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Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat

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Summary. Genomic in situ hybridization was used to identify alien chromatin in chromosome spreads of wheat, *Triticum aestivum* L., lines incorporating chromosomes from *Leymus multicaulis* (Kar. and Kir.) Tzvelev and *Thinopyrum bessarabicum* (Savul. and Rayss) Löve, and chromosome arms from *Hordeum chilense* Roem. and Schult, *H. vulgare* L. and *Secale cereale* L. Total genomic DNA from the introgressed alien species was used as a probe, together with excess amounts of unlabelled blocking DNA from wheat, for DNA:DNA in-situ hybridization. The method labelled the alien chromatin yellow-green, while the wheat chromosomes showed only the orange-red fluorescence of the DNA counterstain. Nuclei were screened from seedling root-tips (including those from half-grains) and anther wall tissue. The genomic probing method identified alien chromosomes and chromosome arms and allowed counting in nuclei at all stages of the cell cycle, so complete metaphases were not needed. At prophase or interphase, two labelled domains were visible in most nuclei from disomic lines, while only one labelled domain was visible in monosomic lines. At metaphase, direct visualization of the morphology of the alien chromosome or chromosome segment was possible and allowed identification of the relationship of the alien chromatin to the wheat chromosomes. The genomic in-situ hybridization method is fast, sensitive, accurate and informative. Hence it is likely to be of great value for both cytogenetic analysis and in plant breeding programmes.

Key words: Genomic probing – In situ hybridization – Interphase cytogenetics – Physical mapping – *Triticum*

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Introduction

The wild and cultivated relatives of bread wheat, *Triticum aestivum* L. emend Thell., form a large pool of genes of value to wheat breeders for yield improvement, disease resistance, and adaptation to extreme, stressful, or changing environmental conditions (Porceddu et al. 1988). Intercrossing of wheat with its relatives has enabled many useful traits to be incorporated into wheat cultivars (see Law 1981; Fatih 1983; Gale and Miller 1987; Islam and Shepherd 1991a). Examples include many high yielding cultivars that incorporate a chromosome segment from rye, *Secale cereale* L. (see Zeller 1973), the leaf-rust resistance gene *Lr9* in North American cultivars that was introduced from *Aegilops umbellulata* Zhuk. (Sears 1956; McIntosh 1988), the stem-rust resistance gene *Sr26* from *Thinopyrum ponticum* (Podp.) Barkworth and Dewey (Knott 1961) that is present in several Australian cultivars (see McIntosh 1988), and drought resistance in some Russian cultivars that has been attributed to *Th. intermedium* (Host) Barkworth and Dewey (Fatih 1983).

The introduction of alien genes into wheat requires hybridization with an alien species, followed by production of an amphiploid and backcrossing. Alien chromosome addition and substitution lines can then be produced, and translocation or recombinant lines can arise spontaneously, or be derived using ionizing radiation, or through manipulation of the genetic control of homoeologous chromosome pairing (see Gale and Miller 1987). Subsequent selection aims to retain useful genes while minimising the number of deleterious characters from the alien. Markers, including the useful genes themselves, must be used to identify and follow the alien chromosome or chromosome segment during back crossing and

selection. Plant morphology is often informative (e.g., Sears 1977; Miller and Reader 1987) but isozymes (e.g., Hart et al. 1980; Koebner et al. 1986; Forster et al. 1987; Islam and Shepherd 1991 b) and restriction fragment length polymorphism (RFLP) analysis (Sharp et al. 1988; Gale et al. 1989; Rogowsky et al. 1991) are now widely applied, and will be of increasing importance. In general, any satisfactory method for detecting alien chromosomes or chromosome segments needs to be efficient and applicable to many situations.

Cytological methods, including C-banding, have been used to look directly at the alien chromosomes and chromosome segments in wheat lines (e.g., Friebe et al. 1989, 1991). In situ hybridization to chromosome spreads with labelled cloned DNA probes can also identify alien chromosome segments. If the probe contains a species-specific sequence dispersed throughout the alien genome, then only the alien chromosome segment will show a hybridization signal (Rayburn and Gill 1987; Guidet et al. 1991). Alternatively, probes that give species-specific hybridization patterns (bands) also enable alien chromosomes to be identified (Lapitan et al. 1986; Friebe et al. 1991). However, the isolation and characterization of species-specific or informative repetitive clones is time consuming and uncertain, particularly in a programme where a range of different alien species is used.

Total genomic DNA can be labelled and used as a probe to identify alien chromosomes in a wheat background by in situ hybridization (Heslop-Harrison et al. 1988; Le et al. 1989) and may be of potentially wide application in plant breeding programmes (Heslop-Harrison et al. 1990; Mukai and Gill 1991). This technique has identified the parental origin of each chromosome in hybrids of *Hordeum chilense* Roem. and Schult and *H. vulgare* L. with *S. africanum* Stapf. and in the hybrid *H. vulgare* × *H. bulbosum* L. (Schwarzacher et al. 1989, 1992; Leitch et al. 1990), as well as alien chromosomes and chromosome segments from *S. cereale* and *H. vulgare* in hexaploid wheat cultivars (Heslop-Harrison et al. 1990; Mukai and Gill 1991) and triticale (Le and Armstrong 1991). Labelled genomic DNA may also be used as a probe on Southern blots of genomic DNA digests to distinguish related species and to identify wheat lines with alien chromosomes (Anamthawat-Jónsson et al. 1990; Heslop-Harrison et al. 1990). The limitation of genomic in-situ hybridization, in terms of the size of the segment that can be identified and the relationships between the species, have not yet been explored. As well as giving information about the presence of the alien chromosomes, in-situ hybridization shows the physical size and numbers of the chromosomes or chromosome segments, which is difficult to discover from gel analyses (Heslop-Harrison 1991).

In the present paper, we analyse various wheat addition, substitution and translocation lines carrying alien chromatin from *H. vulgare*, *H. chilense*, *Th. bessarabicum* (Savul. and Rayss) Löve, *Leymus multicaulis* (Kar. and Kir.) Tzvelev and *S. cereale*. We aimed to test whether total genomic DNA from the different alien species could be used as a probe to identify the presence of alien chromatin by in-situ hybridization.

Materials and methods

Plant material

The following wheat lines with alien chromosome additions and translocations were used:

(1) *Th. bessarabicum* (Savul. and Rayss) Löve: monosomic ($2n=6x+1=43$) and disomic ($2n=6x+2=44$) $5E^b$ (synonym 5J) addition to *T. aestivum* L. cv. Chinese Spring (Forster et al. 1988).

(2) *L. multicaulis* (Kar. and Kir.) Tzvelev: the amphidiploid with *T. aestivum* cv. Chinese Spring was backcrossed to cv. Chinese Spring and then to cv. Feng Kang 7 (Dong et al. 1986; J. Z. Jia, unpublished), and selfed for several generations. Individuals with $2n=6x+1=43$ were grown and plants exhibiting $21''+1'$ at metaphase-I of male meiosis were selected. Seeds obtained from these presumptive monosomic additions were used in the present study.

(3) *H. chilense* Roem. and Schult: selfed progeny of monosomic ($2n=6x-1=41$; $20''+1'$) $1BL/1H^{ch}S$ translocation in *T. aestivum* cv. Chinese Spring (mentioned as "T.E. Miller and S. M. Reader unpublished" in Gale and Miller 1987, and entry 857 in Shepherd and Islam 1988).

(4) *H. vulgare* L. cv. Betzes: disomic ($2n=6x=42$; $21''$), translocation of 4HL to a group 4 chromosome arm in *T. aestivum* cv. Chinese Spring. The line was isolated during screening for wheat-barley recombinant chromosomes in the progeny of a double monosomic plant having 20 pairs of wheat chromosomes and a single dose of barley chromosome 4H and wheat 4A (Islam and Shepherd, unpublished).

(5) *S. cereale* L. cv. King II: disomic ($2n=6x=42$; $21''$) 5AS/5RL spontaneous translocation in *T. aestivum* cv. Chinese Spring (mentioned as "T.E. Miller unpublished" in Gale and Miller 1987, entry 824 in Shepherd and Islam 1988).

Preparation of cells

Seeds were germinated on moist filter paper for 48 h at 25 °C, left for 24–48 h at 4 °C and then between 24 and 30 h at 25 °C to synchronize cell division. The seedlings, or excised root-tips, were transferred to ice water at 0 °C, or 0.05% (w/v) colchicine at 4 °C, for 24 h to accumulate metaphases, and fixed in 3:1 (v/v) 100% ethanol:acetic acid.

Root-tip spread preparations essentially followed the methods described previously by Schwarzacher et al. (1989). Briefly, fixed root-tips were partially digested with cellulase and pectinase before squashing in 45% acetic acid. Cover slips were removed after freezing with dry ice, and the slides dried. Preparations were used immediately or else kept refrigerated for up to 3 months before in-situ hybridization.

In some cases, somatic tissue from anthers was used. The anthers were excised, fixed in 45% acetic acid for 5–10 min and squashed. Coverslips were removed, the slides dried and refixed in 3:1 (v/v) 100% ethanol:acetic acid for 1 h, followed by two changes of 30 min each in 100% ethanol and then air dried. Preparations were kept for a maximum of 5 days before in-situ hybridization.

Table 1. Amount of probe and blocking DNA used in the in situ hybridization experiments. The stringency was calculated for hybridization at 37 °C and a GC content of 45.5% (Swanson and Webster 1975) according to Meinkoth and Wahl (1984) and is dependent on the concentration of formamide and SSC. In all experiments, post-hybridization washes were carried out at a stringency of 80–85%

Slide	Probe DNA	Block DNA	Block/probe ratio	Formamide (v/v)	SSC	Volume	Stringency at 37 °C
Th. bessarabicum							
80-3	200 ng Thb	3.9 μg CS	20	40%	1 ×	35 μl	75%
80-5	150 ng Thb	4.8 μg CS	30	40%	1 ×	40 μl	75%
L. multicaulis							
S33-1	100 ng Lm	3.6 μg CS + SS	35	50%	2 ×	30 μl	75%
H. chilense							
K8-6	300 ng Hch	4.0 μg R + SS	15	60%	2 ×	50 μl	80%
S15-6	170 ng Hch	5.0 μg CD	30	40%	2 ×	40 μl	70%
S29-7	360 ng CS	6.0 μg Hch + SS	15	35%	0.7 ×	30 μl	75%
H. vulgare							
R143-2	100 ng Hv	3.4 μg CS + SS	35	50%	2 ×	30 μl	75%
S. cereale							
86-2, -5	100 ng Sc	3.6 μg	35	50%	2 ×	30 μl	75%

Thb = *Th. bessarabicum*; Lm = *L. multicaulis*; Hch = *H. chilense*; Hv = *H. vulgare* cv. Sultan; Sc = *S. cereale* cv. Petkus; CS = *T. aestivum* cv. Chinese Spring; CD = *T. aestivum* cv. Capelle Desprez; R = *T. aestivum* cv. Rendezvous; SS = 5–8 μg Salmon sperm

Probe preparation and in situ hybridization

Total genomic DNA from *H. chilense, H. vulgare* cv. Sultan, *L. multicaulis, S. cereale* cv. Petkus, *T. aestivum* cv. Chinese Spring or *Th. bessarabicum* was mechanically sheared to 10–12 kb fragments and labelled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation for use as the in situ probe. Total genomic DNA from *T. aestivum* or *H. chilense* was fragmented to pieces about 250 bp long by autoclaving for 5 min and used as blocking DNA (Anamthawat-Jónsson et al. 1990; Heslop-Harrison et al. 1988, 1990). Table 1 shows the probe and blocking DNA concentrations and the composition of the hybridization mixtures.

The method of in situ hybridization and probe detection followed Leitch et al. (1991) with minor modifications. The probe mix contained formamide (v/v), SSC (20 ×: 3M sodium chloride, 0.3 M sodium citrate, pH 7), 10% (w/v) dextran sulphate, 0.05–0.3% (w/v) sodium dodecyl sulphate and in some experiments salmon sperm DNA (see Table 1). The probe mixture was denatured at 70 °C for 10 min, chilled on ice, 30–50 μl applied to the slides, and covered with a plastic coverslip. The slides were denatured by heating to 85–90 °C for 10 min in a water bath. Hybridization was carried out overnight at 37 °C followed by stringent washes in 50% (v/v) formamide in 2 × SSC or 20% (v/v) formamide in 0.1 × SSC at 40–42 °C.

Sites of probe hybridization were detected using fluoresceinated (FITC) sheep anti-digoxigenin (Boehringer Mannheim, 10–20 μg/ml in 4 × SSC containing 0.2% Tween and 5% bovine serum albumin) and amplified using fluoresceinated rabbit anti-sheep (F-135, DAKOPATTS, 1:100 dilution in 4 × SSC containing 0.2% Tween and 5% goat or rabbit serum). Chromosomes were counterstained with propidium iodide (5 μg/ml) and DAPI (2 μg/ml, 4′, 6′-diamidino-2-phenylindole). Slides were mounted in antifade mountant (AF-2, Citifluor Ltd., London, UK) and analysed on an epi-fluorescence Leitz Aristoplan microscope. Photographs were taken on Fujicolor 400 colour print film and printed on Mitsubishi colour print paper.

Results

Genomic in-situ hybridization

The micrographs in Figs. 1 and 2 show root-tip chromosome preparations from five different wheat lines with alien introgressions from *Th. bessarabicum, L. multicaulis, H. chilense, H. vulgare* and *S. cereale*. Genomic in situ hybridization (Schwarzacher et al. 1989; Anamthawat-Jónsson et al. 1990) was used to identify and characterize the numbers and sizes of the alien chromosomes or chromosome arms (Figs. 1 a, c–e, 2 a, c–i). Probe hybridization sites were detected by FITC-linked antibodies that fluoresced green under blue light excitation and allowed visualization of the alien chromosome material (Fig. 1 e). The wheat chromosomes showed very little probe hybridization. In most preparations, chromosomes were counterstained with propidium iodide and fluoresced orange-red with the same excitation wavelength. As strongly labelled alien chromosomes were also counterstained, the green FITC label and orange-red counterstain fluorescence interacted to give yellow or yellow-green fluorescence (e.g., Fig. 1 a, c, d). The chromosomes were always counterstained with DAPI which fluoresced blue under UV excitation and enabled quick scanning of the slides, the location of metaphases, and showed the chromosome morphology more clearly than propidium-iodide fluorescence (Figs. 1 b, 2 b).

Table 1 lists the hybridization mixtures used in the different experiments. Mostly, conditions during hybridization allowed sequences with 75% homology to

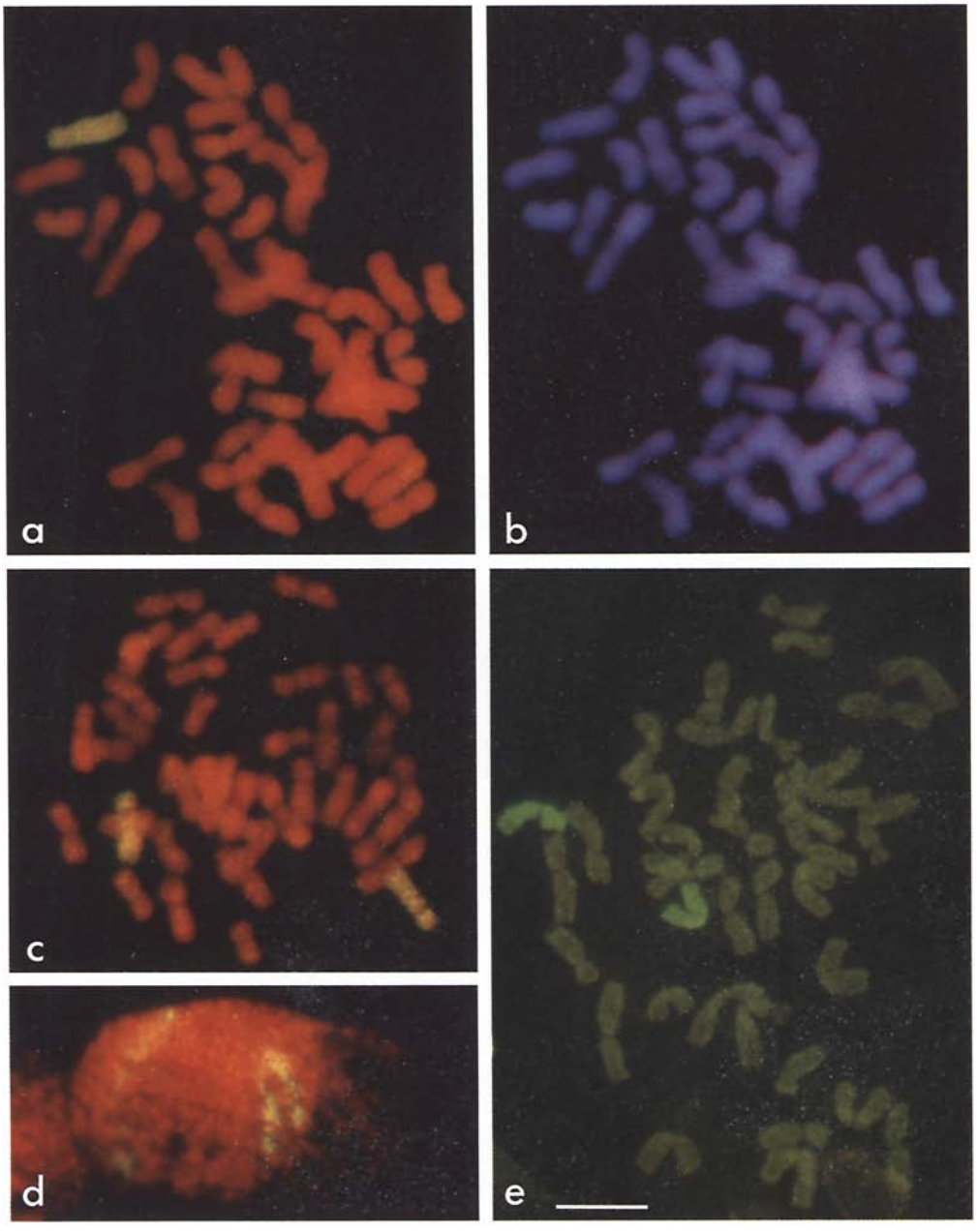
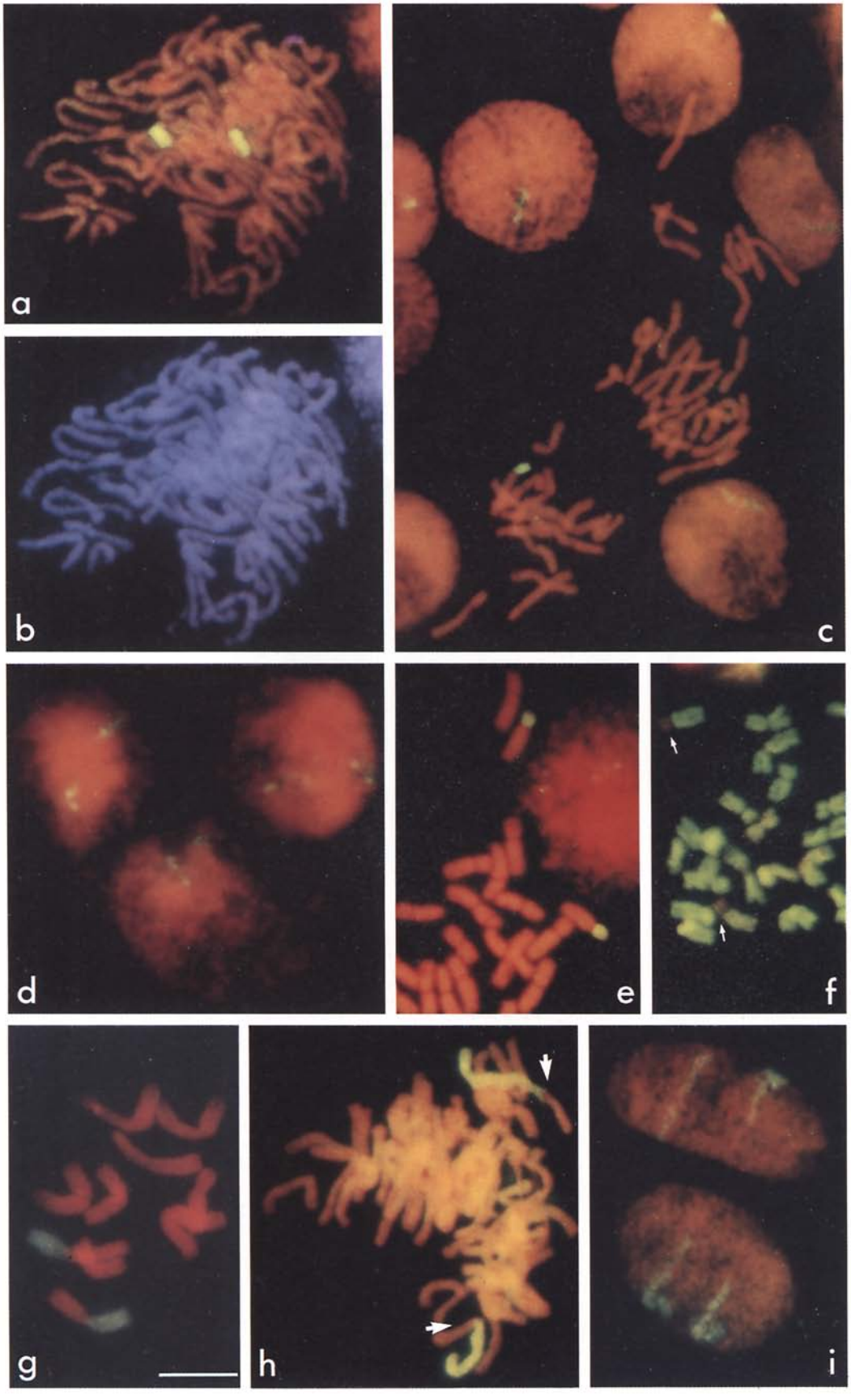


Fig. 1 a–e. Fluorescent micrographs of root-tip chromosome preparations from three different alien-wheat addition lines after genomic in situ hybridization. The presence and number of the alien chromosomes is shown by yellow-green fluorescence at metaphase (**a**, **c**) and interphase (**d**) while the unlabelled wheat chromosomes appear orange-red with the counterstain propidium iodide. Without the counterstain propidium iodide the alien chromosomes appear green and wheat chromosomes are pale green (**e**). Simultaneous staining with the DNA-specific dye DAPI shows uniform blue fluorescence and the alien chromosome(s) cannot be distinguished (compare **a** with **b**). For detailed in situ hybridization conditions see Table 1. **a, b** The same metaphase of monosomic *Th. bessarabicum* addition line, 2n = 43 (slide 80-5) after in-situ hybridization (**a**) and DAPI staining (**b**). **c** Metaphase of a disomic *Th. bessarabicum* addition line, 2n = 44 (slide 80-3). **d, e** Disomic *L. multicaulis* addition line, 2n = 44 (slide S33-1) at interphase (**d**) and in a partial metaphase (**e**). Bar 10 μm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form hybrids, and slightly more stringent conditions were used during the post-hybridization washes. We have not noted any significant difference when slightly higher or lower stringencies were used, either during hybridization or washing. However, probe concentration and blocking ratio can influence the hybridization results.

Detecting alien chromatin

The lines used here were taken from crossing programmes aimed at producing new wheat lines with alien chromosomes or chromosome segments.

(1) Th. bessarabicum *addition.* Addition lines were produced from an amphiploid between *T. aestivum* and *Th. bessarabicum* in a programme to make salt-tolerant wheat lines (Forster et al. 1987, 1988). The group 5 homoeology for the line was determined by morphological characters including broad leaves, clavate ears and coarse grain (Forster et al. 1988). Root-tip metaphases of a monosomic and a disomic plant following genomic in situ hybridization are shown in Fig. 1a–c. One (Fig. 1a) or two (Fig. 1c) alien chromosomes from *Th. bessarabicum* are easily visible, although the sizes and morphologies of the alien chromosomes are similar to the wheat chromosomes and they could not be distinguished by DAPI staining alone (Fig. 1b).

(2) L. multicaulis *addition.* At male meiosis in the parent, 21 bivalents and one univalent were found. As the line originated from an amphiploid with *L. multicaulis*, it was probable that the univalent was of *L. multicaulis* origin. No other evidence for alien introgression was available. In situ hybridization using total genomic *L. multicaulis* DNA verified the presence of alien chromosomes. The root-tip preparations in Fig. 1 show two distinct yellow-green fluorescing domains at interphase (Fig. 1d) and two yellow-green chromosomes of *L. multicaulis* origin at metaphase (Fig. 1e), and hence come from a disomic addition.

(3) H. chilense *1BL/1H^{ch}S translocation.* The translocation line occurred spontaneously in the progeny of a monosmic 1B monosomic 1H^{ch} hybrid (Miller and Reader, unpublished). A monosomic line is shown in Fig. 2c with one short yellow-green fluorescing chromosome arm translocated onto an orange-red fluorescing wheat chromosome. At interphase one yellow-green fluorescing domain is visible. The root-tips shown in Fig. 2b–f were germinated from half-grains where most of the endosperm had been removed for electrophoretic studies that detected the presence of *H. chilense* gliadins but no wheat gliadins. The gliadin loci are located on the short arm of homoeologous group 1 chromosomes (McIntosh 1988). After genomic in-situ hybridization, two yellow-green chromosome arms originating from *H. chilense* could be detected at prophase (Fig. 2a) and interphase (Fig. 2d). The translocation breakpoint was at, or very close to, the centromere (Fig. 2e). The use of total genomic DNA from *T. aestivum* as a labelled probe and *H. chilense* DNA as a block, labelled all the wheat chromosomes yellow-green and left the short chromosome arm from *H. chilense* red (Fig. 2f).

(4) H. vulgare *translocation line.* This line had been characterized by isozyme studies that showed the presence of at least part of the long arm of chromosome 4H. The *β-Amy-H1* gene controlling barley β-amylase isozymes from 4HL was present and the *Adh-H1* gene controlling barley alcohol dehydrogenase isozyme from 4HS was missing (Islam and Shepherd, unpublished). Genomic in situ hybridization with total genomic DNA from *H. vulgare* identified the alien translocation (Fig. 2g) as a centric fusion of the wheat and barley chromosomes.

(5) S. cereale *5AS/5RL translocation line.* The plant examined carries the rye character hairy neck, *Hp* (Gale and Miller 1987), that is located on the long arm of chromosome 5R. Meiotic pairing in crosses with telocentric lines established 5A as the translocated wheat chromosome arm (Miller and Reader, unpublished). Figure 2h and i show somatic cells from an anther wall of a disomic line after genomic in situ hybridization using total genomic rye DNA as a probe. At metaphase, a sharp border between labelled rye chromatin and unlabelled wheat chromatin is visible at the centromere of

Fig. 2a–i. Genomic in situ hybridization to chromosome preparations of wheat lines carrying alien translocations. Total genomic DNA from the alien species (**a**, **c–e**, **g–i**) or from wheat (**f**) was used as probe for in-situ hybridization (see Fig. 1). The number of alien chromosome arms can be counted at prophase and interphase where they form one or two distinct domains (**a**, **c**, **d**, **i**). At metaphase (**c**, **e–h**), the size and breakpoint of the translocation can be estimated. Note the sharp border between labelled and unlabelled chromatin (*arrow* in **h**). For detailed in-situ hybridization conditions see Table 1. **a**, **b**, **d**, **e** Disomic *H. chilense* translocation line, 2n=42 (Slide S15-6), root-tip chromosome preparations showing a prophase (**a** in situ hybridization; **b** DAPI), three interphases (**d**) and a partial metaphase (**e**). **f** Partial root-tip metaphase of a disomic *H. chilense* translocation line, 2n=42 (Slide S29-7). **c** Monosomic *H. chilense* translocation line, 2n=41 (slide K8-6) root-tip preparation showing interphases and a complete metaphase. **g** Selected metaphase chromosomes from a root-tip of a disomic *H. vulgare* translocation line, 2n=42 (slide R143-2). **h**, **i** Somatic cells of anthers of a disomic *S. cereale* translocation line, 2n=42 at metaphase (**h**, slide 86-5) and interphase (**i**, slide 86-2). Bar 10 μm

the translocated chromosome (Fig. 2h). At interphase the two homologous rye chromosome arms are visible as separate, parallel, domains running through the nucleus (Fig. 2i).

Discussion

Total genomic DNA as a probe was able to identify alien chromosomes or chromosome segments in situ in all the wheat lines described (Figs. 1 and 2). Whole chromosome additions and the translocation of small or large chromosome arms from various alien genera, including *Hordeum, Secale, Thinopyrum* and *Leymus*, were detected and could be analysed. No knowledge of the homoeologous group of the alien chromosome was required, and isolation, characterization and screening of markers was unnecessary. Furthermore, root-tips from young seedlings could be used for screening so plants did not need to be planted out and grown for DNA extraction or kept until maturity to assess morphological characters. For some of the *H. chilense* translocation lines, root-tips from half-grains were used for in-situ hybridization (Fig. 2a, d–f), while part of the endosperm was analysed by protein electrophoresis. Tissues from adult plants, such as anther walls from the rye translocation line (Fig. 2h–i), could also be analysed. So far, we have not encountered any wheat addition or substitution line involving chromosomes from alien genomes, where the genomic in situ hybridization method was not successful.

Three of the lines investigated here had been previously characterized by isozyme and pairing analysis at meiosis. The results from genomic in-situ hybridization showed that the presumed alien chromosome situation was correct. The line including *L. multicaulis* had not been characterized and the results presented here were the first to show that it was a disomic addition line.

In all the lines investigated here, we knew the species origin of the alien introgression and, therefore, isolated DNA from the appropriate species. To increase the differentiation between the alien and wheat chromatin, we used excess amounts of unlabelled DNA from wheat (Table 1) to block cross-hybridization of the alien labelled genomic probe to the wheat chromosomes (see also Anamthawat-Jónsson et al. 1990; Heslop-Harrison et al. 1990). The experiment where we used labelled wheat DNA as the probe indicates that prior knowledge of the alien genome involved may not be essential to show that a wheat line contains alien chromosomes or chromosome segments (Fig. 2f). Examination of some intergeneric and interspecific hybrids shows that the addition of blocking DNA is not essential to differentiate the parental chromosomes where the species are distantly related (e.g., *S. africanum* and *H. chilense*; Schwarzacher et al. 1989), but it is essential when they are closely related (e.g., *H. vulgare* and *H. bulbosum*; Anamthawat-Jónsson et al. 1990; Schwarzacher et al. 1992). Even if the origin of the alien chromosome is not known, DNA from a species related to the postulated alien could be used for blocking in the genomic wheat in situ hybridization experiment, as similar sequences are common between groups and genera within the Triticeae (Flavell et al. 1981). Thus, although the illustration shown (Fig. 2f) included DNA from the alien species, *H. chilense*, as blocking DNA (Table 1), some differentiation is obtained using no block (Schwarzacher et al. 1989), and good differentiation resulted with blocking DNA from another *Hordeum* species (Anamthawat-Jónsson et al. 1990, and unpublished data).

In all the lines examined here, the morphology of the alien chromosome or chromosome arm could be clearly visualized by differential fluorescence at metaphase. Hence complete metaphases were not essential for analysis of the length and morphology of alien chromosomes or chromosome arms, including detection of the breakpoint in the translocations (Fig. 2e–h). At interphase, the alien chromatin could usually be identified as one or two labelled domains (Figs. 1d, 2c, d, i). The fact that individual interphase chromosome arms tend to lie in single domains has been shown for both animals and plants and is an important aspect of nuclear architecture (see Heslop-Harrison and Bennett 1990). Accurate and reliable counts of the alien chromosomes can be made from interphases and prophases, and counts are possible without the necessity of complete metaphases or dividing tissues. Interphase cytogenetics is likely to be an increasingly important tool for both plant and animal, including human, studies, since metaphase preparation invariably requires skilful preparation methods using dividing cells, and automation of metaphase analysis is difficult (see Cremer et al. 1988; Maluszynska and Heslop-Harrison 1991; Tkachuk et al. 1991).

Genomic in situ hybridization is widely applicable, since it does not involve screening and characterization of markers, and can be used with many alien species without modification. The method can be used for the initial characterization of recombinant lines that contain an alien chromosome segment, and, following identification of genes for an improved agronomic trait, can be used in each subsequent generation to select progeny carrying a particular alien segment. The present work shows that the genomic in situ hybridization method is fast, sensitive, accurate and gives unique information about alien chromatin in a wheat background. Where such information is valuable, it is likely to be useful for both cytogenetic analysis and in plant breeding programmes (1) to show that a line includes an alien chromosome or chromosome segment; (2) to count the number of alien chromosomes or chromosome segments; and (3) to show the size and morphology of the alien chromo-

some and any recombination with chromosomes from the host genome.

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