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Physical mapping of translocation breakpoints in a set of wheat-*Aegilops umbellulata* recombinant lines using in situ hybridization

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Abstract *Aegilops umbellulata* Zhuk. carries genes at *Glu-U1* loci that code for a pair of high-molecular-weight glutenin subunits not found in common wheat, *Triticum aestivum*. Wheat-*Ae. umbellulata* recombinant lines were produced with the aim of transferring genes coding for glutenin subunits from *Ae. umbellulata* into wheat with minimal flanking material. We used fluorescent genomic in situ hybridization to evaluate the extent of recombination and to map physically the translocation breakpoints on 11 wheat-*Ae. umbellulata* recombinant lines. In situ hybridization was able to identify alien material in wheat and showed breakpoints not only near the centromeres but also along chromosome arms. To characterize and identify chromosomes further, including deletions along the 1U chromosome, we used simultaneous multiple target in situ hybridization to localize a tandemly repeated DNA sequence (pSc119.2) and the 18*S*-25*S* and 5*S* rRNA genes. One line contained an *Ae. umbellulata* telocentric chromosome and another two had different terminal deletions, mostly with some wheat chromosome rearrangements. Although from six independent original crosses, the other eight lines included only two types of intercalary wheat-*Ae. umbellulata* recombination events. Five occurred at the 5*S* rRNA genes on the short arm of the *Ae. umbellulata* chromosome with a distal wheat-origin segment, and three breakpoints were proximal to the centromere in the long arm, so most of the long arm was of *Ae. umbellulata* origin. The results allow characterization of recombination events in the context of the karyotype. They also facilitate the design of crossing programmes to generate lines where smaller *Ae. umbellulata* chromosome segments are transferred to wheat with the potential to improve bread-making quality by incorporating novel glutenin subunits without undesirable linked genes.

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Introduction

The yield of hexaploid wheat, *Triticum aestivum* L. emend Thell, has been increasing for many years. Today wheat is the major cereal grown throughout the world and attempts to widen and improve the gene pool by the introduction of desirable genes from alien species are well documented (reviewed in Sharma and Gill 1983; Gale and Miller 1987). The production of recombinant lines allows novel genes from related species within the Triticeae to be transferred to wheat through a programme of crosses coupled with the manipulation of the homoeologous chromosome pairing-control system (Riley 1966; Sears 1972, 1976). Using this approach, several genes which confer resistance to fungal and viral diseases and to pests have been transferred from various *Aegilops*, *Agropyron* and *Secale* species into wheat (for review see Damania 1993). Although conferring resistance to diseases such as wheat stem rust, some of the recombinant lines produced, particularly the 1BL-1RS wheat-rye recombinant, have a poor quality dough unsuitable for bread-making because other genes have been exchanged between wheat and the alien species.

Wheat is widely used for baking bread, and plant breeders are interested in improving the bread-making quality of this cereal. In the bread-making process, glutenin gives strength and elasticity to the dough (Bietz and Wall 1973). The glutenin protein is formed from a heterogeneous complex of polypeptide chains, which can be divided into high-molecular-weight (HMW) and the low-molecular-weight (LMW) glutenin subunits (Payne and Corfield 1979). Although the endosperm storage proteins, viz. glutenin and gliadins, play a vital role in bread-making quality, the HMW glutenin subunits have proved to be of special importance (Payne et al. 1979; Hamer et al. 1992). Bread-wheat cultivars possess three to five HMW glutenin sub-

units, two coded by chromosome 1D, one or two coded by chromosome 1B, and only one or none by chromosome 1A (Payne et al. 1981; Payne and Lawrence 1983).

The wild relatives of wheat include a large pool of genes that provide a useful reservoir of variation for the introduction of genes for protein content and quality improvement. *Ae. umbellulata* Zhuk. (2 n=2x=14) carries a gene(s) at the locus *Glu-1U*, on the long arm of chromosome 1U (homoeologous to wheat group 1), that codes for a very high-molecular-weight glutenin subunit and which seems not to be present in any of the bread wheats (Law and Payne 1983). The novel proteins from *Ae. umbellulata* have been introduced into wheat by substituting chromosome 1U for each of the three group-1 chromosomes (Shepherd 1973; Chapman et al. 1975). However, each of these substitution lines reduces yield by about 50% (Law et al. 1984). For bread-making quality, the SDS-sedimentation test showed that only chromosome-1A substitution enhanced the sedimentation volume compared to the reference wheat variety Chinese Spring (Law et al. 1984). Since chromosome 1U also reduces yield, it is important to exploit the beneficial effects of this chromosome on bread-making quality, and at the same time remove genes leading to reduced yield.

Allosyndetic recombination between chromosome 1U and wheat chromosomes has been induced to produce wheat-*Ae. umbellulata* recombinant lines (Law et al. 1984; Koebner and Shepherd 1987). Islam-Faridi (1988) undertook a series of crosses in order to transfer the *Glu-U1* gene(s) from *Ae. umbellulata* into the wheat variety Chinese Spring. The successful transfer of the endosperm protein gene, *Glu-U1*, into wheat was made possible through induced allosyndetic recombination between chromosome 1U and a group-1 chromosome from wheat and provided the lines used in the present study. The programme used wheat lines with the 1U chromosome substituted for either chromosome 1A, CS 1U(1A), or 1B, CS 1U(1B). The transfer of the genes for the 1U HMW glutenin subunits was brought about by inducing recombination between the 1U chromosome and the homoeologous wheat chromosomes by manipulation of the gene *Ph1* controlling chromosome pairing and recombination at meiosis.

In the past, the most commonly used procedures to detect and characterize recombinant lines included meiotic pairing analysis, gene markers, chromosome C-banding and protein, isozyme and RFLP analysis. The non-radioactive in situ hybridization technique has recently proved to be a powerful and efficient technique for detecting alien genetic material in wheat (Heslop-Harrison et al. 1988, 1990; Schwarzacher et al. 1992; Jiang and Gill 1994) and, when used together with the other methods of analysis, allows an accurate characterization of recombinant lines. Total genomic DNA can be used as a molecular probe and is particularly useful for differentiating closely related species and detecting introgressions of chromosomes or chromosome segments.

Genomic in situ hybridization using alien DNA as one probe combined with a second probe for the identification of different chromosomes can lead to accurate characterization of the chromosome complement of recombinant lines. Tandemly repeated sequences are particularly valuable for this propose. In wheat the physical location of the rRNA genes within the nucleolus organizer regions (NORs) has been obtained from in situ hybridization experiments (Appels et al. 1980; Hutchinson and Miller 1982); the 18*S*-5.8*S*-25*S* rRNA genes (18*S*-25*S* rDNA) were found on chromosomes 1B, 6B, 5D and 1A, subsequently also on the long arm of chromosome 7D (Mukai et al. 1991). Six pairs of 5*S* rRNA loci were mapped on the chromosomes of homoeologous group 1 (arms 1AS, 1BS, 1DS) and group 5 (arms 5AS, 5BS, 5DS). (Mukai et al. 1990).

In Chinese Spring, the probe pSc119.2 (a highly repeated DNA sequence from rye) hybridizes to 13 chromosomes [4A, 2D, 3D, 5D and all seven B-genome chromosomes (Rayburn and Gill 1985) and 5A and 4D (Mukai et al. 1993a)]. The B genome shows multiple pSc119.2 sites allowing the individual identification of chromosomes. In *Ae. umbellulata* both the 18*S*-25*S* and the 5*S* rDNA are located on the short arm of chromosomes 1U and 5U and pSc119.2 hybridizes with all the *Ae. umbellulata* chromosomes, mainly at the telomeres except on chromosomes 6U and 7U where intercalary sites are also observed (Castilho and Heslop-Harrison 1995).

In the present investigation we applied multiple target in situ hybridization, using as probes the total genomic DNA from *Ae. umbellulata* together with specific cloned DNA sequences (the 18*S*-25*S* and 5*S* rDNA and a repeat from rye, pSc119.2). This enabled physical mapping of the translocation breakpoints in 11 recombinant lines and also the identification of the wheat chromosome involved in the initial translocation.

Material and methods

Plant material

In this investigation we studied 11 *Glu-U1*$^{+}$/*Gli-U1*$^{-}$ wheat-*Ae. umbellulata* recombinant lines selected from those produced by Islam-Faridi (1988); their designation and parentage are listed in Table 1.

Electrophoretic methods

In order to ensure that the plants used in this study were carrying the 1U HMW glutenin subunits, their total endosperm proteins were fractionated according to their molecular weight using one-dimensional 10% sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) under reducing conditions as described by Payne et al. (1981), although the 2-mercaptoethanol was replaced by dithiothreitol (1 mM) as the reducing agent in the sample buffer. Seeds were divided into two halves and the endosperm part not including the embryo was crushed. Only embryos from seeds shown to be carrying the high-molecular-weight proteins from *Ae. umbellulata* were selected to be analysed by in situ hybridization. These could either be heterozygous or homozygous for the recombinant chromosome.

Preparation of cells for in situ hybridization

Half seeds containing the embryo were germinated in distilled water on filter paper in Petri dishes at 25°C for about 2 days. When the

Table 1 List of the wheat-*Ae. umbellulata* recombinant lines showing the original crosses made by Islam-Faridi (1988). Since then, stocks have been maintained by selfing and selection based on protein markers

Line	Parentage[b]
A10	[(CS N5B T5D × CS 1U(1B)) × CS N5B T5D] × CS N5D T5B
A30	[(CS 1U(1B) × CS N5B T5D) × CS N5B T5D] × CS
A34	[(CS 1U(1B) × CS N5B T5D) × CS *ph1ph1*] × CS N5D T5B
A37	[(CS 1U(1A) × CS N5B T5D) × CS N5B T5D] × CS N5D T5B
A47	[(CS N5B T5D × CS 1U(1A)) × CS *ph1ph1*] × CS
248[a]	[(CSM5B × CS 1U(1A))] × CS *ph1ph1*
A56	CS × 248
A58	CS × 248
79[a]	[(CS 1U(1A) × CS N5B T5D)] × CS *ph1ph1*
A62	CS × 79
A64	CS × 79
A65	[(CSM5B × CS 1U(1B) × CS *ph1ph1*] × CS
A94	CS × [(CSM5B × CS 1U(1A)) × CS *ph1ph1*]

[a] Intermediate hybrid line
[b] CS=Chinese Spring, N5BT5D and similar nomenclature=Nullisomic for 5B and tetrasomic for 5D, M5B=Monosomic for 5B, CS1U (1A) and similar nomenclature=CS with 1U substituting for 1A and CS*ph1ph1*=CS 5B *Ph1b* mutant/deletion (Sears 1976)

root-tips were a few mm long, the Petri dishes were transferred to 4°C for 24 h and then back to 25°C for 26–29 h in order to synchronize cell division. The excised root-tips were transferred to ice water at 0°C for 24 h and finally fixed in 3:1 (v/v) 100% ethanol:acetic acid. Root-tip spreads were performed as described by Schwarzacher et al. (1989).

Probe preparation

The physical location of the 18*S*-25*S* and 5*S* rDNA and a highly tandem repeated DNA sequence was performed on wheat-*Ae. umbellulata* recombinant lines using the following labelled probes:

pTa71, containing a 9-kb *Eco*RI fragment of the 18*S*-25*S* rDNA isolated from *T. aestivum* (Gerlach and Bedbrook 1979) and re-cloned in pUC19. The DNA was directly labelled with Fluorored, rhodamin-14-dUTP (Amersham), by nick translation.

pTa794, containing a 410-bp *Bam*HI fragment of the 5*S* rDNA isolated from embryos of *T. aestivum* (Gerlach and Dyer 1980) and cloned in pBR322. The DNA was amplified and labelled by PCR with either digoxigenin-11-dUTP (Amersham) or Biotin-11-dUTP (Sigma).

pSc119.2, containing a 120 bp tandem repeated sequence unit of DNA isolated from rye, *Secale cereale* (Bedbrook et al. 1980), subcloned by McIntyre et al. (1988). The DNA was labelled by nick translation with digoxigenin-11-dUTP.

The physical location and identification of the alien DNA segment in the recombinant wheat-*Ae. umbellulata* lines, was carried out using genomic in situ hybridization. Total genomic DNA from *Ae. umbellulata* was isolated and purified as described by Sharp et al. (1988), then sonicated to produce 10–12-kb fragments and labelled by nick translation with different non-radioactive haptens.

Prior to in situ hybridization, probes were mixed in a solution containing 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS (sodium dodecyl sulphate), 5 ng/µl of autoclaved salmon sperm DNA and 2×SSC (hybridization stringency of 76%). The probe concentration for cloned DNA was 60–100 ng/slide, and 100–200 ng/slide for total genomic DNA. The total genomic DNA from wheat was fragmented into units of about 250 bp long by autoclaving for 5 min and was used in the hybridization mixture as a blocking DNA, together with the labelled DNA from *Ae. umbellulata*. In this way, predominantly, the sequences specific for the target are available for hybridization (Anamthawat-Jónsson et al. 1990). The unlabelled blocking DNA was used at a concentration of 35–50× the genomic probe concentration.

In situ hybridization

Before the DNA:DNA hybridization, the slides were pre-treated as described by Heslop-Harrison et al. (1991) and air dried. Just before being added to the slides, the hybridization mixture was denatured in a 70°C bath for 10 min and incubated on ice for 5 min. The hybridization mixture was added to the slides (30–50 µl/slide) and the chromosomes, together with the probe, were denatured for 5 min at 70°C, using a programmable temperature controller (Hybaid Omnislide, Heslop-Harrison et al. 1991). The hybridization was carried out in a 37°C incubator overnight (in a humid chamber). After hybridization, the slides were washed as described by Heslop-Harrison et al. (1991) and the hybridization sites of digoxigenin- and biotin-labelled probes were detected using a fluorochrome marker, anti-digoxigenin conjugated to FITC (Fab fragment, Boehringer) and anti-biotin conjugated to Cy3 (Sigma), respectively. The detection of both digoxigenin and biotin hybridization sites were carried out by incubating the slides for 1 h at 37°C in a solution of 1% (v/v) anti-digoxigenin FITC, 0.025% (v/v) anti-biotin Cy3 in 5% (w/v) BSA. After detection the slides were washed, chromosomes were stained with DAPI and mounted, following the procedures of Heslop-Harrison et al. (1991). When the direct label systems were used, no detection was needed. The probe hybridization sites were visualized on a Leitz epifluorescent microscope following excitation with light of the right wavelength using appropriate light filters. Photomicrographs were taken on Fujicolor Super HG400 colour print film.

Results and discussion

Endosperm proteins

A sample of recombinant seeds were screened for the presence of their *Glu-1* and absence of their *Gli-1* proteins on 10% SDS-PAGE. Althought the gliadin proteins are better separated by acid polyacrylamide-gel electrophoresis (A-PAGE: Bushuk and Zillman 1978), some characteristic *Gli-1* bands can still be identified in 10% SDS-PAGE. Figure 1 shows the HMW glutenin subunit and some of the gliadin bands in one example of each line. All the lines carry both the *Glu-B1* and *Glu-D1* genes except for lines A34 and A65 where the *Glu-B1* proteins are missing. A65

also lacked the *Gli-B1* phenotype indicating that this line is probably nullisomic for the 1B chromosome. Line A34 shows a *Glu-B1*$^{-}$/*Gli-B1*$^{+}$ phenotype and thus represents either a misdivision of the centromere or a product of homoeologous recombination. Lines A34, A37 and A62 were still segregating for the presence of the *Ae. umbellulata* glutenins and hence for the recombinant chromosome (A34 and A62 are now stabilized). About 100 screened seeds of the other lines all carried the *Ae. umbellulata* HMW glutenin subunits.

Multiple target in situ hybridization

Figures 2–7 show root-tip chromosome preparations from the wheat lines with alien chromosome segments introgressed from *Ae. umbellulata*. Genomic in situ hybridization was used to identify and characterize the number and size of the alien segments, thus identifying heterozygous and homozygous recombinant lines. We physically mapped the location and number of 18*S*-25*S* rDNA (pTa71) and 5*S* rDNA (pTa794). We also used the hybridization pattern of the probe pSc119.2 on Chinese Spring (Mukai et al. 1993a) and *Ae. umbellulata* (Castilho and Heslop-Harrison 1995) to identify chromosomes from the wheat complement and, in particular, the chromosome involved in the translocation with 1U. Since these recombinant lines were obtained by manipulation of the homoeologous pairing-control system, single or multiple translocations could have taken place between homoeologous wheat chromosomes. In fact, although the hybridization pattern of pSc119.2 allowed the identification of several wheat chromosomes, in some lines (e.g. A56 and A58) results were often difficult to interpret, indicating the probable presence of translocations or other chromosomal rearrangements.

To avoid exhaustive presentation of photomicrographs, only limited examples of each type of translocation found amongst the 11 recombinant lines studied in this investigation are shown.

A10

Figure 2 illustrates the results of multiple target in situ hybridization on the A10 recombinant line. The genomic in situ hybridization shows that this is a ditelosomic addition line (2n=42+2 t) with the alien chromosome present as a pair of 1U long-arm telocentric chromosomes. Four major wheat 18*S*-25*S* rDNA sites (Fig. 2a) and ten 5*S* rDNA sites, all of wheat origin, were detected (Fig. 2 b, c). The pSc119.2 hybridization (Fig. 2d) on the alien chromosome was, as expected, telomeric on the long arm of chromosome 1U (Castilho and Heslop-Harrison 1995). The SDS-PAGE analysis (Fig. 1) showed that this line carries both 1B and 1D HMW glutenins and gliadins, suggesting that these chromosomes were, most probably, not involved in the recombination event. The characteristic pSc119.2 pattern on the 1B chromosome (Fig. 8) allowed it to be identified (Fig. 2d), and the four major 18*S*-25*S* rDNA sites (1B and 6B chromosomes) detected (Fig. 2a) showed that this line is disomic for the 1B chromosome. On the other hand, only 10 out of 12 5*S* rDNA sites were detected indicating that a wheat chromosome or chromosome segment must be missing in this line, as 12 sites were consistently detected in wheat under the same hybridization conditions. The results presented suggest that the missing 5*S* rRNA sites are most likely to be those located on the short arm of chromosome 1A since we know that 1B is disomic and both *Glu-D1* and *Gli-D1* protein markers are present, although group 5 also carries a 5*S* rRNA locus.

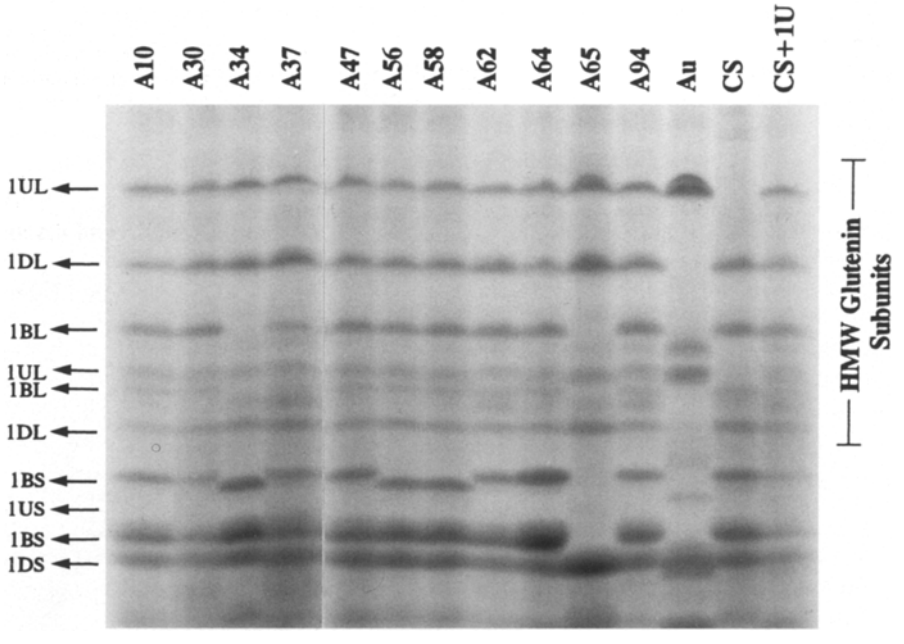

Fig. 1 Electrophoretic pattern of the total endosperm proteins from Chinese Spring wheat (*CS*), *Ae. umbellulata* (*Au*), a 1U addition line (*CS+1U*) and the 11 recombinant lines (*A10 to A94*) after fractionation by 10% SDS-PAGE. The HMW glutenin subunits appear well separated on the upper half of the gel and some relevant gliadin bands can also be distinguished in the lower half of the gel. The figure also indicates the chromosome arms responsible for the bands

A30, A34, A37, A47 and A64

Multiple target in situ hybridization allowed the physical mapping of the translocation breakpoints on A30, A34, A37, A47 and A64 (Fig. 3). These lines had all been obtained independently, from different series of crosses (see Table 1), but the alien segment transferred to wheat is similar in length in all of them. Lines A34 and A37 were monosomic for the translocated chromosome, while all the other lines were disomic. In situ hybridization allowed us to map the translocation breakpoint on the short arm of chromosome 1U proximal to the 1U 5*S* rDNA site. Figure 3 shows examples of results obtained for this group of recombinants. Comparing the genomic hybridization signal with the morphology of the DAPI stained chromosomes (Fig. 3e, f) it is possible to distinguish a segment of wheat origin on the end of the short arm of the translocated chromosome. The SDS-PAGE (Fig. 1) and the multiple target in situ hybridization results (data not shown) suggest that in line A34 chromosome 1B is probably involved in the translocation with 1U. The analysis of the endosperm protein markers showed that this line is phenotypically *Glu-U1*$^{+}$, *Glu-B1*$^{-}$*/Gli-U1*$^{-}$, *Gli-B1*$^{+}$ and we could identify only four major NORs and 12 5*S* rDNA sites, two of each located on the *Ae. umbellulata* segment in the translocated chromosome. In the other recombinant lines we have mapped six major sub-terminal NORs, four on wheat chromosomes (1B and 6B) and two on the *Ae. umbellulata* chromosome segment (Fig. 3a, b). All 12 5*S* rDNA sites were detected, ten being of wheat origin and two from *Ae. umbellulata* (Fig. 3c, d). It seems, therefore, that the 1U segment transferred to wheat includes the complete long arm as well as the short arm up to and including the 5*S* rDNA. The SDS-PAGE (Fig. 1) shows that the lines are carrying both endosperm markers on chromosomes 1B and 1D and that pSc119.2 hybridizes with 13 wheat chromosome pairs (Fig. 3e) allowing the identification of the 1B and group-5 chromosomes. Therefore the wheat segment of the translocated chromosome is most likely to belong to the short arm of chromosome 1A.

A56 and A58

We found that lines A56, A58 and A62 are near centromeric translocations (Fig. 4). The multiple target in situ hybridization suggests the presence of long arm/long arm translocations, although comparison of the genomic signal with the morphology of the chromosome indicates that the translocation breakpoint is located just beyond the centromere; a section of wheat arm is visible between the centromere and the alien segment, suggesting a complete 1U long arm is not present. All the short arm 1U gene markers tested are missing. The SDS-PAGE results (Fig. 1) showed, as for the lines discussed above, that chromosome 1B and 1D are most likely present as disomics. The pSc119.2 pattern (Fig. 4c), together with the 18*S*-25*S* rDNA (Fig. 4b) and the 5*S* rDNA (Fig. 4a) numbers, exclude chromosome 1B from being involved in the translocation. Lines A56 and A58 result from the same intermediate hybrid line (see Table 1) and hence they may not involve two different translocation events. The pSc119.2 hybridization pattern on the translocated chromosome from these lines is complex, showing one telomeric site on the alien segment and an intercalary site on the wheat segment (Fig. 4c). The results presented indicate that the short arm of chromosome 1A may be missing and the long arm is the wheat segment present in the translocated chromosome. However, the translocated chromosome carries a pSc119.2 site on its wheat arm, which is not present in the long arm of chromosome 1A (Mukai et al. 1993a). It is therefore possible that a chromosome other than a group-1 chromosome is involved in the translocation: neither the 1B nor 1D long arm is involved, as chromosome 1B is clearly identified by its pSc119.2 hybridization pattern and chromosome 1D also does not carry a pSc119.2 site on the long arm.

A62

The results obtained for this recombinant line are similar to those presented above. However, the translocated chromosome in the A62 line shows a different pSc119.2 hybridization pattern with only one telomeric site on the 1U chromosome segment (Fig. 5).

A65 and A94

A65 (Fig. 6) and A94 (Fig. 7) are characterized by partial deletions of the short arm of chromosome 1U. Genomic in situ hybridization on both lines showed that the alien chromosome has no detectable wheat segment.

Line A65 was obtained originally from crosses involving the CS1U(1B) substitution line. The number of 18*S*-25*S* rDNA (Fig. 6a, b) and 5*S* rDNA (Fig. 6d, e) sites, the pSc119.2 pattern (Fig. 6c), and the SDS-PAGE show that chromosome 1B is still missing in this line. The 1U chromosome transferred to wheat includes the long arm and the short arm up to and including part of the 18*S*-25*S* rDNA site. The breakpoint occurred within the ribosomal rRNA gene cluster and the hybridization signal (Fig. 6b) reveals the presence of a minor 1U 18*S*-25*S* rDNA instead of the expected major NOR. This type of deletion has also been reported by Payne et al. (1984) on the wheat 1B chromosome.

Line A94 is also a deletion of the 1U chromosome. However the breakpoint has been mapped proximal to the NOR and so all the 1U short-arm marker genes are missing. In this line we were able to map four major NORs (Fig. 7a) and ten 5*S* rDNA sites (Fig. 7b, c) all of wheat origin. The pSc119.2 hybridization pattern in this line (Fig. 7d) shows, as expected, a telomeric site in the alien chromosome segment. For the same reasons already discussed for other lines, the results of multiple target in situ hybridization in these two lines demontrate that both 1B (in the A94 recombinant line) and 1D are disomic and, as only ten (A94) and eight (A65) of 12 wheat 5*S* rDNA sites are present, the 5*S* rDNA from chromosome 1A must be missing.

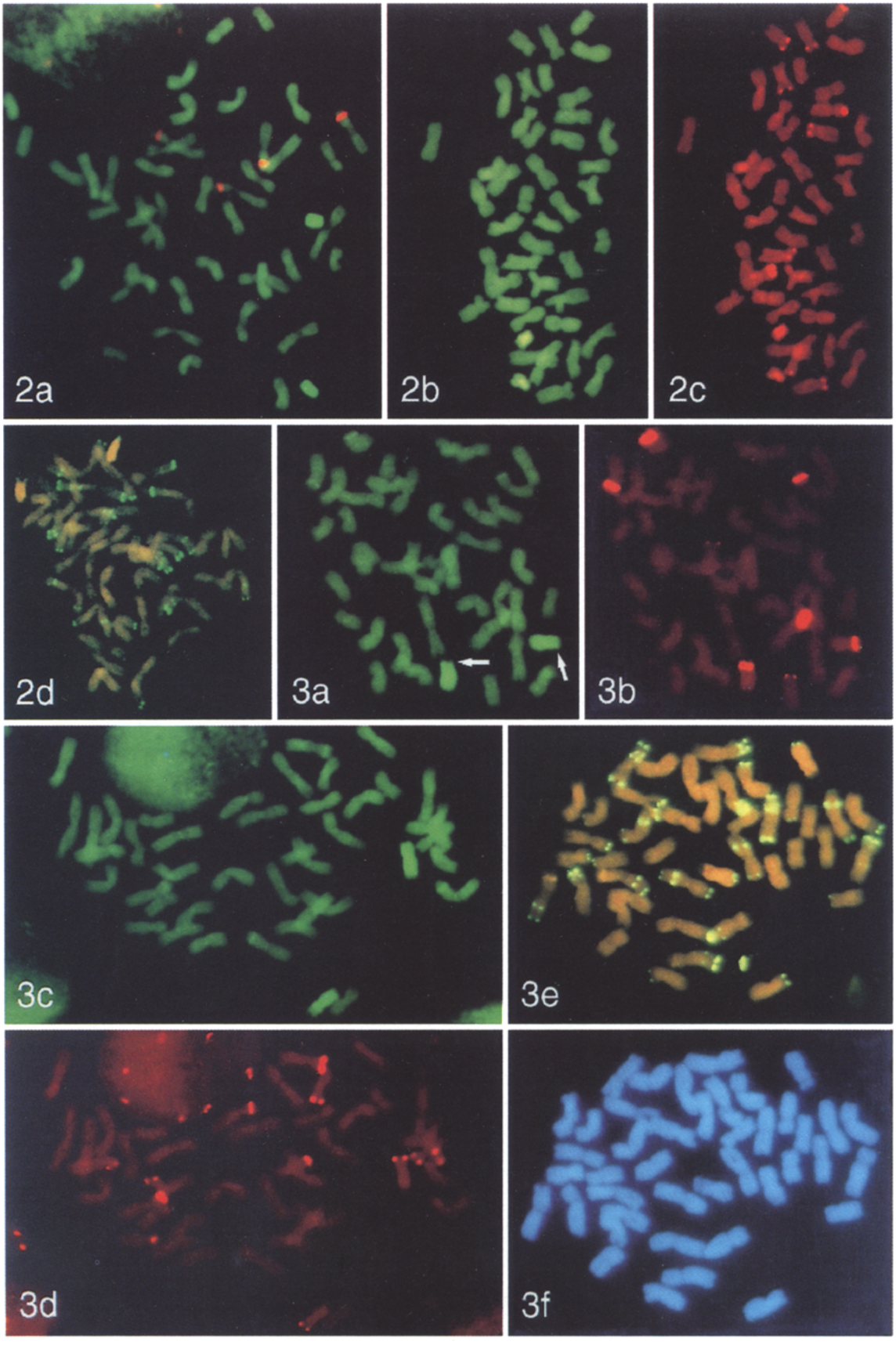

Fig. 2–3
Legend of figure 2–7 please see on page 823

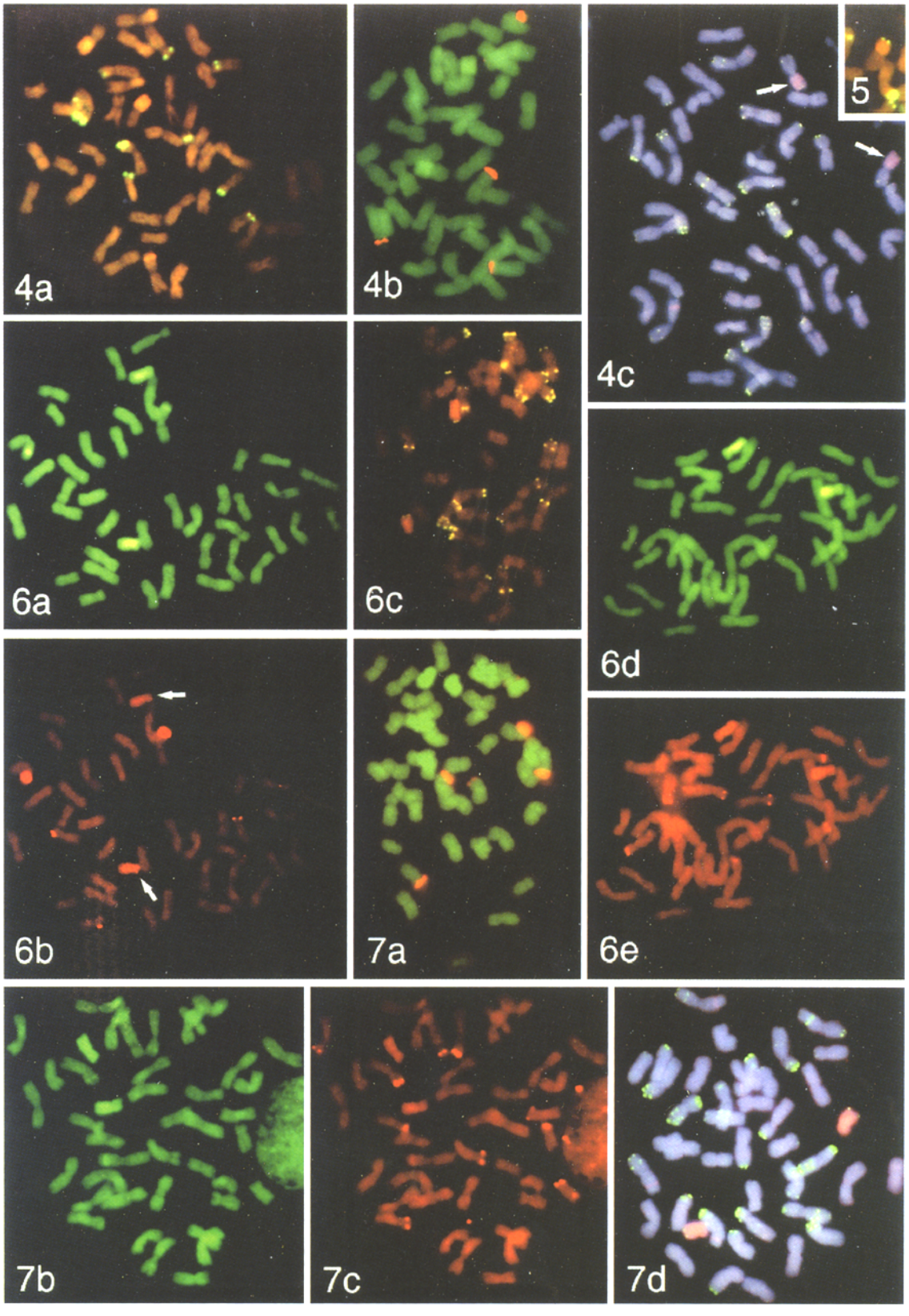

Fig. 4–7

Figs. 2–7 Double target in situ hybridization on somatic metaphase cells from root-tips of wheat-*Ae. umbellulata* recombinant lines. *Blue* shows DAPI fluorescence from DNA staining, *yellow to green* colour shows FITC fluorescence and *red* shows rhodamine or CY3 fluorescence-detecting sites of hybridization. 18*S*-25*S* rDNA is always labelled red with rhodamine and pSc119.2 sites are green, detecting digoxigenin label; other fluorochromes are described in individual legends. Colours vary slightly with different fluorescence filters and superimposition of fluorochromes. **Fig. 2** Line A10 with telocentric alien chromosomes. **a** In situ hybridization of total genomic DNA from *Ae. umbellulata* labelled with digoxigenin, detected green, and 18*S*-25*S* rDNA. Micrograph taken with a bandpass filter allowing simultaneous visualization of four major 18*S*-25*S* rDNA hybridization sites of wheat origin, the 1U chromosome segment (bright green telocentric chromosome pair; right) and the 42 wheat chromosomes (dull green). **b** Genomic in situ hybridization with *Ae. umbellulata* DNA labelled with digoxigenin, showing two telocentric *Ae. umbellulata* chromosomes (bright yellow-green; lower left) and **c** 5*S* rDNA (biotin label: bright red bands) at ten sites on chromosomes of wheat origin (dull red). **d** Hybridization pattern of the tandem repeated DNA sequence (pSc119.2; green dots) and genomic DNA from *Ae. umbellulata* labelled with biotin (orange telocentric chromosome pair; top). **Fig. 3** Examples of lines A30, A34, A37, A47 and A64 with wheat-alien recombination proximal to the *Ae. umbellulata* 5*S* rRNA locus. **a** Genomic in situ hybridization with *Ae. umbellulata* DNA labelled with digoxigenin (bright green) (line A30); wheat-alien recombinant chromosomes are *arrowed* at the breakpoint, and have a small, dull green terminal segment of wheat origin, while in **b** the 18*S*-25*S* rDNA probe shows four major wheat and two *Ae. umbellulata*-origin rDNA sites (line A47). **c** Genomic in situ hybridization with *Ae. umbellulata* DNA (bright green, right centre) (line A30) and **d** the sites of 5*S* rDNA (biotin-label: red bands) on ten wheat-origin and two *Ae. umbellulata*-origin chromosome segments (line A30). **e, f** Hybridization pattern of pSc119.2 (green dots) and genomic DNA from *Ae. umbellulata* labelled with biotin (bright orange, top right and centre) (line A64). The dull wheat-origin terminal segment is seen on the short arm of the recombinant chromosome that also has a green, terminal pSc119.2 band on the long arm, both undetectable by DAPI staining alone (**f**). **Fig. 4** Examples of lines A56 and A58 with wheat-alien recombination near the centromere of the *Ae. umbellulata*-origin long-arm chromosome segment. **a** In situ hybridization using 5*S* rDNA (digoxigenin label; yellow) and total DNA from *Ae. umbellulata* (biotin label; orange, top left and centre). 18*S*-25*S* rDNA sites are also visible by orange fluorescence, while wheat-origin chromosomes and the segments on the recombinant chromosomes are dull orange-brown The sites of ten 5*S* rRNA genes, all of wheat origin, and the translocations near the centromere are visible. **b** Genomic in situ hybridization with *Ae. umbellulata* DNA (digoxigenin label; green) and 18*S*-25*S* rDNA, photographed with a triple bandpass filter allowing the simultaneous visualization of four major 18*S*-25*S* rDNA sites of wheat origin and the 1U chromosome segments (centre). **c** Hybridization pattern of pSc119.2 (yellow dots) and genomic *Ae. umbellulata* DNA (biotin label, detected pink, arrows), showing pSc119.2 sites on both ends of the recombinant chromosome and demonstrating that the long arm of *Ae. umbellulata* chromosome 1U is present **Fig. 5** Example of line A62 with wheat-alien recombination near the centromere of the *Ae. umbellulata*-origin long-arm chromosome segment (pSc119.2, yellow, and *Ae. umbellulata*-origin chromosome segment, orange). A terminal pSc119.2 band is present on the *Ae. umbellulata*-origin segment but not the wheat-origin segment of the recombinant chromosome. **Fig. 6** Line A65 showing absence of the terminal segment of *Ae. umbellulata* chromosome 1U short arm from within the 18*S*-25*S* rDNA site to the end of the chromosome. **a** In situ hybridization of total DNA from *Ae. umbellulata* (digoxigenin label: green) and **b** 18*S*-25*S* rDNA. The *Ae. umbellulata*-origin chromosome has no wheat segment, and the 18*S*-25*S* rDNA hybridization is terminal and weak (*arrows*; cf. strength in Fig. 3b) showing that the terminal segment of the chromosome, starting within the rDNA locus, is deleted. **c** Hybridization pattern of pSc119.2 (yellow dots) and *Ae. umbellulata* genomic DNA (biotin label, bright orange chromosome segments). **d** Hybridization of *Ae. umbellulata* genomic DNA (digoxigenin label, green) and **e** 5*S* rDNA (biotin, red dots). The eight 5*S* rDNA sites (red dots) are all of wheat origin and the locus is deleted from the *Ae. umbellulata* origin chromosome. **Fig. 7** Line A94 showing absence of the terminal segment of the short arm including the 18*S*-25*S* and 5*S* rRNA loci. **a** In situ hybridization genomic *Ae. umbellulata* DNA (digoxigenin label, green) and 18*S*-25*S* rDNA. Micrograph taken with a triple filter allowing simultaneous visualization of four 18*S*-25*S* rDNA sites, all of wheat origin, and the deleted 1U chromosomes (lighter-green, top centre). **b** In situ hybridization of *Ae. umbellulata* (digoxigenin; green) and **c** 5*S* rDNA (red). There are ten 5*S* rDNA sites, all of wheat origin. **d** Hybridization of pSc119.2 (digoxigenin, yellow) and total genomic DNA (biotin, red). Microphotograph taken with a triple filter

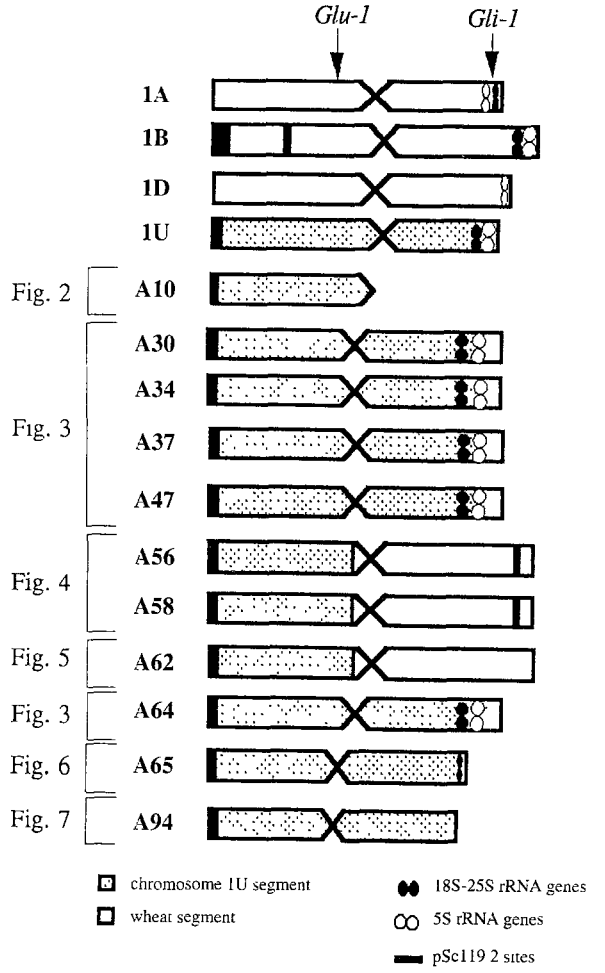

Fig. 8 Diagrammatic representation of the wheat homoeologous group-1 chromosomes, the *Ae. umbellulata* 1U chromosome, and translocated chromosomes in each recombinant line. The figure shows, in each recombinant line, the relative size of the *Ae. umbellulata* 1U chromosome segment with the origin and relative location of the 18*S*-25*S* and 5*S* rRNA genes and the location of pSc119.2 bands

Conclusions

This investigation shows that multiple target in situ hybridization using a combination of genomic DNA and repetitive DNA probes provides a sensitive, informative and accurate method to characterize recombinant chromosomes in wheat. The additional information obtained makes it possible to distinguish between monosomic and disomic recombinant lines and also allows a better understanding of the chromosomal complement in the recombinant lines.

The effort involved in using in situ hybridization is considerable, and would be prohibitive for the analysis of an extensive crossing programme involving many hundreds of potential recombinants. However, clear marker loci are often available for the pre-selection of potential recombinants: in the present case, all lines tested showed the HMW glutenins but not the gliadins of *Ae. umbellulata* origin, and lines including both *Ae. umbellulata* genes were discarded by Islam-Faridi (1988). Analysis of the genetic constitution and rearrangements using either molecular markers or conventional cytological analysis of somatic or meiotic chromosome preparations would require considerably more effort than the present analysis, and key results about the presence and nature of the recombinant chromosomes would have been obscured by the other karyotypic rearrangements and translocations found within the lines. Using in situ hybridization we could directly visualize the morphology of the alien chromosomes or chromosome segments, thus identifying the number and size of the alien segments, translocations, and aspects of the structure of wheat chromosomes.

The results obtained from the 11 recombinant lines are summarized in Fig. 8. The idiograms of the translocated chromosomes indicate the physical locations of the breakpoints, the short arm markers of the 18*S*-25*S* and 5*S* rDNAs and the repetitive DNA probe pSc119.2. Although the lines, a sample from those produced by Islam-Faridi (1988), were obtained from nine series of crosses using different parental lines (Table 1), we show that the progeny include only two types of wheat-*Ae. umbellulata* translocations, along with two deletion and one telocentric addition line.

An alternative method to induce recombination between wheat and alien chromosomes uses X-rays. This treatment has been extensively used in alien genetic transfers since the first report of Sears (1956), who transferred a leaf rust resistance gene (*Lr9*) from *Ae. umbellulata* to the long arm of wheat chromosome 6B (see Mukai et al. 1993b). All radiation-induced wheat-alien transfers identified so far are terminal chromosome translocations, but as Mukai et al. (1993b) have demonstrated they are randomly located with respect to C-bands. Their experiments have generated a series of wheat-rye lines and recombinants giving Hessian fly resistance that have been analysed by in situ hybridization. In contrast to radiation-induced recombinants, the recombinants induced by the manipulation of chromosome pairing reported here have occurred either near to, but not at, the centromere, or near or within the ribosomal genes in the short arm of chromosome 1U. Spontaneous translocations may often involve centromeric recombinants, although non-centromeric reciprocal translocations have occurred frequently during evolution in the Triticeae. Homoeologous pairing can occur between wheat and rye chromosomes in hybrids and these may be of considerable significance for plant breeding (Koebner and Shepherd 1985, 1986a, b); Miller et al. (1994) advocate the use of in situ hybridization to identify the wheat genotypes that are most effective for inducing homoeologous chromosome pairing and for identifying the best strategy for obtaining alien introgression into wheat.

Ae. umbellulata-wheat recombinant lines are required for transferring the *Glu-U1* loci from *Ae. umbellulata* into wheat with only small flanking regions, as the HMW glutenins are related to bread-making quality (Law et al. 1984). It is likely that the lines A56, A58 and A62 are most valuable for this purpose since the wheat-*Ae. umbellulata* translocation breakpoint is close to the *Glu-U1* genes. We therefore suggest that these are used for further crossing to induce a breakpoint on the other side of the locus. A targeted strategy using the *Ph* pairing-manipulation system to generate small intercalary rye chromosome segments in a wheat background, starting from the 1BS-1RL centromeric translocation, has been used with considerable success by Koebner and Shepherd (1986a, b). It is likely that a similar strategy would prove successful with these lines.

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References

Anamthawat-Jónsson K, Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1990) Discrimination between closely related Triticeae species using genomic DNA as a probe. Theor Appl Genet 79:721–728

Appels R, Gerlach WL, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma 78:293–311

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. Cell 19:545–560

Bietz JA, Wall JS (1973) Isolation and characterization of gliadin-like subunits from glutenin. Cereal Chem 50:537–547

Bushuk W, Zillman RR (1978) Wheat cultivar identification by gliadin electrophoregrams. 1. Apparatus, method and nomenclature. Can J Plant Sci 58:505–515

Castilho A, Heslop-Harrison JS (1995) Physical mapping of 5*S* and 18*S*-25*S* rDNA and repetitive DNA sequences in *Aegilops umbellulata*. Genome 38:91–96

Chapman V, Riley R, Miller TE (1975) Alien chromosome addition and substitution lines. Annu Rep Plant Breed Inst Cambridge 1974, pp 125–126

Damania AB (1993) (ed) Biodiversity and wheat improvement. John Wiley and Sons, UK

Gale MD, Miller TE (1987) The introduction of alien genetic variation in wheat. In: Lupton FGE (ed) Wheat breeding – its scientific basis. Chapman and Hall, London, pp 173–210

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Gerlach WJ, Dyer TA (1980) Sequence organization of the repeating units in the nucleus of wheat which contains 5S-rRNA genes. Nucleic Acids Res 8:4851–4865

Hamer RJ, Weegels PL, Marseille JP (1992) Prediction of the breadmaking quality of wheat: the use of HMW glutenin-A subunit-based quality scoring systems. J Cereal Sci 15:91–102

Heslop-Harrison JS, Schwarzacher T, Leitch AR, Anamthawat-Jonsson K, Bennett MD (1988) A method of identifying DNA sequences in chromosomes of plants. European Patent Application Number 8828130 8 December 2

Heslop-Harrison JS, Leitch AR, Schwarzacher T, Anamthawat-Jonsson K (1990) Detection and characterization of 1B/1R translocations in hexaploid wheat. Heredity 65:385–392

Heslop-Harrison JS, Schwarzacher T, Anamthawat-Jonsson K, Leitch AR, Shi M, Leitch IJ (1991) In situ hybridization with automated chromosome denaturation. Technique 3:109–115

Hutchinson J, Miller TE (1982) The nucleolar organisers of tetraploid and hexaploid wheats revealed by in situ hybridization. Theor Appl Genet 61:285–288

Islam-Faridi MN (1988) Genetical studies of grain protein and developmental characters in wheat. PhD thesis, University of Cambridge

Jiang J, Gill BS (1994) Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. Genome 37:717–725

Koebner RMD, Shepherd KW (1985) Induction of recombination between rye chromosome 1RL and wheat chromosomes. Theor Appl Genet 71:208–215

Koebner RMD, Shepherd KW (1986a) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induced allosyndesis. 1 Isolation of recombinants. Theor Appl Genet 73: 197–208

Koebner RMD, Shepherd KW (1986b) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induced allosyndesis. 2. Characterization of recombinants. Theor Appl Genet 73:209–217

Koebner RMD, Shepherd KW (1987) Allosyndetic recombination between a chromosome of *Ae. umbellulata* and wheat chromosomes. Heredity 59:33–45

Law CN, Payne PI (1983) Genetical aspects of breeding for improved grain protein content and type in wheat. J Cereal Sci 1:79–93

Law CN, Payne PI, Worland AJ, Miller TE, Harris PA, Snape JW, Reader SM (1984) Studies of genetical variation affecting grain protein type and amount in wheat. In: Cereal grain protein improvement. IAEA, Vienna, pp 279–300

McIntyre CL, Clarke BC, Appels R (1988) Amplification and dispersion of repeated DNA sequences in the Triticeae Pl Syst Evol 160:39–59

Miller TE, Reader SM, Purdie KA (1994) Determination of the frequency of wheat-rye chromosome pairing in wheat × rye hybrids with and without chromosome 5B. Theor Appl Genet 89:255–258

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. J Hered 81:290–295

Mukai Y, Endo TR, Gill BS (1991) Physical mapping of the 18S.26S rRNA multigene family in common wheat: identification of a new locus Chromosoma 100:71–78

Mukai Y, Nakahora Y, Yamamoto M (1993a) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome 36:489–494

Mukai Y, Friebe JH, Yamamoto M, Gill BS (1993b) Molecular cytogenetic analysis of radiation-induced wheat-rye terminal and intercalary chromosomal translocations and detection of rye chromatin specifying resistance to Hessian fly. Chromosoma 102:88–95

Payne PI, Corfield KG (1979) Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145:83–88

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-1A*, *Glu-1B* and *Glu-1D* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Payne PI, Corfield KG, Blackman JA (1979) Identification of a high-molecular-weight subunit of glutenin whose presence correlates with the bread-making quality in wheats of related pedigree. Theor Appl Genet 55.153–159

Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theor Appl Genet 60:229–236

Payne PI, Holt LM, Hutchinson J, Bennett MD (1984) Development and characterization of a line of bread wheat, *Triticum aestivum*, which lacks the short-arm satellite of chromosome 1B and the *Gli-B1* locus. Theor Appl Genet 68:327–334

Rayburn AL, Gill BS (1985) Use of biotin-labelled probes to map specific DNA sequences on wheat chromosomes. J Hered 76: 78–81

Riley R (1966) The genetic regulation of meiotic behaviour in wheat and its relatives. Proc 2nd Int Wheat Symp Lund 1963. Hereditas (suppl) 2:395–408

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ hybridization and the architecture of the nucleus. Trans Roy Microscopical Soc 1:669–674

Schwarzacher T, Anamthawat-Jonsson K, Harrison GE, Islam AKMR, Jia JZ, King IP, Leitch AR, Miller TE, Reader SM, Rogers WJ, Shi M, Heslop-Harrison JS (1992) Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat. Theor Appl Genet 84:778–786

Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookaven Symp Biol 9:1–22

Sears ER (1972) Chromosome engineering in wheat. Stadler Symp 4: 23–38

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet 10:31–51

Sharma HC, Gill BS (1983) Current status of wide hybridization in wheat. Euphytica 32:17–31

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Shepherd KM (1973) Homoeology of wheat and alien chromosomes controlling endosperm phenotypes. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, University of Missouri, Columbia, Missouri, pp 745–760