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Starch-Branching Enzymes *Sbe1* and *Sbe2* From Wheat (*Triticum aestivum* cv. Cheyenne): Molecular Characterization, Developmental Expression, and Homoeologue Assignment by Differential PCR*

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Abstract. Starch is the main component of the wheat kernel, and wheat flour is used for hundreds of food and nonfood products. We are exploring ways to improve wheat quality and to develop new uses for wheat based on altered starch characteristics. To understand the molecular basis for variations in the physical and chemical properties of starch, we examined transcripts for starch biosynthetic enzymes. cDNAs encoding 2 isoforms of starch-branching enzyme (*Sbe1, Sbe2*) were isolated from wheat endosperm. The longest *Sbe1* and *Sbe2* cDNAs were 2797 and 2975 bp, respectively, and they shared extensive identity with *Sbe* sequences reported for wheat and other species. With orthologue-specific primer pairs, homoeologue assignments to chromosome 7 were made for *Sbe1*#19 (TRIae:Sbe1A.1) and *Sbe1*#9 (TRIae:Sbe1D.1) using the wheat cv. Chinese Spring nullisomic-tetrasomic-7 lines. This strategy may prove useful for future mapping of expressed sequence tag (EST) data. The *Sbe* cDNAs and a granule-bound starch synthase cDNA (*GbssI*) (from an EST sequencing project) were used to examine the steady-state RNA levels during development of the wheat. Steady-state levels of *Sbe2* mRNA were detectable 5 d postanthesis (DPA) and reached a maximum at 10 DPA. Steady-state levels of *Sbe1* and *GbssI* began to rise at 10 DPA and peaked at 15 DPA. Levels of all messages declined rapidly at 20-25 DPA. Reported here is the first analysis of transcripts for these enzymes in the same RNA pools and demonstration that expression patterns are unique and developmentally regulated.

Full text†: This manuscript, in detail, is available only in the electronic version of the *Plant Molecular Biology Reporter.*

Key words: cDNA sequences, developmental gene expression, homoeologue assignment, steady-state mRNA

*Note: Sequences presented in this article have been deposited into Genbank under the following accession numbers: Sbe1D#9 AF286317, Sbe1A#19 AF286318, Sbe2#30 AF286319, GbssI#3 F286320.

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†Editor's note: Although the scientific content of this paper has been reviewed, the full text WEB document has not been edited in detail.

Abbreviations: AM, amylose; AP, amylopectin; DPA, days postanthesis; EST, expressed sequence tag; GBSS, granule-bound starch synthase; SBE, starch-branching enzyme; SDE, starch-debranching enzyme.

Introduction

Starch constitutes 75% of the wheat kernel and has a direct affect on the nature and quality of flour and on products of wheat. The structure of both the starch molecule and the starch granule plays an important role in product application. The molecular structure is due in part to the nature of amylopectin (AP) branching and the ratio of amylose (AM) to AP in the granules.

In plants, 4 enzymes control starch biosynthesis. The committed step is ADP-glucose pyrophosphorylase, which converts glucose-phosphate to ADP-glucose in the presence of ATP. ADP-glucose then is polymerized into α (1,4)-linked chains by multiple isoforms of starch synthase. Granule-bound starch synthase (GBSS) catalyzes the addition of glucose units to form an essentially linear polymer of AM with very few branches (Nakamura et al., 1995). Soluble starch synthase produces linear polymers that are the substrate for addition of α (1,6) branches to form AP (Ball et al., 1996). Branching of AP is the result of the balanced activities of starch-branching enzymes (SBE) and starch-debranching enzyme (SDE). The SBEs are present in multiple isoforms in all plants examined (Preiss and Sivak, 1996). The isoforms *Sbe1* and *Sbe2* are distinguished by their deduced amino acid sequences (Burton et al., 1995). Each isoform exhibits a unique developmental time of peak activity and a different length of amylose chain transferred (Smith et al., 1995). The exact role and regulation of SDE in AP biosynthesis are not entirely understood (James et al., 1995; Nakamura et al., 1997). The relative activities of starch synthases and the balance of branching and debranching enzymes create the molecular structure of AM and AP (Mouille et al., 1996). The ratio of AM to AP and the structure of AP control many of the physical and chemical properties of the flour and starch, thus affecting their use in food and nonfood products.

The regulation of SBE isoforms during development has been studied in a number of species. In pea embryos (cotyledon + axis), the enzymatic activity of *Sbe2* (SBE peak I) increases early in development when embryos are 0.1 to 0.15 g. It holds steady while a second peak of activity (*Sbe1*) rises because the embryo size increases from 0.35 to 0.7 g (Smith, 1988). *Sbe2* is absent in wrinkled or rugosus peas (Smith, 1988). In maize, *Sbe1* is abundant at 10 d and decreases through the 28th d after pollination (Baba et al., 1991). The SBE isoforms of maize show unique preferences for substrates, with *Sbe1* preferentially transferring longer chains than *Sbe2* (Takeda et al., 1993). In maize, the *Sbe1* isoform has higher activity on amylose, and *Sbe2* isoform has higher activity on amylopectin (Guan and Preiss, 1993). The *Sbe2* of maize is observed as both a soluble and a granule-associated enzyme (Mu-Forster et al., 1996). In rice, SBE (Q-enzyme, *Sbe1*) activity increases 6-13 d after pollination and then begins to decline (Nakamura and Yuki, 1992). In rice, the superior caryopses exhibit higher levels of *Sbe1* (Q-enzyme) activity sooner after pollination than inferior caryopses

(Umemoto et al., 1994). In potato, SBE RNA levels rise 1 d before the onset of tuber swelling during the transition from stolon to tuber (Visser et al., 1994).

In peas (Burton et al., 1995), steady-state levels of *Sbe2* (*SBE I)* mRNA are high early in development, peaking in the 0.1- to 0.2-g stage embryos, and then they decline. *Sbe1 (SBE II)* mRNA levels rise rapidly after the 0.2- to 0.3-g stage, peak at the 0.4- to 0.5-g stage, and then decline. Using RT-PCR, *Sbe1 (SBE II)* was amplified in most pea tissues, with the exception of pods, whereas *Sbe2* (*SBE I)* was only amplified in leaves and pods. Extensive developmental and tissue specific expression studies in maize showed that different isoforms of *Sbe* are independently controlled (Boyer et al., 1978; Gao et al., 1996). The 2 forms of *Sbe2* in maize are *a* and *b.* The *Sbe2b* form appears to be an endosperm-specific isoform, whereas the *Sbe2a* form is present at higher levels in embryo than in endosperm and is expressed in other tissues and organs (Gao et al., 1997). In rice, the steady-state mRNA levels of *Sbe2 (RBE3*) are barely detectable at 5 DPA, they increase dramatically at day 7 and peak at day 15, and they are barely detectable at 20 DPA (Mizuno et al., 1992).

The *Sbe2* genes of wheat are mapped to the chromosome 7 homoeologues (Morell et al., 1997). A conflicting report of the rice *Sbe2 (Rbeiii*) links it to chromosome 2, predicting a syntinous location on chromosome 6 of wheat based on the flanking genetic markers (Harrington et al., 1997).

Our long-term research goals are to create new phenotypes in wheat by transformation and tissue-specific expression of genes encoding starch biosynthetic enzymes. Transgenic plants are expected to result in a new series of novel wheats containing modified starch mediated by overexpression or suppression of enzyme activity by sense and antisense gene constructs. Reduction of starch branching also may be achieved from sense constructs by cosuppression (Jorgensen, 1995). Variation in levels of branching enzymes may result in starch with novel branching patterns. It is anticipated that plants suppressed for expression of an SBE isoform will help elucidate the role of each isoform in the biosynthesis of amylopectin. These changes are anticipated to have a significant impact on the chemical and physical properties of the starch, the starch granule, and the flours and products of these plants (Zeng et al., 1997).

We have cloned sequences corresponding to the mRNA for 2 *Sbe1* and 1 *Sbe2* from *Triticum aestivum* cv. Cheyenne. The *Sbe1* sequences have been compared to those in the database and are tentatively mapped to their respective chromosome 7 homoeologues. The *Sbe2* sequence represents a new allele of 1 of the 2 previously described potential orthologues from wheat. We also identified and sequenced a novel cDNA encoding GBSS, the waxy gene from wheat, in an expressed sequence tag (EST)–sequencing project. The cDNA sequences for *Sbe1*, *Sbe2*, and *GbssI* were used as probes and revealed a staggered pattern of steady-state RNA expression during kernel development.

Materials and Methods

Plant material and treatments

Wheat plants (*T. aestivum*) were grown in the greenhouse. Developing seeds were used to produce a cDNA library (cv. Cheyenne). Leaf material was used to isolate

genomic DNA for PCR (cv. Chinese Spring). For the developmental time course, wheat was grown in a climate-controlled greenhouse that had a maximum daytime temperature of 25°C and a nighttime temperature of 17°C, as described previously (Hurkman et al., 1998).

cDNA library preparation and screening

A wheat endosperm cDNA library was prepared in Lambda ZAP II (constructed by Stratagene) from an mRNA pool isolated from wheat endosperm at 5, 10, 15, 20, 25, and 30 DPA. The original library titer was $1x10^7$ pfu. All screening was done using an amplified library at $1x10^8$ pfu/mL.

Probes for screening the library were prepared from cDNAs of the maize homologues of *Sbe1* (pA2-4) and *Sbe2b* (pMA11) (kindly provided by Mark Guiltinan at the University of Pennsylvania). The entire cDNA inserts were excised by restriction enzyme digestion and labeled using ^{32}P-dCTP (New England Nuclear) and a random primed DNA labeling kit (Boehringer Mannheim Biochemicals). The plating of phage, blotting, probe hybridization, and phagemid isolation were carried out following standard molecular biology protocols (Sambrook et al., 1989) and manufacturer's instructions. Phagemids were released from the phage, subcloned, and amplified according to manufacturers instructions (Stratagene).

Approximately 180,000 plaques were plated, blotted in duplicate, and sequentially screened with the *Sbe1* and *Sbe2b* cDNA sequences. Positively hybridizing plaques then were isolated, replated, and rescreened to obtain single phage plaques. Phagemids were released from all positives of the secondary screen and transformed into *Escherichia coli* DH5α. Plasmid DNA was isolated, digested, separated by gel electrophoresis, blotted, and hybridized to confirm positively hybridizing clones.

cDNA characterization and sequencing

Plasmid DNA from positively hybridizing *Sbe* cDNA clones was used for cycle sequencing with fluorescently labeled dye terminators and analyzed on an Applied Biosystems 310 Genetic Analyzer (Perkin Elmer). The resulting sequences were screened by BLAST searching of the Genbank database for homology to *Sbe* sequences from maize, rice, and other plant species. The cDNA encoding *GbssI* was identified by sequence homology to *Gbss* sequences via Blast searching of the Genbank database with EST sequences from the universal primer sequencing of random wheat endosperm cDNAs. Apparent full-length cDNAs for *Sbe* and *Gbss* isolates were sequenced completely in both directions using synthetic oligonucleotides. Sequences were analyzed by ClustalW alignments.

PCR analysis of cDNA orthologues

DNA was extracted (D'Ovidio et al., 1992) from young seedlings of the wheat cv. Chinese Spring nullisomic-tetrasomic-7 lines. PCR conditions were carried out according to the manufacturer's instructions (Promega) with the following cycling conditions: denaturation at 95°C for 30 s, annealing at 63°C for 1 min, and extension at 72°C for 2 min. These conditions were repeated for 35 cycles. The

forward and reverse primers were as follows: *Sbe1* #19 52F (5′-CTCCTC-CTGGCCCTCGC-3′), and 363R (5′-CTTCAAGGCCCCCTCGTAT-3′); *Sbe1* #9 43F (5′-ACCGCCCCCTCCTGC-3′), and 352R (5′-CTTCAAGGCCTCCTC-GTGC-3′); *Sbe1* #D2 (Rahman et al. 1997) 79F (5′-GCCTCAGCTCCTCTCTCT-TGC-3′), and 378R (5′-ATTCTTCTAGGCTTCCCTCATGT-3′).

Northern blot analysis

RNA was isolated (Hurkman and Tanaka, 1996) from endosperm of developing grains from single heads of wheat (cv. Cheyenne) at different times after flowering (DuPont et al., 1998). RNA blots represent 20 ug of total RNA using digoxigenin-labeled ribo probes (Boehringer Mannheim) from T7 transcription of linearized cDNA templates according to the manufacturer's instructions.

Results and Discussion

Isolation and characterization of starch-branching enzymes

cDNAs encoding SBEs were isolated from a library (prepared from mRNA isolated from wheat endosperm) by sequential screening with maize *Sbe1* and *Sbe2* homologs as probes. Eight putative clones encoding *Sbe1* were isolated. Sequence analysis resulted in the identification of 6 clones (#8, 9, 14, 22, 25) with sequence homology to *Sbe1*. Five of the clones had the same open reading frame as clone #9 but were truncated at different positions in the 5′ ends (Figure 1). Clones #25, 14, and 8 had the same 3′ poly(A) tail. Clone #9 lacked a poly(A) tail but was otherwise identical to #25, 14, and 8. Clone #22 had an open reading frame identical to clone #9 but, similar to clone #19, had a slightly longer untranslated 3′ end before the addition of the poly(A) tail. Clones #9 and 19 were sequenced completely and contained open reading frames predicting preproteins similar to the maize *Sbe1* sequence of 830 and 833 amino acids, respectively.

Five putative clones for *Sbe2* were isolated from the second screen. Sequence analysis resulted in the identification of 2 clones (#30, 36) with homology to *Sbe2*. The *Sbe2* sequence from clone #30 was apparently full length with a methionine start codon and a predicted preprotein of 823 amino acids similar to its maize homolog. The *Sbe2* sequence from clone #36 was truncated by approximately 400 bp at the 5′ ends but was otherwise identical to the sequence from clone #30.

Gene characterization

Because bread wheat is hexaploid, we expect as many as 3 orthologous gene products representing the A, B, and D ancestral genomes. The *Sbe1* cDNA isolates represent 2 classes of potential orthologues. The 5 most similar *Sbe1* isolates were judged to be independent based on length variation at the 5′ and 3′ ends of the cDNAs. The greatest variation was observed in the 3′ polyadenylation site and length or absence of poly(A) tails (Figure 1). Truncation of the 5′ ends most likely resulted from incomplete first-strand synthesis during library construction or from naturally occurring degradation or turnover products present in the RNA used for library construction. The *Sbe1* isolate with the longest 5′ end (#9) lacked a

Figure 1. Alignment of the 3′ ends of *Sbe1* sequences. The final 100 bp of *Sbe1* #19 are aligned with the 3′ ends of the other *Sbe1* sequences. Substitutions in clone # 19 relative to the other *Sbe1* sequences have white shading and the conserved 3′ region between the potential orthologue clones # 19 and 22 is shaded in gray. The consensus polyadenylation signal at the expected position in #19 and #22 is underlined. The alignment suggests the presence of a second nonconsensus polyadenylation signal being used by #14 and possibly #8.

poly(A) tail and was apparently truncated 11 bases from the first of 2 poly(A) addition sites observed for the other 5 *Sbe1* isolates. The nucleic acid sequence of *Sbe1* #9 was 99.6% identical to an *Sbe1* sequence from wheat cv. Fielder (Repellin et al., 1997) and differed by only a single amino acid in the predicted proteins. The nucleic acid sequence of the sixth isolate, *Sbe1* #19, was 97.2% identical to another *Sbe1* sequence from wheat (Rahman et al., 1999) and 96.8% identical to *Sbe1* #9. The nucleic acid sequences of *Sbe1* #9 and *Sbe1* #19 are quite distinct from the published sequence for *Sbe1* #D2 (Rahman et al., 1997) at 71.0% and 70.5% identity to *Sbe1* #9 and *Sbe1* #19, respectively. D2 is now thought to be a transcribed pseudogene (Rahman et al., 1999). The *Sbe1* #D2 sequence was mapped to the D genome and is part of a complex locus with several apparently nonfunctional genes (Rahman et al., 1997). If the functional gene sequence from the D locus is similar to the #D2 gene, then *Sbe1* #9 and *Sbe1* #19 may represent the other 2 orthologous *Sbe1* sequences derived from the A and B genomic ancestors of modern hexaploid wheats.

Isolation of Sbe2

Two isolates were confirmed by sequence identity as *Sbe2* in the library screen. One was apparently full length and contained more 5′ untranslated sequences than its maize homolog, as well as a similar methionine start, transit peptide length, and coding sequence. The first *Sbe2* sequence (#30) contained a complete open reading frame and was 99.5% and 99.9% identical to the nucleic acid and deduced protein sequences, respectively, of the *Sbe2* from wheat reported by Nair et al. (1997). The second *Sbe2* sequence, isolate #36, was truncated by more than 700 nucleotides at the 5′ end but was otherwise identical to the sequence of clone #30. The sequence of clones #30 and *Sbe2* (Nair et al., 1997) had 95% and 98% identity, respectively, to the nucleic acid sequence of another *Sbe2* from wheat (Genbank ACC U66376).

A comparison of the *Sbe1* and *Sbe2* from wheat shows several regions of identity between the deduced amino acid sequences (Figure 2). Conserved amylolytic regions found in other enzymes from other species also are conserved in the wheat sequences. Figure 3 shows the phylogenetic relationship of all known *Sbe1* and *Sbe2* sequences from wheat. The close relationship of *Sbe1* #19 and *Sbe1* from Rahman et al. (1999) suggest an allelic relationship. A similar relationship is observed between *Sbe1* #9 and *Sbe1* from Repellin et al. (1997). The greater

distance observed between these and the other 2 *Sbe1* sequences suggest that these are the other 2 orthologues. Distances between the *Sbe2* sequences also suggest an allelic relationship between isolate *Sbe2* #30 and the *Sbe2* from Nair et al. (1997), whereas the greater differences observed for the other *Sbe2* from wheat (ACC U66376) suggest a potential orthologous relationship.

Isolation of GBSS

We have isolated and characterized a 2289-bp cDNA-encoding GBSSI (*GbssI*) from an EST sequencing project using the same wheat endosperm cDNA library used to isolate the *Sbe* sequences. This clone, *GbssI* #3, was sequenced on both strands and represents a novel allele of wheat GBSS I from wheat cv. Cheyenne. Blast search comparison to *Gbss* sequences from the database confirmed the identity as a *GbssI* isoform. Further comparison with all the available *Gbss* sequences in Genbank suggests that *GbssI* #3 encodes an allele of the *Wx-4A* orthologue. GbssI #3 is 99.9% and 99.5% identical with the nucleotide and amino acid sequences, respectively, reported for the *GbssI Wx-4A* (originating from the 7B translocation) sequence from wheat cv. Chinese Spring (Murai et al., 1999). There is a single nucleotide substitution that results in a conservative substitution with a histidine in #3 and an arginine in *Wx-4A* at position 520.

Homoeologue assignment for Sbe1 isolates

Assignment of *Sbe1* orthologues to their homoeologues was performed using gene-specific oligonucleotides and PCR. Bread wheat is hexaploid (AABBDD), derived from the fusion of 3 ancestral genomes. Assignments of sequences to the A, B, or D ancestral genomes of wheat were determined using the Chinese Spring nullisomic-7-tetrasomic lines and PCR. Homoeologue assignment in wheat usually is carried out by performing RFLP analysis on the nullisomic tetrasomic lines deficient in one homoeologue pair and doubled for 1 or the other 2 remaining homoeologues. When DNA sequences are known, specific orthologues can be mapped using PCR. To determine if the 2 cDNAs isolated for *Sbe1* represented distinct orthologous gene products and not alleles and to determine if these were distinct from the *Sbe1* #D2 isolate in Genbank, a series of orthologue-specific primer sets were synthesized based on divergences in the gene sequences. Forward and reverse primers for *Sbe1* #9, *Sbe1* #19, and *Sbe* #D2 were designed to take advantage of the insertion/deletion observed in the 3′ end between #9 and #19 and nucleotide substitutions downstream in #D2. Reverse primers were designed to take advantage of multiple nucleotide substitutions from the same position of the aligned amino acid sequences (Figure 4).

The results of amplification using orthologue specific primer pairs on DNA from the Chinese Spring nullisomic-7-tetrasomic lines is shown in Figure 5. For example, the results show that the *Sbe1* #19-specific primer pair fails to yield bands in either the nullisomic-7A-tetrasomic-7B (BBBBDD) or the nullisomic-7A-tetrasomic-7D (BBDDDD) lines, but it produces bands in both the nullisomic-7B (AAAADD and AADDDD) and nullisomic–7D (AAAABB and AABBBB) lines. This indicates that sequences required for amplification of the fragment from *Sbe1* #19 are present only in the A genome. Figure 5 also shows slightly

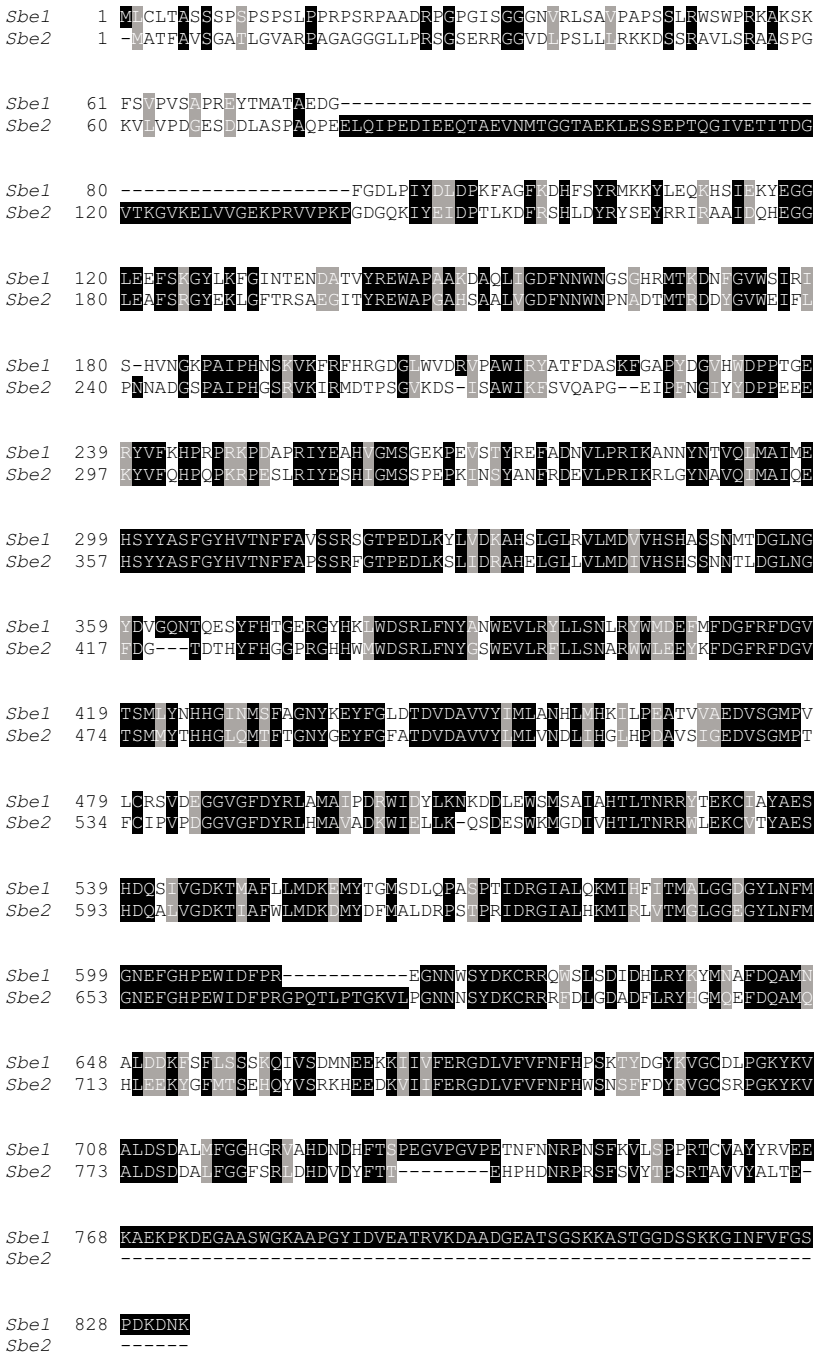

Figure 2. Comparison of deduced amino acid sequences of *Sbe1* and *Sbe2*. Boxshade treatment of ClustalW ver 1.8 (Thompson et al., 1994) alignment of the deduced protein sequences for *Sbe1* #9 and *Sbe2* #30. The black shading indicates identity, the gray shading indicates conservative substitutions, and the white background indicates nonconservative substitutions.

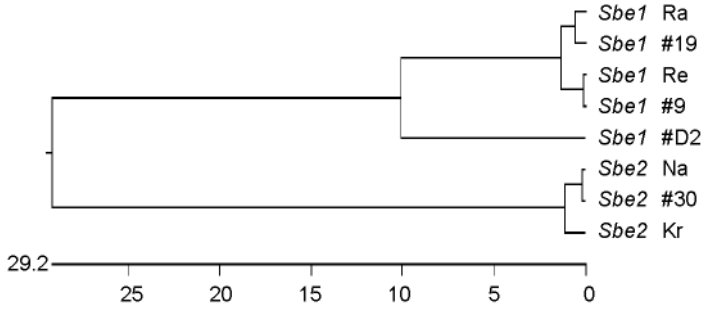

Figure 3. Phylogenetic tree of *Sbe* sequences. Relationships of 2 new *Sbe1* and 1 new *Sbe2* sequences with other known *Sbe* cDNAs from wheat using ClustalW and PAM250 residue weight table (MegAlign, Lasergene, DNAstar). On the tree are *Sbe1* #9 (this paper), *Sbe1* Ra (Rahman et al., 1999), *Sbe1* #19 (this paper), *Sbe1* Re (Repellin et al., 1997), *Sbe1* #D2 (Rahman et al., 1997), *Sbe2* #30 (this paper), *Sbe2* Na (Nair et al., 1997), *Sbe2* Kr (Genbank ACC U66376).

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#9     23 TGCCTCACCGCC---CCCTCCTG----------CTC-GC-CATCTCTCCCGCCGCGC
#19    25 TGCCTCACCGCC---TCCTCCTCGCCCTCGC----CCTC-GC-CCTCTCTCCCGCCGCGC
#2D    25 CGCC-CTCCGCTGTGCCGTCGACGACGATGCTGTGCCTCAGCTCCTCTCTCTTGCCGCGC

#9    328 TGACCAGAAACATTCGATTGAGAAGCACGAGGGAGGCCTTGAAGAGTTCTCTAAAGGCTA
#19   339 TGAACAGAAACATTCGATCGAGAAATACGAGGGGGGCCTTGAAGAGTTCTCTAAAGGCTA
#2D   354 TGAACAGAAACATTTGATTGAGAAACATGAGGGAAGCCTAGAAGAATTCTCTAAAGGCTA
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Figure 4. Location of forward and reverse primers for homoeologue assignment of *Sbe1* orthologues by PCR. Sequence numbering is based on individual cDNA sequences.

stronger amplification whenever 4 copies of chromosome A are present. The predicted fragment sizes calculated from the cDNA sequences are 347 bp for #19, 349 bp for #9, and 342 bp for #D2. However, the bands resulting from amplification were approximately 850 bp for #19, 730 bp for #9, and 1500 bp for #D2. The differences between the predicted and observed sizes of the amplified bands are likely caused by the presence of insertions and variation in introns within the amplified regions of the genomic DNA. According to the structure of the one reported genomic *Sbe1* sequence from Baga et al. (1999), the *Sbe1* #19 primers (conserved in this sequence) are predicted to produce a band of 894 bp. For each gene-specific primer pair a band is amplified in 4 of the 6 available nullisomic-7-tetrasomic lines. Experiments with any single primer on DNA from the Chinese Spring lines failed to produce any bands (data not shown). The band amplified for the #D2-specific primer sets are lighter than for the other primer sets. This may be because of the complexity described for this *Sbe* locus by Rahman et al. (1997), which contained 1 complete and 2 partial *Sbe1* sequences. Multiple priming sites at the D locus, variation in inserts and intron size, and potential base substitutions between *T. tauschii* and *T. aestivum* may be responsible for the reduced signal from these primers.

From these results we can tentatively assign cDNA sequences for *Sbe1* orthologues to their respective homoeologue of chromosome 7. *Sbe1* #19 is derived

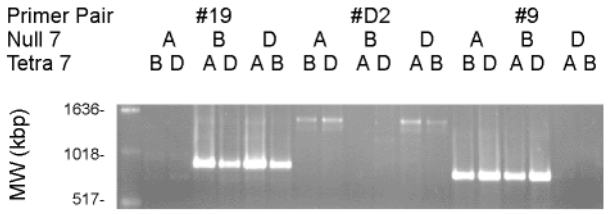

Figure 5. Sbe1 homoeologue assignment. Results of PCR amplification using orthologue-specific primers for *Sbe1* #19, #9 and #D2 (Rahman et al., 1997) using DNA isolated from Chinese Spring nullisomic-7-tetrasomic lines.

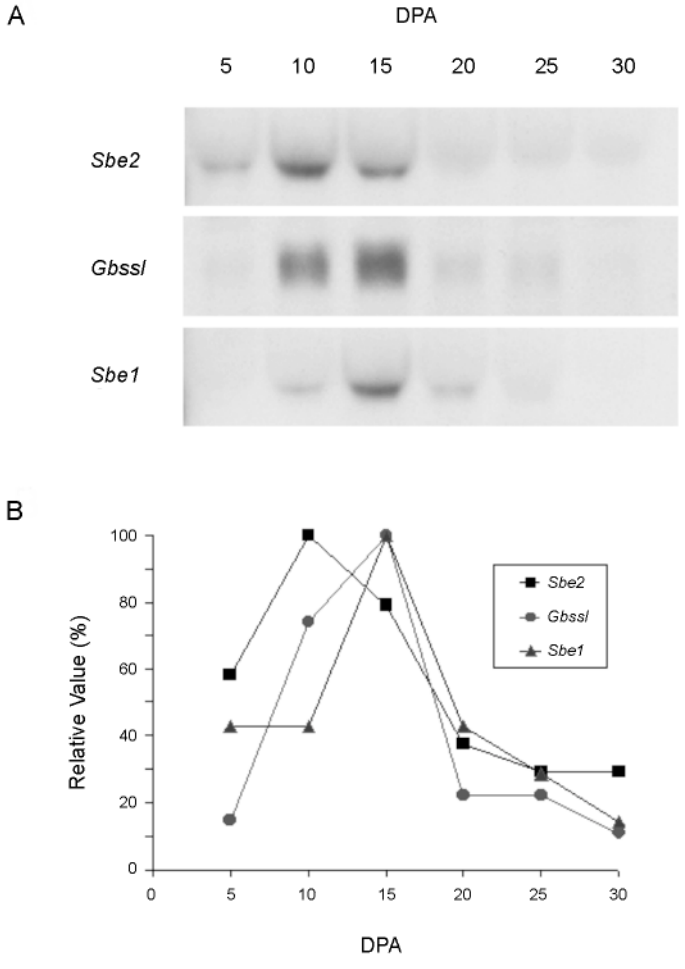

Figure 6. Analysis of steady-state levels of RNA for starch biosynthetic enzymes during seed development. (A) Lanes represent 20 µg of total RNA extracted from a single kernel endosperm at 5, 10, 15, 20, 25, and 30 DPA probed with: *Sbe2*, *GbssI*, and *Sbe1* cDNA sequences. (B) Scanning densitometric representation of RNA blots normalized for each gene probe over the developmental time course.

from the A genome, and *Sbe1* #9 from the D genome. Our data indicate that *Sbe* #D2 is present on the B genome, contrary to a previous report assigning it to the D genome (Rahman et al., 1997).

Developmental analysis

The expression of both *Sbe* isoforms and *GbssI* was examined in the same total RNA isolated from wheat endosperm cv. Cheyenne at different times during development (Figure 6). The steady-state levels of *Sbe2* were readily detectable at 5 DPA, reached a maximum at day 10, and started to decline by day 15. The levels of *GbssI* were almost undetectable at day 5, were strong by day 10, reached a maximum at day 15, and dramatically declined to very low levels by day 20. The levels of *Sbe1* RNA rose lightly at day 10, peaked at day 15, and declined again by day 20.

As observed in other species, the 2 isoforms of *Sbe* are differentially regulated at the steady-state mRNA level (Burton et al., 1995; Gao et al., 1996). As in pea, *Sbe2* levels are high during endosperm development, and *Sbe1* levels increase and peak later in development (Burton et al., 1995). The results do not allow for discrimination of possible multigene family differences in expression from the 3 orthologous gene products for either *Sbe1* or *Sbe2*.

Future experiments

Additional experiments to isolate, identify, and map the full set of orthologous gene products for the SBEs of hexaploid wheat are still required. Evidence is strong for assignment of *Sbe1* sequences to 2 of the 3 ancestral genomes. More information is still required on the genomic sequences of the *Sbe1* orthologues. Homoeologue mapping of the *Sbe2* isoforms and isolation of their genomic sequences are still required to make their placement and determine their structure on the physical map of wheat. This study demonstrates the usefulness of using PCR for the mapping of known orthologous sequences to the homoeologous chromosomes.

Reference to a company and/or product by the USDA is only for purposes of information and does not imply approval or recommendation of the product or the exclusion of others that also may be acceptable.

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