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Characterisation of a gene encoding wheat endosperm starch branching enzyme-I

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Abstract A genomic DNA fragment from *Triticum tauschii*, the donor of the wheat D genome, contains a starch branching enzyme-I (SBE-I) gene spread over 6.5 kb. This gene (designated *wSBE I-D4*) encodes an amino acid sequence identical to that determined for the N-terminus of SBE-I from the hexaploid wheat (*T. aestivum*) endosperm. Cognate cDNA sequences for *wSBE I-D4* were isolated from hexaploid wheat by hybridisation screening from an endosperm library and also by PCR. A contiguous sequence (*D4* cDNA) was assembled from the sequence of five overlapping partial cDNAs which spanned *wSBE I-D4*. *D4* cDNA encodes a mature polypeptide of 87 kDa that shows 90% identity to SBE-I amino acid sequences from rice and maize and contains all the residues considered essential for activity. *D4* mRNA has been detected only in the endosperm and is at a maximum concentration mid-way through grain development. The *wSBE I-D4* gene consists of 14 exons, similar to the structure for the equivalent gene in rice; the rice gene has a strikingly longer intron 2. The 3′ end of *wSBE I-D4* was used to show that the gene is located on group 7 chromosomes. The sequence upstream of *wSBE I-D4* was analysed with respect to conserved motifs.

Key words *Triticum tauschii* · Starch branching enzyme genes · Wheat · Endosperm

Sequences reported here have been submitted to Genbank as Accession numbers AF 076679, AF 076680

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Introduction

Starch is an important constituent of the wheat grain, accounting for approximately 65% of the weight of the grain at maturity. It is produced in the amyloplast of the endosperm by the concerted action of a number of enzymes that include ADPglucose pyrophosphorylase (EC 2.7.7.27), granule-bound and soluble starch synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes (EC 3.2.1.41 and EC 3.2.1.68) (Martin and Smith 1995; Morell et al. 1995). Some of the proteins involved in the synthesis of starch can be recovered from the starch granule (Denyer et al. 1995; Rahman et al. 1995).

Branching enzymes catalyse a transglycosylation reaction in which the reducing terminus produced by the hydrolysis of an α1,4 glucan is linked to the C6 hydroxyl of another α1,4 linked glucosyl residue. This reaction is the only known mechanism for the introduction of α1,6 linkages in starch. There are two types of branching enzymes in plants, starch branching enzyme-I (SBE I) and starch branching enzyme-II (SBE-II), and both are about 85–90 kDa in mass. At the nucleic acid level there is about 65% sequence identity between types I and II in the central portion of the molecules; the sequence identity between SBE I from different cereals is about 80% overall (Burton et al. 1995; Morell et al. 1995). While SBE-I and SBE-II catalyse identical reactions, evidence from mutational and gene suppression experiments demonstrate that the enzymes differ in their roles, and biochemical evidence suggests that they differ in their patterns of action (Guan et al. 1997). In maize (Boyer and Preiss 1981), rice (Mizuno et al. 1993) and pea (Smith 1988), null mutations in SBE-II reduce starch branching and lead to a high amylose phenotype (*amylose extender* in maize). In contrast, the partial suppression by antisense of SBE-I activity in the potato tuber leads to subtle alterations in starch physico-chemical properties but not to

alterations in the amylose/amylopectin ratio (Flipse et al. 1996). Mutants lacking SBE-I activity are not known. Several possible reasons for this can be advanced: (1) SBE-I may be encoded by multiple genes, (2) the null mutation does not leads to a phenotype identified in coarse screens for seed morphology or starch structure or (3) an SBE-I mutant is lethal for reasons which are not as yet evident.

In cereals, genomic DNA encoding SBE-I have so far been reported only for rice (Kawasaki et al. 1993) and wheat (Rahman et al.1997), while cDNA sequences for SBE-I are known from rice (Nakamura et al. 1992), maize (Baba et al. 1991) and wheat (Rahman et al. 1997; Repellin et al. 1997). Our previous report (Rahman et al. 1997) concerned the characterisation of a truncated SBE-I gene (called *wSBE I-D2*) from *Triticum tauschii* (the donor of the D genome to wheat). This gene is probably a transcribed pseudogene as no corresponding protein was found in our analysis of SBE-I isoforms from the endosperm (Morell et al. 1997) although a very closely related mRNA was detected. *wSBE I-D2*- type cDNA lacks approximately 300 nucleotides (encoded over exons 9 and 11–14 in the rice SBE-I gene) that are found in rice SBE-I cDNA. This paper reports the characterisation of a second gene, *wSBE I-D4*, from *T. tauschii* that is full-length and encodes the N-terminus of SBE-I purified from the endosperm of wheat (Morell et al. 1997). The gene *wSBE I-D4* has an intron-exon structure that is similar to that of rice and includes those exons at the 3′ end that are missing in *wSBE I-D2*. In addition, *wSBE I-D4* encodes amino acid motifs that were shown by Svensson (1994) to be present in all members of the α-amylase protein family and by Burton et al. (1995) to be encoded in all SBE cDNAs characterised.

Materials and methods

Plant material

A genomic library was constructed from *Triticum tauschii* var 'strangulata' accession number CPI 110799. Of all the accessions of *T. tauschii* surveyed, the genome of CPI 110799 is the most closely related to the D genome of hexaploid wheat on the basis of conservation of the order of genetic markers (Lagudah et al. 1991).

Genetic stocks of wheat cv 'Chinese Spring' with various chromosome additions and deletions were kindly supplied by Dr. E. Lagudah (CSIRO Plant Industry, Canberra) and are derived from stocks described in Sears and Miller (1985).

Hexaploid wheat (*Triticum aestivum* L. cv 'Hartog') was grown in a glasshouse at 24°C (day) and 16°C (night) with a photoperiod of 12 h light and 12 h dark. Wheat leaves, florets and endosperm were collected over the period 5–22 days after anthesis, immediately frozen in liquid nitrogen and stored at −80°C until required.

Construction of genomic library and isolation of plaques

The genomic library used in this study has been described in Rahman et al. (1997). Positive plaques in the genomic library were selected as those hybridising with the 5′ end of a maize SBE-I cDNA (from nucleotide 1–1200) (Baba et al. 1991) using moderately stringent conditions (hybridisation in 25% formamide, 6 × SSC, 0.1% SDS, 42°C, 16 h ,wash at 65°C, 2 × SSC, 0.1% SDS, 3 × 1 h).

Preparation of total RNA from wheat

Total RNA was isolated from indicated tissues essentially as described in Higgins et al. (1976). RNA was quantified by UV absorption and by separation in 1.4% agarose-formaldehyde gels which were then visualized under UV light after staining with ethidium bromide (Maniatis et al. 1982).

DNA and RNA analysis

DNA was isolated and analysed using established protocols (Maniatis et al. 1982). Approximately 20 μg of DNA was transferred to reinforced nitrocellulose membranes as described in Rahman et al. (1997).

RNA analysis was performed as follows. Ten micrograms of total RNA was separated on a 1.4% agarose-formaldehyde gel, transferred to a nylon Hybond N+ membrane and hybridised with a cDNA probe at 42°C as described in Maniatis et al. (1982). A fragment containing a 3′ region from the wheat SBEI cDNA (called wSBE I-*D4*3, see Table 1) was labelled with the Rapid Multiprime DNA Probe Labelling Kit (Amersham) and used as probe. After washing at 60°C with 2 × SSC, 0.1% SDS three times, each time for about 1–2 h, the membrane was visualised by overnight exposure at − 80°C with X-ray film, Kodak MR.

Construction and screening of the cDNA library

A cDNA library was constructed from RNA from the endosperm of the hexaploid cultivar 'Rosella' and has been described in Rahman et al. (1997). The library was screened with the 5′ end of a maize SBE I cDNA (bases 1–1200) (Baba et al.1991) using the conditions described above. A second library was also constructed from RNA from the endosperm of cv 'Rosella' using identical conditions and reagents as the first. This second library was screened with the indicated probes using the same conditions as above. Both libraries were constructed using oligo dT primers.

Cloning of specific cDNA regions of wheat SBE-I using reverse transcription-polymerase chain reaction (RT-PCR)

The first-strand cDNAs were synthesized from 1 μg of total RNA (derived from endosperm 12 days after pollination) as decribed by Maniatis et al. (1982) and then used as templates to amplify two specific cDNA regions of wheat SBE-I by PCR. Two pairs of primers were used to obtain the cDNA clones HEX1 and HEX3 (Table 1). Primers used for cloning of HEX3 were the degenerate primer BEIB (5′ GGC NAC NGC NGA G/AGA C/TGG 3′; the 5′ end is at position 151 of *D4* cDNA, see Table 1) based on the N-terminal sequence of wheat SBE-I (Morell et al. 1997) and BI (5′ TAC ATT TCC TTG TCC ATCA 3′; the 5′ end is at position 1601 of *D4* cDNA, see Table 1) derived from the conserved regions of the nucleotide sequences of HEX5 and the maize and rice SBE-I cDNAs. For clone HEX1, the primers used were BEC5′ (5′ ATC ACG AGA GCT TGC TCA, the 5′ end is at position 1 of *D4* cDNA, see Table 1; the sequence was based on *wSBE I-D4*) and BEC3′ (5′ CGG TAC ACA GTT GCG TCA TTT TC 3′; the 5′ end is at position 354 of *D4* cDNA, see Table 1; the sequence was based on HEX 3). The cycle used was 95°C for 2 min, 1 cycle; then 95°C for 30 s, 60°C for 1 min,

Table 1 Location of probes and structural features within *wSBEI-D4* sequence and the *D4* cDNA sequence

Sequence name	wSBE I-*D4* sequence	*D4* cDNA sequence
Putative initiation of translation	4900	11
N-terminal sequence of SBE I	5550	124
End of translated SBE I sequence	10225	2431
End of *D*4 cDNA sequence	10461	2687
wSBE I-*D45*	4870, 5860	1,354
wSBE I-*D43*	10116, 10435	2338,2657
E 1.1	5680, 6400	380,630
HEX1	Not referred to	1,354
HEX2		169,418
HEX3		151,1601
HEX4		867,2372
HEX5		867,2687

72°C for 2 min, 35 cycles; followed by 25°C, for 1 min, 1 cycle. The reaction mix contained 0.4 µl AmpliTaq, 1 × AmpliTaq buffer (both supplied by Perkin-Elmer), 125 µ*M* of each of dATP, dCTP, dGTP, dTTP, 2 pmol of each primer and approx 10 ng cDNA in a total volume of 20 µl.

DNA sequencing and analysis

Sequencing was performed using the automated ABI system using dye terminators as described by the manufacturers. DNA sequences were analysed using the GCG suite of programmes (Devereaux et al. 1984).

Results and discussion

Identification of a gene encoding SBE-I from the endosperm

The isolation of two classes of SBE-I genomic clones from *T. tauschii* was reported by Rahman et al. (1997). One class contained two genomic clone isolates, and one example of this class, λE7, has been characterised in some detail (Rahman et al. 1997). The complete gene contained in λE7 was termed *wSBE I-D2*; there were also partial SBE-I-like genes at either ends of the cloned DNA, designated *wSBE I-D1* and *wSBE I-D3*. A second class of hybridising clones contained nine isolates. Of these, λE1 was the first one purified. Preliminary experiments suggested that it was likely to contain sequences that extended beyond the SBE-I gene in both the 5′ and 3′ directions, and it was decided to analyse it further. The restriction map of λE1 is given in Fig 1. The SBE-I gene within λE1 was called *wSBE I-D4*.

A detailed analysis of λE1 was carried out. Fragments E 1.1 (0.8 kb) and E 1.2 (2.1 kb) were completely sequenced, and 4.8 kb and 3 kb of sequence were obtained from fragments E 1.7 and E 1.5, respectively

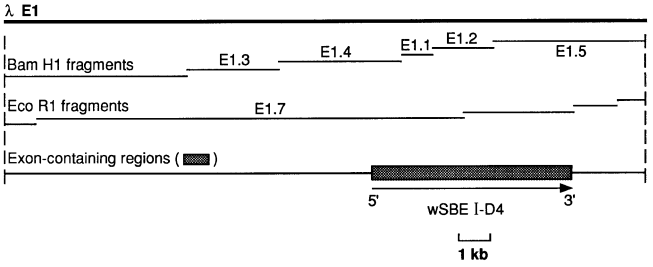

Fig. 1 Restriction map of clone λE1 containing the *wSBE I-D4* gene. The fragments obtained from λE1with *Eco*RI and *Bam*HI are indicated. The fragments sequenced are E1.1, E1.2 and a part of E1.7 and a part of E1.5

(Fig. 1). Fragment E 1.7 was found to encode the N-terminus of the SBE-I found in the endosperm as described in Morell et al. (1997) (Fig. 2).

All 11 SBE-I genomic clones from the two classes referred to above were investigated by hybridisation with probes derived from fragments E1.7 and E1.5 (wSBE I-*D45* and wSBE I-*D43*, see Table 1). The hybridisation results obtained were consistent with the same *wSBE I -D4* gene being isolated in different fragments in 9 different clones, and this conclusion was confirmed by the sequencing of PCR products using primers that amplify near the 5′ end of the gene (data not shown). The 2 clones containing the *wSBE I-D2*-type gene (λE7 and λE22) did not hybridise with either of the probes, although they did hybridise with a probe from the central portion of λE1 (probe E1.1, see Table 1) (Rahman et al. 1997).

Isolation of cDNA for starch branching enzyme-I

Using the maize SBE-I cDNA as a hybridisation probe (Baba et al. 1991), we recovered 10 positive plaques by screening approximately 10^5 plaques from a hexaploid wheat endosperm cDNA library. Upon purification and sequencing of these plaques it was clear that even the longest clones (HEX4, HEX5) did not encode the N-terminal sequence of wheat SBE-I obtained from protein analysis (Morell et al. 1997). Degenerate primers based on wheat SBE-I N-terminal (primer BE IB) and the sequence from HEX5 (primer B I) were then used to amplify the 5′ region from hexaploid wheat endosperm mRNA: this produced the cDNA clone termed HEX3 (Table 1 and Fig. 3). This cDNA clone overlapped extensively and had 100% sequence identity with HEX5 and HEX4 (Fig. 3). As almost the entire SBE-I N-terminal sequence had been included in the primer sequence design this did not provide independent evidence of the selection of a cDNA sequence in the endosperm that encoded the protein sequence of SBE-I. Use of HEX3 to screen a second cDNA library produced HEX2, which is shorter than HEX3 but

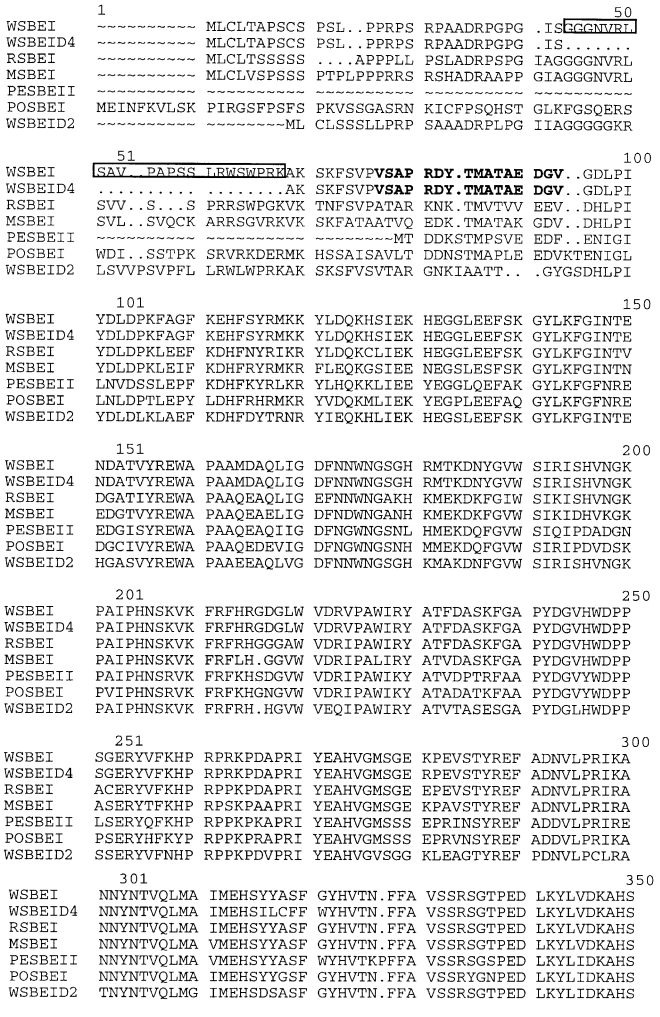

Fig. 2 Alignment of deduced amino acid sequences for SBE-I from wheat (WSBE I, Repellin et al. 1997), wheat (WSBE I-D4, from the cDNA described in this paper), rice (RSBEI, Nakamura et al. 1992), maize (MSBEI, Baba et al. 1991), pea SBE-II (PESBEII, equivalent to maize SBE-I, Burton et al. 1995), potato (POSBEI, Cangiano et al. 1993), and the truncated wheat SBE-I derived from wSBE I-*D2* (Rahman et al. 1997). Note that the *boxed sequence* in wheat SBE-I occurs only in the sequence reported by Repellin et al. (1997); the *bold sequence* is the N-terminal sequence of SBE-I as determined by Morell et al. (1997); the *vertically boxed residues* are considered to be essential for catalytic activity by Jesperson et al. (1993) and occur in all amylolytic enzymes.

confirmed the HEX3 sequence at 100% identity between positions 169 and 408 (Fig. 3 and Table 1). In addition, the entire cDNA sequence for HEX3 could be detected at a 100% match in the genomic clone λE1.

A primer based on the putative transcription start point combined with a primer based on the incomplete cDNAs recovered were then used to obtain a PCR product from total endosperm RNA by reverse transcription. This led to the isolation of the cDNA clone, HEX1, whose size is 354 bp (Fig. 2). By analysing this product, we again obtained a sequence that matched exactly the corresponding region of the genomic clone λE1 and which overlapped precisely with HEX3. The sequence of HEX1 encodes the N-terminal amino acid sequence of SBE-I (Fig. 2) (Morell et al. 1997).

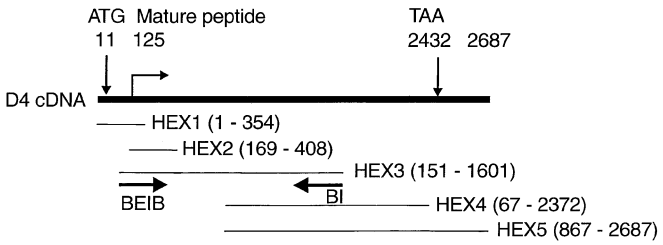

Fig. 3 Alignment of cDNA clones to obtain the sequence represented by *D4*. HEX 1, 2 and 3 were obtained by RT-PCR using defined primers. HEX4 and HEX5 were obtained from screening the cDNA library with maize *SBE I* (Baba et al. 1991)

The 5 cDNA clones (HEX1–5) were sequenced, and their sequences were assembled into one contiguous sequence (Devereaux et al. 1984); this sequence was designated *D4* cDNA. The assembled cDNA sequence is 2687 bp and contains one large ORF which starts at nucleotide 11 and ends at nucleotide 2434. *D4* cDNA encodes a polypeptide of 807 amino acids; the mature product has a predicted molecular mass of 87 kDa, consistent with the 88 (A- and D-genome forms) and 87 (B-genome form) kDa forms of SBE-I present in the wheat endosperm (Morell et al. 1997). This molecula mass is similar to proteins encoded by maize (Baba et al. 1991) and rice (Nakamura et al. 1992). Comparison of the amino acid sequence encoded by the *D4* cDNA sequence with that encoded by maize and rice SBE-I cDNAs showed that there is 75–80% identity between any two of these sequences at the nucleotide level and almost 90% at the amino acid level (Fig. 2). Alignment of these three polypeptide sequences, along with the deduced sequences for pea, potato and *wSBE I-D2*-type cDNA indicated that the sequences in the central region are highly conserved and sequences at the 5′ (about 80 amino acids) and 3′ (about 60 amino acids) ends are variable (Fig. 2). The conservation of sequence is especially marked in the amino acid sequence encoded by exon 6 (see Fig. 4 and below). This long exon contains the first seven β-strands and α-helices of the $(\beta\alpha)_8$ barrel domain structure proposed for starch branching enzymes by Jespersen et al. (1993) and further developed by Burton et al. (1995), and contains residues considered to be conserved in the active site.

The *wSBE I-D4* cDNA is distinct to the *wSBE I-D2*-type cDNA described previously which encoded a 74-kDa protein (Rahman et al. 1997). Svensson et al. (1994) indicated that there were several invariant residues in the α-amylase super-family of proteins which are present in all SBE-I-type sequences (Burton et al. 1995); these residues have been underlined in Fig. 2 and are all encoded in the *wSBE I-D4* sequence (Fig. 2), further supporting the view that this gene encodes a functional enzyme. This is in contrast to results with the *wSBE I-D2* gene, where three of the conserved motifs appear not to be encoded and expression in *E. coli* failed to complement a *glg*B mutation or produce an active branching enzyme (Rahman et al. 1997).

The deduced protein sequence of *D4* cDNA and the sequence of Repellin et al. (1997) are 97% identical over the mature peptide (Fig. 2). However, there is clearly a 23 amino acid insertion in the sequence reported by Repellin et al. (1997) relative to the sequence in *D4* cDNA (Fig. 2) in the putative transit peptide, and so the mature forms of SBE-I [based on either the *D4* cDNA or the wheat SBE-I cDNA reported by Repellin et al. (1997)] should have identical N-terminii. We failed to detect an alternative form of a *wSBE I-D4*-type gene in either wheat or *T.tauschii* by PCR amplification of the region corresponding to exon 1 to exon 3 of *wSBE I-D4*. In contrast we could detect two size classes of *wSBE I-D4* type mRNA in the wheat endosperm (Li et al. unpublished observations) and, furthermore, Morell et al. (1997) detected two mass isoforms of SBE-I that could be assigned to the D genome of wheat. This raises the possibility of alternative splicing of the *wSBE I-D4* transcript and also the question of the relative efficiency of translation/transport of the two putative forms of SBE-I. The possibility of alternative splicing in both rice and wheat has been discussed for soluble starch synthase (Baba et al.1993; Rahman et al. 1995) and for barley ADPglucose pyrophosphorylase (Thorbjornsen et al. 1996). The length of the proposed transit peptide for barley ADPglucose pyrophosphorylase is 24 amino acids, which is shorter than the putative transit peptide deduced from *D4* cDNA. The consequence of the difference between the *D4* cDNA sequence and that of Repellin et al. (1997) is under further investigation

Intron-exon structure

A comparison of the SBE-I cDNA sequence of Repellin et al. (1997) or of the *D4* cDNA sequence with that of *wSBE I-D4* allows us to deduce the intron-exon structure of *wSBE I-D4*. However, the sequence reported by Repellin et al. allows the detection of an extra exon (exon 2) in the structure of *wSBE I-D4* and this is shown in Fig. 4. The number, positions and sizes of the exons in rice are very similar to those in *wSBE I-D4*. The sequence identity over introns is lower (about 60%) than over exons (about 80%) (see Fig. 4). The main difference in the structure of the gene is in the length of intron 2 in rice, which is considerably longer (2.5 kb) than the corresponding introns in *wSBE I-D4* (370 bp) and *wSBE I-D2* (1.2 kb). The sequence identity between *wSBE I-D4* and *wSBE I-D2* is similar to their sequence identity with rice over regions that are common to all three genes; however, *wSBE I-D2* lacks sequences corresponding to exons 9,12,13 and 14 of rice (Rahman et al. 1997). A sequence corresponding to rice exon 11 is found in the gene but not in *wSBE I-D2*-type cDNA.

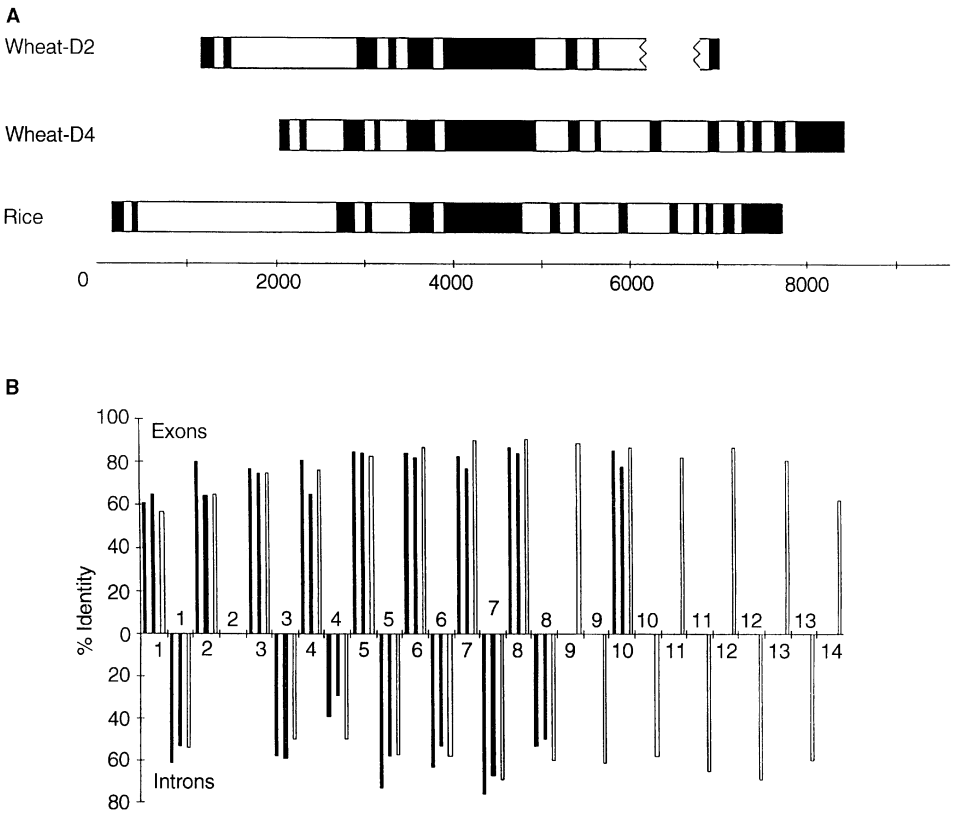

Fig. 4A, B Intron-exon structure of *wSBE I-D2* (Rahman et al. 1997) (*D2*), *wSBE I-D*4 (*D4*) and rice *SBE-I* (rice) (Kawasaki et al. 1993) and a comparison of their sequence identities. The intron-exon structure of *wSBE I-D4* is deduced by comparison with the wheat SBE-I sequence reported by Repellin et al. (1997). **A** The *dark rectangles* correspond to the exons and the *open rectangles* to the introns for the three genes compared. **B** The sequence identities of indicated exons and introns common to the three genes are compared. *Black*, *shaded* and *open columns* correspond to percentage identity between *D4*/*D2*, *D2*/rice and *D4*/rice, respectively

Analysis of promoter sequence

Comparison of the *D4* cDNA sequence with that of *wSBE I-D4* indicates that the putative start of translation of *D4* cDNA corresponds to position 4900 of *wSBE I-D4*. There are putative CAAT and TATA motifs at positions 4870 and 4830, respectively, of the *wSBE I-D4* sequence.

The 4.9-kb sequence available that was 5′ to *wSBE I-D4* was analysed. Forde et al. (1985) compared prolamin promoters and suggested that the presence of a motif approximately −300 bp upstream of the transcription start point, called the endosperm box, was responsible for endosperm-specific expression. The endosperm box was subsequently considered to consist of two different motifs: the endosperm motif (EM) (canonical sequence TGTAAAG) and the GCN 4 motif (canonical sequence G/ATGAG/CTCAT). The GCN4 box is considered to regulate expression according to nitrogen availability (Muller and Knudsen 1993). The *wSBE I-D4* promoter contains a number of imperfect EM-like motifs at approximately −100, −300 and −400 as well as further upstream. However, no GCN4 motifs could be found. Comparison of the upstream regions for *wSBE I-D4* and *D2* (Rahman et al. 1997) indicate that although there are not extensive sequence homologies there is a region of about 140 bp starting at position 4660 of the *wSBE I-D4* sequence (this is 240 bp before the translational start codon in *wSBE I-D4*) where the homology is 61% between the two promoters. In particular, there is almost perfect match in the sequence over 20 base pairs (CTCGTTGCTTCC/TACTCCACT) (position 4723–4742 of *wSBE I-D4* sequence), but the significance of this is hard to gauge as it does not occur in the rice promoter for *SBE I*. The availability of more promoters for endosperm and starch biosynthetic enzymes may allow firmer conclusions to be drawn.

Tissue specificity and expression during endosperm development

The 320 bp of largely untranslated sequence at the 3′ end of *D4* cDNA does not show any homology with the *wSBE I-D2*-type cDNA (see Fig. 6) or the *wSBE I-D1* and *-D3* genes that we have described earlier and which are contained in λE7 (see Fig. 4). We have called this sequence *wSBE I-D43* (Table 1). It seemed likely that *wSBE I-D43* would be a specific probe for *D4*-type sequences, and so it was used to investigate tissue specificity. The results are shown in Fig. 5. An RNA species of about 2700 bases in size hybridised. This is consistent with the size of the *D4* cDNA sequence. In glasshouse-grown material where the grain development rate is accelerated we could detect RNA

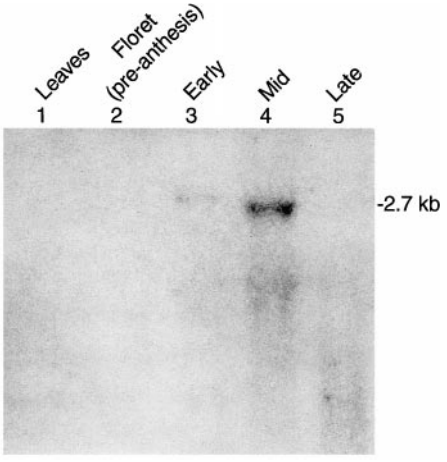

Fig. 5 Expression of SBE-I-type sequences during endosperm development. The probe used was *wSBE I-D43*, corresponding largely to the untranslated 3′ end of *D4* cDNA. Note that there is no hybridisation to RNA extracted from leaves or florets prior to anthesis

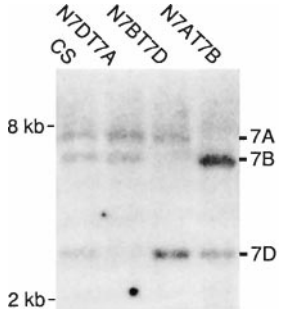

Fig. 6 Hybridisation of wheat DNA from chromosome-engineered lines using wSBE I-*D43* (corresponding largely to the untranslated 3′ end of *D4* cDNA). Wheat DNA was digested with *Dra*I, electrophoresed and transferred to nitrocellulose. *N7AT7B* No 7A chromosome, four copies of 7B chromosome; *N7BT7D* no 7B chromosome, four copies of 7D chromosome; *N7DT7A* no 7D chromosome, four copies of 7A chromosome. The chromosomal origin of hybridising bands is indicated, as are 8-kb and 2-kb size estimates

sequences hybridising to *wSBE I-D43* at an early stage of grain development (5–8 days). It is clearly more abundant mid-way through grain filling (10–15 days); there seems to be at least a tenfold increase in this RNA during this period. At the late stage (18–22 days after anthesis) the RNA is undetectable; this corresponds in this glasshouse-grown material to grains that were in the final stages of grain filling and beginning to dessicate.

The sequence contained within the *wSBE I-D4* gene was expressed only in the endosperm (Fig. 5) in the tissues examined. We could not detect any expression in the leaf by RNA hybridisation experiments. This could be because wheat typically produces very little starch in the leaves and therefore may not contain detectable levels of mRNAs for the starch biosynthetic enzymes under the conditions we have used. It is also possible that another isoform is expressed in the leaves. When Ainsworth et al. (1993) analysed the expression of granule-bound starch synthase (GBSS) mRNA in wheat leaves they also failed to detect the expression of a species of the same size although they did detect a RNA species that was considerably smaller and had only moderate sequence identity to the GBSS probe. Nair et al. (1997) detected the expression of RNA for SBE-II in wheat leaves by the use of poly A + RNA and prolonged exposure, suggesting that the RNA abundance for SBE-II was about 1% of that seen in the grain.

Localisation of genes

Sequences from the 3′ (*wSBE I-D43*) region of *wSBE I-D4* were used to probe transfers of *Dra*I-digested DNA from chromosome-engineered lines of wheat (Fig. 6). With the *wSBE I-D43* probe single bands could be assigned to each of chromosomes 7A, 7B and 7D (Fig. 6). This region did not hybridise to genomic clones containing *wSBE I-D2* (data not shown), which is to be expected as *wSBE I-D2* lacks these sequences. Thus, the results clearly show that *wSBE I-D4* is located on chromosome 7; we have previously shown that *wSBE I-D2* is also located on chromosome 7. Hybridisations with the 5′ end of *wSBE I-D4 (wSBE I-D45)* were difficult to interpret because of high background, possibly due to homology with unknown repetitive sequences, although bands could be assigned to chromosome 7B and 7D (data not shown).The size of the hybridising D-genome fragments with both probes is consistent with the sequence of *wSBE I-D4*.

Rahman et al. (1997) used probes based on exons 4, 5 and 6 of *wSBE D2* and *wSBE I-D4* to probe Southern blots of wheat and *T. tauschii* genomic DNA (cut with *Pvu*II and *Bam*HI, respectively). This region is highly conserved within rice *SBE I*, *wSBE I -D2* and *wSBE I-D4* and hybridisation revealed 10 bands with wheat DNA and 5 with *T. tauschii* DNA. Neither *Pvu*II nor *Bam*HI cut within the probe sequences, suggesting that each band represented a single type of SBE-I gene. We have described four SBE-I genes from *T. tauschii*: *wSBE I-D1, -2, -3* and *-D4* (Rahman et al. 1997 and this paper) and so have accounted for most of the genes in *T. tauschii* and, by extension, the genes from the D genome of wheat.

The co-location on wheat chromosome 7 of the genes for enzymes related to starch biosynthesis is striking. The genes for SBE-I, granule-bound starch synthase (GBSS), ADP-glucose pyrophosphorylase (ADPG PP) and soluble starch synthase (SSS) have been located there (Devos and Gale 1997). Only one class of SBE genes is on chromosome 2 (Sharp 1997); these are

SBE- II genes (Rahman et al. unpublished) and represent the only starch biosynthetic genes to date not located on chromosome 7. It remains to be seen if there is any evolutionary advantage to this co-location or whether it reflects the assimilation of the ability to store starch from some ancient organism into the wheat progenitor.

In summary, this paper has described the structure of a SBE-I gene from *T. tauschii*, *wSBE I-D4*. The wheat homeologue can be reasonably expected to be very similar. *wSBE I-D4* type genes are only on group 7 chromosomes. While *wSBE I-D4* has similarities with rice *SBE-I* there are also considerable differences, especially in the 5′ half of the gene. It is clear, however, that *wSBE I-D4* encodes a functional SBE-I that contains the N-terminal sequence present in the wheat endosperm.

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