Equivalent locations of sucrose synthase genes in chromosomes 7D of wheat, 7Ag of *Agropyron elongatum*, and 7H of barley

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Wheat/*Agropyron* 7D/7Ag recombinant chromosomes were analysed by Southern blotting, using cDNA radioactive probes corresponding to the *Ss1* and *Ss2* sucrose synthase genes of wheat. The genes were located in the central segments of the short arms of both chromosomes 7D and 7Ag. A similar analysis of wheat/barley addition lines demonstrated that DNA sequences that cross-hybridized with the wheat probes were located in chromosome 7H of barley.

Sucrose synthase gene; (Wheat, *Agropyron*, Barley)

1. INTRODUCTION

Genomes, chromosomes or chromosomal segments from related taxa are considered to be homologous if they can be traced to a common ancestral counterpart, having retained enough of the original genetic structure for the common origin to be recognised, even if they have lost their ability to pair at meiosis under normal conditions. Homology can be assessed by the ability of an alien chromosome or chromosomal segment to compensate for the loss of phenotypic traits, such as vigor or fertility, caused by the corresponding deletions in the genome of the recipient species or, in certain cases, by studying meiotic pairing in hybrids under appropriate experimental conditions. A more detailed analysis of homology can be derived through investigation of the chromosomal location of genes.

We have recently reported the existence of two types of sucrose synthase genes, *Ss1* and *Ss2*, which are linked in the short arms of group 7 chromosomes of the A, B and D genomes of cultivated wheat [1]. Here, we present evidence that these genes are similarly located in equivalent central segments of the short arms of chromosomes 7D from wheat and 7Ag from *Agropyron elongatum*. We also report the existence of cross-hybridizing DNA sequences in chromosome 7H from barley.

2. MATERIALS AND METHODS

2.1. *Genetic stocks*

Hexaploid wheat, *Triticum aestivum* cv. Chinese Spring, and its aneuploid nulli7D-tetra7A, the 7D/7Ag wheat/*Agropyron* substitution line, and homologous transfer lines carrying segments of chromosome 7Ag were the gift of E.R. Sears (Columbia, MO). Disomic addition lines of chromosomes 2H, 3H, 4H, 5H (formerly 7), 6H, and 7H (formerly 1) from barley, *Hordeum vulgare* cv. Betzes, on a wheat cv. Chinese Spring background were provided by K.W. Shepherd (Adelaide, Australia).

2.2. *DNA blotting and hybridization*

Recombinant plasmid DNA was isolated from bacterial cultures using the alkaline lysis method of Birnboim and Doly [2] and purified by CsCl-gradient ultracentrifugation when required. Genomic DNA was isolated from 7-day-old dark-grown seedlings essentially as described by Murray and Thompson [3]. Restriction digestion, agarose gel electrophoresis and Southern blotting to nylon membranes (Hybond, N., Amersham) were performed according to Maniatis et al. [4] and to the manufacturer's instructions. Sucrose synthase cDNA probes from wheat, P1 and P2, have been previously described [1]. Hybridization to nick-translated probes was in 5 × SSPE (0.9 M

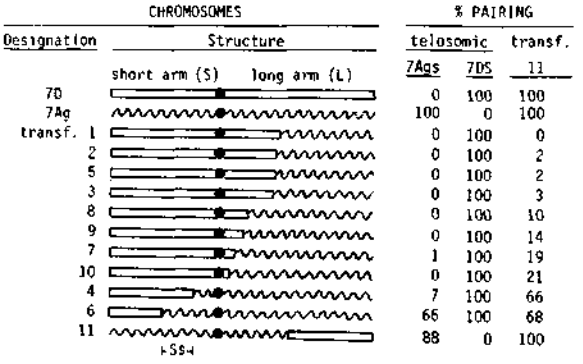
NaCl, 0.05 M NaH_2PO_4, pH 7.4, 0.005 M EDTA), 2×Denhardt's (0.04% polyvinylpyrrolidone, 0.04% BSA, 0.04% Ficoll), 0.2% SDS, 100 μg/ml salmon sperm DNA, at 65°C.

3. RESULTS

3.1. *Analysis of 7D/7Ag wheat*/Agropyron *recombinant chromosomes*

Transfer lines carrying the 7D/7Ag wheat/*Agropyron* recombinant chromosomes indicated in fig.1 were used in this study. These lines were obtained by Sears, taking advantage of the homologous recombination that takes place when the diploidizing gene *Ph* in chromosome 5B of *T. aestivum* is absent or inactive [5,6]. The extent of introgression in the recombinant chromosomes was estimated by the effectiveness of meiotic pairing with appropriate ditelosomic chromosomes (fig.1) and through the distribution of biochemical chromosome markers [7–9].

Two radioactive probes, P1 and P2, respectively corresponding to the two types of sucrose synthase genes in wheat, *Ss1* and *Ss2*, and representing approximately equivalent regions of their 3′-ends [1], were used to hybridize Southern-blotted DNAs from the wheat euploid, the nulli7D-tetra7A line, which lacks chromosome 7D, and the 7D/7Ag substitution line, in which chromosome 7D has been replaced by chromosome 7Ag from *A. elongatum*. Four hybridization bands, at 12, 7.5, 5.9 and 3.4 kb could be assigned to chromosome 7Ag with either probe (fig.2A), which is in contrast with the hybridization bands corresponding to chromosome 7D, that could be classified into two types according to their preferential hybridization with either P1 or P2 [1]. However, the fact that the sum of the four 7Ag DNA fragments (~28.8 kb) is similar to that of the four previously assigned to chromosome 7D of wheat (~27.7 kb), where they correspond to at least two genes, indicates that this is also the case in the *Agropyron* chromosome. Probe P1 was used to hybridize the DNAs from the recombinant chromosomes because it gave a clearer contrast between the 7D and 7Ag patterns (fig.2A). All transfer lines had the complete wheat pattern, except lines 6 and 11, which reproduced the pattern of the substitution line (fig.2B), indicating that both the 7D and 7Ag DNA fragments hybridizing with the probe must be located in the same central region of the short arms of the two chromosomes between the recombination points of the chromosomes in lines 4 and 6 (fig.1).



Designation	7Ags	7DS	transf. 11
7D	0	100	100
7Ag	100	0	100
transf. 1	0	100	0
2	0	100	2
5	0	100	2
3	0	100	3
8	0	100	10
9	0	100	14
7	1	100	19
10	0	100	21
4	7	100	66
6	66	100	68
11	88	0	100

Fig.1. Schematic representation of wheat/*Agropyron* 7D/7Ag recombinant chromosomes based on meiotic pairing data of hybrids between each of the transfer lines and the indicated stocks (based on [9]): ditelosomic line carrying the short arm of chromosome 7Ag (7Ags); ditelosomic line carrying the short arm of chromosome 7D (7DS); transfer line 11 (transf. 11). The region between the recombination points of transfers 4 and 6 is indicated (Ss).

3.2. *Analysis of wheat-barley addition lines*

The chromosomal location of sucrose synthase genes was investigated in the wheat/barley disomic addition lines, obtained by Islam et al. [10] for 6 of the 7 chromosomes of Betzes barley on a wheat cv. Chinese Spring genetic background. In this case, the relative intensity of the hybridization signals for the different bands from Betzes DNA digested with *Eco*RI was not the same with the two probes: a band at 1.9 kb was the most intense with probe P1, whereas a band of 15.5 kb was the most prominent in the case of probe P2 (fig.3A). The band at 1.9 kb was detected in the 7H addition line (fig.3B), whereas the band at 15.5 kb could not be detected in the wheat background because fragments of the same mobility are contributed by *Ss2* genes located in at least two group-7 chromosomes in wheat [1].

4. DISCUSSION

The observations concerning the wheat/*Agropyron* recombinant chromosomes have at least three implications: (i) The postulated identity of the *Agropyron* chromosome, which was established at the cytological level (see [6]), is further con-

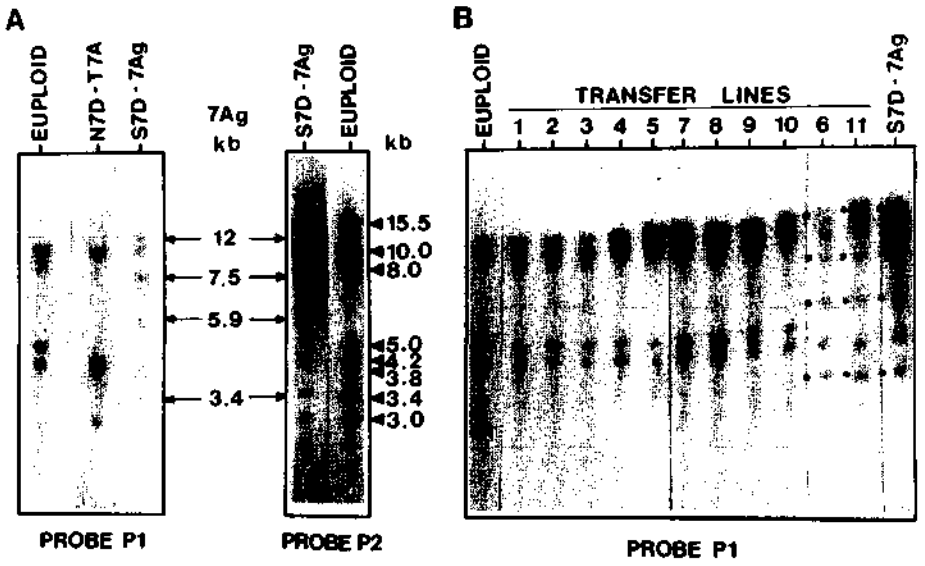


Fig.2. Southern-blot analyses of wheat/*Agropyron* recombinant chromosomes. DNAs were digested with endonuclease *Eco*RI. (A) Chinese Spring wheat euploid; nulli7D-tetra7A line (N7D-T7A); wheat/*Agropyron* 7D/7Ag substitution line (S7D–7Ag). (B) Euploid; transfer lines 1–11; substitution line (S7D–7Ag). Probes P1 and P2, respectively corresponding to genes *Ss1* and *Ss2* from wheat, were used as indicated under each panel. Hybridization bands corresponding to the 7Ag *Agropyron* chromosome are indicated by horizontal arrows (A) or by dots (B).

firmed at the DNA level. (ii) Structure has been conserved in the homologous chromosomes 7D and 7Ag to the extent that similar genes occupy equivalent positions in the two chromosomes. (iii)

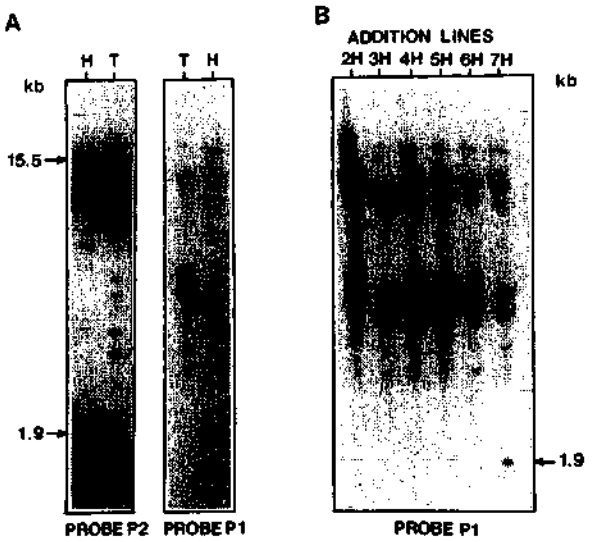


Fig.3. Southern-blot analyses of wheat/barley addition lines. DNAs were digested with restriction endonuclease *Eco*RI. (A) Wheat (T) and barley (H). (B) Disomic addition lines for chromosomes 2H to 7H.

Homologous chromosome recombination, in the absence of the diploidizing gene *Ph*, must take place in a regular manner, as judged by the absence of recombinant chromosomes with either deletions or duplications for the investigated loci. These conclusions are in line with our previous assignment of genes encoding CM-proteins in wheat and in *Agropyron* to this chromosome region [7], as well as with the inheritance of sterol ester patterns [7,11,12]. The synthesis of sterol palmitate is controlled by the *Pln* allele at a locus in the short arm of chromosome 7D from wheat [11,12], and chromosome 7Ag restores the esterification activity lost in stocks lacking chromosome 7D [7], so the observation that all the transfer lines had the normal level of sterol palmitate indicated that the gene was at equivalent positions in the chromosomes from the two species [7].

Addition line D of Islam et al. [10], which carried the barley chromosome previously designated as 1, has been found by various workers to carry biochemical chromosome markers which in wheat are associated with group 7 chromosomes and consequently, this chromosome 1 has been newly designated as 7H (see [13]). The present finding further supports this conclusion.

The two types of wheat sucrose-synthase genes, *Ss1* and *Ss2*, seem to correspond to the Sh1 and *Css* or *Ss2* genes of maize [1]. The maize genes are also linked and located in chromosome 9 [14], so it will be of interest to investigate whether other maize genes associated with this chromosome have their counterparts in group 7 chromosomes of wheat, barley and *Agropyron*.

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