns of mitotic metaphase cells of lines T4, T7, T24, and T25, using labeled total genomic *Ag. intermedium* DNA as a probe, are shown in Fig. 1. All these lines have *Ag. intermedium* chromosome segments translocated to chromosomes of wheat. With the exception of line T7, which had 2n = 44, all the other lines had a chromosome number of 2n = 42.

The GISH pattern of T4 showed that this line has a pair of translocation chromosomes which were both labeled in the distal halfes of their long arms (Fig. 1 a). Chromosome measurements revealed that the proximal 46% of the long arm was unlabeled and derived from wheat, whereas the distal 54% of this arm was labeled and derived from *Ag. intermedium* (Table 1).

Line T7 carried two pairs of wheat-*Ag. intermedium* translocation chromosomes. One pair of chromosomes was submetacentric and was labeled in the distal 68% of the long arm, whereas the proximal 32% of this arm was unlabeled. In addition, this line also had a pair of telocentric chromosomes, where the proximal 40% was labeled and the distal 60% was unlabeled (Fig. 1 b, Table 1).

The wheat-*Ag. intermedium* translocation chromosome pair of line T24 was labeled in the distal 65% of the long arm. The proximal 35% of this arm was unlabeled (Fig. 1 c, Table 1).

Line T25 had a pair of submetacentric translocation chromosomes in which the proximal 59% of the long arm was unlabeled and the remaining distal 41% was labeled and thus derived from *Ag. intermedium* (Fig. 1 d, Table 1).

A pair of submetacentric chromosomes in T33 was labeled in the distal region of the long arm and derived from *Ag. intermedium* whereas the proximal unlabeled region was derived from wheat.

Line T2 was a chromosome addition line (2n = 44) carrying a small submetacentric *Ag. intermedium* chromosome pair that was labeled on both arms (Fig. 2).

C-banding analysis

C-banding analysis of T4, T7, T24, T25, and T33 confirmed the presence of wheat-*Ag. intermedium* chromosome translocations. Furthermore, the wheat and *Ag. intermedium* chromosomes involved were identified. The C-banding analysis showed that the translocated *Ag. intermedium* segments were derived from the *Ag. intermedium* chromosome designated 7Ai#2 present in the 7Ai#2(7A) and 7Ai#2(7D) chromosome substitution lines W52 and W44, respectively (Friebe et al. 1992a). C-banded karyotypes of lines T4, T7, T24, and T25 are shown in Figs. 3–6, and a comparison of the C-banding and GISH patterns of the critical wheat, *Ag. intermedium*

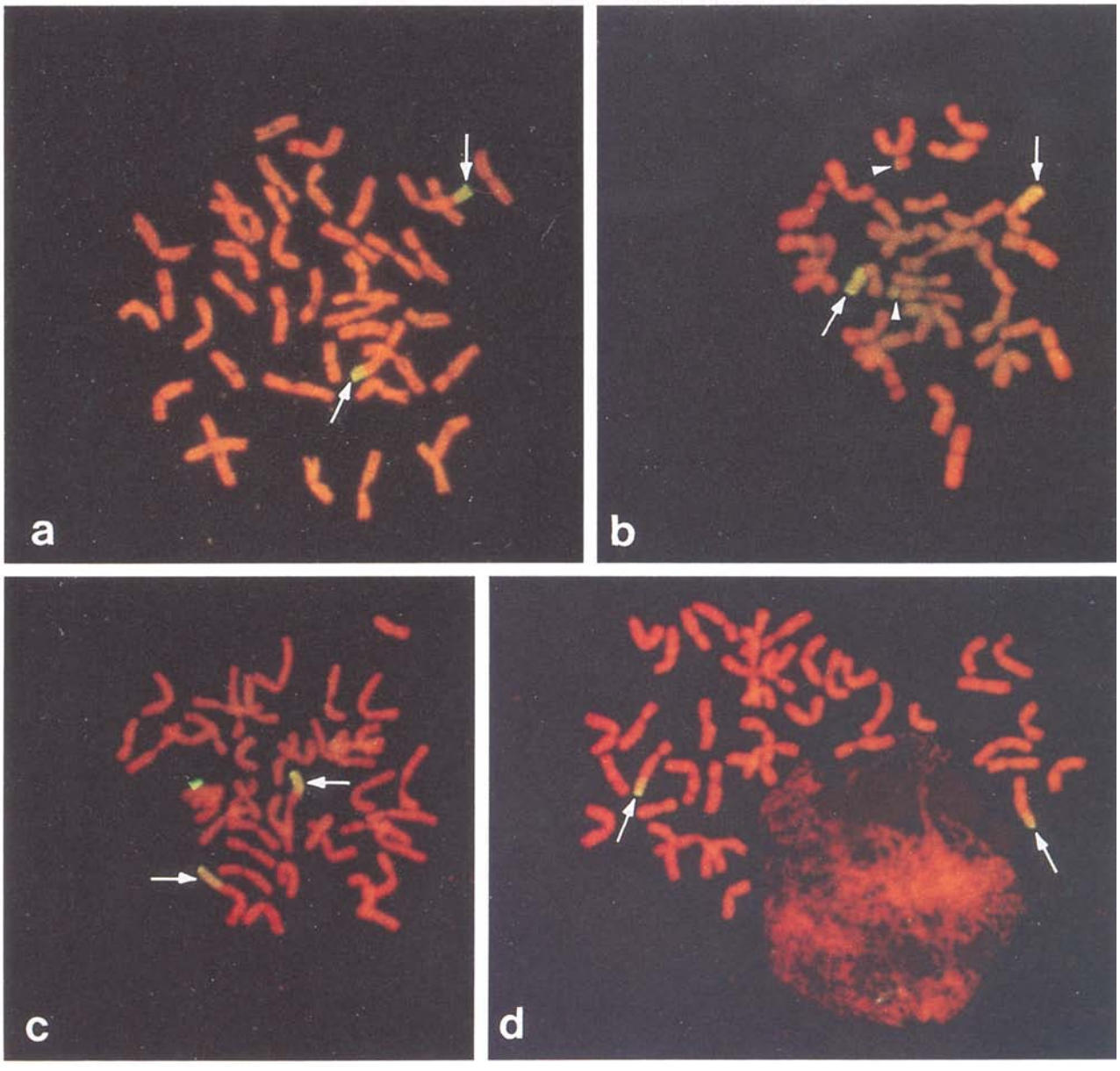

Fig. 1 a–d. Genomic in-situ hybridization patterns of mitotic metaphase chromosomes of radiation-induced wheat-*Ag. intermedium* chromosome translocation lines resistant to leaf rust. **a** T4 (carrying T3DL · 3DS-7Ai#2L); **b** T7 (carrying T6DS · 6DL-7Ai#2L and T6DL-7Ai#2S); **c** T24 (carrying T5AL · 5AS-7Ai#2L); **d** T25 (carrying T1DS · 1DL-7Ai#2L) (*arrows* point to the 7Ai#2L segments, *arrowheads* point to the 7Ai#2S segments)

and wheat-*Ag. intermedium* translocation chromosomes is given in Fig. 7. Measurements were carried out on mitotic metaphase chromosomes for identifying translocation breakpoints and for estimating the sizes of the transferred *Ag. intermedium* segments. The results are summarized in Table 1.

Line T4 carried a segment derived from the long arm of the *Ag. intermedium* chromosome 7Ai#2 translocated to the short arm of wheat chromosome 3D. The translocation arm showed one proximal and another interstitial faint C-band that are characteristic for the 3DS arm of wheat (Figs. 3, 7). In the translocated chromosome, the latter C-band is at an interstitial position, followed by an unbanded euchromatic segment with a prominent C-band at the telomere. This segment is derived from the distal region of 7Ai#2L, and the translocation chromosome can be described as T3DL · 3DS-7Ai#2L (or T3DL · 3DS1.6: :7Ai#2L1.4 according to the nomenclature system proposed by Gill et al. 1991). Based on C-banding and GISH data, the breakpoint is very close,

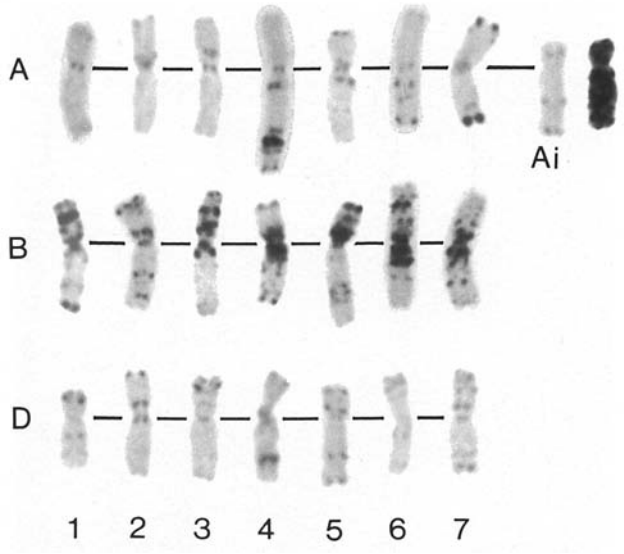

Fig. 2. C-banded karyotype of the wheat-*Ag. intermedium* addition line T2 (the genomic in-situ hybridization pattern of the added *Ag. intermedium* (*Ai*) chromosome is shown on the right)

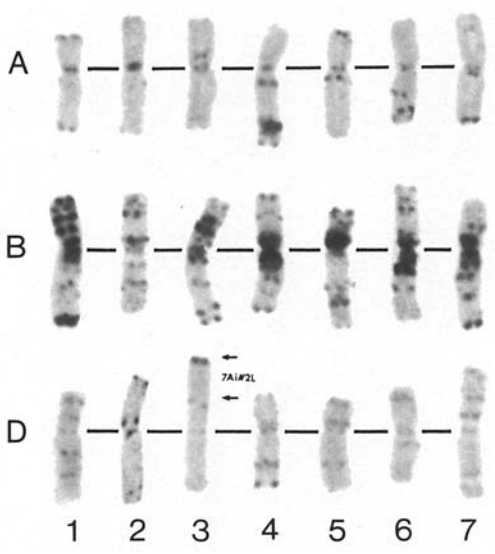

Fig. 3. C-banded karyotype of the T3DL · 3DS-7Ai#2L wheat-*Ag. intermedium* translocation line T4

Table 1. Chromosome lengths (S = short arm, L = long arm), standard deviations (σ), arm ratios, translocation breakpoints given as fraction lengths from the centromere, sizes of the transferred 7Ai#2L segments, and sizes of the missing wheat segments, in radiation-induced wheat *Agropyron imtermedium* translocation lines resistant to leaf rust

Line	Chromosome	Chromosome length (σ) in µm		Arm ratio L/S	Fraction length of translocation breakpoints (σ)	Size of the 7Ai#2L segment in µm (percent of 7Ai#2L)	Size of the missing wheat segment in µm (percent of the corresponding wheat arm)
		S	L				
7Ai#2(7D) (=W44)[a]	7Ai-2	4.46 (0.45)[b]	5.00 (0.62)	1.1			
'Heine IV'	3D	3.04 (0.37)	4.33 (0.50)	1.4			
T4	T3DL · 3DS-7Ai#2L	5.15 (0.22)	4.42 (0.35)	0.9	0.46 (0.08)	2.78 (56)	0.67 (22)
'Heine IV'	6D	3.16 (0.33)	3.42 (0.36)	1.1			
T7	T6DS · 6DL-7Ai#2L	3.12 (0.31)	6.16 (0.64)	2.0	0.32 (0.07)	4.19 (84)	1.45 (42)
T7	T6DL-7Ai#2S	2.46 (0.31)			0.40 (0.08)	0.98 (7Ai#2S)	
'Heine IV'	5A	3.14 (0.43)	5.64 (0.87)	1.8			
T24	T5AL · 5AS-7Ai#2L	6.46 (0.64)	5.86 (0.71)	0.9	0.35 (0.09)	4.20 (84)	0.88 (28)
'Heine IV'	1D	2.46 (0.32)	4.50 (0.64)	1.8			
T25	T1DS · 1DL-7Ai#2L	2.37 (0.28)	6.23 (0.77)	2.6	0.59 (0.08)	2.55 (51)	0.82 (18)
7Ai#2(7D) (=W44)[a]	2A	4.26 (0.59)	5.36 (1.00)	1.3			
T33 (=W49)[a]	T2AS · 2AL-7Ai#2L	3.95 (0.42)	6.38 (0.56)	1.6	0.62	2.42 (48)	1.40 (26)

[a] Data taken from Friebe et al. (1992)[b]

[b] Total chromosome length of 7Ai#2 corresponds to 84 percent of the total chromosome length of 3B present in the 7Ai#2 (7D) substitution line W44

and distal, to the interstitial 3DS C-band at a fraction length of 0.46 in the longer arm of this chromosome.

In line T7, the long arm of the translocated chromosome had a faint proximal C-band typical for 6DL (Figs. 4, 7). The region distal to this 6DL C-band included the large interstitial C-band up to the telomeric C-band derived from 7Ai#2L. The translocation chromosome is described as T6DS · 6DL-7Ai#2L (or T6DS · 6DL1.4 ::7Ai#2L1.2). The breakpoint in this translocation is located between the proximal C-band of 6DL and the large interstitial C-band derived from 7Ai#2L at a fraction length of 0.32.

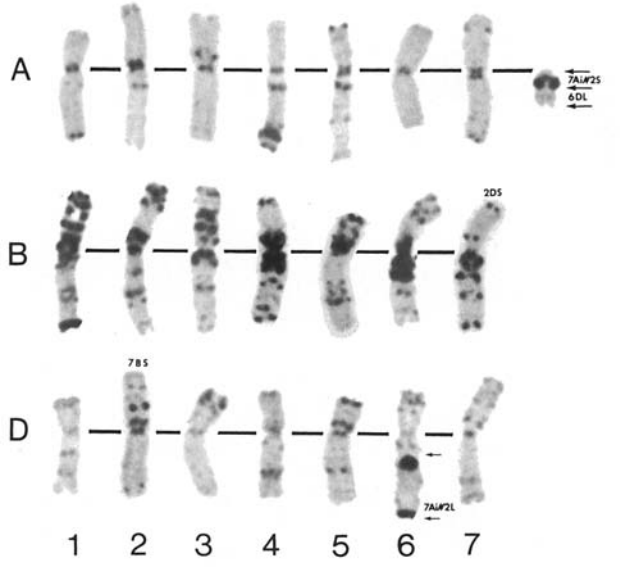

Fig. 4. C-banded karyotype of the T6DS · 6DL-7Ai#2L wheat-*Ag. intermedium* translocation line T7 carrying in addition the telocentric translocation chromosomes T6DL-7Ai#2S. This line also carries the wheat-wheat translocation chromosomes T2DS · 7BL and T7BS · 2DL

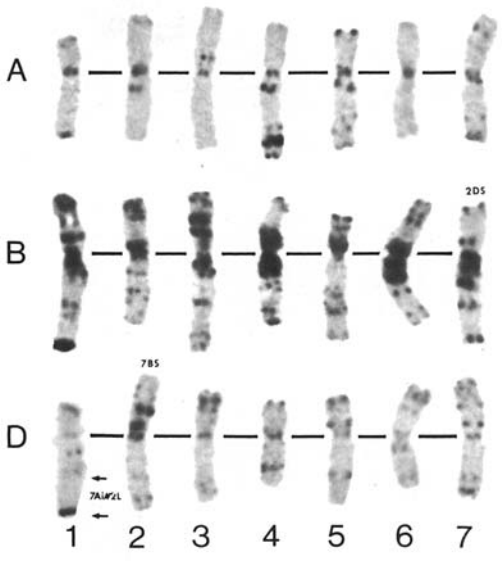

Fig. 6. C-banded karyotype of the T1DS · 1DL-7Ai#2L wheat-*Ag. intermedium* translocation line T25. This line also carries the wheat-wheat translocation chromosomes T2DS · 7BL and T7BS · 2DL

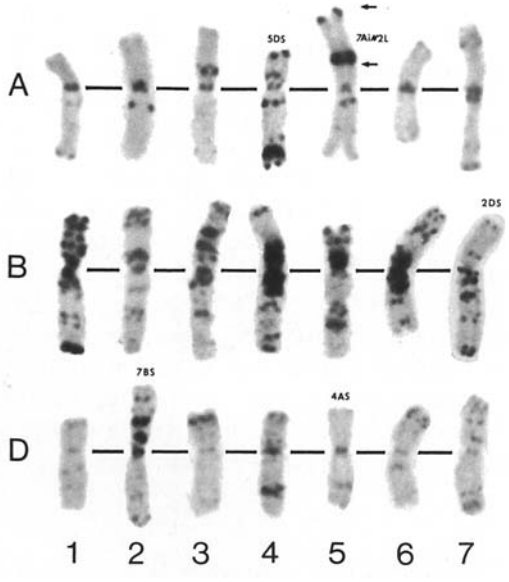

Fig. 5. C-banded karyotype of the T5AL · 5AS-7Ai#2L wheat-*Ag. intermedium* translocation line T24. This line also carries the wheat-wheat translocation chromosomes T2DS · 7BL, T7BS · 2DL, T5DS · 4AL, and T4AS · 5DL

In addition, T7 carried a pair of telocentric chromosomes. The GISH data showed that the proximal 40% of each telocentric chromosome, corresponding to the proximal large C-band, was derived from *Ag. intermedium*. The distal 60% of the telocentric chromosome was unlabeled and derived from wheat. The C-banding pattern of the wheat segment is similar to the distal region of 6DL which is missing in T6DS · 6DL-7Ai#2L. The proximal C-banded region of the telocentric chromosome is probably derived from the proximal region of the short arm of the chromosome 7Ai#2. Therefore, the second wheat-*Ag. intermedium* translocation chromosome of T7 can be described as T6DL-7Ai#2S (or T6DL1.4: :7Ai#2S1.4).

Furthermore, chromosomes 7B and 2D in T7 were involved in a reciprocal translocation, with the breakpoint within the centromeric region. The translocation chromosomes can be described as T7BS · 2DL and T2DS · 7BL, respectively.

In T24, almost all of the 7Ai#2L arm has been translocated to the short arm of wheat chromosome 5A (Figs. 5, 7). The translocation chromosome is described as T5AL · 5AS-7Ai#2L (or T5AL · 5AS1.2: :7Ai#2L1.2). Combining these data with the GISH analysis, the breakpoint is located at a fraction length of 0.35 in the T5AS-7Ai#2L arm close, and proximal, to the large interstitial C-band of 7Ai#2L.

In addition to the T7BS · 2DL and T2DS · 7BL translocation, line T24 was homozygous for another reciprocal wheat-wheat translocation involving the complete arms of chromosomes 4A and 5D. The resulting translocation chromosomes are described as T4AS · 5DL and T5DS · 4AL.

C-banding analysis of line T25 (Figs. 6, 7) identified the wheat-*Ag. intermedium* translocation chromosome as T1DS · 1DL-7Ai#2L (or T1DS · 1DL1.6: :7Ai#2L1.4). The long arm of this chromosome showed two interstitial

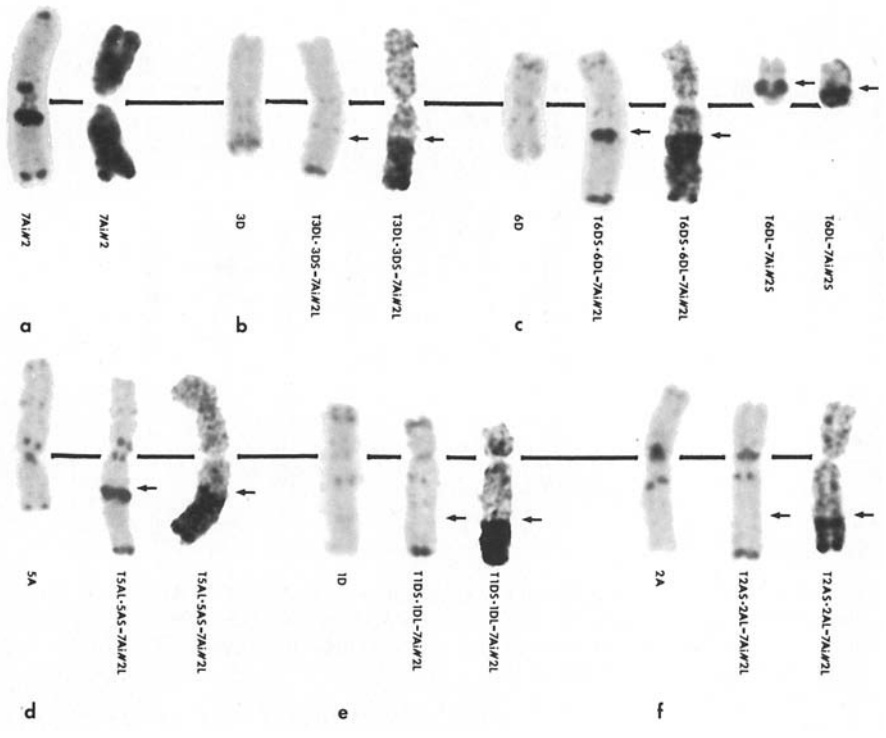

Fig. 7. C-banding (left) and genomic in-situ hybridization patterns (right) of the critical wheat, *Ag. intermedium* and wheat-*Ag. intermedium* translocation chromosomes present in radiation-induced translocation lines resistant to leaf rust: **a** W44, **b** T4, **c** T7, **d** T24, **e** T25, **f** T33 (*arrows* point to the translocation breakpoints)

C-bands derived from 1DL of wheat and, in addition, a prominent telomeric C-band derived from 7Ai#2L. C-banding and GISH analysis locate the breakpoint at a fraction length of 0.59, distal to the second interstitial C-band of 1DL. Line T25 was also homozygous for T7BS · 2DL and T2DS · 7BL.

Line T33 carried a terminal segment derived from 7Ai#2L translocated to the long arm of wheat chromosome 2A. This translocation chromosome is identical in C-banding and GISH pattern to the T2AS · 2AL-7Ai#2L (or T2AS · 2AL1.4::7Ai#2L1.4) wheat-*Ag. intermedium* translocation present in line W49 described previously by Friebe et al. (1992a).

C-banding analysis of line T2 confirmed the GISH results that this line is not a translocation but a chromosome addition line (Fig. 2). The added *Ag. intermedium* chromosome differed in morphology and C-banding pattern from the *Ag. intermedium* chromosome 7Ai#2. It is submetacentric and has a subtelomeric C-band in the short arm and an interstitial and telomeric C-band in the long arm. The homoeologous relationship of the added *Ag. intermedium* chromosome remains to be established. Chromosomes 7B and 2D of T2 were normal and chromosome arm 3BL was different compared to 3BL of 'Heine IV', indicating that this line is not in a pure 'Heine IV' background.

C-banding and GISH analysis of lines T6 and T28 revealed no evidence for the presence of wheat-*Ag. intermedium* translocations. Line T28 was homozygous for T7BS · 2DL and T2DS · 7BL, whereas no translocation was observed in T6.

Meiotic pairing analysis

Meiotic chromosome pairing was analyzed a metaphase-I in PMCs of line T7. A total to 59 PMCs were analyzed and in all cases the telocentric chromosomes were either paired as a rod bivalent (58 PMCs, Fig. 8) or appeared as univalents (one PMC). No pairing was observed between the telocentrics and any other chromosome of the complement. This result is further evidence that the wheat segment in the telocentric translocation chromosome is derived from the distal region of 6DL, which is missing in T6DS · 6DL-7Ai#2L.

Resistance analysis

Lines T4, T7, T24, and T33, all of which have *Ag. intermedium* chromosome segments, gave a; infection type of the leaf rust isolates (Table 2). This resistance must be due to gene *Lr38* (Friebe et al. 1992a). The level of resistance to race 30 in T6 was not as good as in the other lines and must be due to a gene or genes other than *Lr38*. Line T28 was heterozygous for rust reaction. The origin of the resistance in T6 and T28 is not known. *Ag. intermedium* chromosome segments were not found in these lines. An intermediate type of leaf rust resistance was found in the addition line T2 and must be different from that found in the other lines.

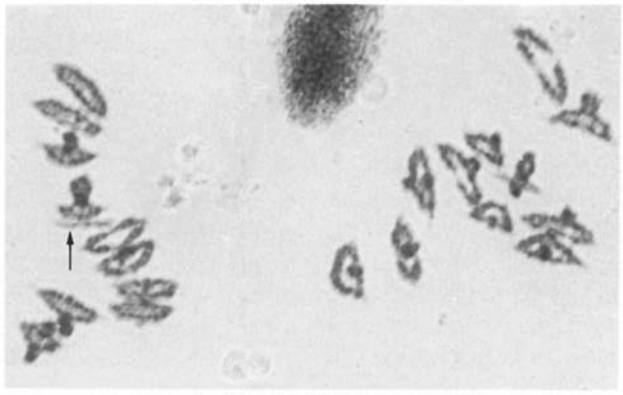

Fig. 8. Aceto-carmine stained meiotic metaphase cell (PMC) of line T7 (*arrow* points to the T6DL-7Ai#2S rod bivalent)

Table 2. Seedling reaction[a] of the wheat-*Ag. intermedium* derived lines to isolates of *P. recondita* tritici and P. *graminis* tritici

Line	P. *recondita* tritici			P. *graminis* tritici			
	Race 1 122-54	Race 30 176-58	91 mix[b]	C17	C10	69-MD 193A	85AF10 -1
T2	2	2	2	1^+	1^+	1^+	3^+
T4	;	;	;	3^+	3^+	3^+	3^+
T6	;	;1	–	3^+	3^+	3^+	3^+
T7	;	;	;	3^+	3^+	3^+	3^+
T24	;	;	;	4	3^+	3^+	3^+
T25	;	;	;	3^+	3^+	3^+	3^+
T28	;&3^+[c]	;&3^+[c]	3^+	4	4	3^+	3^+
T33	;	;	;	4	3^+	3^+	3^+

[a] Infection types: ;=no uredia but hypersensitive flecks; 1=small uredia often surrounded by chlorosis or necrosis; 2=small to medium uredia often surrounded by chlorosis or necrosis, 3=medium uredia often surrounded by chlorosis; 4=large uredia without chlorosis or necrosis; $^+$=uredia somewhat larger than normal

[b] Mixture of isolates of *P. recondita* tritici used in the Winnipeg rust nursery epidemic

[c] This line was heterozygous for the two infection types

– Not tested

All of the lines were susceptible to stem rust except the addition line T2. It was resistant (1^+ infection type) to three of the four isolates. The virulent isolate (85AF10-1) is also virulent on *Sr24* which is completely linked with *Lr24* (McIntosh et al. 1977) and is derived from *Ag. elongatum*. It is unlikely that the genes in the addition line are the same as *Sr24*/*Lr24* since the leaf rust of *L24* is usually a fleck reaction and not the type 2 observed here.

Discussion

GISH and C-banding analyses revealed the presence of wheat-*Ag. intermedium* translocation chromosomes in lines T4, T7, T24, T25, and T33, that were identified as T3DL · 3DS-7Ai#2L, T6DS · 6DL-7Ai#2L, T5AL · 5AS-7Ai#2L, T1DS · 1DL-7Ai#2L, and T2AS · 2AL-7Ai#2L, respectively.

The *Ag. intermedium* segments in these translocations derived from a group 7 chromosome which was designated 7Ai#2 because it differed from 7Ai#1 present in the wheat-*Ag. intermedium* chromosome addition line L1 (=TAF2) produced by Cauderon (Friebe et al. 1992a, b). Chromosome 7Ai#2 compensates for the loss of wheat chromosomes 7A and 7D in 7Ai#2(7A)- and 7Ai#2(7D)-derived chromosome substitution lines [W52 and W44 of Friebe et al. (1992a)]. In addition, 7Ai#2 carried an isozyme marker (endopeptidase-1, EP-1) that further indicates its homoeology to group 7 of the Triticeae.

The present results show that line T2, although described as a translocation line earlier (Wienhues 1973), was a wheat-*Ag. intermedium* chromosome addition line. Morphology, GISH, and C-banding pattern of the added *Ag. intermedium* chromosome present in T2 showed that this chromosome is not related to 7Ai#2. Differences in the leaf rust and stem rust reactions of T2 and lines T4, T7, T24, T25, and T33 further indicate that the resistance gene(s) of line T2 is different from *Lr38* present in the wheat-*Ag. intermedium* translocation lines. The homoeology of this chromosome remains to be established. Furthermore, GISH and C-banding analyses revealed no evidence for the presence of *Ag. intermedium* chromatin in lines T6 and T28. These lines had probably lost their translocation chromosomes during their propagation. The leaf rust resistance of these lines is different from that associated with 7Ai#2 and *Lr38*.

GISH analysis allowed determination of the breakpoints in these translocations and an estimation of the sizes of the transferred *Ag. intermedium* segments (Fig. 9). The breakpoints of all the translocations were not located within the centromeric regions, indicating that they were radiation-induced. T6DS · 6DL-7Ai#2L in line T7 and T5AL · 5AS-7Ai#2L in line T24 have almost the complete 7Ai#2L arm (4.19 μm and 4.20 μm). The *Ag. intermedium* segments present in T3DL · 3DS-7Ai#2L in line T4, T1DS · 1DL-7Ai#2L in line T25, and T2AS · 2AL-7Ai#2L in line T33, derived from the distal region of the 7Ai#2L arm, are 2.78 μm, 2.55 μm, and 2.48 μm in length, respectively. The wheat-*Ag. intermedium* translocation chromosome of line T33 was identical in morphology, C-banding, and GISH pattern to T2AL · 2AL-7Ai#2L, present in line W49, and has been described earlier (Friebe et al. 1992a).

Except in line T4, all the other wheat-*Ag. intermedium* translocation lines were homozygous for a reciprocal T7BS · 2DL and T2DS · 7BL translocation. This translocation was not radiation-induced since it is also present in the parent wheat cv 'Heine IV' (Friebe et al. 1992a). In

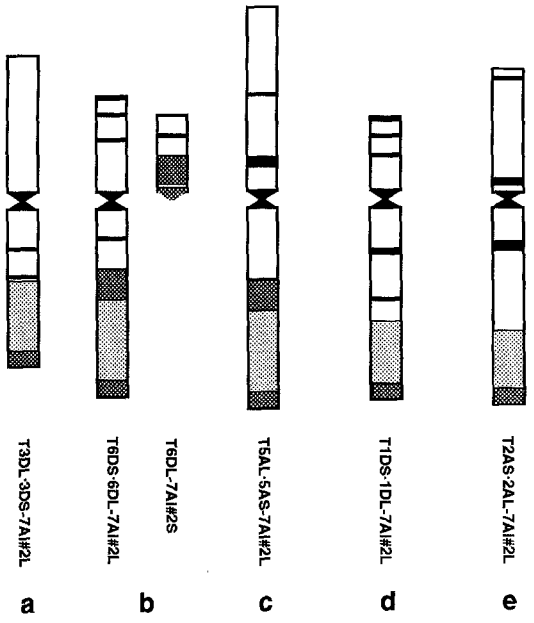

Fig. 9. Idiograms of the radiation-induced wheat-*Ag. intermedium* translocation chromosomes: **a** T4, **b** T7, **c** T24, **d** T25, and **e** T33 [*Ag. intermedium* segments are shown in *light* (unbanded regions) or *dark* (C-banded regions) *hatching*]

addition, line T24 was homozygous for another reciprocal wheat-wheat translocation involving the complete arms of chromosomes 4A and 5D. The T4AS · 5DL and T5DS · 4AL translocation was not present in 'Heine IV' and may, therefore, have occurred during the production of these lines.

All five wheat-*Ag. intermedium* translocation lines were resistant to leaf rust, but susceptible to stem rust. The leaf rust resistance gene in these lines was derived from the *Ag. intermedium* chromosome 7Ai#2 and was designated *Lr38* (Friebe et al. 1992a). 7Ai#2 also conditions resistance to stem rust and stripe rust (Friebe et al. 1992a). The present data indicate that *Lr38* is located in the distal region of the 7Ai-2L arm. Since all five translocation lines were susceptible to stem rust, the corresponding resistance gene(s) has to be located in the short arm of the *Ag. intermedium* chromosome 7Ai#2. The present study shows that line T33 carries the same T2AS · 2AL-7Ai#2L wheat *Ag. intermedium* translocation chromosome as line W49 described earlier (Friebe et al. 1992a). Since W49 was resistant to leaf rust but susceptible to stripe rust and stem rust this locates the stripe rust resistance either in the proximal half of 7Ai#2L or in the 7Ai#2S arm.

Isozyme analysis showed the Ep-Ai1 marker band to be lacking in all of the wheat-*Ag. intermedium* translocation lines (B.P. Forster, personal communication). The *Ep-1* locus is known to be located on the long arm of group 7 chromosomes. Line T7, with almost all the complete 7Ai#2L arm and the proximal region of the 7Ai#2S arm, lacked the Ep-Ai1 isozyme. Therefore, the *Ep-Ai1* locus is located in the chromosome segment distal to the proximal C-band of the 7Ai#2S arm. Furthermore, this indicates that at least part of the shorter arm of 7Ai#2 is homoeologous to the long arm of group 7 chromosomes. A similar situation was reported recently for wheat chromosome 7D. Here, RFLP analysis showed that the shorter arm of 7D is homoeologous to the long arms of chromosomes 7A and 7B (Werner et al. 1992).

The chromosomes involved in all these wheat-*Ag. intermedium* translocations belong to different homoeologous groups. Thus, the transferred *Ag. intermedium* chromatin cannot compensate for the missing wheat segments in these lines. The sizes of the missing wheat segments range from 0.67 μm in T3DL · 3DS-7Ai#2L up to 1.45 μm in T6DS · 6DL-7Ai#2L which may cause a reduction in plant vigor.

X-ray treatment, as a method to induce small wheat-alien chromosome translocations, was first used by Sears (1956) to transfer a leaf rust resistance gene (*Lr9*) from *Aegilops umbellulata* to wheat. Later, radiation-induced transfers of resistance genes were also reported from *Agropyron elongatum* (*Sr25, Sr26, Lr19*) (Knott 1964; Sharma and Knott 1966), *Agropyron intermedium* (*Lr38, Wsm1*) (Friebe et al. 1991a, 1992a) and from cultivated rye, *Secale cereale* (*Lr25, Pm7, Pm17, H25*) (Driscoll 1968; Heun et al. 1990; Friebe et al. 1991b). Although several wheat-alien chromosome translocations were recovered in these experiments, only those involving homoeologous chromosomes were favorable (Sharma and Knott 1966; Sears 1972).

Most radiation-induced wheat-alien transfers analyzed in detail were identified as terminal translocations. So far only one case of a radiation-induced intercalary translocation was reported where an 0.7 μm segment derived from rye chromosome arm 6RL, and carrying the Hessian fly resistance gene H25, was inserted into the long arm of wheat chromosome 4A (Friebe et al. 1991b; Mukai et al. 1993). The available data, including those reported here, indicate that radiation-induced breakpoints occur at random. They do not occur more frequently in homoeologous chromosomes nor is their intrachromosomal distribution related to the presence or absence of constitutive heterochromatin.

Another approach for introducing alien genetic material to wheat was first used by Riley et al. (1968), who transferred a yellow rust resistance gene (*Yr8*) from *Aegilops comosa* to wheat by induced homoeologous pairing and recombination. In the absence of the *Ph1* gene, homoeologous pairing and recombination can occur among wheat and alien chromosomes of related species (Sears 1973, 1977). One advantage of induced homoeologous recombination is that all the resulting wheat-alien translocations are between homoeologous chromosomes. Therefore, the transferred alien chromat-

in can compensate for the loss of the missing wheat segments. However, this approach seems to be work only for genes that are located in the distal half of chromosome arms. As has been shown cytologically (Curtis et al. 1991) and by RFLP analysis (Werner et al. 1992), crossing over in wheat is greatly reduced in the proximal half of chromosome arms. Thus, the chances of transferring a proximally located alien gene by induced homoeologous recombination are very low. In such cases, radiation treatment, which produces randomly distributed translocation breakpoints, might be the better choice. However, most of the resulting wheat-alien translocations will be noncompensating and appropriate protocols must be developed to favor selection for compensating types of translocations (Sears 1993).

Acknowledgements. We thank John Raupp and Duane Wilson for their excellent technical assistance. Cooperative investigations of the Kansas Agricultural Experiment Station, the USDA-ARS, and Agriculture Canada. Contribution no. 92-620-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan. This research was supported in part by a USDA-CSRS special research grant 'Wheat Genetics Resource Center' at Kansas State University.

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