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Homologous sucrose synthase genes in barley (*Hordeum vulgare*) are located in chromosomes 7H (syn. 1) and 2H

Evidence for a gene translocation?

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The chromosomal location of the two types of sucrose synthase genes, Ss1 and Ss2, has been investigated in barley by Southern blot analysis of wheat–barley addition lines using non-cross-hybridizing-specific probes corresponding to the C-terminal regions of their respective cDNA clones (≈250 bp). The Ss1 gene, whose cDNA of 2,667 bp has been entirely sequenced, is located in the β-arm of chromosome 7H (syn. 1), while that corresponding to the homologous Ss2 is in the short arm of 2H, suggesting the existence of a translocation event between these two chromosomes in cultivated barley after an initial gene duplication and divergent evolution.

Barley; cDNA cloning; DNA mapping; Sucrose synthase gene; Wheat–barley addition line

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. A detailed analysis of homology can be derived through comparison of gene sequences and investigation of the chromosomal location of genes.

Sucrose is the primary form of translocated carbon in plants, from photosynthetic mature leaves to sink-cells such as the developing endosperm [1-3]. In barley and other cereals, the endosperm accumulates high levels of starch synthesized from the transported sucrose in its amyloplasts. During germination of the seed, the endosperm becomes a source of carbohydrates, and degradation of accumulated starch provides carbon and energy for the development of the new plantule. In all these processes, the enzyme, sucrose synthase (EC 2.4.1.13), that catalyzes the reversible reaction, sucrose + UDP → UDP-glucose + fructose, plays a key role.

Duplicate genes in chromosome arms, 9S and 9L, encoding sucrose synthase (Sh1 and Ss2) have been found in maize [4–6]. In hexaploid wheat, *Triticum aestivum* L. (genomes AABBDD; 2n = 42) we have described the existence of two types of sucrose synthase genes [7], Ss1 and Ss2, corresponding to maize Sh1 and Ss2, respectively. The wheat cDNA clones have been used for chromosomal mapping and in gene expression studies [7,8], as well as for detecting cross-hybridizing sequences in related species, such as *Agropyron elongatum* and *Hordeum vulgare* L. [9]. The wheat genes were originally described to be linked in the short arms of chromosomes 7A, 7B and 7D, but recent investigations by us and others (M. Gale pers. commun.) using type-specific probes, indicate that while Ss1 genes are actually located in the chromosomes mentioned above, the Ss2 ones are in a different location.

We report here the complete cDNA cloning and sequencing of type 1 sucrose synthase (Ss1) in barley, and the chromosome mapping of both types of sucrose synthase (Ss1, Ss2), using as specific probes the non-cross-hybridizing C-terminal portions of their corresponding cDNAs. Evidence for a translocation event between chromosomes 7H (syn. 1) and 2H in barley is discussed.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X65871.

2. MATERIALS AND METHODS

2.1. *Biological material*

Developing barley endosperm from *Hordeum vulgare* L. cv Abyssinian 2231 was the source of the poly(A)$^+$ mRNA for the construction of the cDNA library in the lambda vector, NM 1149. Hexaploid wheat, *Triticum aestivum* cv. Chinese Spring, diploid barley, *Hordeum vulgare* L. cv. Betzes, and the disomic addition lines of chromosomes 2H, 3H, 4H, 5H (formerly 7), 6H and 7H (formerly 1) from barley cv.

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Betzes on a wheat Chinese Spring background were obtained from K.W. Shepherd and A.K.M.R. Islam [10]. The ditelosomic addition lines corresponding to chromosomes 2H and 7H were also analysed: 2HS (short arm of 2H), 2HL (long arm of 2H); 7Hα (probably long arm of 7H), 7Hβ (probably short arm of 7H). These lines were used for the chromosomal location of the sucrose synthase genes.

2.2. *cDNA cloning and Southern blot mapping*

The cDNA library was screened with an equimolar mixture of labelled cDNA inserts from the plasmids, pST3 and pST8 [7], corresponding to the two types of wheat sucrose synthase genes. To complete the 5′ end of the cDNA corresponding to the barley Ss1 gene, a PCR strategy was adopted [11]. Amplification was carried out under standard conditions using as template cDNA from developing barley endosperm with primers (5′ATGGCTGCCAAGCTGAC3′) and (5′CTTCTCCAAGCTGGCAG3′) derived from the genomic sequence of barley Ss1 [12] and an appropriate region of the incomplete cDNA, respectively.

Plant total DNA for chromosome mapping was isolated from 7-day-old etiolated seedlings, essentially as described [7]. Barley sucrose synthase type 1-specific probe was the *SalI/Eco*RI C-terminal fragment of 269 bp, including the last 8 amino acids plus the 3′ non-coding region. An analogous 257 bp C-terminal fragment specific for barley Ss2 was a gift from O. Martinez de Ilarduya (unpublished). All other molecular biology manipulations were according to standard procedures [13].

3. RESULTS AND DISCUSSION

3.1. *Isolation and characterization of barley sucrose synthase cDNA clones*

A cDNA library from developing barley endosperm was screened at 65°C with a mixture of cDNA probes (pST3 and pST8) corresponding to the two types of sucrose synthase genes from wheat [7]. Three positive plaques were identified, which hybridized more strongly with wheat pST8 (type 1 sucrose synthase cDNA) than to the pST3 probe. Sequencing of the longest recombinant phage of the three (λSC1), revealed a truncated

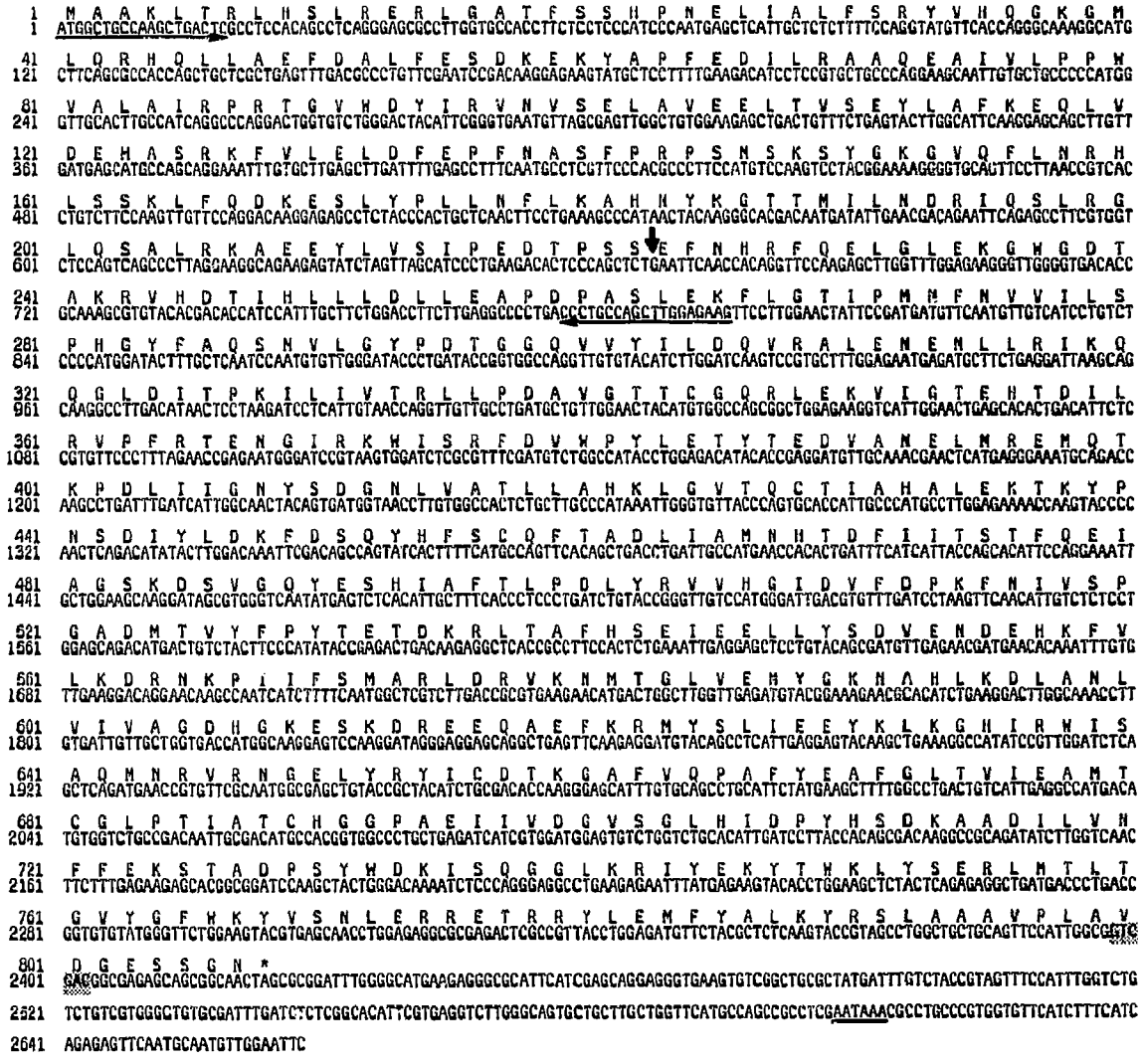

Fig. 1. Nucleotide and amino acid-derived sequence of type 1 sucrose synthase (Ss1) cDNA from barley. A vertical arrow indicates the 5′ end of the longest truncated clone obtained from a cDNA library. Horizontal arrows correspond to the regions from which PCR primers were derived in order to complete the cDNA sequence. The 3′ end of the clone, up to the shaded *SalI* site, was used as a specific probe for Ss1 in chromosome mapping experiments. The polyadenylation signal is underlined.

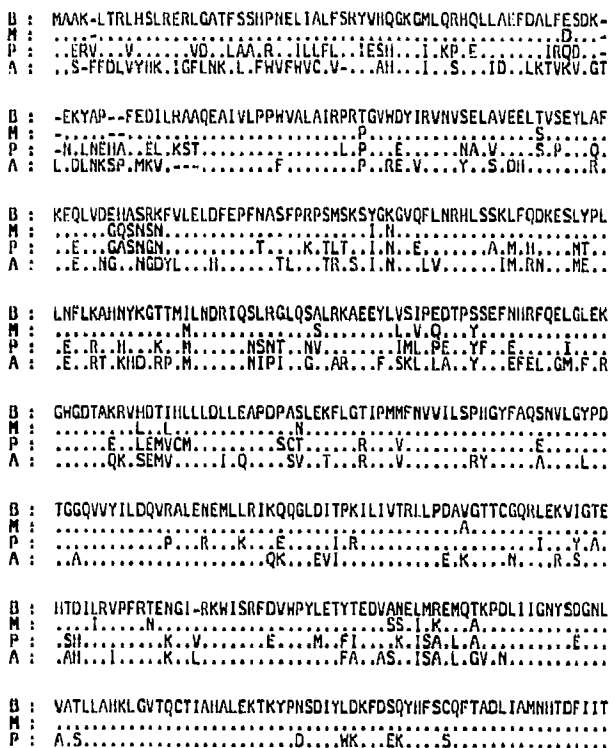

Fig. 2. Alignment of deduced protein sequences of type 1 sucrose synthases from barley (B), wheat (W) and maize (M). The only reported type for potato (P) and *Arabidopsis* (A) sucrose synthases are also included. Dots represent perfect matches with respect to the barley sequence, and deletions are indicated by dashes (note that the wheat sequence is only partial) [7].

cDNA of 2,001 bp with its 5′ end extending up to a region homologous to that corresponding to exon 6 in the maize Sh1 gene [6]. To complete the sequence of the Ss1 cDNA, the missing 5′ end fragment was amplified by PCR. The availability of a genomic clone corresponding to type 1 barley sucrose synthase, sequenced in its promoter region and 1st exon [12], permitted us to design a perfectly matching sense primer of 17 nucleotides, beginning at the ATG translation initiation codon. The antisense primer was derived from a favourable sequence in the 5′ region of the truncated cDNA (Fig. 1). This figure also displays the complete cDNA sequence for type 1 sucrose synthase from developing barley endosperm. The deduced protein sequence corresponding to this cDNA has been aligned with the sequences for type 1 sucrose synthases of wheat and maize and for the only type described so far in potato and *Arabidopsis* in Fig. 2. Binary comparisons of all these sequences expressed as percentages of identical residues appear in Table I. As expected, the three type 1 cereal genes are more closely related (>88% matches per compared length) than barley Ss1 is to potato and *Arabidopsis* (73% and 65% similarity, respectively).

Table I

Percentage of coincident residues between sucrose synthase protein sequences from different origins

	BARLEY*	WHEAT*	MAIZE*	POTATO+
WHEAT*	(96.8)			
MAIZE*	92.7	(88.8)		
POTATO+	73.4	(70.9)	69.9	
ARABIDOPSIS+	65.5	(69.0)	66.5	67.3

*: Sequences correspond to type 1 sucrose synthases.
+: Only one type reported in the EMBL library.
( ): Comparisons involving wheat refer to the partial sequence reported [7].

3.2. *Chromosomal location of sucrose synthase genes in barley*

Total DNA from the different genetic stocks described in Materials and Methods, was separately digested with the restriction enzymes, *Hin*dIII and *Bgl*II, and used in Southern blot experiments using specific non-cross-hybridizing probes (data not shown) for each type of barley sucrose synthase gene. The result of the hybridization is shown in Fig. 3. The Ss1 probe showed one band of ca. 1.3 kb that appears in the 7H addition line and in the parental barley cv. Betzes. The weaker band at 0.9 kb corresponds to wheat type 1 sucrose synthase that cross-hybridizes with its homologue from barley (>90% coincident residues), and appears in the parental wheat and in all the addition lines as expected (Fig. 3A). When a specific probe for Ss2 is used, a *Bgl*II band of 1.7 kb that appears only in the 2H addition line and in the parental Betzes barley is detected (Fig. 3C). A similar analysis of the ditelosomic addition lines reveals that the short arm of 2H, 2HS, carries the gene for Ss2, while the gene for the Ss1 gene is in the β-arm of 7H (syn. 1) (Fig. 3B,D).

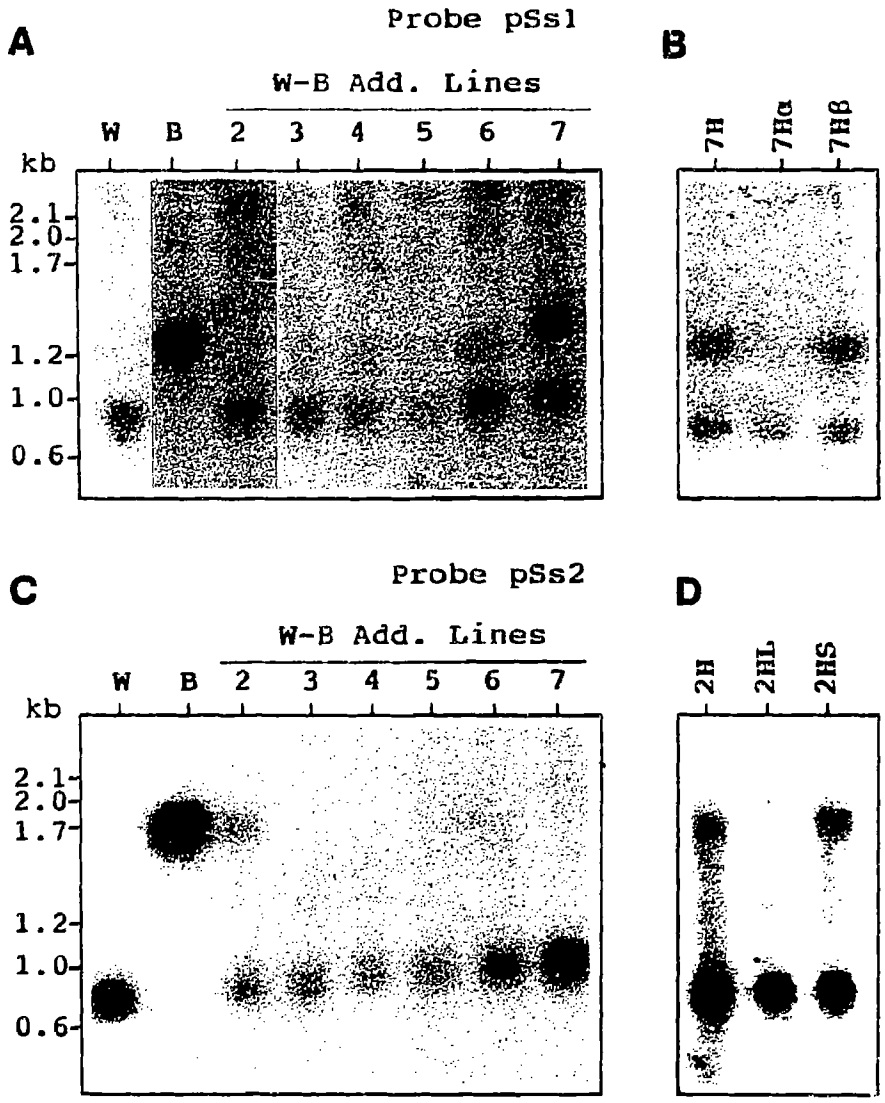

Fig. 3. Chromosomal location of genes corresponding to barley sucrose synthases, Ss1 and Ss2. Southern blot hybridization of the following genotypes: hexaploid wheat, *Triticum aestivum* cv. Chinese Spring (W), diploid barley, *Hordeum vulgare* cv. Betzes (B), Chinese Spring wheat–Betzes barley addition lines (2,3,4,5,6 and 7) and Chinese Spring wheat–Betzes barley ditelosomic addition lines (7Hα, 7Hβ, 2HL and 2HS). Addition line 1 was not available. (A and B) Hybridization patterns of total DNA digested with *Hin*dIII using the Ss1-specific probe. The band of 1.3 kb in Betzes barley is present only in addition line 7H and ditelo 7Hβ. (C and D) Hybridization patterns of total DNA digested with *Bgl*II using a Ss2-specific probe. The band of 1.7 kb in Betzes barley is present only in addition line 2H and ditelo 2HS.

Since the DNA segments assigned to the Ss1 and Ss2 genes are located in different chromosomes (2H and 7H), these two types of sucrose synthases are non-allelic and non-linked. In maize, two non-allelic, linked genes on chromosome 9 have been described for sucrose synthase. The high degree of conservation between Ss1 and Ss2 in maize, wheat and barley seems to indicate that both sucrose synthase genes are derived from a common ancestor and probably originated after a gene duplication followed by divergent evolution. In contrast with maize, their different chromosomal location in barley suggests that a translocation event could have taken place afterwards. In the Triticeae, translocations are well documented between chromosomes 4 and 7 [14] in wheat, 2R and 6R in rye [15], and 2H and 6H in barley [16]. To our knowledge, this is the first molecular evidence of a translocation event implicating chromosomes 7Hβ (syn. 1) and 2HS. In this context it would be worth exploring if other genes mapped to the short arm of chromosome 2H in barley have homologous counterparts in the β-arm of 7H (syn. 1).

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