Plant Molecular Biology **30**: 1223–1232, 1996.

Evolutionary conservation and expression patterns of maize starch branching enzyme I and IIb genes suggests isoform specialization

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Received 25 May 1995; accepted in revised form 10 January 1996

Key words: gene evolution, gene expression, *Zea mays* L., starch biosynthesis, starch branching enzyme

Abstract

Expression of the maize (*Zea mays* L.) starch branching enzyme (SBE) genes *Sbe1* and *Sbe2* were characterized during kernel development and in vegetative tissues. The onset of *Sbe1* and *Sbe2* expression during endosperm development was similar to that of other genes involved in starch biosynthesis (*Wx*, *Sh2* and *Bt2*). However, the expression of *Sbe2* peaked earlier than that of *Sbe1* in developing endosperm and embryos resulting in a shift in the ratio of *Sbe1* to *Sbe2* relative message levels during kernel and embryo development. Transcripts hybridizing to the *Sbe2* probe were not detectable in leaves or roots which nonetheless have SBEII enzymatic activity, suggesting that there may be another divergent SBEII-like gene(s) in maize. A similar expression pattern is shared between the maize genes and related genes in pea, which together with their evolutionary conservation, suggests that the SBE isoforms may play unique roles in starch biosynthesis during plant development.

Introduction

Starch biosynthesis in plants involves the activity of three groups of enzymes, ADPG pyrophosphorylase, starch synthase and starch branching enzyme (reviewed by [27, 30, 35]). Recent molecular cloning of the *sugary1* locus in maize has also revealed the importance of starch debranching enzyme in the determination of starch composition in endosperm [18]. Starch is composed of two structurally different glucans, amylose and amylopectin. Amylose is an essentially linear polymer of α-1,4-linked glucosyl residues while amylopectin is a branched polymer of α-1,4-linked glucosyl residues with α-1,6 linkages at branch points. Starch branching enzyme (SBE) hydrolyzes α-1,4 bonds and reattaches the released α-1,4 glucan segments to the same or another glucosyl chain by an α-1-6 linkage. The reaction creates not only branch points but also new nonreducing ends for further elongation of the α-1,4 glucan branches.

Multiple forms of SBE have been characterized in many species, including spinach leaf, pea embryo, potato tuber, maize endosperm and leaf [9, 29, 30], and recently in rice seed and other vegetative tissues [24, 25, 40]. The SBE isoforms in maize [8], rice [26] and pea [34] have been classified into two groups, SBEI and II, based on their chromatographic properties. In maize, two

isoforms of SBEII (IIa and IIb) have been further resolved [7]. The biochemical differences of the SBE isoforms in maize have been well characterized [30]. In maize, SBEI is quite different from SBEIIa and IIb in molecular size, chromatographic and enzyme kinetic properties and immunological reactivity, whereas SBEIIa and IIb are quite similar, but not identical, in most biochemical properties (reviewed by [30]). However, the particular role of each SBE isoform in starch biosynthesis remains unknown.

The recent isolation of genes encoding various SBE isoforms in several plant species has greatly facilitated the investigation of the role of SBE isoforms in starch biosynthesis. The first SBE cDNA was isolated for the gene encoding pea SBEI at the *rugosus* (*r*) locus ([5], EMBL accession number 80009), used by Mendel in his study on the law of inheritance [5, 22]. cDNAs encoding SBE have been isolated from potato tubers [20, 28], rice seed [19, 23, 24, 26] and cassava [31]. The developmental expression patterns of several cloned SBE genes have been well characterized in potato [20, 31, 38], rice [19, 23, 24] and in pea embryo [4, 9]. In maize, a partial cDNA encoding SBEI was first isolated by Baba *et al.* [2]; a full-length cDNA encoding maize SBEI was later isolated by Fisher *et al.* [16]. Our laboratory first reported the isolation of a full-length cDNA (*Sbe2*) encoding SBEIIb using the pea *Sbe1* cDNA as a heterologous hybridization probe [13]. The identity of this cDNA as the product of the *amylose-extender* gene in maize was subsequently verified by transposon tagging [36].

Sequence comparisons of the various SBE genes and their encoded proteins have revealed that two distinct classes of genes encoding SBE may be clearly distinguished [9, 35]. As listed in Table 1, we refer to these classes by the prototypic family member names maize SBEI and SBEII as recommended by the Commission on Plant Gene Nomenclature [10]. In order to gain insight into the role of each maize SBE isoform in starch synthesis, we examined the expression of the cloned *Sbe1* and *Sbe2* genes at different developmental stages. We present evidence that the *Sbe1* and *Sbe2* genes are expressed differentially during maize kernel development. The onset of *Sbe1* and *Sbe2* mRNA accumulation in developing endosperm is similar to that of other genes involved in starch biosynthesis (*Wx*, *Sh2* and *Bt2*). However, the timing of their maximal expression levels differ, resulting in a shift of the ratio of *Sbe1* to *Sbe2* mRNAs during development, similar to that described for corresponding family members during pea embryo development [9]. Furthermore, while SBEI and II enzymatic activity can be detected in maize leaves and roots, we show that only the *Sbe1* cDNA hybridizes to mRNA from these tissues, indicating that there could be another divergent gene encoding vegetative SBEII activity in maize. The evolutionary conservation of the genes encoding SBEI and II in pea and maize and their distinct expression patterns sug-

Table 1. Nomenclature system for plant starch branching enzyme genes based on enzymological precedence of the maize endosperm isoforms as suggested by the Commission on Plant Gene Nomenclature [10], and categorization of individual SBE family members from various species. In this manuscript, protein designations are in capitals and Roman numerals, all genes, cDNAs and mRNAs are designated in italics with first-letter capitals only and Arabic numerals. A previously published nomenclature system is also listed [9]. cDNAs from maize have been categorized based on encoded sequence identity to N-terminal amino acid sequence data of purified SBEI and SBEII protein. All other cDNAs can be categorized into either the SBEI family or the SBEII family based on sequence relatedness to the maize cDNAs as reported [10, 15], although their published designations may not correspond numerically.

Enzymological precedence	SBEI family EC 2.2.4.1.18:1	SBEII family EC 2.2.4.1.18:2
Commission on Plant Gene Nomenclature [10]	Individual family members maize *Sbe1* pea *Sbe2* pea *Sbe1* rice BE-1 potato *Sbe* cassava *Sbe*	Individual family members maize *Sbe2b* rice *Rbe3* arabidopsis *Sbe2.1*, *Sbe2.2*
Previously proposed nomenclature system [9]	Family B	Family A

gest specific roles of the SBE isoforms in starch biosynthesis.

Materials and methods

Plant materials

Maize (*Zea mays* L.) inbred W64A was field-grown, and inbred B73 was grown in 4-liter pots outside from late in May to late October of 1993. Plants were self or sib-pollinated and developing kernels were harvested at various days after pollination (DAP). Kernels at later developmental stages were hand-dissected into endosperm and embryo fractions. Leaves and primary roots from W64A seedlings were sampled at various days after emergence (DAE). The seedlings were germinated and grown in perlite in a growth chamber at 30 °C under a 14 h light/10 h dark photoperiod regime. Leaf blades and sheathes at different positions, stem segments of the top most 5 cm and the next 5 cm and a tassel just before anthesis were harvested from one maize B73 plant grown in a 4 liter pot outside. All of the samples were immediately frozen in liquid nitrogen and stored at −70 °C.

cDNA cloning

Our laboratory reported the cloning of a *Sbe2b* cDNA by screening maize λgt10 cDNA libraries constructed from endosperm poly(A)$^+$ RNA (14, 22, 29 DAP) with the pea *Sbe1* cDNA [5] as a probe [13]. A full-length cDNA clone of the maize *Sbe1* gene was isolated by screening the same maize endosperm cDNA libraries with a PCR-amplified 426 bp fragment of the 5′ coding region of *Sbe1* from maize genomic DNA [16]. It should be noted that the *Sbe1* and *Sbe2b* genes exist as multigene families in the maize genome with two to three members each (M. Guiltinan, unpublished data). While the cDNA probes for each isoform class used in this study do not hybridize to each other, they do cross-hybridize to each of the related genes in their respective families and thus are expected to hybridize with mRNA from each. Thus, the transcript levels presented here represent mRNA from one or multiple genes in a given family, representing the total message for a given isoform class.

Northern blotting and RNA quantification

Total RNAs from all samples were extracted as described [21] and fractionated on a 2.2 M formaldehyde, 1.2% (w/v) agarose gel. All experiments were repeated on RNA extracted from independent ears. Total RNA was dried in a SpeedVac (Savant), and denatured at 65 °C for 15 min in 30 μl of loading buffer containing 50% (v/v) formamide, 2.2 M formaldehyde, 1 × MOPS buffer (20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA pH 7.0), 4% (v/v) glycerol, 0.8% (w/v) bromophenol blue and 1 μg ethidium bromide. Denatured RNA samples were chilled on ice for 3–5 min, loaded and allowed to rest in wells for 5–10 min before running. They were run at 2–3 V/cm for 4–5 h until bromophenol blue neared the bottom of the gel. Fractionated RNA was blotted onto nylon membranes (Hybond N, Amersham) according to the manufacturer's instructions, and fixed by a 30 s exposure under a UV lamp. Membranes were prehybridized and hybridized at 65 °C in 10 ml of the buffer containing 6 × SSC (0.9 M NaCl, 0.09 M sodium citrate pH 7.0), 0.5% (w/v) SDS, 100 μg/ml salmon sperm DNA, and 5 × Denhardt's solution (0.1% (w/v) Ficoll, 0.1% (w/v) PVP, 0.1% (w/v) BSA). The probes were labeled with [32P]dCTP using a random-primed DNA labeling kit (Boehringer Mannheim Biochemica, catalog No 1004760), and purified with Sephadex G-50 spun columns [32]. Membranes were washed with 2 × SSC for 5 min, 2 × SSC and 0.1% (w/v) SDS twice for 10 min each at room temperature, and 0.1 × SSC and 0.1% (w/v) SDS at 65 °C for 15 min. Membranes were exposed to X-ray film with one intensifying screen at −70 °C. Radioactivity was directly quantified with a β-scope (Betagen, Mountain View, CA). Membranes were stripped of the radioactive

probes in a boiling 0.1% (w/v) SDS solution and allowed to cool to room temperature. Membranes were then sequentially hybridized with other probes. The actual amount of the total RNA loaded was based on the total RNA concentration calculated from spectrophotometric readings (absorbance at 260 nm). Minor RNA loading differences between samples from the same tissue or organ were calibrated using a tomato cDNA probe hybridizing to the 26 S rRNA. The relative mRNA level (RL) of each band on a membrane was then calculated and expressed as the percentage of the peak expression level in the same tissue at a different developmental stage or the control as specified in figure legends, based on β-scope data.

Results

Expression of Sbe1 *and* Sbe2 *genes during maize kernel development*

To investigate the roles of the *Sbe1* and *Sbe2* genes in starch biosynthesis, we characterized their expression patterns during maize kernel development and compared them with those of several other starch biosynthetic genes, including *Wx*, encoding the granule bound starch synthase [33], and *Sh2* [6] and *Bt2* [3], encoding the large and small subunits of ADPG pyrophosphorylase, respectively. Total RNA was extracted from whole kernels, endosperm and embryos of kernels at various developmental stages and analyzed by northern blotting. Total RNA from endosperm of kernels at mid or later stages were used in the blots shown in Fig. 1. Because of the difficulty of separating the endosperm and embryo of very young kernels, total RNA was extracted from whole kernels at the early stages of development. The endosperm tissue takes up much of the volume of kernels at 12–20 DAP, and thus in a practical sense, the transcript levels in total RNA from kernels at these early stages is quantitatively representative of the endosperm of the same age (data not shown).

The *Sbe1* gene was expressed at a very low level in W64A kernels at 6 and 8 DAP and began

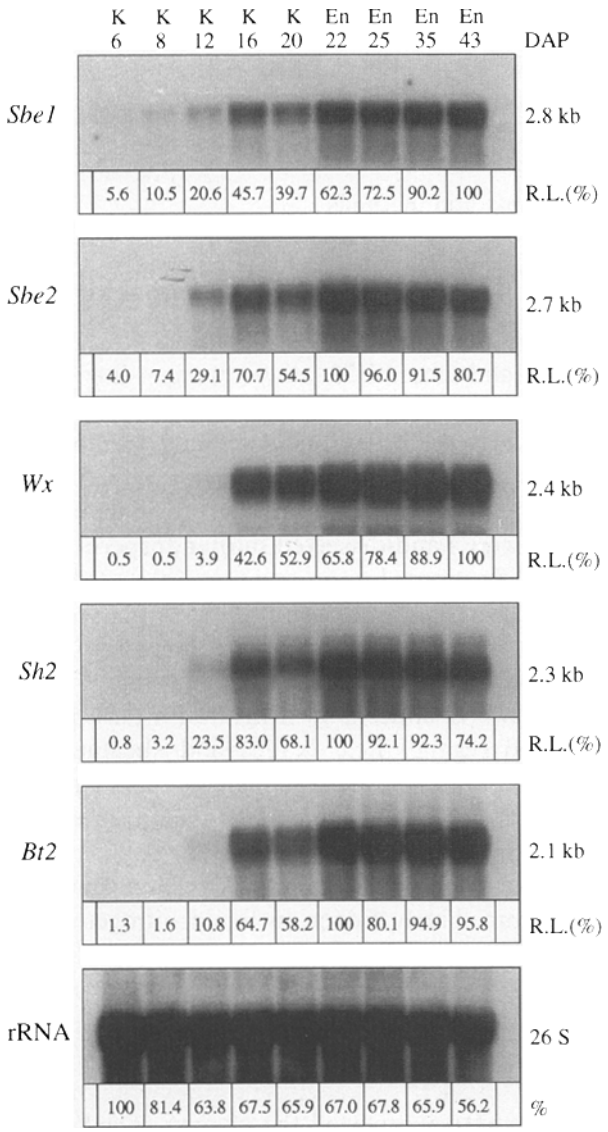

Fig. 1. Northern blot analysis of the expression of starch biosynthetic genes during development of W64A maize kernels. 7.5 μg of total RNA extracted from kernels (K) and endosperm (En) at various days after pollination (DAP) was fractionated, blotted and probed consecutively with radioactively labeled *Sbe1* [16], *Sbe2* [13], *Sh2* [6], *Bt2* [3], and *Wx* [33] cDNAs. The relative mRNA level (RL) of each gene in a sample was expressed as the percentage of its maximal transcript level in a particular sample (e.g., 22 DAP endosperm for *Sbe2*), calibrated for the 26S rRNA levels in the two samples. RL for the *Sbe1*, *Sbe2* and *Sh2* genes represent the average of three or four repeated blots with the absolute value of standard error less than 9%. The data shown below *Bt2* and *Wx* are the average from two repeated blots with the absolute value of standard error less than 15.3%. The data below the 26S rRNA bands are the percentage of the maximal 26S rRNA level on the particular blot. All repeat experiments were performed on RNA extracted from independent ears.

to accumulate to high levels after 12 DAP (Fig. 1). Similarly, the *Sbe2* transcript level was first detectable at 6 DAP, and continued to rise thereafter. The expression of *Sbe1* peaks in endosperm at a developmental stage at or later than 43 DAP. The transcript level of the *Wx* gene likewise increased throughout kernel development. Thus, the developmental timing of the *Sbe1* gene expression parallels that of the *Wx* gene except that *Wx* mRNA was not significantly detectable in kernels before 12 DAP. Very similar expression patterns for the two genes were also observed in B73 kernels during development (data not presented).

The *Sbe2* expression pattern was very similar to those of the *Sh2* and *Bt2* genes. As shown in Fig. 1, although barely detectable at earlier stages, the message levels of all three genes first accumulated to significant levels in kernels at 12 DAP, then gradually increased until peaking at 22 DAP. They dropped slightly in endosperm older than 22 DAP, but were maintained at a high level as the endosperm matured. Very similar developmental expression patterns for all three genes were also observed in B73 kernels except that the expression peaked later at about 30 DAP (data not presented). This difference between the inbreds examined is likely due to differences in the environmental conditions during the growth of the plants. It should be noted that quantitative levels of different transcripts can not be compared since the specific activity, length and the hybridization times were slightly different for each probe. However, qualitatively, it is valid to compare the developmental timing of the peak expression of the five genes, based on these data. Significantly, the peak expression of *Sbe1* and *Wx* genes occurred later than those of *Sbe2*, *Sh2* and *Bt2* in developmental timing.

To see if the *Sbe1* and *Sbe2* genes also exhibit differential expression patterns in developing embryos, total RNA from embryos of both inbreds were analyzed by northern blot. As in endosperm RNA, the *Sbe1* message level peaked later than that of *Sbe2* in developing embryos of both inbreds (Fig. 2). As indicated by the relative transcript level, the expression of *Sbe2* peaks at or before 22 DAP in W64A embryos and at about

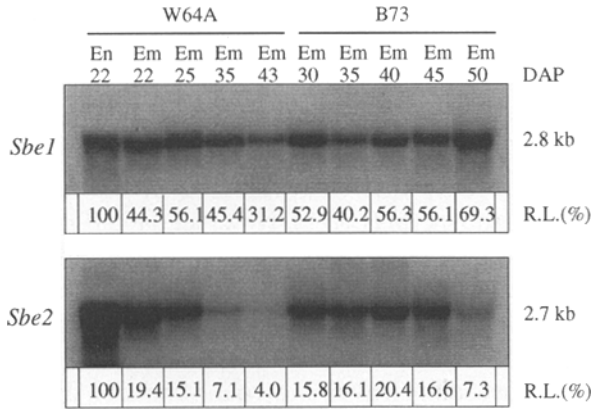

Fig. 2. Northern analysis of the expression of *Sbe1* and *Sbe2* in developing embryos of W64A and B73 maize inbreds. 15 μg of total RNA extracted from embryos (Em) at various days after pollination was fractionated, blotted and probed consecutively with the *Sbe1* and *Sbe2* cDNAs. 7.5 μg of total RNA from 22 DAP endosperm of W64A plants was used as a positive control. After calibration of minor loading differences between embryo RNA samples with a rDNA clone, the relative message level (RL) of each gene in embryos at various developmental ages was calculated and expressed as a percentage of that in the control.

40 DAP in B73 embryos, whereas *Sbe1* was expressed maximally in W64A embryos at 25 DAP and B73 embryos at 45–50 DAP. Although we cannot make a quantitative comparison of the *Sbe1* and *Sbe2* message level, we can compare the relative expression levels of each class between embryos and endosperm. The highest level of the *Sbe1* transcript in embryos was about 50 to 70% of the *Sbe1* transcript level in 22 DAP W64A endosperm on a per unit RNA basis. The *Sbe2* gene was expressed in embryos at about 20% of the *Sbe2* transcript level in 22 DAP W64A endosperm on a per unit RNA basis. A very similar expression pattern of corresponding family members in developing pea embryos has also been observed [9]. The significance of the differential timing of expression levels of the *Sbe1* and *Sbe2* genes in starch biosynthesis in endosperm and embryo remains to be investigated.

Expression of Sbe1 *and* Sbe2 *in other maize organs*

To investigate the tissue specificity of expression of the *Sbe1* and *Sbe2* genes and the possible in-

volvement of the products of both genes in starch biosynthesis in organs other than kernels, we characterized their expression in other maize tissues. The *Sbe1* gene was expressed in seedling leaves and young roots of W64A plants at a low and constant level of about 10% of that in 22 DAP endosperm (Fig. 3). No essential difference in the level of *Sbe1* transcript was observed among adult leaf blades from upper, middle and lower parts of a B73 plant and in the leaf sheath (Fig. 4). By contrast, the *Sbe2* transcript was not detectable in seedling leaves and young roots of W64A plants (Fig. 3), even under low stringency hybridization and with increased loading of the leaf total RNA up to 50 μg (data not shown). Similarly, the *Sbe2* transcript was not detectable in adult leaf blades and sheath of a B73 plant (Fig. 4). This does not rule out the possibility that the *Sbe2* transcript may be expressed at very low levels in leaf, undetectable by this method, or that a separate, divergent, SBEII-like gene is expressed in these tissues. RT-PCR of leaf mRNA using PCR primers designed from highly conserved regions of SBEII family members did not result in the amplification of *Sbe2b* sequences to our detection limits, although large numbers of such clones were obtained from similar reactions using endosperm RNA. This is consistent with previous biochemical evidence of a lack of SBEIIb activity in maize leaves [11, 12] and supports the conclusion that the *Sbe2b* gene is not expressed to significant levels in leaf (data not shown).

The *Sbe1* gene was expressed in the top most 10 cm of the stem at about 6–8% of that in 35 DAP endosperm. The *Sbe2* gene was also expressed at an extremely low level (1–2% of that in 35 DAP endosperm) in the upper stem segments. Both *Sbe1* and *Sbe2* genes were expressed in the tassel just before anthesis (Fig. 4). The *Sbe1* transcript level in the tassel corresponded to about 13% of that in 35 DAP endosperm of B73 plants on a per unit RNA basis. The *Sbe2* gene was expressed in the tassel at about 16% of that in 35 DAP endosperm. A faint *Sbe1* hybridizing band at about 3.1 kb was reproducibly detected in the tassel and the young stem just above the 2.8 kb *Sbe1* band. A similarly sized 3.1 kb transcript in the tassel total RNA was also detected with the *Sbe2* cDNA although *Sbe1* and *Sbe2* cDNAs do

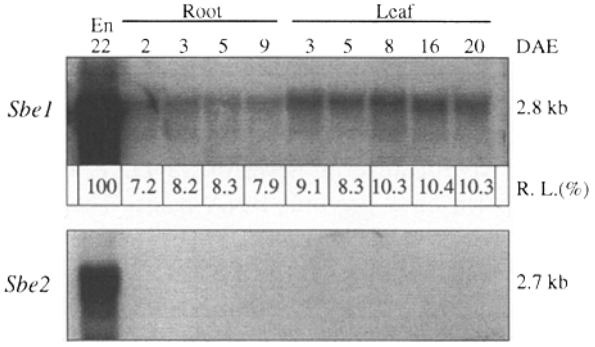

Fig. 3. Northern blot analysis of the expression of *Sbe1* and *Sbe2* in primary roots and seedling leaves of W64A plants. 15 μg of total RNA from primary roots and seedling leaves collected at various days after emergence were fractionated, blotted and probed consecutively with *Sbe1* and *Sbe2* cDNAs. 15 μg of total RNA from 22 DAP endosperm of W64A plants was loaded in the first lane as a control. The minor loading differences between leaf RNA samples, and between root RNA samples, respectively, were calibrated with a rDNA clone. The relative message level (RL) of each gene in leaves and roots of various developmental age was expressed as the percentage of that in the control.

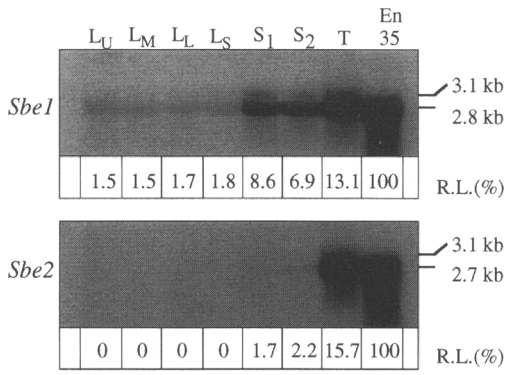

Fig. 4. Northern blot analysis of the expression of *Sbe1* and *Sbe2* in the leaf, the young stem and the tassel of a B73 plant just prior to anthesis. 30 μg each of total RNA from leaf blades at upper (LU), middle (LM) and lower (LL) parts, leaf sheath (LS), stem segments of top most 5 cm (S1) and the next 5 cm (S2), and a tassel before anthesis of a B73 plant were fractionated, blotted and probed consecutively with *Sbe1* and *Sbe2* cDNA clones. 7.5 μg of total RNA from 35 DAP endosperm of B73 plants was used as a control. The minor loading differences among the RNA samples of the leaf blades and sheath were calibrated with a rDNA clone. The relative message level (RL) is expressed as the percentage of that in the control.

not cross-hybridize significantly (data not shown). The 3.1 kb transcript(s) was not detected with either the *Sbe1* or the *Sbe2* probe in maize kernels, young roots and leaves, and it's identity remains to be further investigated.

Discussion

The maize *Sbe1* gene was expressed in all maize tissues or organs examined, including endosperm, embryos, leaf blade and sheath, young stem, young roots and tassel. Moreover, the expression remained at the same low level in young leaves and roots from their emergence. These results suggest that the *Sbe1* gene is constitutively expressed in vegetative tissues, however since our probe cross-hybridizes to two or three *Sbe1* genes in the genome, we cannot rule out the possibility that different genes are expressed with tissue specific and complementary distributions.

Unlike in other organs, the *Sbe1* transcript level steadily increased in kernels from an early stage (12 DAP for W64A) until at least 43 DAP (the oldest kernel sampled). Thus, the *Sbe1* gene is strongly induced in developing kernels (mostly in endosperm). The *Sbe1* and *Wx* genes shared a very similar expression pattern in kernels older than 16 DAP, suggesting that the induced expression of *Sbe1* and the expression of *Wx* may be coordinately regulated. The rice *rbe1* cDNA is most similar to the maize *Sbe1* cDNA [19]. Consistent with our results, it was reported that the *Rbe1* gene is also constitutively expressed at low levels in most tissues, and is strongly induced during seed development [19]. Interestingly, the timing of its expression is also similar to that of the rice *Wx* gene. The SBE from potato tuber has a high degree of immunological similarity with maize SBEI, in both native and denatured forms [39], and cDNA sequence is most similar to the maize *Sbe1* and the rice *Rbe1* cDNAs. Similar to maize *Sbe1* and rice *Rbe1*, the potato *Sbe* gene is expressed in a fashion similar to that of the two genes encoding a granule bound starch synthase and the small subunit of ADPG pyrophosphorylase in potato tubers [38]. It is also expressed at an extremely low level in other potato organs, and was strongly induced in leaves by metabolizable carbohydrates such as sucrose [20].

The expression pattern of the *Sbe1* gene during development of the W64A inbred kernel differs from that reported for the Oh 43 inbred kernels [1]. It was reported that the expression of the *Sbe1* gene peaked early in kernel development (10–14 DAP) and dropped quickly thereafter. We also examined the expression pattern of the *Sbe1*, *Sbe2*, *Sh2*, *Bt2* and *Wx* genes in the maize B73 inbred. The expression pattern of all five genes in B73 was similar to that observed in W64A. The *Sbe1* gene was maximally expressed at a later stage, and the expression of *Sbe2* peaked at an earlier stage during development of endosperm (data not shown) and embryo (Fig. 2) of the B73 inbred kernels. It is possible that the differences in timing of expression observed in the two inbred genotypes reflects the effects of different growing conditions on endosperm development. In addition, we never detected a 1.5 kb transcript of the *Sbe1* gene previously reported [2].

The maize *Sbe1* and *Sbe2* genes are differentially expressed in endosperm and embryos during kernel development. As summarized in Fig. 5, the expression of the *Sbe1* gene peaked later than that of the *Sbe2* gene in both developing endosperm and embryos (Figs. 1 and 2). This is similar to what has been reported for the genes encoding corresponding family members in pea embryo development [9]. The maize SBEI and IIb isoforms show differences in the length of chains synthesized by recombinantly expressed proteins (11–14 chain length glucans and 8–9 chain length glucans respectively) [37]. If the SBE enzymatic activities are reflected by the *Sbe* mRNA levels during endosperm development, the differential expression of the *Sbe1* and *Sbe2* genes may result in starch with on average, longer chain lengths later in development. Although evidence for such a shift in chain length over development has been reported for pea embryos [9], similar data have not been reported in maize endosperm.

DNA sequence comparison of plant SBE cDNAs show that they fall into 2 classes corresponding to cDNAs encoding SBEI and II maize

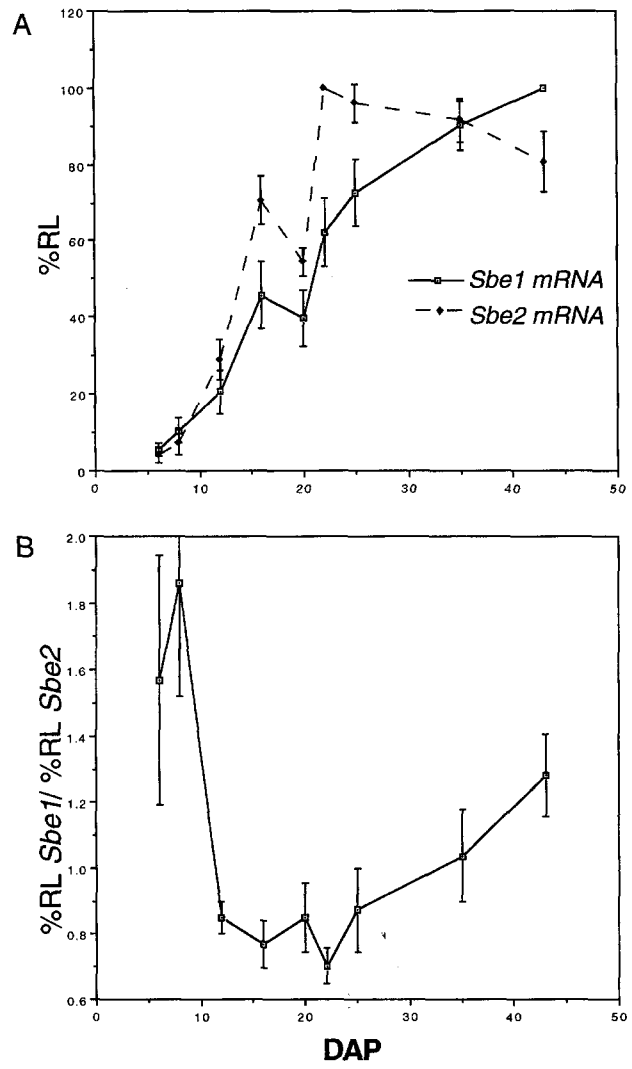

Fig. 5. Relative transcript levels for *Sbe1* and *Sbe2* during kernel development. A. Mean relative transcript levels (RL) for *Sbe1* and 2 were measured in four replicate experiments as described in Fig. 1. Error bars represent 1 standard error of the mean. B. Mean ratios of *Sbe1* RL to *Sbe2* RL during kernel development. Standard errors of the mean ratios were determined from the same data in Fig. 1 and A from ratios determined independently from each of the replicate experiments.

isoforms (Table 1 and [9]). Although the N- and C-terminal domains of these proteins have diverged substantially, the internal sequences (from amino acids 1–595 of the mature SBEI protein) show high conservation within the groups. The SBEI class of genes includes maize *Sbe1*, rice *Rbe1*, pea *Sbe2* and potato *Sbe*, which share 76 to 92% amino acid similarity in the conserved central region (determined using Clustal analysis with a PAM250 table). The SBEII family includes maize *Sbe2*, rice *Rbe3*, pea *Sbe1* and arabidopsis *Sbe2* genes [15] which share 81 to 92% amino acid similarity. The percentage similarity in this central region is much lower between the two classes of genes (55–60%). This suggests that while the *Sbe1* and *Sbe2* genes are related, they had evolved their distinctive features prior to the monocot-dicot divergence. The evolutionary conservation of these sequences along with the similarities in expression patterns noted above, strongly suggests that these enzymes play unique and essential roles in plant development.

The following characteristics may apply to all genes encoding the SBEI isoforms: (1) they are constitutively expressed in most vegetative tissues, but strongly induced during the development of storage tissues and embryos; (2) their timing of their expression is similar to the gene encoding granule bound starch synthase during the development of storage tissue; and (3) for species with multiple *Sbe* genes, maximal expression of genes encoding the SBEI isoforms occurs later in development of storage and embryo tissues than those encoding SBEII.

As discussed above, a very similar expression pattern can also be observed among the genes encoding SBEII family members. The maize *Sbe2* gene is maximally expressed earlier in both developing endosperm and embryos than the maize *Sbe1* gene (Fig. 5). The pea *Sbe1* gene also reaches its maximal expression level earlier in developing pea embryos than the pea *Sbe2* gene [4, 9]. The maize *Sbe2* and pea *Sbe1* are thus very similar not only in sequence but also in their expression pattern. The expression of the maize *Sbe2* gene in endosperm is very similar in developmental timing with the *Sh2* gene encoding the large subunits of ADPG pyrophosphorylase (Fig. 1). It would be interesting to see if the expression of other *Sbe2* class genes are also similar to those of genes encoding ADPG pyrophosphorylase in the corresponding species.

In addition to endosperm and embryos, the maize *Sbe2* gene is also highly expressed in young tassels (Fig. 4). An extremely low level of the *Sbe2* transcripts was also detected in young stem tissues below the tassel (Fig. 4). No *Sbe2* transcripts were detectable in young leaves and roots (Fig. 3), even under low stringency condition. The same *Sbe2* gene was also cloned by transposon tagging from the maize *amylose extender1* locus and no transcript was detectable in seedling leaves [36]. Consistent with our results, the rice *Sbe3* gene (similar in cDNA sequence to the maize *Sbe2* gene) was demonstrated to be seed-specific [19]. Thus it appears that the *Sbe2* gene, and perhaps other similar genes, are not expressed at detectable levels in leaves or other vegetative tissues. However, the major starch branching enzyme in maize leaves was shown to be similar to the endosperm SBEIIa enzyme, and no SBEIIb activity was detected in maize leaves [11, 12]. Thus SBEIIa and IIb may be the products of different genes [11, 12, 17]. Our results indicating the lack of detectable expression of the *Sbe2* gene in leaves and roots which have significant SBEIIa activity, implicate another, yet to be identified gene encoding SBEII in maize. We have recently extended these observations by molecular analysis of 16 allelic amylose extender isogenic lines which also indicates the presence of a separate gene encoding SBEIIa [14].

Acknowledgements

This work was supported by a grant from Pioneer Hi-Bred Int'l Inc. to M.J.G., J.C.S. and Charles Boyer and by the Penn State Agricultural Experiment Station (project 3303). We would like to thank Dr L. Curtis Hannah at University of Florida for providing the *Sh2* and *Bt2* cDNA clones, and Dr Susan R. Wessler at University of Georgia for providing the *Wx* cDNA clone. We also appreciate Dr Charles Boyer's support of this project and Drs Jill Deikman and Richard N. Arteca for reviewing the article.

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