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# Analysis of a set of homoeologous group 1 wheat-*Aegilops umbellulata* recombinant chromosome lines using genetic markers

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**Abstract** Eleven wheat-*Ae. umbellulata* recombinant lines involving chromosome 1U, including an important high-molecular-weight glutenin locus, were characterized by protein and RFLP markers. Four 1U-1A recombinants, one 1U-1B recombinant, two 1U deletions with either nullisomy for chromosome 1A or 1B and a 1U ditelosomic addition line were detected, while 3 recombinant lines involved 1U and non-homoeologous wheat chromosomes. Similar recombination events were found in independent lines, and no small segmental translocations of *Ae. umbellulata* chromatin were detected. Correlation of the markers with physical maps of the wheat-*Ae. umbellulata* breakpoints obtained using in situ hybridization enabled the marker order to be established on chromosomes 1A, 1B and 1U. The short arm of chromosome 1A probably differs from both 1U and 1B by one inversion. As now being found to be universal in the Triticeae, clustering of the genetical map in the distal physical regions of the group 1 chromosomes was found.

**Key words** Glutenin loci · Mapping · Evolution · Genome organization · Wheat · *Aegilops umbellulata*

## Introduction

The relatives of wheat, *Triticum aestivum* L., offer a valuable source of genes for improvement of the crop, including bread-making quality. High-molecular-weight (HMW) glutenin subunits, coded by genes located proximally on the long arm of chromosomes from homoeologous group 1 (Payne et al. 1981), have been found to account for a large proportion of the variation in bread-making quality (Rogers et al. 1987). Considerable variation in glutenin subunits exists amongst wheat varieties, but more "high quality" protein subunits are of interest. The wheat relative *Aegilops umbellulata* Zhuk. carries genes at the *Glu-U1* locus that code for a pair of HMW glutenin subunits not found in bread wheat, which could be used to improve bread-making quality. Law et al. (1984) and Islam-Faridi (1988) initiated a programme to transfer these novel proteins from *Ae. umbellulata* into wheat by inducing recombination between chromosome 1U and the group 1 wheat chromosomes through the manipulation of chromosome pairing and recombination at meiosis (Islam-Faridi 1988). Eleven of these wheat-*Ae. umbellulata* recombinant lines were determined by means of SDS-PAGE to be carrying *Glu-U1* but lacking *Gli-U1*, and these were subsequently characterized by multiple target in situ hybridization to evaluate the extent of recombination and to localize physically the translocation breakpoints (both near the centromeres and also along chromosome arms) (Castilho et al. 1996).

Restriction fragment length polymorphisms (RFLPs), in combination with protein and other markers, provide a powerful tool in plant breeding programmes, useful for parental selection, improved backcross breeding efficiency, direct introgression of novel genes from alien germplasm and gene isolation by map-based cloning (Devos and Gale 1993), leading to improvements in selection efficiency in breeding for both qualitative and quantitative traits. Within the past few years the density of maps from all of the major crops has been increased by up to 50% (reviewed in Schwarzacher 1994). In the investigation presented here, our aim was to map the breakpoints between the wheat and *Ae. umbellulata* group 1 chromosomes using RFLP and protein markers. Although no genetic map is available for *Ae. umbellulata*, synteny between species in the Triticeae indicates that markers from the wheat map can be used to predict gene order in *Ae. umbellulata* (Moore et al. 1995). The data were analysed to show the extent of the genetic chromosome segments involved in the translocations and enable correlation of physical and genetical maps of wheat.

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## Materials and methods

Eleven wheat-*Ae. umbellulata* recombinant lines produced by Islam-Faridi (1988) were used in this investigation. Characterization of the lines by in situ hybridization to detect major genomic reorganizations showed that 1 line (coded A10) contains an *Ae. umbellulata* telocentric chromosome addition and that another 2 (A65 and A94) have distinguishable terminal deletions on the 1U chromosome. The other 8 lines, produced from six independent crosses, included only two different types of terminal recombination events: one near the centromere in the long arm of the 1U chromosome (A56, A58 and A62) and the other near the *5S-Rrna* locus on the short arm of 1U with a distal wheat segment (A30, A34, A37, A47 and A64) (Castilho et al. 1996).

The endosperm half of the seed was used for the extraction of triticin proteins, which were separated by unreduced SDS-PAGE (Singh and Shepherd 1985). The embryo half was germinated and plants grown for genomic DNA extraction. Beside the 11 recombinant lines, genomic DNA and triticin proteins were also extracted from *Ae. umbellulata*, a wheat-1U chromosome addition line (CS+1U) and two different lines of 'Chinese Spring' (CS) wheat, one from E. R. Sears and the other a euploid extracted from a monosomic 1B line including a deletion of part of chromosome 1D that was used as a parent in the original crosses.

The RFLP markers used, listed in Table 1, have been mapped on the chromosomes of homoeologous group 1, usually in wheat, rye and barley (Gale et al. 1995). Techniques of DNA extraction, restriction enzyme digestion, Southern transfer, probe labelling and hybridization were as described by Devos et al. (1992) except for the *Nor* and *5S-Rrna* loci, which were analysed by non-radioactive Southern hybridization (ECL; following Anamthawat-Jónsson et al. 1990).

## Results

Figure 1 shows the triticin proteins from CS as a triplet of bands in an unreduced SDS-PAGE gel. The bands indicated were assigned to chromosomes by analysis of wheat group 1 aneuploids in agreement with Singh and Shepherd (1985). The analysis of the results on the recombinant lines showed that lines A30, A47, A64 and A94 do not carry the *Tri-A1* locus. The 1U triticin homotetramer has indistinguishable mobility relative to that of the 1D product.

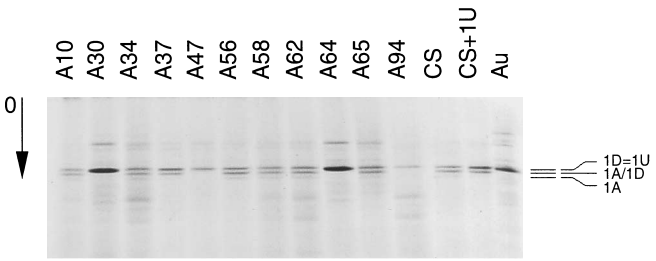


**Fig. 1** Electrophoretic pattern of the triticin proteins using unreduced samples of 'Chinese Spring' wheat (*CS*), *Ae. umbellulata* (*Au*), 1U addition line (CS+1U) and the 11 recombinant lines (A10–A94) fractionated by 10% SDS-PAGE. Chromosomes responsible for specific protein bands are indicated. This gel includes one line, A37, that is heterozygous for the wheat and recombinant chromosomes (determined by in situ hybridization to root-tip metaphases from the same seed). Disomic recombinant plants showed only the upper band

Table 2 summarizes the results for all of the informative markers, along with the number of 5S rDNA sites, glutenin and some gliadin proteins analysed previously (Castilho et al. 1996). Five further RFLP probes tested, *Xpsr13*, *Xpsr596*, *Xpsr949*, *Xpsr95* and *Xpsr162*, hybridized to *Ae. umbellulata* DNA but gave no useful polymorphisms between the wheat and 1U loci with the enzymes tested. Glutenin results obtained using the RFLP probe (Table 2) were consistent with those obtained by SDS-PAGE (Castilho et al. 1996). All markers from wheat chromosome 1D and some *Ae. umbellulata* 1U markers were present in all recombinant lines. Lines A10, A56, A58 and A62 showed all of the wheat group 1 and 1U long-arm loci, while other lines were missing some wheat group 1 loci and carried markers from the short arm of 1U.

The *Adpg*2 probe showed the absence of 1BL and 1AL loci in some lines (Table 2). The probe hybridized with two fragments in the *Ae. umbellulata* parental track; as these fragments were absent in the CS+1U addition line and the recombinant lines, no *Adpg2* locus was present on chromosome 1U.

**Table 1** Description of the RFLP markers

| Clone | Locus | Function | Type of clone | Arm location[a] | Copy number per genome | Reference |
|---|---|---|---|---|---|---|
| PSR161 | *Xpsr161* | Unknown | cDNA | 1S | 1 | Chao et al. 1989 |
| PSR381 | *Xpsr381* | Unknown | Genomic | 1S | 2 | Harcourt 1992 |
| PSR957 | *Xpsr957* | Unknown | Genomic | 1L | 1 | Devos et al. 1992 |
| pTag1290 | *Glu-1* | HMW glutenin subunits | cDNA | 1L | 2 | Thompson et al. 1983 |
| PSH2.25 | *Adpg2* | ADP-glucose pyrophosphorylase | cDNA | 1L | 1 | Ainsworth et al. 1995 |
| pTa71 | *Nor* | Ribosomal proteins | Genomic | 1BS, 6BS, 5DS, 1US, 5US[b] | Multiple | Gerlach and Bedbrook 1979 |
| pTa794 | *5S-Rrna* | Ribosomal proteins | Genomic | 1S+5S[c] | Multiple | Gerlach and Dyer 1980 |

[a] S=short arm, L = long arm
[b] Major sites analysed, showing polymorphisms in Southern analysis
[c] Fragments assigned to wheat A, B and D genomes and *Ae. umbellulata* (U) chromosomes

**Table 2** Summary of marker loci present in the wheat-*Ae. umbellulata* recombinant lines. + indicates the presence of a locus, – the absence, while ? indicates uncertainty, usually because of low polymorphism in size of fragments between or within the parents

| Genetical map[a] | Markers | Arm location | Recombinant lines | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A10 | A30 | A34 | A37 | A47 | A56 | A58 | A62 | A64 | A65 | A94 |
| Short arm telomere | | | | | | | | | | | | | |
| | *Gli-1*[b] | 1US | – | – | – | – | – | – | – | – | – | – | – |
| 7 cM | | 1BS | + | + | + | + | + | + | + | + | + | – | + |
| | *Tri-1*[c] | 1AS | + | – | + | – | – | + | + | + | – | + | – |
| | *5S-Rrna*[d, h] | 1US | – | + | + | + | + | – | – | – | – | – | – |
| 30 cM | | Wheat sites[f] | 12 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 10 |
| | *Nor*[d] | 1BS | + | + | – | + | + | + | + | + | + | – | + |
| | | 6BS | + | + | + | + | + | + | + | + | + | + | + |
| | | 1US | – | + | + | + | + | – | – | – | + | +? | – |
| 8 cM | | | | | | | | | | | | | |
| | *Xpsr381*[e, g] | 1US | – | + | + | + | + | – | – | – | + | +? | – |
| | | 1AS | + | – | + | –? | –? | + | + | + | – | + | – |
| | | 1BS | + | + | – | + | + | + | + | + | + | – | + |
| 3 cM | | | | | | | | | | | | | |
| | *Xpsr161*[e] | 1US | – | + | + | + | + | – | – | – | + | +? | + |
| | | 1AS | + | – | + | – | – | + | + | + | – | + | – |
| | | 1BS | + | + | – | + | + | + | + | + | + | – | + |
| Centromere | | | | | | | | | | | | | |
| 3 cM | | | | | | | | | | | | | |
| | *Xpsr957*[e] | 1UL | + | + | + | + | + | + | + | + | + | + | + |
| | | 1AL | + | –? | + | –? | –? | ? | + | + | –? | + | –? |
| | | 1BL | + | + | – | + | + | + | + | + | + | – | + |
| 23 cM | | | | | | | | | | | | | |
| | *Glu-1*[b, e] | 1UL | + | + | + | + | + | + | + | + | + | + | + |
| | | 1AL | + | – | + | – | – | + | + | + | – | + | – |
| | | 1BL | + | + | – | + | + | + | + | + | + | – | + |
| 52 cM | | | | | | | | | | | | | |
| | *Adpg2*[e] | 1AL | + | – | + | – | – | + | + | + | – | + | – |
| | | 1BL | + | + | – | + | + | + | + | + | + | – | + |
| Long arm telomere | | | | | | | | | | | | | |

[a] Recombination distances are approximate and averages of those in chromosomes 1A, 1B and 1D (Gale et al. 1995). The order of the *5S-Rrna* and *Nor* loci is the same on chromosome 1B and 1U but is reversed on chromosome 1A (Castilho and Heslop-Harrison 1995); the recombination distances between the *Nor* and adjacent loci are based on those for the 1B chromosome
[b] Results obtained by SDS-PAGE analysis (from Castilho et al. 1996)
[c] Results obtained by unreduced SDS-PAGE (Fig. 1)
[d] Results obtained by RFLP analysis with non-radioactive Southern hybridization
[e] Results obtained by RFLP analysis with radioactive Southern hybridization
[f] Number of sites on wheat chromosomes detected by in situ hybridization (from Castilho et al. 1996)
[g] The locus has only been mapped in barley and the data was extrapolated to the wheat map (K. M. Devos, personal communication)
[h] *5S-Rrna* locus has not been mapped

## Discussion

All of the group 1 markers tested, originating from wheat, were present in *Ae. umbellulata*, thereby showing that these loci are widely conserved in the Triticeae (Fig. 1 and Table 2) and are useful for the determination and mapping of breakpoints, even in species where complete maps are not available. About half of the markers showed informative polymorphisms between wheat and *Ae. umbellulata*.

All of the protein and informative RFLP markers were present in at least some of the recombinant lines, except for the distal long arm marker, *Adpg*2, which was found to be absent from chromosome 1U. Koebner (1987) showed that chromosome 1U carries a 1U-5U translocation relative to wheat on the long arm. No evidence for inter-chromosome rearrangements involving group 1 has been found in wheat and rye (Devos et al. 1993). However, other *Ae. umbellulata* chromosomes are involved in translocations (Sharp et al. 1989), including a reciprocal translocation between chromosomes 4U and 7U (King et al. 1994). Intra-chromosomal rearrangements involving the order of the *5S-Rrna* and *Nor* loci on 1U and 1A relative to 1B have also been demonstrated (Castilho and Heslop-Harrison 1995).

In situ hybridization showed that all lines, except A10, include a single pair of wheat-*Ae umbellulata* recombinant chromosomes (Castilho et al. 1996). The marker results were consistent with line A10 being a 1UL telocentric addition line to wheat. In the other lines, marker data, in combination with the in situ hybridization results, enabled identification of the wheat chromosome segments that were replaced with segments of chromosome 1U, showed the genetic location of the wheat-*Ae. umbellulata* breakpoint and demonstrated the deletion of loci from 1U.

The recombinant lines shown to be nullisomic 1B (A65) or to include a 1U-1B translocation (A34) were derived from 1U(1B) substitution lines (see Castilho et al. 1996, Table 1 for detailed crossing scheme), which is consistent with the parentage and homoeologous chromosome recombination involving the 1B chromosome. Similarly, lines derived from a 1U(1A) substitution include A37, A47, A64 (all 1U-1A translocations) and A94 (nullisomic 1A). Line A30, derived from a 1U(1B) substitution, has a 1U-1A recombinant chromosome that is probably derived from complex pairing involving group 1 chromosomes at meiosis during the crossing programme. The other 3 lines [derived from 1U(1A) substitutions; A62 and the siblings A56 and A58] include translocations between 1U and wheat chromosomes other than group 1 since none of the wheat group 1 loci examined here were missing. A pair of wheat 5S-rDNA sites (found on all six pairs of wheat group 1 and 5 chromosomes) are absent in the 3 lines, indicating deletion of the short arm of a group 5 chromosome (since group 1 loci were present). Some lines had indistinguishable breakpoints, suggesting that particular chromosomal regions may be prone to breakage and homoeologous recombination; we know that in rye chromosome breakage and rejoining in chromatid-type breakage-fusion-bridge cycles occurs at particular sequence motifs (Vershinin et al. 1995).

Figure 2 shows the correlation between the genetic maps reported for wheat chromosomes and the physical maps based on breakpoints between wheat and *Ae. umbel-*

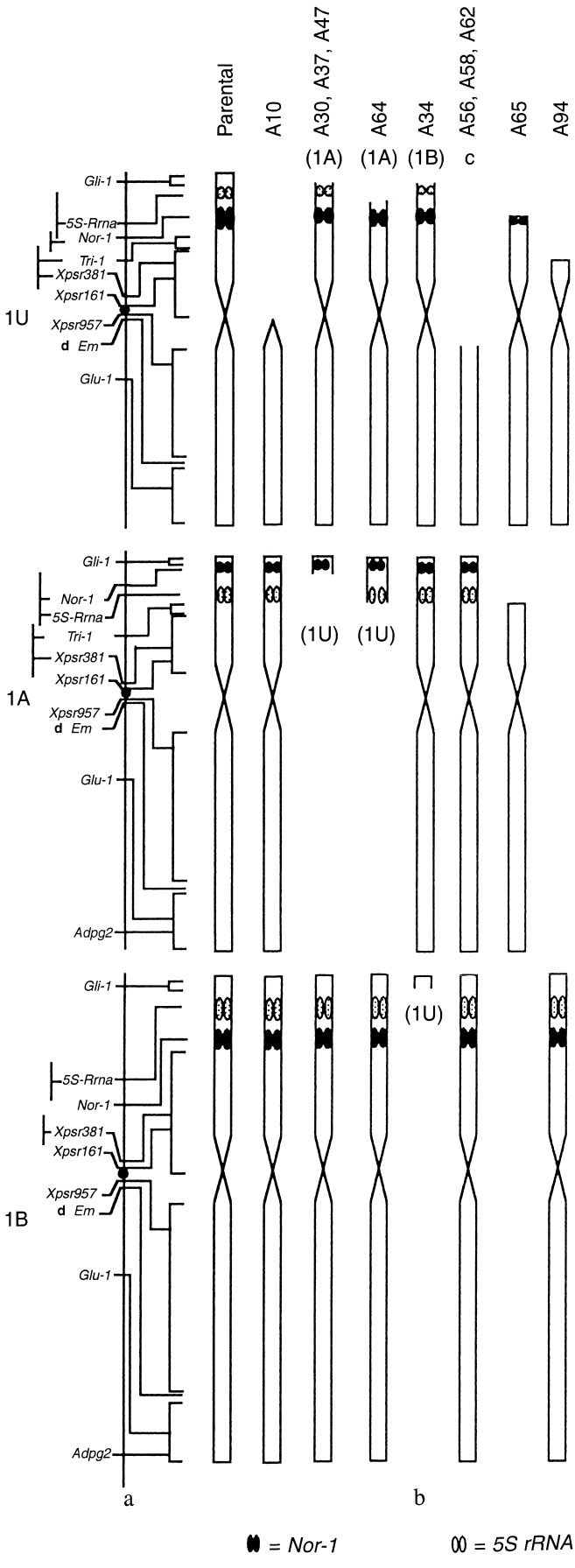


**Fig. 2** Diagrammatic representation of the chromosome constitution of each class of recombinant line. As well as the chromosome pairs illustrated, each line includes the 19 chromosome pairs from the A-, B- and D-genome homoeologous groups 2–7, and chromosome 1D, with some background translocations. *Left side* genetic map of the loci analysed. Chromosomes show the morphology determined by in situ hybridization (Castilho et al. 1996) and the marker loci (Table 2) at their inferred physical positions. **a** Genetical map of the RFLP probes used. Genetical distances are based on Gale et al. (1995) for the wheat group 1 chromosomes. The order of the *5S-Rrna* and *Nor-1* markers is reversed on wheat chromosome 1A, compared with 1B and 1U chromosomes. The *Xpsr381* locus has not been mapped in wheat, but its position in the map has been extrapolated from the results in barley K. M. Devos (personal communication). The vertical bars, on the *left-hand side* connect adjacent loci and indicate ranges of map interval; where possible, gene order is inferred from the chromosome maps *b*. **b** Physical maps of the wheat-*Ae. umbellulata* lines assigning the RFLP probes on the recombinant chromosome and on the background wheat group 1 chromosomes. All lines carry a pair of 1D chromosomes. **c** Unknown wheat chromosome segment, see Discussion. **d** *Em* physical map location from Leitch et al. (1994)

*lulata* group 1 chromosomes. As has been widely reported by others (Curtis and Lukaszewski 1991; Heslop-Harrison 1991; Kota et al. 1993; Gill et al. 1993), the results here also show that well-distributed genetic markers are physically clustered in distal regions of chromosomes, a region of high meiotic recombination. Even using large mapping populations, few recombination events were found between some markers, so there is a degree of uncertainty about gene order in the genetic maps (Fig. 2a). The analysis of the breakpoints presented here enables the unequivocal ordering of some of the genetic markers in the A, B and U genomes (Fig. 2b). Chromosomes 1A and 1U differ in their *5S-Rrna* and *Nor* order, and both have the *Tri-1* and *Xpsr381-1* loci more proximal than the ribosomal loci. Analysis of the orientation of the marker sequences with respect to the chromosome would be able to test the nature of evolutionary inversions.

In conclusion, the data indicate that wheat-alien chromosome breakpoints can be identified and mapped using syntenic RFLP and protein markers. Without prior cytogenetical characterization of the lines studied here, which enables selection of candidate molecular markers, it would have required a more extensive map of the alien species and the use of more markers to determine the breakpoints and chromosomal rearrangements present in the lines. A small number of markers were able to identify most of the chromosomes involved in the translocations. The reason why not translocation involved only small segments of alien chromosomes remains enigmatic, although obtaining small segments remains an important challenge for plant breeders wishing to transfer useful genes between species without the involvement of many linked deleterious characters.

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