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Sucrose synthase genes in barley*

cDNA cloning of the Ss2 type and tissue-specific expression of Ss1 and Ss2

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A cDNA of 2,708 bp encoding type 2 sucrose synthase (Ss2) from barley has been sequenced. Similarity of this cDNA with respect to that of type 1 (Ss1) is high in the coding region (75% identical positions), but low (41% identical residues) in the 3′ non-coding region. Type-specific non cross-hybridizing probes for Northern blot analysis have been obtained from the 3′ ends. The Ss1 type is highly expressed in developing endosperm and in roots and, at lower levels, in coleoptiles and aleurone. The Ss2 mRNA is abundant in endosperm, low in aleurone, and undetected in coleoptiles and roots.

Barley; cDNA cloning; Gene expression; Northern blot; Specific probe; Sucrose synthase gene

1. INTRODUCTION

The enzyme sucrose synthase (EC2.4.1.13.) catalyzes the reaction sucrose + UDP ⇆ UDP-glucose + fructose that mediates the cleavage of phloem-transported sucrose. This enzyme controls the flow of carbon into starch biosynthesis in the amyloplasts of sink organs such as the developing endosperm of cereals. Although it was first described in wheat germ by Leloir and coworkers [1], the molecular characterization of the enzyme has been carried out mainly in maize, where a shrunken endosperm mutant (*sh sh*) has been shown to have a low starch content through the inactivation of one gene for sucrose synthase . The native enzyme is a tetramer composed of either pure or mixed sets of two different types of subunits, Sh1 and Ss2. It has been claimed that Sh1 is the structural gene for the major endosperm form that is anaerobically induced in roots and shoots, while Ss2 (previously designated Css and Sus) is the structural gene for the constitutively expressed isoenzyme in embryo and other tissues [2–5].

In hexaploid wheat, *Triticum aestivum* L. (genomes AABBDD; $2n = 42$) we have described two types of sucrose synthase genes, Ss1 and Ss2, corresponding to maize Sh1 and Ss2, respectively, that are differentially induced in response to anaerobiosis, light or cold temperatures [6,7]. Diploid barley, *Hordeum vulgare* L. (genome HH; $2n = 14$), is a simpler system to study sucrose synthase in connexion with starch accumulation because this species has only one gene of each type per haploid genome, while hexaploid wheat has triplicate sets of each type of gene. We have recently characterized a cDNA clone for barley Ss1 [8] and report here the characterization of a Ss2 cDNA, as well as the differential expression of Ss1 and Ss2 in barley tissues, using type-specific probes derived from the non-coding portions of their corresponding cDNAs.

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*The nucleotide sequence data reported will appear in the EMBL-Gen Bank and DDBJ Nucleotide Sequence Databases under the accession number X69931.

2. MATERIALS AND METHODS

2.1. *Biological material*

Diploid barley, *Hordeum vulgare* L. cv Sundance was the source of the poly(A)$^+$mRNA for the endosperm cDNA synthesis and of total RNAs from different tissues for the Northern blot analysis.

2.2. *cDNA cloning and Northern-blot analysis*

A cDNA library from developing barley endosperm was screened with an equimolar mixture of labelled cDNA inserts from plasmids pST8 and pST3, respectively, corresponding to the Ss1 and Ss2 wheat genes [6,8]. The PCR amplification [9] was carried out for 35 cycles (2 min, 94°C; 1.5 min, 55°C; 4 min, 72°C) in a Perkin-Elmer apparatus using as primers the oligonucleotides (5′-GAAGAGGACAGCAATGG-3′) and (5′-GCATGACACTCTCCGAT-3′) respectively derived from the 5′ region of the Ss2 cDNA from rice [10] and the 3′ region of an incomplete barley Ss2 cDNA.

DNA sequencing was performed by the dideoxy chain termination method [11]. Analysis and sequence comparisons were done with the Beckman Microgenie software and the EMBnet/CNB computer facilities.

Plant total RNA for Northern blottings was isolated from immature endosperms collected at different developmental stages, aleurone, etiolated coleoptiles and roots, at the times indicated in the legend to Fig. 3. The extraction procedure was essentially as described by Lazarov et al. [12].

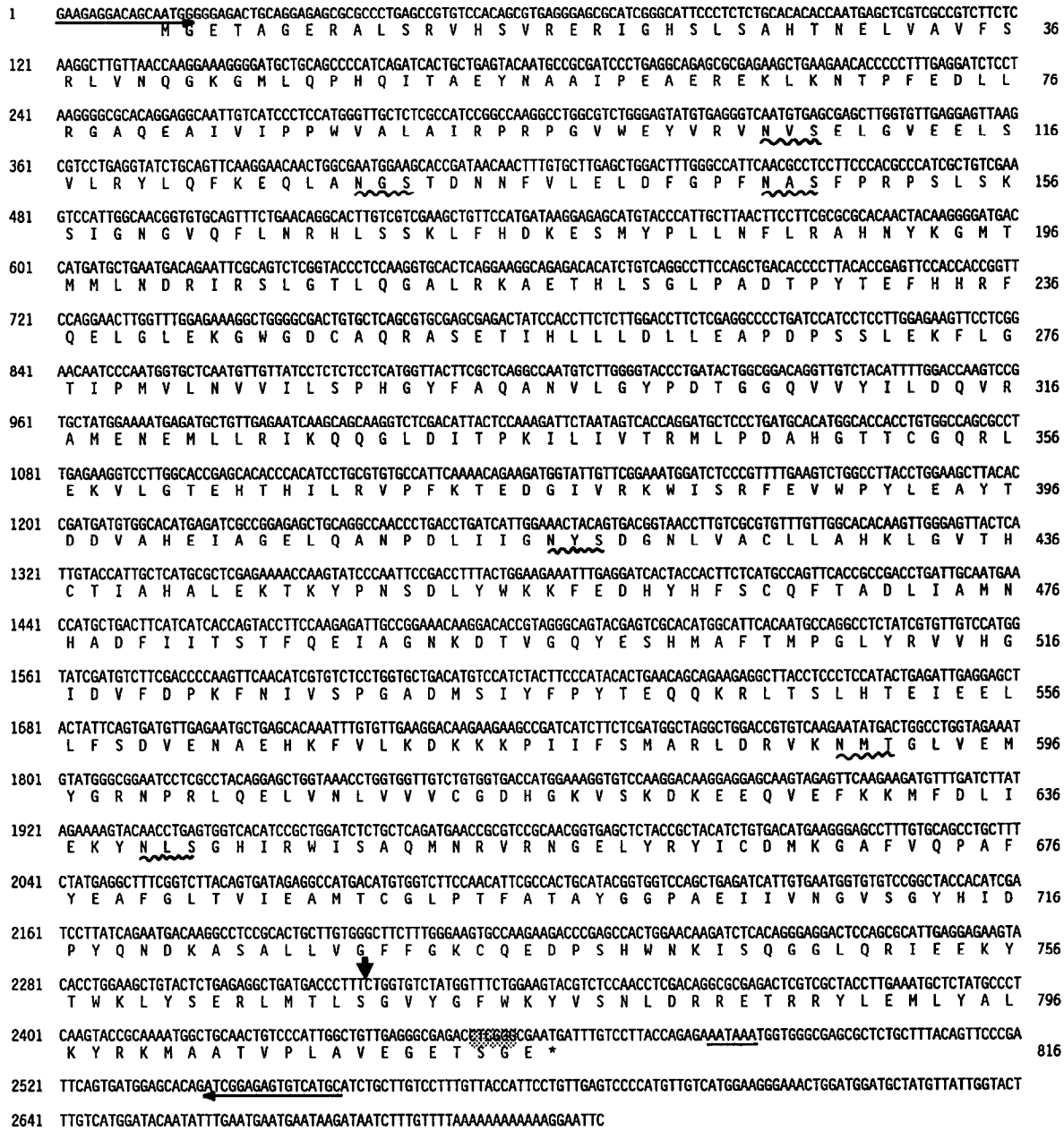
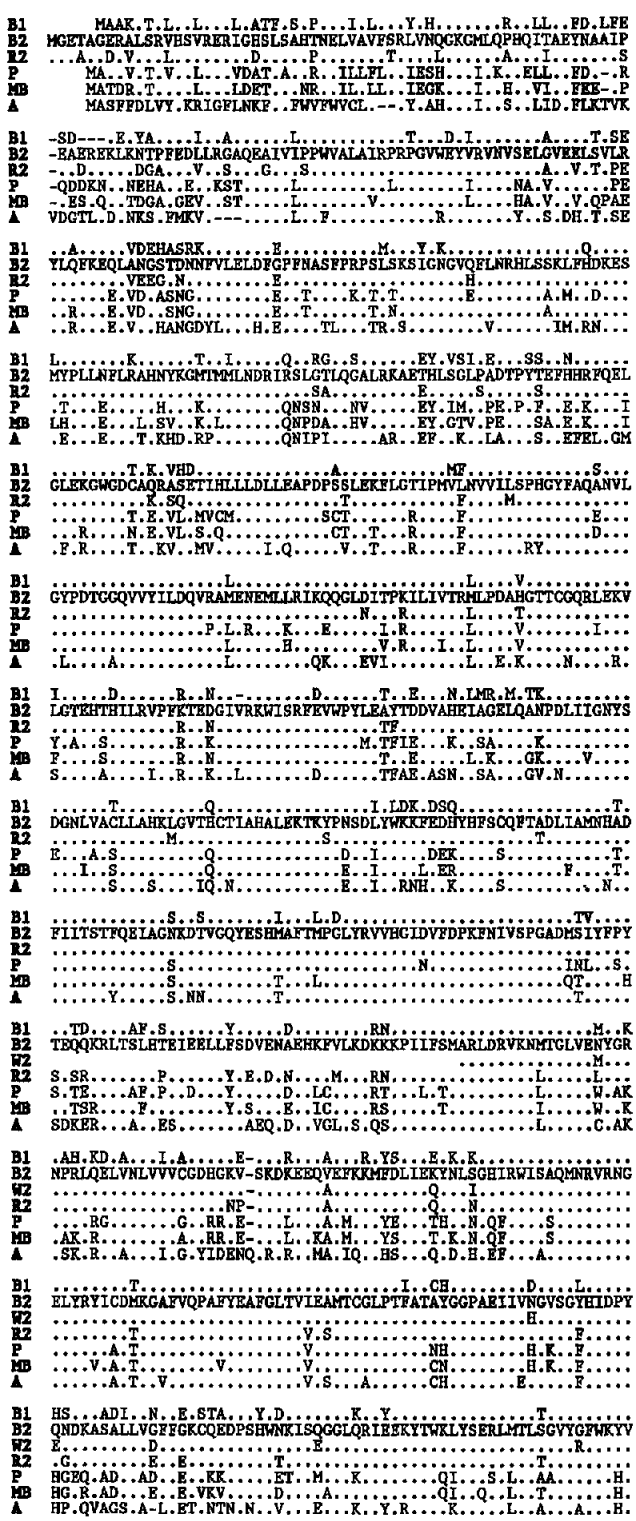


Fig. 1. Nucleotide and amino acid-derived sequences of type 2 sucrose synthase (Ss2) cDNA from barley. A vertical arrow indicates the 5′ end of the truncated clone obtained after hybridization of the cDNA library with a probe corresponding to the wheat Ss2 cDNA. Horizontal arrows underline the regions from which PCR primers were derived. The 3′ end of the clone up to the shaded *Ava*I site was used as a specific probe for Ss2 in Northern blot experiments. The six putative N-glycosylation sites (NXS/T) are underlined with a wavy line. The polyadenylation signal is underlined.

3. RESULTS AND DISCUSSION

3.1. *Characterization and sequencing of a Ss2 cDNA clone from barley endosperm*

The screening of a barley cDNA library from developing endosperm with the wheat sucrose synthase probes, pST3 and pST8 [6], allowed the identification of a cDNA of 401 bp with 85% identical residues with respect to the corresponding fragment from wheat Ss2 cDNA. To obtain the complete coding sequence of the barley Ss2 cDNA, a PCR strategy was adopted. Amplification was carried out using as template ss-cDNA from developing barley endosperm collected at 18 days after pollination (dap). In Fig. 1, the nucleotide sequence of the Ss2 cDNA is shown together with the deduced amino acid sequence corresponding to its longest open-reading frame. The putative Ss2 protein would have 816 amino acid residues (9 more than Ss1) and a



molecular weight of 92,522 Da. Six putative N-glycosylation sites (NXS/T) are found (Fig. 1).

In Fig. 2, the amino acid sequence of the barley Ss2 protein has been aligned with type-2 sucrose synthases from rice [10] and wheat [6], with barley Ss1 [8], and with the only type described so far in mung bean [13], potato [14] and Arabidopsis [15]. The maize Ss2 sequence has not been included in this comparison since it has not been published or included in the EMBL-GenBank or DDBJ nt sequence data bases. The percentages of identical residues for all binary comparisons of these sequences, which appear in Table I, were calculated using the Microgenie Sequence Analysis program (Beckman). The barley Ss2 sequence is more closely related to the Ss2 sequences from the other species (> 89% matches) than to the Ss1 sequence from the same species (79% matches), in a similar manner as previously described for the Ss1 enzymes [8]. These data suggests that a duplication followed by divergent evolution of an ancestral gene took place prior to the evolutionary branching-out between rice and the ancestor of barley and wheat. Sucrose synthase sequences from mung-bean, potato, and Arabidopsis are about equidistant with respect to the cereal Ss1 and Ss2 types (65–75%) and quite divergent from them, as expected from their greater evolutionary separation. It remains to be investigated whether one or two gene types for sucrose synthase are present in mung-bean, potato and Arabidopsis, all dicot species.

3.2. *Differential expression of Ss1 and Ss2 in barley tissues*

Northern-blot analysis of total RNAs from developing endosperm, aleurone, coleoptiles, and roots was carried out using type-specific probes. These probes were derived from the more divergent 3′ ends of the Ss1 and Ss2 cDNAs: a 269 bp *SalI*/*Eco*RI fragment from the Ss1 cDNA, which included the last 8 coding triplets and the 3′ non-coding region [8], and a 257 bp fragment spanning from an *Ava*I site near the stop codon to the 3′ terminus of the Ss2 cDNA (Figs. 1 and 3A). A single band with an apparent size of about 3 kb under denaturing conditions was observed in all expressing tissues (Fig. 3B). Loading equal amounts of the RNAs in electrophoresis, the Ss1 signal was strong in 20-dap endosperm and in young roots, faint in 20 dap aleurone and coleoptiles, and undetected in ovules and early endosperm (5 dap). In contrast, the Ss2 signal was strong

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Fig. 2. Alignment of deduced amino acid sequences of type 2 sucrose synthases from barley (B2), wheat (W2) and rice (R2). The only reported type for potato (P), mung bean (MB) and Arabidopsis (A), as well as type 1 sucrose synthase from barley (B1) are also included. Dots represent matches with respect to the barley B2 sequence and deletions are indicated by dashes. Note that the gene sequence for wheat Ss2 (W2) is only partial [7].

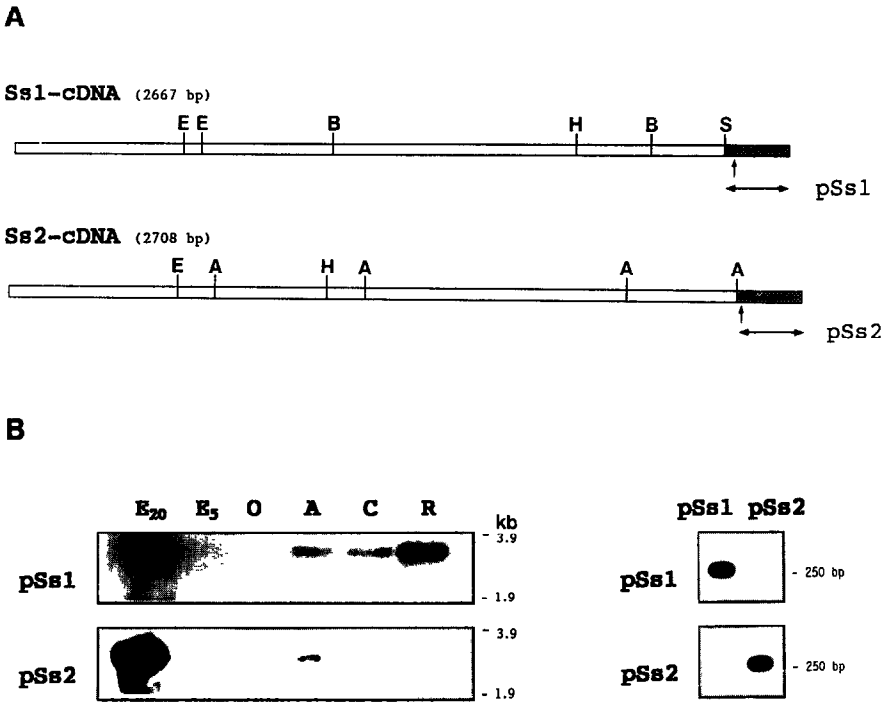

Fig. 3. (A) Restriction maps of cDNAs for barley sucrose synthases Ss1 [8] and Ss2. 3′ Terminal regions used as specific probes are shaded. The stop codon is indicated by a vertical arrow.A, *Aval*; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I. (B) Northern blot analysis using specific probes, pSs1 and pSs2. E, endosperm (E_5 and E_{20}, 5 and 20 dap); O, ovules; A, aleurone (20 dap); C, etiolated coleoptiles (7 days); R, roots (7 days). 5 μg of total RNA were applied per slot.

in 20-dap endosperm and faint in aleurone, but undetected in roots and coleoptiles (Fig. 3B). These barley data, which essentially corroborate our previous observations in wheat [7], show that sucrose synthase is highly expressed in sink organs although with different tissue-specificity patterns: both types Ss1 and Ss2, are expressed in endosperm but only type 1 is expressed in roots. In green (or green-to-be) tissues such as aleurone or coleoptile the expression is faint or undetectable.

In maize, it has been proposed that sucrose synthase Ss2 would act as a constitutive enzyme and that Sh1 (Ss1) would be the inducible counterpart, in tissues other than endosperm, providing the extra activity needed in certain situations such as anaerobiosis. In this context, McCarty et al. [5] described that Ss2 is expressed in roots at approximately the same level as Ss1 and that Ss2 is 3–4 times more abundant than Ss1 in maize coleoptiles. However, the probes used in this study were not type-specific and they relied on a maize Sh1 deletion mutant (sh1 bz-m4) for the analysis. Data from our lab, both in barley and in wheat, using type-specific non cross-hybridizing probes, could not detect the Ss2 mRNA in roots and the expression in coleoptiles, although fainter than in roots, shows that Ss1 mRNA is more abundant than Ss2 mRNA ([7] and this paper).

The complexity of these data suggest that the mechanisms underlying sucrose synthase expression and regulation are far from being totally understood. Further studies on barley sucrose synthase expression as well as

Table I

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

	Barley Ss2	Wheat Ss2	Rice Ss2	Barley Ss1	Mung-bean*	Potato*
Wheat Ss2	(95.7)					
Rice Ss2	89.3	(90.2)				
Barley Ss1	78.6	(80.0)	78.6			
Mung bean*	74.0	(74.0)	75.3	75.5		
Potato*	72.9	(73.6)	73.5	73.7	81.4	
Arabidopsis*	65.1	(65.7)	66.5	66.4	67.0	67.0

*Only one type reported in the EMBL library. ( ) = Comparisons involving wheat refer to the partial sequence reported [7].

the sequencing and functional characterization of the corresponding promoters are currently under way in our lab in order to have a better understanding about the mechanisms of control of this gene expression.

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