Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat

Uwe Hohmann, Katia Badaeva, Winfried Busch, Bernd Friebe, and Bikram S. Gill

Abstract: Nine families of bread wheat (TC5, TC6, TC7, TC8, TC9, TC10, TC14, 5395-(243AA), and 5395) with resistance to barley yellow dwarf virus and containing putative translocations between wheat and a group 7 chromosome of *Agropyron intermedium* (L1 disomic addition line, 7Ai#1 chromosome) induced by homoeologous pairing or tissue culture were analyzed. C-banding, genomic in situ hybridization (GISH), and restriction fragment length polymorphism (RFLP) in combination with repetitive *Agropyron*-specific sequences and deletion mapping in wheat were used to determine the relative locations of the translocation breakpoints and the size of the transferred alien chromatin segments in hexaploid wheat–*Agropyron* translocation lines. All homoeologous compensating lines had complete 7Ai#1 or translocated 7Ai#1–7D chromosomes that substitute for chromosome 7D. Two complete 7Ai#1 (7D) substitution lines (5395-(243AA) and 5395), one T1BS–7Ai#1S·7Ai#1L addition line (TC7), and two different translocation types, T7DS–7Ai#1S·7Ai#1L (TC5, TC6, TC8, TC9, and TC10) and T7DS·7DL–7Ai#1L (TC14), substituting for chromosome 7D were identified. The substitution line 5395-(243AA) had a reciprocal T1BS·1BL–4BS/T1BL–4BS·4BL translocation. TC14 has a 6G (6B) substitution. The RFLP data from deletion mapping studies in wheat using 37 group 7 clones provided 10 molecular tagged chromosome regions for homoeologous and syntenic group 7 wheat or *Agropyron* chromosomes. Together with GISH we identified three different sizes of the transferred *Agropyron* chromosome segments with approximate breakpoints at fraction length (FL) 0.33 in the short arm of chromosome T7DS–7Ai#1S·7Ai#1L (TC5, TC6, TC8, TC9, and TC10) and another at FL 0.37 of the nonhomoeologous translocated chromosome T1BS–7Ai#1S·7Ai#1L (TC7). One breakpoint was identified in the long arm of chromosome T7DS·7DL–7Ai#1L (TC14) at FL 0.56. We detected some nonreciprocal translocations for the most proximal region of the chromosome arm of 7DL, which resulted in small duplications.

Key words: C-banding, genomic in situ hybridization (GISH), physical mapping, translocation mapping, RFLP analysis.

Résumé : Les auteurs ont analysé neuf familles de blé tendre (TC5, TC6, TC7, TC8, TC9, TC10, TC14, 5395-(243AA) et 5395) montrant une résistance au VJNO (virus de la jaunisse nanisante de l'orge) suite à des translocations putatives entre le blé et un chromosome du groupe 7 de l'*Agropyron intermedium* (lignée d'addition disomique L1, chromosome 7Ai#1). Ces translocations auraient été induites par recombinaison homéologue ou par suite de la culture de tissus. Afin de déterminer la taille et l'emplacement du segment de chromatine étrangère chez ces lignées, les auteurs ont réalisé des colorations des bandes C, des hybridations génomiques in situ (GISH) et des analyses RFLP. Toutes les lignées possédaient soit un chromosome 7Ai#1 entier ou un chromosome 7Ai#1–7D à la place du chromosome 7D. Deux lignées de substitution 7Ai#1 (7D) (5395-(243AA) et 5395), une lignée d'addition T1BS–7Ai#1S·7Ai#1L (TC7) ainsi que deux types de translocations, T7DS–7Ai#1S·7Ai#1L (TC5, TC6, TC8, TC9, et TC10) et T7DS·7DL–7Ai#1L (TC14) ont été identifiées. La lignée de substitution 5395-(243AA) contient une translocation réciproque T1BS·1BL–4BS/T1BL–4BS·4BL. La lignée TC14 a une substitution 6G (6B). Les résultats des analyses RFLP avec 37 clones du groupe 7 ont permis d'identifier 10 régions chromosomiques homéologues et syntènes chez les chromosomes du groupe 7 chez le blé et l'*Agropyron*. Grâce à l'hybridation in situ, trois segments chromosomiques d'*Agropyron* de tailles différentes ont pu être identifiés. Les points d'échange chez ces translocations sont situés à 0,33 (fraction de la longueur) du bras court du chromosome T7DS–7Ai#1S·7Ai#1L (TC5, TC6, TC8, TC9, et TC10) et à 0,37 sur la translocation non-homéologue T1BS–7Ai#1S·7Ai#1L (TC7). Un point d'échange a été identifié à 0,56 sur le bras long du

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chromosome T7DS·7DL–7Ai#1L (TC14). Quelques translocations non-réciproques ont également été identifiées dans les régions les plus proximales du bras 7DL, donnant ainsi lieu à de petites duplications.

Mots clés : bandes C, hybridations génomiques in situ (GISH), cartographie physique, cartographie de translocations, analyses RFLP.

[Traduit par la Rédaction]

Introduction

The domestication and cultivation of wheat as one of the most important crop species in the world for more than 10 000 years and its ongoing improvement continually require the transfer of genetic traits from alien species into wheat breeding populations. The genetic variation from *Agropyron* introduced into wheat has substantially contributed towards extending the wheat gene pool by accumulating resistance or tolerance genes to rusts (Sharma and Knott 1966; Wienhues 1966; Cauderon et al. 1973; Friebe et al. 1992*b*, 1993, 1994; Jiang et al. 1994), wheat streak mosaic (Friebe et al. 1991; Jiang et al. 1993) and barley yellow dwarf viruses (Brettell et al. 1988; Larkin et al. 1995; Sharma et al. 1984, Sharma et al. 1995; Xin et al. 1988), and salt stress (Dewey 1960; McGuire and Dvořák 1981; Forster et al. 1988; Littlejohn 1988).

Worldwide, barley yellow dwarf virus (BYDV), vectored by several aphid species, is likely the most destructive disease of wheat and other cereal crops (for review see Pike 1990). All wheat is vulnerable to BYDV infection and crop losses, and only one gene that confers tolerance (reduced infection) has been reported (Singh et al. 1993). However, several perennial Triticeae species within *Agropyron* contain potent resistance genes to BYDV infection that work by interfering with virus replication (Sharma et al. 1984). Brettell et al. (1988) reported a major BYDV resistance on the *Agropyron intermedium* (syn. *Thinopyrum intermedium* (Host) Barkworth and Dewey) chromosome transferred to wheat in the form of a disomic addition line, L1, produced by Cauderon (1966). This chromosome was designated as 7Ai#1 (Friebe et al. 1992*a*).

Recently, Banks et al. (1995) reported the transfer of 7Ai#1 chromatin specifying BYDV resistance to wheat chromosomes by tissue culture. They described eight lines (TC5, TC6, TC7, TC8, TC9, TC10, TC14, and 5395) with resistance to BYDV that contained putative wheat–*Agropyron* translocations. Although these lines were characterized by cytological and molecular analysis, more detailed molecular cytogenetic characterization of the lines has been found to be necessary in terms of identifying the agronomically most promising material.

Several detection systems are used to assay transferred alien chromatin in wheat. C-banding distinguishes the chromosomes of *Agropyron* and wheat (Gill et al. 1991; Friebe et al. 1992*a*). However, small chromosome segment transfers lacking C-bands are difficult to detect, especially when they replace unbanded chromosome regions in the wheat complement. Therefore, alternative techniques, for example, nonisotopic in situ hybridization (ISH) (Rayburn and Gill 1985), genomic in situ hybridization (GISH) (Anamthawat-Jónsson et al. 1990; Mukai and Gill 1991; Schwarzacher et al. 1992), and fluorescence in situ hybridization (FISH) (Leitch et al. 1991, 1994) have been developed and used to characterize alien chromatin transfers. Hybridization with dispersed "species-specific" or "species-amplified" sequences by Southern analysis (Appels et al. 1986; Guidet et al. 1991; McNeil et al. 1994; Zhang and Dvořák 1990*a*) or by ISH (Lapitan et al. 1986) and chromosome region specific RFLP (restriction fragment length polymorphism) markers (Kim et al. 1993; Banks et al. 1995) are helpful in identifying small introgressed alien chromatin segments.

This paper describes the detailed physical location and size of the transferred BYDV resistant *A. intermedium* chromosome segments in nine independently produced families of bread wheat (Banks et al. 1995) by means of C-banding, *Agropyron*-amplified repetitive DNA sequences, RFLP, comparative physical deletion mapping, and GISH.

Materials and methods

Plant materials

Seeds of nine heterozygous and (or) homozygous BYDV resistant lines (TC5, TC6, TC7, TC8, TC9, TC10, TC14, 5395-(243AA), and 5395), wheat varieties 'Sunstar', 'Millewa', Vulcan cms, and Restorer R37532E, and the ditelosomic *A. intermedium* addition line 7Ai#1L were kindly provided by P.M. Banks, P.J. Larkin, and R.A. McIntosh and are designated as mentioned by Banks et al. (1995). The TC lines were regenerated from tissue-cultured immature embryos or spikes of monosomic additions of *A. intermedium* chromosome 7Ai#1 to wheat; the other two lines were selected subfamilies from *ph*-induced recombination experiments. A morphological short-arm marker (red coleoptile), repetitive DNA sequences, RFLP markers and an ELISA (enzyme-linked immunosorbent assay) test to screen for BYDV resistance were used to identify *A. intermedium* translocation chromosomes. The source of the BYDV resistance is a group 7 *A. intermedium* chromosome that was transferred to wheat by Cauderon and coworkers (Cauderon 1966; Cauderon et al. 1973). Banks et al. (1995) claimed that the portion of the translocated alien chromatin was smaller than a chromosome arm based on a missing RFLP marker and loss of the red coleoptile marker, as well as the reduced hybridization to a repetitive *Agropyron* amplified DNA sequence. All lines, except TC7, were the result of a translocation between chromosome 7Ai#1 and chromosome 7D of wheat. The C-banding of the 7Ai#1 chromosome was performed on the L1 addition line (accession No. TA3647, Wheat Genetics Resource Center (WGRC), Kansas State University, Manhattan, Kans.) and the double ditelosomic lines 7Ai#1S (accession No. TA3656 from WGRC; TAF2D from Y. Cauderon) and 7Ai#1L (120.N.I.5, produced and provided by P.M. Banks and P.J. Larkin).

GISH

Seed germination and the drop preparation of chromosomes followed the protocol of Busch et al. (1994). The probes were labelled by nick translation with biotin-16-dUTP (Boehringer Mannheim) following the instructions of the supplier. The in situ hybridization signal detection was performed according to

Fig. 1. Detection of *Agropyron* chromatin using GISH. (*a*) T1BS–7Ai#1S·7Ai#1L wheat – *A. intermedium* addition line TC7. (*b*) T7DS–7Ai#1S·7Ai#1L wheat – *A. intermedium* translocation line TC8. (*c*) T7DS·7DL–7Ai#1L wheat – *A. intermedium* translocation line TC14. (*d*) 7Ai#1 (7D) wheat – *A. intermedium* substitution line 5395. (*e*) Ditelosomic addition line of *A. intermedium* 7Ai#1L. Scale bar = 8 μm.

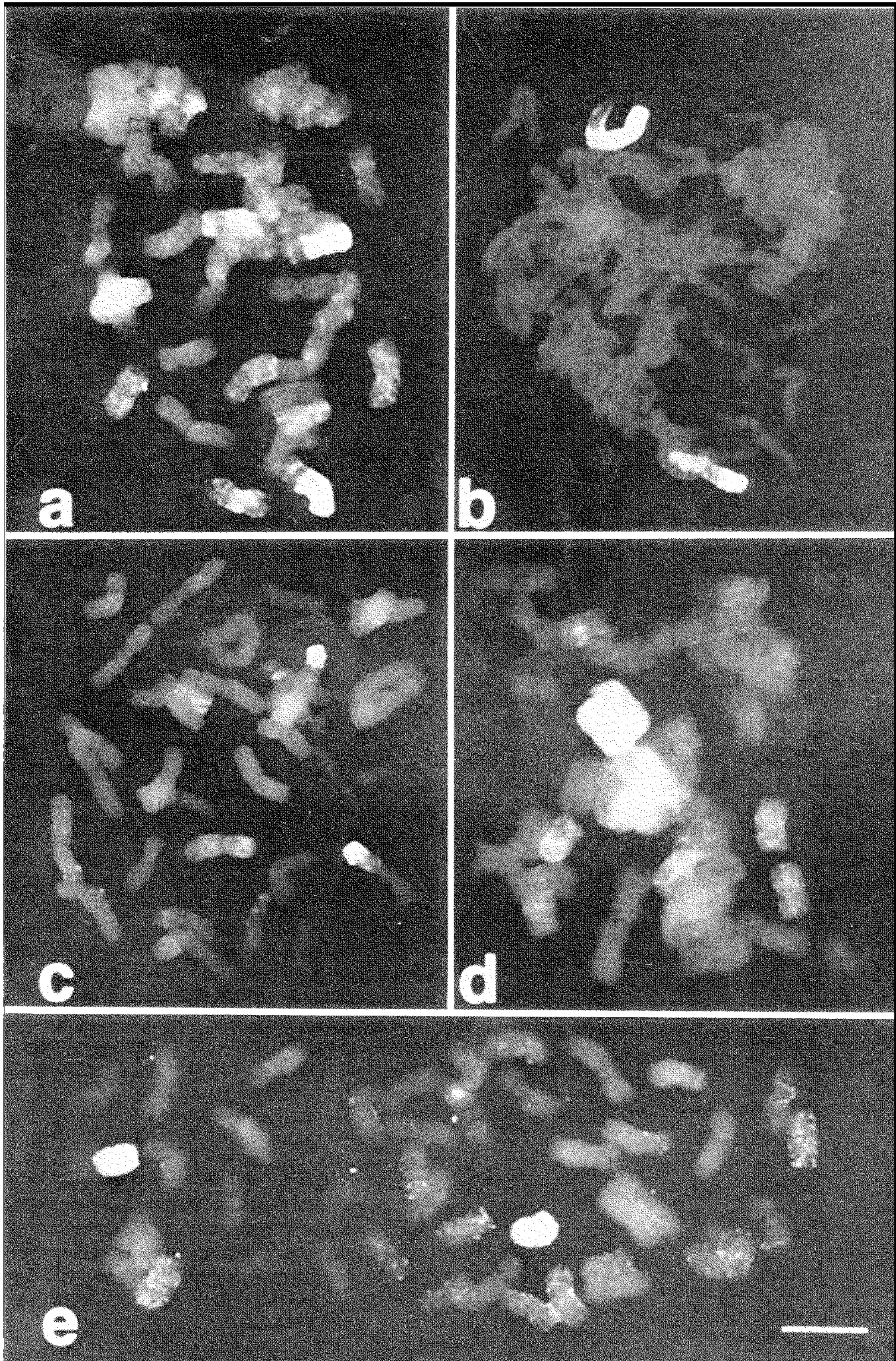
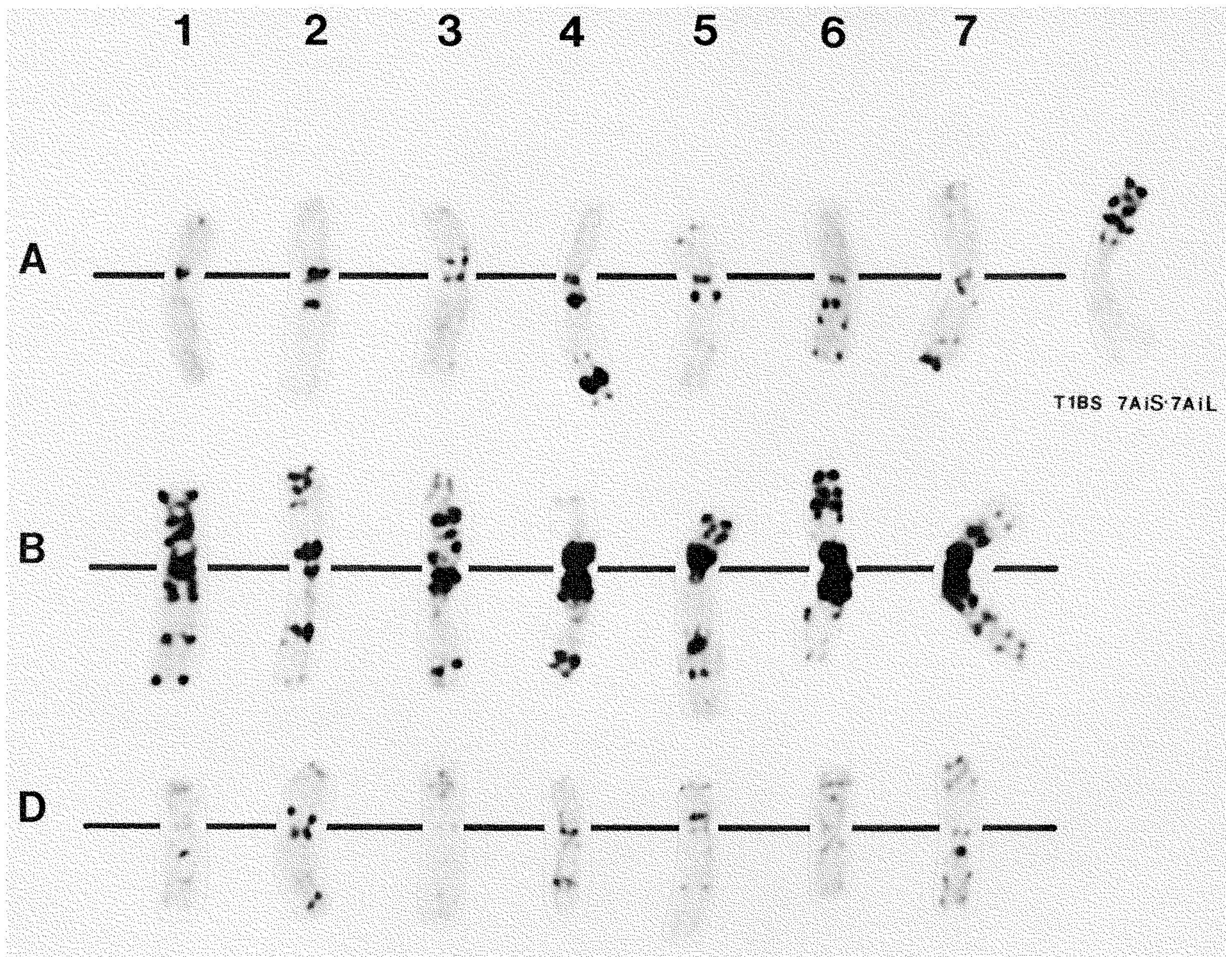


Fig. 2. C-banded karyotype of the T1BS–7Ai#1S·7Ai#1L wheat – *A. intermedium* addition line TC7.



Busch et al. (1995) with some minor modifications. The temperature for chromosome denaturation was lowered to 60°C to improve the chromosome morphology. The hybridization mixture (15 μL; 60% formamide, 10% dextran sulfate, 2× SSC (1× SSC: 0.15 M NaCl plus 0.015 M sodium citrate)) contained 4 ng/μL of labelled *A. intermedium* genomic DNA and a 100-fold excess of unlabelled sonicated 'Chinese Spring' DNA. The post-hybridization washes allowed binding of the probe with homologies of approximately 70 or 80%. The stringency conditions were calculated according to Meinkoth and Wahl (1984). The specimens were finally mounted in Vectashield (Vector) antifading medium. The breakpoint was determined by measuring the labelled region of the translocated chromosome arm of from five to eight metaphases. The length was calculated as a percentage of the length of the opposite chromosome arm, as the 7Ai#1 chromosome is almost metacentric with an arm ratio of 1.1 (Friebe et al. 1992*a*).

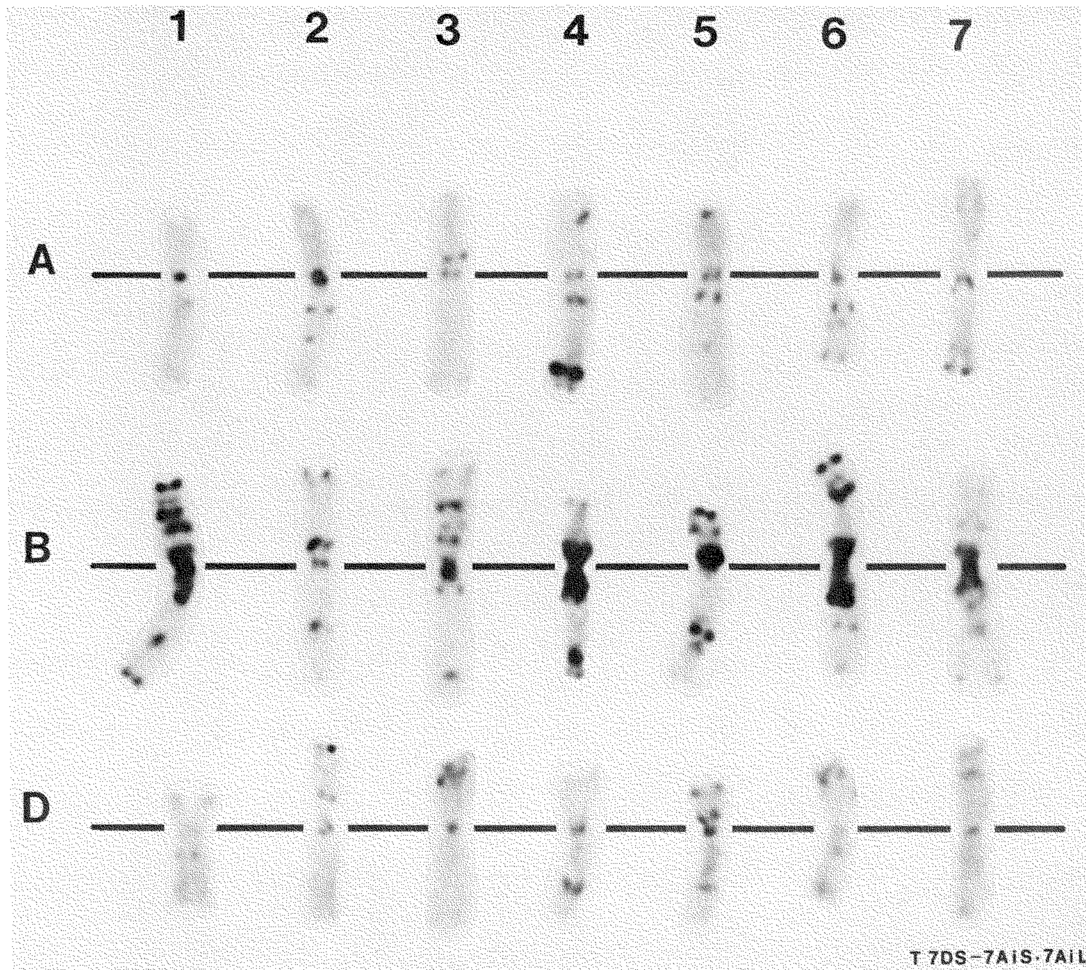
Microscopy
Specimens were examined using a Zeiss Axioplan epifluorescence microscope equipped with the filter sets 487901 (DAPI (4′,6-diamidino-2-phenylindole)), 487917 (FITC (fluorescein isothiocyanate)), and a Cy3-specific filter set supplied by AHF analysentechnik (Tübingen). Pictures of counterstained chromosomes and probe signals were taken separately with a CCD (charge coupled device) camera (Photometrics, Tucson, Ariz., U.S.A.) and processed using the IPLAB SPECTRUM and MULTIPROBE software (Signal Analytics Corp., Vienna, Va., U.S.A.).

C-banding
The chromosomes of all lines were C-banded and identified according to Gill et al. (1991). The physically shorter arm of 7D (7DS) is genetically 7DL and is homoeologous to chromosome arms 7AL and 7BL. In the present paper the generally accepted genetic nomenclature is used to describe the group 7 arms.

Genomic DNA extraction and Southern hybridization
The extraction of genomic DNA from leaf tissue of the deletion and aneuploid stocks followed the procedure of Appels and Moran (1984). Ten-microgram samples of total genomic DNA digested with *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII restriction endonucleases were electrophoresed on 1% agarose gels and blotted onto Nylon membranes. Random priming and hybridization were performed as previously described (Hulbert et al. 1990; Lagudah et al. 1991).

RFLP probes were generously provided by M. Gale, Cambridge Laboratory, Norwich, U.K. (designated PSR), K.S. Gill, Kansas State University, Manhattan, Kans., U.S.A. (designated KSU), M. Sorrells, Cornell University, Ithaca, N.Y., U.S.A. (designated BCD, CDO, and WG), E.S. Lagudah, Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia (designated CS), and A. Graner,

Fig. 3. C-banded karyotype of the T7DS–7Ai#1S·7Ai#1L wheat – *A. intermedium* translocation line TC8.



Institut für Resistenzgenetik, Grünbach, Germany (designated MWG). The plasmid A600 carries a dispersed repetitive sequence that is amplified in *Agropyron* relative to wheat (U. Hohmann, R. Appels, H. Ohm, and L. Hogie, in preparation).[1]

Results

GISH

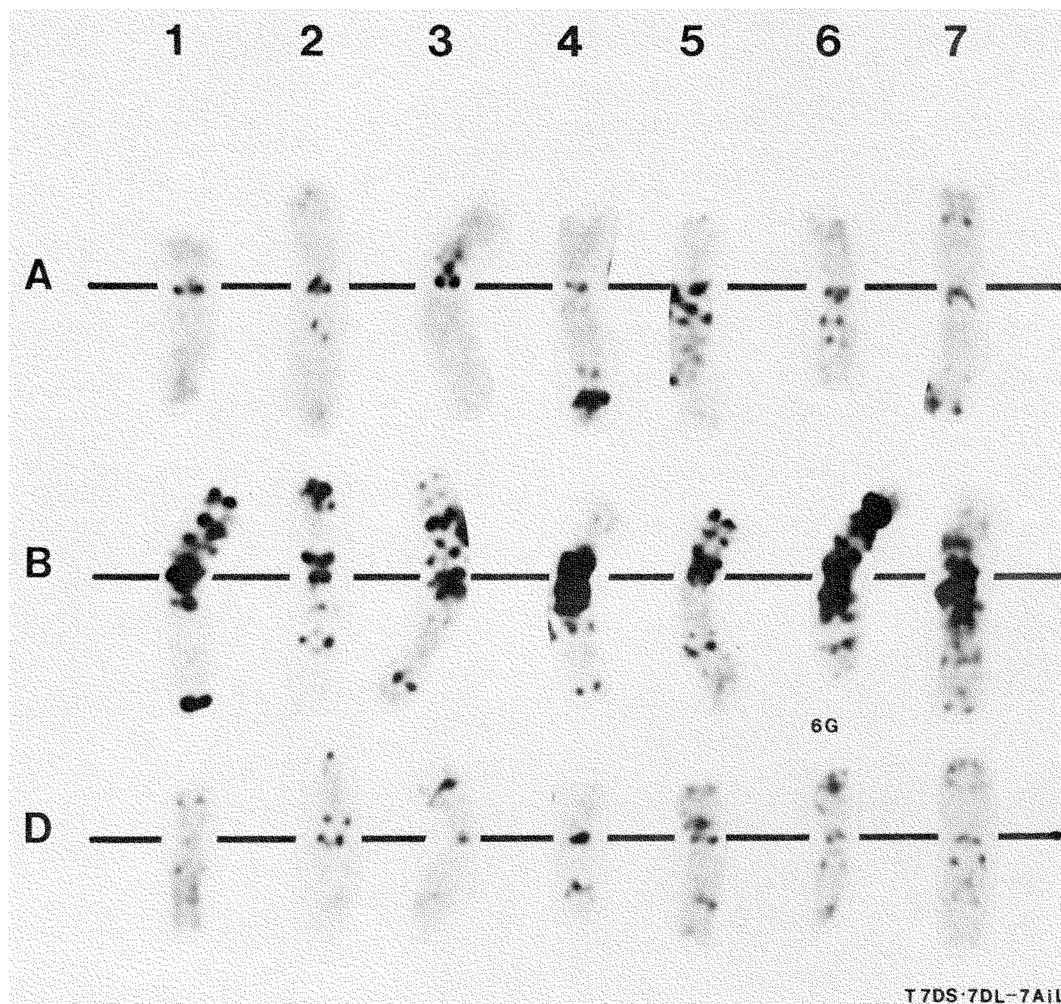
The concentrations of labelled *Agropyron* DNA and unlabelled blocking DNA of wheat were critical for obtaining a clear differentiation between wheat and *Agropyron* DNA. In all lines examined, *Agropyron* chromatin was present. The line TC7 carries a translocated chromosome with the breakpoint at fraction length (FL) 0.37 in the short arm (Fig. 1*a*). The proximal part of the short arm and the entire long arm was uniformly labelled. In line TC8 the breakpoint of the chromosome is also located in the short arm, but at FL 0.33 (Fig. 1*b*). A characteristic feature of this chromosome is the heavily labelled distal part (48%) of the long arm, whereas the labelling of the remaining *Agropyron* chromatin is less intense. In line TC14, the breakpoint is located at FL 0.56, which indicates that the distal 44% of the long arm originates from *A. intermedium* (Fig. 1*c*). In line 5395 a completely labelled pair of *Agropyron* chromosomes substitutes for a pair of wheat chromosomes (Fig. 1*d*). The ditelosomic long arm of 7Ai#1 can be easily identified in a wheat background, but one set of small wheat chromosomes shows weak labelling as well (Fig. 1*e*).

C-banding analysis

The C-banding of TC7, TC8, TC14, and 5395 showed that the 7Ai#1 chromosome was involved in translocations with chromosome 7D. The C-banding pattern is in good agreement with the allocation of the breakpoints from the GISH experiments. The line TC7 was identified as an addition–translocation line with a translocated T1BS–7Ai#1S·7Ai#1L chromosome (Fig. 2). The five lines TC5, TC6, TC8, TC9, and TC10 each carried a pair of T7DS–7Ai#1S·7Ai#1L translocation chromosomes substituting for chromosome 7D

[1] U. Hohmann, R. Appels, H. Ohm, and L. Hogie. Amplification of DNA sequences in wheat and its relatives. II. The Acc2 family of *Agropyron* (syn. *Thinopyrum*) amplified sequences and their use for tracing *Agropyron*-derived BYDVR genes in wheat. In preparation.

Fig. 4. C-banded karyotype of the T7DS·7DL–7Ai#1L wheat – *A. intermedium* translocation line TC14.



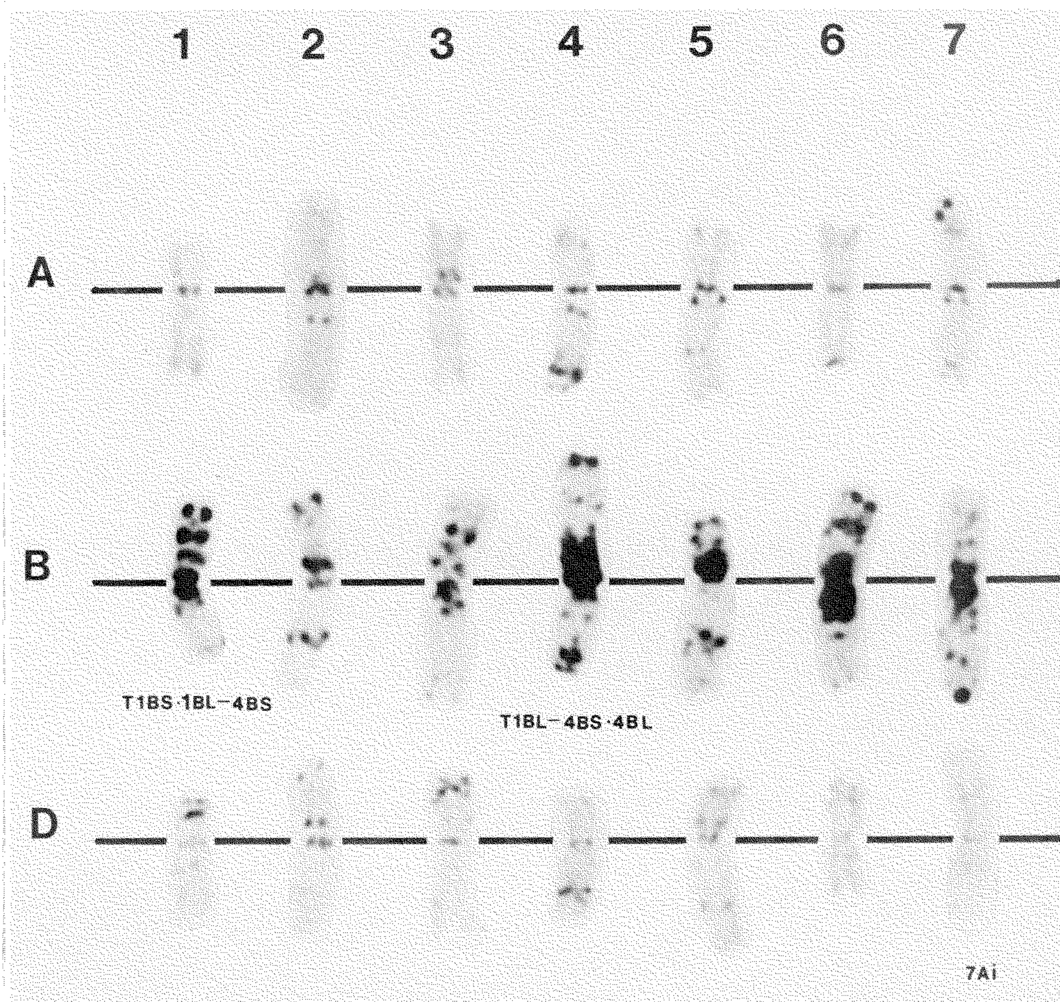
(Fig. 3). The C-banding pattern of the chromosome was identical in all five lines. A translocation T7DS·7DL–7Ai#1L chromosome substituted for chromosome 7D in line TC14 (Fig. 4). This line has a pair of *Triticum timopheevii* chromosomes, 6G, which was most likely introgressed during the backcross procedure and which substitutes for chromosome 6B. Lines 5395-(243AA) and 5395 are 7Ai#1 (7D) substitution lines. One substitution line (5395-(243AA)) had a reciprocal T1BS·1BL–4BS/T1BL–4BS·4BL translocation (Fig. 5). This translocation is not present in line 5395 (Fig. 6). The C-banding of the L1 line chromosomes proved that the 7Ai#1 chromosome is almost metacentric (arm ratio 1.1). Very small C-bands were present in the proximal half of the long arm and the distal half of the short arm (Fig. 7) of this chromosome.

RFLP analysis

Any *Agropyron* chromatin in the wheat background was identified with RFLP analysis and the use of the *Agropyron* dispersed repetitive DNA sequence A600. The probe hybridizes to one wheat and one *Agropyron* band in Southern blots of total genomic plant DNA digested with *Eco*RV (Fig. 8*a*). The upper diagnostic wheat band can be used as an internal standard for measuring the amount of DNA transferred to the membrane. The intensity of the *Agropyron* band reflects the relative amount of introgressed *Agropyron* chromatin, as this band is almost absent in wheat. Compared with all other lines, the band in TC14 is less intense. The A600 sequence is capable of identifying subchromosomal *Agropyron* chromatin in wheat.

Thirty-seven low-copy RFLP probes of homoeologous group 7 chromosomes were tested to produce restriction fragments characteristic for *Agropyron*. Ten of the genomic or cDNA clones from *Triticum aestivum*, *Triticum tauschii*, *Hordeum vulgare*, or *Avena sativa* revealed polymorphism by generating a single restriction fragment specific for each of the chromosomes 7A, 7B, 7D, and 7Ai#1. The precise comparison of the band intensities in Southern analysis proved or allowed estimation of the nullisomic, monosomic, or disomic chromosome constitution in the tested lines. The clones were assigned to chromosomes or chromosome segments by the analysis of aneuploid lines

Fig. 5. C-banded karyotype the 7Ai#1 (7D) wheat – *A. intermedium* substitution line 5395.



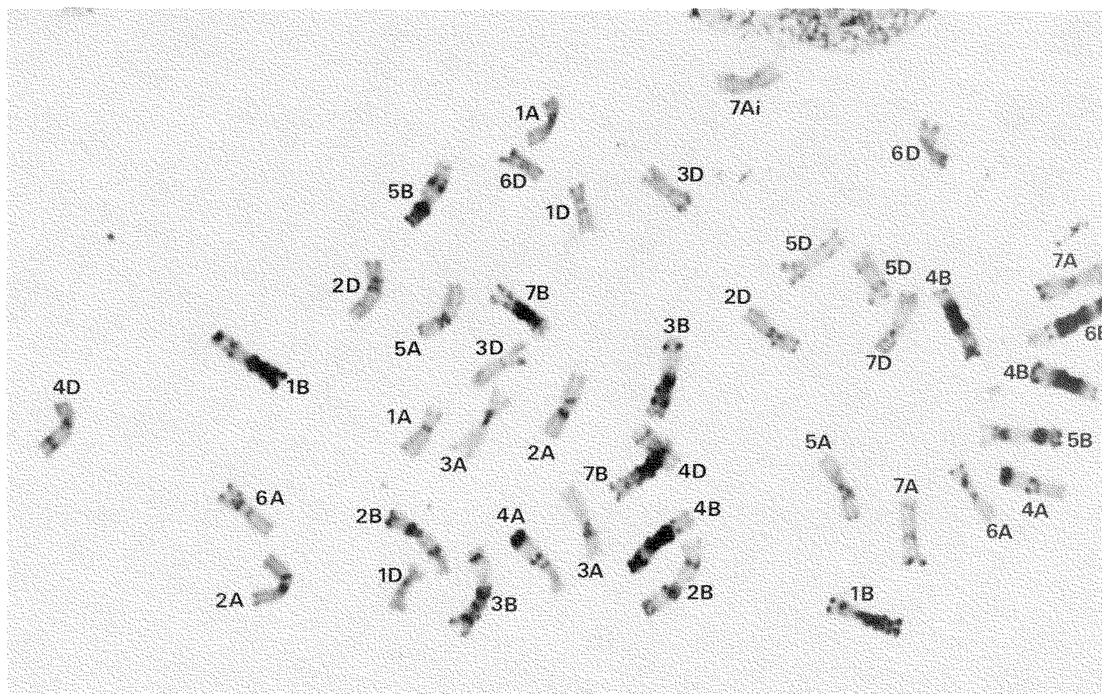
and deletion stocks in wheat. Three probes that were polymorphic for chromosome 7Ai#1 were allocated in three different proximal or terminal chromosome regions in the short arm of chromosomes 7A, 7B, and (or) 7D (Fig. 9). The remaining seven long arm markers were evenly distributed to chromosome regions along the arm. Based on the general synteny among the Gramineae it became evident that chromosome 7Ai#1 of *A. intermedium* is syntenic to group 7 chromosomes of hexaploid wheat. However, none of the markers that were mapped in the interstitial chromosome region between FLs of approximately 0.37 and 0.88 (short arm) or 0.45 and 0.76 (long arm) in wheat were found to be polymorphic for 7Ai#1. In comparison with C-banding, the physically shorter arm of 7Ai#1 is genetically 7Ai#1L and is homoeologous to chromosome arms 7AL and 7BL. The RFLP analysis of F_1 recombinant lines TC5, TC6, TC7, TC8, TC9, TC10, and 5395 and 26 subfamilies revealed no clear differences between these lines except for lines 5395 and TC7. In the TC7 line some plants were segregating for the *Agropyron*-specific RFLP obtained with BCD1338. Therefore, we analyzed four different homozygous lines, TC7, TC8, 5395, and TC14, in detail.

Detailed molecular characterization of the lines

In line TC7 all chromosome specific restriction fragments for the A and D genomes were detected in disomic conditions. The interstitial region of the long arm of chromosome 7B that carries the two markers WG466 (Fig. 8*b*) and WG719 was only present in one copy. The deficiency is compensated for by an additional translocated wheat–*Agropyron* chromosome (Fig. 2). This chromosome is highly recombined with two copies of the proximal short arm and distal long arm segments from 7Ai#1 and the one copy of the interstitial long arm region (Fig. 10). The distal short arm region of the chromosome is 1B. Deletion mapping allocated the breakpoint in the short arm region to between FL 0.0 and FL 0.37. GISH analysis showed that the location of the breakpoint is at FL 0.37.

Line TC8 has a T7DS·7Ai#1S–7Ai#1L (7D) substitution. From deletion mapping, the location of the breakpoint was found to be in the short arm proximal to FL 0.84 (Fig. 10). GISH showed that the actual breakpoint is at FL 0.33. Two long arm chromosome regions of 7D, which contain the loci for the molecular markers WG466 (Fig. 8*b*) and CDO1199 (Fig. 8*c*), harbor both the *Agropyron* specific and

Fig. 6. C-banded mitotic metaphase chromosomes from a $2n$ = 42 chromosome plant of a F_1 hybrid *T. aestivum* cv. Sunstar × 5395.

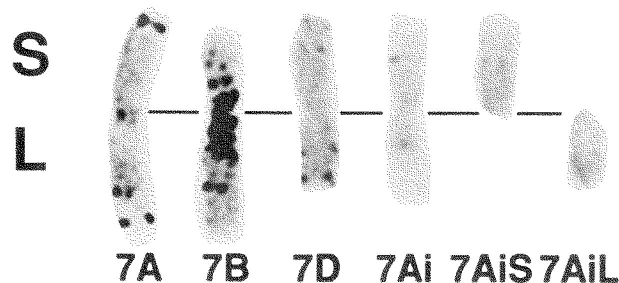


D genome specific RFLP markers. These interstitial regions of chromosomes 7D and 7Ai#1 were present in the translocated chromosome as "duplications." The *Agropyron* specific restriction fragment for the clone KSUD2 is lacking but that for the most distal marker, CSIH81, is present in line TC8. Therefore, a small deletion in the chromosome region of FL 0.94–0.99 in the long arm is possible. The differences in GISH intensities in the long arm of the chromosomes support the possibility of a recombined chromosome arm that carries chromatin from 7Ai#1L and introgressed chromatin from 7DL via unequal crossing-over events.

TC14 carries the smallest *Agropyron* segment of the translocation lines. All short arm markers and the proximal long arm markers of chromosome 7D were present. The molecular marker WG466, which mapped in the chromosome region of FL 0.30–0.61 on the long arm of chromosome 7D, was present (Fig. 8*b*), whereas the marker PSR129 that mapped in the interval FL 0.76–0.82 was absent (not shown). The distal chromosome region was replaced by the syntenic *Agropyron* chromosome segment. Therefore, the translocation breakpoint must have been in the region between FL 0.30 and 0.82. GISH verified the location of the breakpoint at FL 0.56. The only modification detected in homoeologous group 7 chromosomes with molecular markers was the absence of the restriction fragment for BCD1338, which is characteristic for chromosome 7A in *Dra*I-digested genomic DNA of TC14 (not shown).

Line 5395 has a complete 7Ai#1 (7D) substitution. All 10 molecular markers, including the distal short arm marker MWG905 (Fig. 8*d*), detected *A. intermedium* chromatin that replaced chromosome 7D. However, the interstitial chromosome region of 7D (FL 0.30–0.61) that carries the long arm marker WG466 was observed in one copy (Fig. 10).

Fig. 7. C-banding of the wheat chromosomes 7A, 7B, 7D, *A. intermedium* chromosome 7Ai#1, and the telosomes 7Ai#1S and 7Ai#1L.

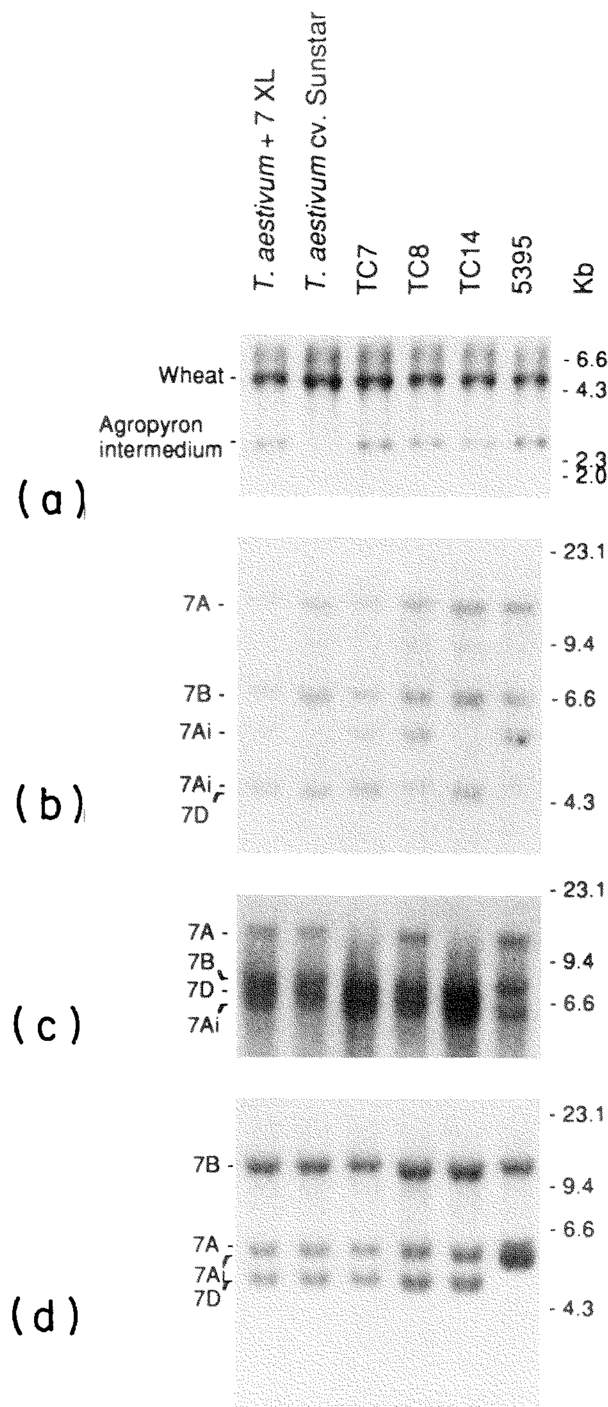


Discussion

Assaying *Agropyron* chromatin in wheat

For the improvement of wheat it is necessary to limit the size of transferred alien chromatin. The restriction fragments specific for *Agropyron* segments can be used as markers to identify the chromatin related to the BYDV resistance gene(s) introduced into wheat breeding populations, especially in the absence of disease or virulence in the environment. The evaluation of landmark loci for homoeologous group 7 chromosomes (Hohmann et al. 1995) covering the entire *Agropyron* chromosome makes it possible to analyze any breeding material with a limited number of RFLP probes and restriction assays of genomic DNA. Southern analysis with repetitive *Agropyron* amplified sequences will allow a rough estimation of the size and chromosomal compensation of any transferred *Agropyron* chromatin into

Fig. 8. Hybridization to genomic DNA using the *Agropyron* amplified sequence A600 (*a*), wheat genomic clone WG466 (*b*), oat cDNA clone CDO1199 (*c*), and barley genomic DNA clone MWG905 (*d*). DNA of the ditelosomic long arm addition line of chromosome 7Ai#1 (7Ai#1L; *T. aestivum* + 7XL), *T. aestivum* cv. Sunstar, TC7, TC8, TC14, and 5395 was digested with the restriction endonucleases *Eco*RV (*a*), *Bam*HI (*b*), *Hin*dIII (*c*), and *Eco*RI (*d*). The chromosome origin of the bands was determined from nullitetrasomic lines and the *A. intermedium* 7Ai#1 addition line to wheat.



wheat. For the molecular marker assisted selection of resistance genes introduced from wild species into wheat (Kim et al. 1993; Autrique et al. 1995), the synteny among the Triticeae will be an extremely helpful tool, as demonstrated in the present paper, by constructing integrative physical maps using the syntenic relationships of homoeologous chromosomes between different Triticeae species (Hohmann et al. 1995).

The presence and absence of chromosome-specific RFLPs indicated that the tissue-culture induced wheat–*Agropyron* breakpoints occurred in interstitial regions of the short and long arms in eight lines. It is noteworthy, that there was no difference in the translocated chromosome T7DS–7Ai#1S·7Ai#1L in the analyzed lines TC5, TC6, TC8, TC9, and TC10. A recombined chromosome having most of the *Agropyron* chromatin was of advantage under the selection pressure during the development of the substitution lines. The material was selected against the red coleoptile marker on chromosome 7Ai#1S. As the translocated chromosomes in TC7 and TC8 have breakpoints at FL 0.37 and 0.33, respectively, the morphological marker is distal to FL 0.37. However, line 5395, which has a whole 7D (7Ai#1) substitution, has also lost the red coleoptile marker. At the moment, we have no explanation for this phenomenon. GISH, C-banding, and RFLP analysis gave no evidence for the presence of a small intercalary deletion in the 7Ai#1 chromosome in line 5395. In addition, we have evidence for the presence of small intercalary duplications in the translocated long arm of line TC8. These duplications may have occurred by unequal crossing-over events in the subsequent backcrosses of the material and were possibly useful in retaining the translocated chromosomes in the population. The duplicated and deleted RFLP markers show indirectly the distribution of possible crossing-over events between wheat and the *Agropyron* chromosome 7Ai#1. We can not exclude that the *Agropyron* segments of 7Ai#1 for which duplicated wheat segments were identified have a reduced compensating capability. Compensating exchanges between homoeologous DNA segments are advantageous over the introgression of non-homoeologous DNA segments, which often are associated with duplications or deletions. In summary, chromosome 7Ai#1 has, on the one hand, large homoeologous regions and a good compensating capability for chromosome 7D but, on the other hand, group 7 chromosomes of *Agropyron* are known to have a meiotic drive (Marais 1990; Zhang and Dvořák 1990*b*).

GISH analysis showed that the labelled *A. intermedium* DNA weakly hybridized to a set of small wheat chromosomes that are most likely chromosomes of the D genome. The use of *Agropyron*-amplified sequences (U. Hohmann and R. Appels, unpublished data) has revealed that the *Agropyron* genome shares more homologies with the D than with the A or B genome of wheat. In addition, the transfer of *Agropyron* chromatin specifying resistances against wheat streak mosaic virus, barley yellow dwarf virus, and leaf and stem rusts into wheat has shown that, in compensating substitution or translocation lines, often chromosomes of *Agropyron* and the D genome were involved (Friebe et al. 1991, 1992*b*; Kim et al. 1993; Banks et al. 1995).

Fig. 9. Physical maps of C-banded group 7 chromosomes 7A, 7B, 7D, and 7Ai#1. FL measurements are shown on the left and are based on deletion mapping (chromosomes 7A, 7B, and 7D) or translocation mapping (chromosome 7Ai#1). Markers that were polymorphic for all chromosomes were allocated to specific chromosome regions (shown on the right). Note that the most proximal short arm marker, MWG905, is not shown on chromosome 7B. This marker is present on the long arm of chromosome 4A that is involved in the cyclical translocation with chromosomes 5A and 7B in hexaploid wheat (Gill and Chen 1987; Naranjo et al. 1987; Liu et al. 1992).

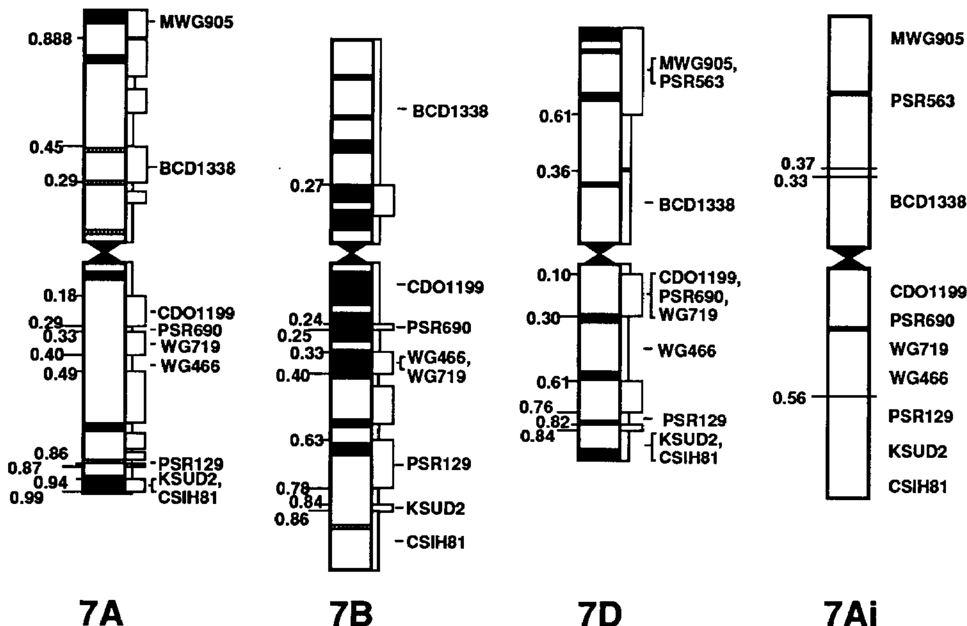
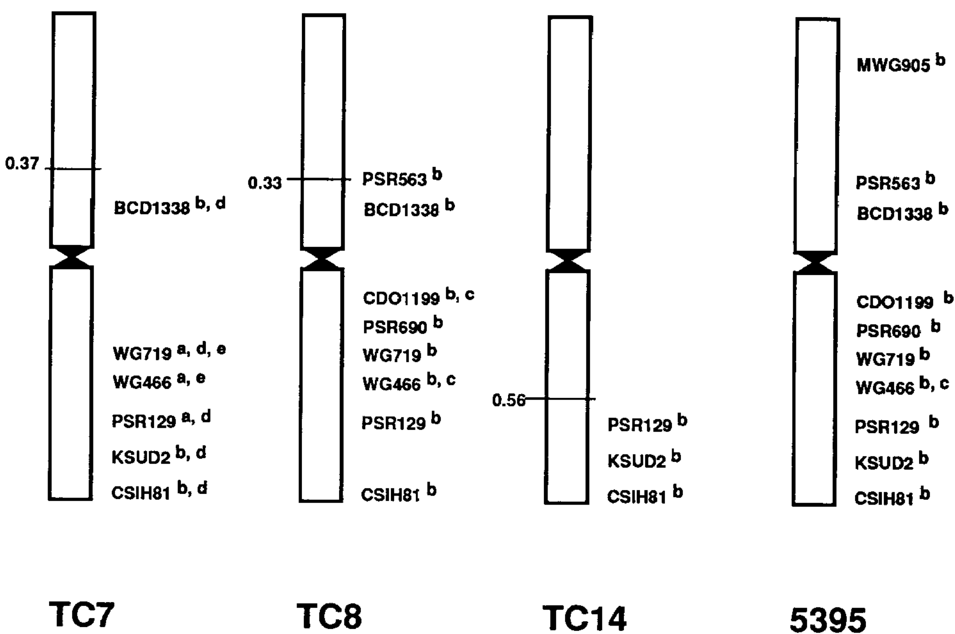


Fig. 10. Constitution of wheat–*Agropyron* translocation chromosomes in wheat lines TC7, TC8, TC14, and 5395 with respect to the presence of 7Ai#1 chromosome specific RFLP bands. 7Ai#1 RFLP markers were present in monosomic (*a*) or disomic (*b*) constitution. 7D RFLP markers were present in monosomic (*c*) or disomic (*d*) constitution. 7B RFLP markers were present in monosomic (*e*) constitution.



The present investigation has shown some discrepancies in the lines described earlier by Banks et al. (1995). We have shown that the tissue culture derived BYDV resistant lines TC5, TC6, TC7, TC8, TC9, and TC10 carry more than a chromosome arm of 7Ai#1. Banks et al. (1995) calculated from Southern hybridization with the dispersed repetitive probe pEleAcc2 that all lines carry less than a chromosome arm of *Agropyron*. The hybridization signal for the long arm of 7Ai#1 was more than twice that of the short arm, although both arms are approximately the same size. Therefore, they underestimated the amount of the *Agropyron* chromatin that was introgressed into the lines. The pEleAcc2 probe is dispersed in *Agropyron* but occurs in clusters in subtelomeric regions of every *Agropyron* chromosome arm (U. Hohmann and R. Appels, unpublished data). The hybridization signals occur predominantly from these clusters and the dispersed sequences along the chromosome do not contribute significantly to the intensity of the *Agropyron*-specific restriction fragment. Therefore, the differences in the hybridization intensities are related to the different number of introgressed chromosome arms, rather than to the different sizes of chromosome segments. The repetitive probe A600 (U. Hohmann, R. Appels, H. Ohm, and L. Hogie, see footnote 1) used in the present study has a more uniformly dispersed character and is more useful than pEleAcc2 in quantifying the *Agropyron* chromatin.

The line TC7 was believed to carry all group 7 markers and resulted from a recombination with an unidentified group 7 chromosome (Banks et al. 1995). We have shown that TC7 or the subline studied here is an addition line with a highly recombined wheat–*Agropyron* chromosome. However, in other sublines of TC7, the translocated chromosome T1BS–7Ai#1S·7Ai#1L substitutes for chromosome 1B. These 42-chromosome plants can show regular meiosis with 21 bivalents as stated by Banks et al. (1995). The last example demonstrates that in one translocation "family" there can exist different sublines that will be identified in the future with a combination of techniques of higher resolution.

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