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Molecular characterization and expression of four cDNAs encoding sucrose synthase from green bamboo *Bambusa oldhamii*

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Summary

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• Bamboo is distinguished by its rapid growth. To investigate sucrose metabolism in this plant, we cloned the cDNAs encoding sucrose synthase (SuS) from *Bambusa oldhamii* and investigated their expression in growing shoots and leaves.
• Four cDNA clones, *BoSus1*, *BoSus2*, *BoSus3* and *BoSus4*, were isolated by screening a cDNA library from etiolated bamboo shoots. Recombinant BoSuS proteins were produced in *Escherichia coli* and purified by immobilized metal affinity chromatography and ultrafiltration. Semi-quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) was used to determine the abundance of the transcript of each gene.
• *BoSus1* and *BoSus3* may be duplicate or homeologous genes, the sequences of which show high identity. Similarly, *BoSus2* shows high identity with *BoSus4*. Kinetic analysis showed that the two BoSuS isoforms of each type had similar michaelis constant (K_m) values for sucrose, but different values for UDP. The four genes were expressed in various bamboo organs but were differentially regulated. The increase in the abundance of their mRNA paralleled the growth rate of the bamboo.
• The results suggest that, in bamboo, SuS is encoded by at least four genes, each with a specific role in providing substrates for the polysaccharide biosynthesis and/ or energy production necessary to support the rapid growth of this species.

Introduction

Bamboo belongs to the grass family Poaceae (Gramineae). Propagation of bamboo is usually vegetative because of its infrequent flowering. Under appropriate conditions, shoots emerge from buds on the rhizome and develop into new culms. Growth of bamboo is generally rapid, faster than that of other woody plants, although the rate varies greatly among different species. For example, the average elongation rate of culm is 24.5, 9.7 and 8.3 cm d^{-1}, respectively, for *Phyllostachys makinoi*, *Bambusa oldhamii* (synonyms *Leleba oldhami* and *Dendrocalamopsis oldhami*, respectively) and *Leleba dolicho-merothalla* (Lin, 1958), and can be over 100 cm d^{-1} for *Phyllostachys edulis* (Ueda, 1960). Before a new culm is able to assimilate carbon, its growth depends mainly on a supply of sucrose from mature plants through the rhizome; cleavage of that translocated sucrose serves as the starting point of its carbohydrate metabolic pathway.

In plants, sucrose cleavage is catalysed either by invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) or by sucrose synthase (UDP-glucose:D-fructose 2-α-D-glucosyl transferase, EC 2.4.1.13, SuS). The former hydrolyses sucrose into fructose and glucose, while the latter converts sucrose and UDP into fructose and UDP-glucose. Hexoses produced by

these two enzymes are converted into hexose phosphates and then channeled into glycolysis, the pentose phosphate pathway, and so on. The UDP-glucose produced by SuS is a direct, or indirect, glucose donor for synthesis of polysaccharides such as cellulose (Amor *et al.*, 1995; Carlson & Chourey, 1996; Chourey *et al.*, 1998; Nakai *et al.*, 1999; Salnikov *et al.*, 2001), hemicelluloses (Konishi *et al.*, 2004) and starch (Chourey, 1981; Déjardin *et al.*, 1997). It can also be channeled into the hexose-phosphate pool after being converted to glucose-1-phosphate by UDP-glucose pyrophosphorylase (Kleczkowski *et al.*, 2004). Research on the green bamboo *Bambusa oldhamii* has shown that starch does not accumulate in rapidly growing shoots and young culms while synthesis of cell wall polysaccharides is highly active (Su, 1969). Among the various sugar nucleotides that are required for synthesis of the diverse array of cell wall polysaccharides, UDP-glucose is the most abundant in green bamboo shoots (Su, 1965). Bamboo shoots are also rich in SuS (Su *et al.*, 1977). It has been estimated that SuS accounts for nearly 1% of the total soluble protein in bamboo shoot extracts (Su *et al.*, 1977), which underscores the importance of SuS for providing substrates to support the rapid growth of bamboo.

SuS occurs as isoforms and is encoded by at least two genes in many plants. For example, three sucrose synthase (*Sus*) genes or cDNAs have been cloned from maize (*Zea mays*; Geiser *et al.*, 1982; Gupta *et al.*, 1988; Shaw *et al.*, 1994; Carlson *et al.*, 2002), pea (*Pisum sativum*; Barratt *et al.*, 2001) and citrus (*citrus unshiu*) (Komatsu *et al.*, 2002) and the genome of *Arabidopsis thaliana* contains six *Sus* genes (Barratt *et al.*, 2001; Baud *et al.*, 2004). The genome of rice (*Oryza sativa*) may also contain six members of the *Sus* family (Yu *et al.*, 1992; Huang *et al.*, 1996; Feng *et al.*, 2002; Kikuchi *et al.*, 2003). Expression of different *Sus* genes is spatially and temporally regulated, and is differentially modulated in response to sugar concentration, anaerobiosis, temperature and osmotic stress (Wang *et al.*, 1999; Winter & Huber, 2000 and references therein; Barratt *et al.*, 2001; Carlson *et al.*, 2002; Liao & Wang, 2003; Koch, 2004 and references therein). Although the reaction catalysed by SuS is reversible, it is thought that the enzyme mainly acts in the cleavage direction. In addition to providing substrates for polysaccharide synthesis, SuS activity has been linked to responses to sink strength (Sun *et al.*, 1992; Zrenner *et al.*, 1995), phloem loading/unloading (Yang & Russell, 1990; Martin *et al.*, 1993; Nolte & Koch, 1993; Wächter *et al.*, 2003), and nitrogen fixation in the nodules of legumes (Gordon *et al.*, 1999).

Although bamboo is the fastest growing woody plant on earth, heretofore no molecular studies of sucrose metabolism have been carried out. In the present study, four cDNAs encoding SuS from the green bamboo *B. oldhamii* were cloned and characterized. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis indicated that the four genes are differentially regulated and play important roles in bamboo growth. To our knowledge, *Sus* is the first gene involved in carbohydrate metabolism ever to be cloned from bamboo, and this is also the first report demonstrating that SuS is physiologically crucial to the rapid growth of bamboo. This study adds significant insights to current understanding of sucrose metabolism in this remarkable plant.

Materials and Methods

Plant material

Fresh shoots and leaves of the bamboo *Bambusa oldhamii* (Munro) were collected from a bamboo farm in Taipei, Taiwan, in July 2000, 2001 and 2002. Samples were collected from 07:00 to 09:00 h. Flowers were collected from flowering bamboo in the Taipei Tai-An Forest Park in August 2003. For construction of a cDNA library, small shoots that were still underground (etiolated shoots) were used. After the outermost sheath had been removed, the shoots were cut into pieces and immediately frozen in liquid nitrogen. For expression studies, shoots at three different growth stages, including etiolated shoots (15 cm tall on average) and two groups of green shoots (30 cm and 100 cm tall on average), were collected. After the outer layers of the sheaths had been removed, each shoot was divided into three parts for separate analysis: the top, middle and base regions. In addition, unexpanded leaves, mature leaves and leaf sheaths were collected from mature bamboo plants. Each sample was immediately frozen in liquid nitrogen and stored at −80°C until used.

RNA isolation

The frozen bamboo samples were ground to a fine powder in liquid nitrogen, after which total RNA was extracted using the method of Chomczynski & Sacchi (1987), except that the homogenization buffer contained a higher concentration of guanidine thiocyanate (6 M guanidine thiocyanate, 35 mM sodium citrate, 0.75% sodium sarcosyl and 0.15 M 2-mercaptoethanol). In addition, the RNA was precipitated with isopropanol in the presence of 0.4 M sodium citrate and 0.6 M NaCl to remove polysaccharides. Poly(A)$^+$ RNA was isolated from the total RNA by oligo(dT) cellulose chromatography.

Construction and screening of a cDNA library

To prepare a DNA probe for *Sus*, a pair of primers (Table 1) was designed based on the conserved regions found within plant *Sus* sequences. These primers were then used in RT-PCR to amplify a partial sequence of *Sus* cDNA using, as a template, total RNA from the entire etiolated shoot. The identity of the amplified 1-kb DNA fragment was confirmed by sequencing.

Poly(A)$^+$ RNA isolated from the entire etiolated bamboo shoot was used for construction of a cDNA library using the SuperScript Choice System (Invitrogen, Carlsbad, CA, USA) and the Lambda ZAP II RI Library Construction Kit (Stratagene,

Table 1 Primers used for cloning of the *Bambusa oldhamii* sucrose synthase (*BoSus*) cDNAs by reverse transcriptase–polymerase chain reaction (RT-PCR), for synthesis of gene-specific probes used in Southern hybridization, and for semiquantitative RT-PCR

Purpose	Sequence	Position[2]
cDNA cloning	F[1] : 5′-CAAGTTGTITACATIITIGATCAAGT-3′	1016–1041[3]
	R[1] : 5′-ACICGGTTCATCTGIGIIGAIATCCA-3′	2036–61[3]
Hybridization probes		
BoSus1	F: 5′-GGCTGAAACGTGGAAGAAGGGAGC-3′	2607–2630
	R: 5′-ATGCTACTATTATTTCAGAAACGG-3′	2843–2866
BoSus2	F: 5′-AAGAGGCGCGTTTAGCGGGAG-3′	2474–2494
	R: 5′-GAGGCAACAAAATATTGCATTGAA-3′	2652–2675
BoSus3	F: 5′-GGCTGAAACGTGGAAGAAGGGAGC-3′	2617–2640
	R: 5′-ATGCTACTATTATTTCAGAAACGG-3′	2854–2877
BoSus4	F: 5′-ACAGAGGCGCCTTCAGCGGGGG-3′	2543–2564
	R: 5′-GATAAAACACCCCGAACAGCGGGA-3′	2726–2749
Semiquantitative RT-PCR		
BoSus1	F: 5′-ATGGCCAGCAAGACCACAG-3′	2338–2356
	R: 5′-AACTTCCACATCCACCGAG-3′	2721–2739
BoSus2	F: 5′-AAGAGGCGAGTTTAGCGGGAG-3′	2474–2494
	R: 5′-CTGAAAGATAAAAACACCACGAAC-3′	2626–2649
BoSus3	F: 5′-AGTGCCAGCAAGACCCCCGC-3′	2348–2366
	R: 5′-CACTCCCACATCAATCTTAAT-3′	2731–2751
BoSus4	F: 5′-TTCTCAATGGCTCGTCTT-3′	1818–1835
	R: 5′-GAGCTAACAGTCCATGAC-3′	2624–2641
BoActin	F: 5′-TGGCATCACACCTTCTACAA-3′	262–281[4]
	R: 5′-ACCTGGATCTTCATGCTGCT-3′	973–992[4]

[1]F, forward primer; R, reverse primer.
[2]The nucleotide position of the primer in the corresponding mRNA.
[3]The nucleotide position in rice (*Oryza sativa*) *RSus1* mRNA.
[4]The nucleotide position in rice actin *OsRAc1* mRNA.

La Jolla, CA, USA). The unamplified library, which contained 1.5×10^6 recombinant plaque forming units, was then screened using the 1-kb RT-PCR product as a probe.

DNA sequencing and sequence analysis

Both DNA strands were sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit with an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Analysis and alignment of the amino acid sequences were carried out using Vector NTI Suite 9 Sequence Analysis Software (Invitrogen). A phylogenetic dendrogram was generated using the program GROWTREE in the GCG Wisconsin Package (Accelrys, Inc., San Diego, CA, USA).

DNA isolation and Southern analyses

Genomic DNA was isolated from bamboo shoots according to the method of Murray & Thompson (1980), after which 12.5-μg samples were digested to completion with restriction enzymes and separated on 0.8% [weight/volume (w/v)] agarose gels. After electrophoresis, the nucleic acids in the gels were transferred to nylon membranes (Immobilon-Ny+; Millipore, Bedford, MA, USA). The DNA probes used for Southern hybridization were synthesized by PCR using the four *BoSus* cDNAs as templates with primers selected from

the 3′-untranslated region (3′-UTR) of each cDNA (Table 1). After hybridization, the blots were exposed to phosphor-imaging plates, and the captured images were analysed with a Bio Imaging Analyzer (Fujix BAS1000; Fuji Photo Film, Tokyo, Japan).

Semiquantitative RT-PCR

Reverse transcriptase reactions were carried out using total RNA isolated from the various bamboo samples collected. The resultant cDNAs were then used as templates for PCR with gene-specific primers (Table 1) to amplify each *BoSus* cDNA, after which the amplified cDNA was sequenced to confirm the specificity. In addition, for each cDNA, the linearity of the amplification was confirmed by carrying out the PCR for different numbers of cycles. An actin fragment was also amplified as an internal control using primers (Table 1) designed from the conserved regions of plant actin sequences. The cDNA bands resolved in the agarose gels were then quantified using the UVP BioImaging System (UVP Inc., Upland, CA, USA) and LabWork Image Acquisition and Analysis Software (Version 4.5; UVP Inc., Upland, CA, USA).

Construction of expression plasmids

The expression plasmid carrying the coding region of *BoSus1* was constructed by inserting the *Bam*HI-*Eco*RI fragment of

BoSus1 cDNA between the *Bam*HI and *Eco*RI sites of plasmid pTrcHisA (Invitrogen), yielding pHisBoSus1. To construct the expression plasmid for *BoSus2*, the 420-bp 5′ region of the *BoSus2* coding sequence was synthesized by PCR using *BoSus2* cDNA as the template. The amplified DNA was digested with *Bam*HI and *Eco*RI and then ligated with *Bam*HI-*Eco*RI-digested pTrcHisA. The resultant plasmid was digested with *Eco*RI and ligated with the 3′-*Eco*RI fragment (2270 bp) of *BoSus2* cDNA to form pHisBoSus2. To construct the expression plasmid for *BoSus3*, the 1771-bp *Bam*HI-*Eco*RI fragment containing the 3′ region of *BoSus3* cDNA was inserted into the *Bam*HI-*Eco*RI site of pTrcHisA, which was then digested with *Bam*HI and ligated with the 5′ *Bam*HI fragment (1027 bp) of *BoSus3* cDNA to form pHisBoSus3. Finally, to construct the expression plasmid for *BoSus4*, the 720-bp *Bam*HI-*Eco*RI fragment containing the 5′ region of the *BoSus4* coding sequence was PCR-amplified, digested with *Bam*HI and *Eco*RI, and inserted into the *Bam*HI-*Eco*RI site of pTrcHisA, which was then digested with *Eco*RI and ligated with the 3′-*Eco*RI fragment of *BoSus4* cDNA to form pHisBoSus4.

Production and purification of recombinant BoSuS in *Escherichia coli*

E. coli TOP10 harbouring pHisBoSus1, pHisBoSus2, pHisBoSus3 or pHisBoSus4 was grown in Luria-Bertani (1% w/v Bacto-tryptone, 0.5% w/v Bacto-yeast extract and 1% w/v NaCl) containing 100 µg ml^{-1} ampicillin at 37°C. Production of His-tagged recombinant BoSuS proteins was induced at mid-log phase by adding Isopropylthio-β-D-galactoside (IPTC) to a final concentration of 0.25 mM. After growing at 37°C for 6 h, the cells were harvested by centrifugation, washed with PB-7 (50 mM sodium phosphate, pH 7.0, 1 mM EDTA and 1 mM 2-mercaptoethanol), suspended in PB-7, disrupted by sonication and then centrifuged at 12 000 *g* for 15 min. The resultant supernatant was applied to a column packed with 5 ml of cobalt-based immobilized metal affinity chromatography (IMAC) resin (BD TALON Resin; BD Biosciences Clontech, Palo Alto, CA, USA), after which the column was eluted first with wash buffer (50 mM sodium phosphate, 300 mM NaCl and 10 mM imidazole, pH 8.0) and then with elution buffer (50 mM sodium phosphate, 300 mM NaCl and 100 mM imidazole, pH 8.0). The fractions containing SuS activity were pooled, concentrated and desalted by ultrafiltration (Amicon Centriplus YM-30; Millipore). The purified enzyme solution was stored at 4°C until used. The enzyme was stable at 4°C for at least 2 wk.

Enzyme assay and protein determination

Throughout the purification, SuS activity was assayed at pH 6.0 in the presence of 100 mM sucrose and 0.5 mM UDP by a UDP-glucose dehydrogenase coupling assay as described by Su *et al.* (1977). One unit of enzyme was defined as the amount needed to catalyse the production of 1 µmol of UDP-glucose per minute. To determine the kinetic parameters, SuS was assayed in the presence of different concentrations of one substrate and a saturated concentration of a second substrate (1.5 mM UDP or 300 mM sucrose). The initial velocities at different substrate concentrations were measured at different time intervals to obtain the linear rate. The michaelis constant (K_m) values were estimated from the double reciprocal plot. The protein concentration was determined by the dye-binding method of Bradford (1976) using bovine serum albumin as the standard.

Polyacrylamide gel electrophoresis and western analysis

Proteins were separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), after which they were stained with Coomassie Blue R-250 or transferred to PVDF membranes (Millipore) for immunodetection using a polyclonal antibody against recombinant RSuS1 (Tsai, 2003).

Results

Isolation and characterization of sucrose synthase cDNA from bamboo shoots

RT-PCR was carried out with a set of *Sus* primers (Table 1) to amplify a partial sequence of *Sus* cDNA from bamboo shoots, and a major product of the expected size (1 kb) was obtained. Nucleotide sequence analysis showed that the amplified product contained at least two different cDNA sequences (data not shown), and their deduced amino acid sequences showed 81.3–96.8% identity with SuS from wheat (*Triticum aestivium*), maize and rice. To clone the full-length cDNA encoding SuS, we used the 1-kb RT-PCR product as a hybridization probe to screen an unamplified bamboo shoot cDNA library, which yielded 650 positive clones from 165 000 screened recombinant plaques. The large number of positive clones is indicative of the high abundance of SuS mRNA in the bamboo shoot. Fifty positive clones were then randomly selected for *in vivo* excision, but only clones with inserts larger than 2.5 kb were analysed further. From 17 analysed clones, four different classes were identified based on their restriction maps and the sequences of their 3′-UTR, after which the longest representative from each class was sequenced and characterized. These four cDNAs were 2891, 2690, 2910 and 2774 bp in length, and were designated *BoSus1*, *BoSus2*, *BoSus3* and *BoSus4*, respectively. The characteristics of these four cDNAs and their encoded proteins (BoSuS1, BoSuS2, BoSuS3 and BoSuS4) are summarized in Table 2. *BoSus1* and *BoSus3* showed 95% identity at the nucleotide level and 97.8% identity at the amino acid level. However, their deduced amino acid sequences showed only 80% identity with those of *BoSus2* and *BoSus4*. Similarly, *BoSus2* and *BoSus4* exhibited a high degree of

Table 2 Summary of the *Bambusa oldhamii* sucrose synthase (*BoSus*) cDNA sequences

Gene name	Accession number	cDNA			Polypeptide		
		Length (bp)	5′-UTR (bp)	3′-UTR (bp)	Amino acid	Molecular mass (kDa)	pI
BoSus1	AF412036	2891	140	300	816	92.86	6.03
BoSus2	AF412038	2690	39	224	808	92.25	6.04
BoSus3	AF412037	2910	150	309	816	92.97	6.10
BoSus4	AF412039	2774	108	239	808	92.12	5.99

UTR, untranslated region.

identity with each other, both at the nucleotide (95%) and at the amino acid (97.5%) levels. Alignment of the amino acid sequences of the four BoSuS isoforms and SuS isoforms from rice revealed that the sequences of BoSuS1, BoSuS3 and RSuS1 are highly conserved, as are those of BoSuS2, BoSuS4 and RSuS2 (Fig. 1). Phylogenetic analysis of SuS sequences from monocot plants (Fig. 2) also showed that both *BoSus1* and *BoSus3* are closely related to the *Sus1*-type sequences of rice and maize, while *BoSus2* and *BoSus4* have *Sus2* (*Sh1*)-type sequences.

Southern analysis of bamboo DNA

For Southern analysis of the genomic DNA, part of the 3′-UTR of each *BoSus* cDNA was synthesized by PCR for use as a probe. Initially, we determined the specificity of each probe by evaluating its capacity for cross-hybridization with the four *BoSus* cDNAs. It was found that there was a low level of cross-hybridization between the *BoSus2* probe and *BoSus4* cDNA, between the *BoSus3* probe and *BoSus1* cDNA, and between the *BoSus1* probe and *BoSus3* cDNA (data not shown). Consistent with these findings, when blots of restriction-enzyme-digested genomic DNA were hybridized with these probes, a few faint bands were obtained in addition to the major bands (Fig. 3). Nevertheless, the differences in the patterns of the major bands suggested that these four *Sus* cDNAs were derived from different genes. Moreover, given that there are no restriction sites for the selected enzymes in the 3′-UTR of these cDNAs, our results indicate that the bamboo genome contains two copies of each *BoSus* gene.

Expression and functional identification of *BoSus*-encoded proteins in *E. coli*

To test the functionality of the cloned sequences, the coding regions of the four cDNAs were cloned into a His-tag fusion vector, pTrcHisA, after which the chimeric plasmids were used to transform *E. coli* cells. After the transformed cells had been induced with IPTG for 6 h, maximal SuS activity was detected in the soluble fraction of *E. coli* lysate (data not shown). The four recombinant His-tagged BoSuS proteins (rBoSuS1, rBoSuS2, rBoSuS3 and rBoSuS4) were purified to

Table 3 Kinetic parameters of the recombinant *Bambusa oldhamii* sucrose synthase (BoSuS)

Enzyme	$K_m^{sucrose}$ (mM)	K_m^{UDP} (mM)
BoSuS1	21.1	0.131
BoSuS2	33.1	0.018
BoSuS3	26.0	0.036
BoSuS4	37.0	0.127

SuS activity was assayed in the presence of different concentrations of one substrate and a saturated concentration of a second substrate (1.5 mM UDP or 300 mM sucrose). The michaelis constant (K_m) values were estimated from the double reciprocal plot.

apparent homogeneity using cobalt-based IMAC and subsequent ultrafiltration (Fig. 4). The specific activities of purified rBoSuS1, rBoSuS2, rBoSuS3 and rBoSuS4 were 0.115, 2.397, 0.804 and 0.306 U mg^{-1}, respectively. Kinetic analysis of sucrose cleavage showed that rBoSuS1 and rBoSuS3 had similar affinity for sucrose, whereas rBoSuS3 had substantially greater affinity for UDP than did BoSuS1 (Table 3). Similarly, rBoSuS2 and rBoSuS4 had similar K_m values for sucrose and different values for UDP.

Expression of *BoSus* genes in bamboo

The abundance of transcripts from each of the four *BoSus* genes was determined in different organs. Selected samples included sink leaves (unexpanded leaves), source leaves (mature leaves), leaf sheaths, flowers and shoots at the three growth stages. Because bamboo shoots are comprised of heterogeneous tissues, each shoot was divided into three parts: the base, the middle and the top regions (Fig. 5a). The base is an area 5–10 cm above the point where the shoot joins the rhizome, the middle consists of the developing nodes and internodes, and the top consists of a series of overlapping sheaths surrounding the developing nodes and internodes. Because some cross-hybridization was observed between *BoSus* cDNAs and the probes selected from the 3′-UTRs (see above), we used semiquantitative RT-PCR instead of northern analysis to

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BoSuS1  MGEAAGDRVLSRLHSVRERIGDSLSAHPNELVAVFTRLVNLGKGMLQPHQIIAEYNNAIPEAEREKLKDGAFEDVLRAAQEAIVI  (85)
BoSuS3  MGETAGDRVLSRLHSVRERIGDSLSAHPNELVAVFTRLVNLGKGMLQPHQIIAEYNNAIPEAERDKLKDGAFEDVLRAAQEAIVI  (85)
RSuS1   MGEAAGDRVLSRLHSVRERIGDSLSAHPNELVAVFTRLVNLGKGMLQAHQIIAEYNNAISEADREKLKDGAFEDVLRSAQEGIVI  (85)
RSuS3   MGETTGERALTRLHSMRERIGDSLSAHTNELVAAFSRLVNQGKGMLQPHQIIAEYNNAAIPEGEREKLKDSALEDVLRGAQEAIVI (85)
RSuS2   -----MAAKLARLHSLRERLGATFSSHPNELIALFSRYVNHQLLAEFDALI---EADKEKYAPFEDILRAAQEAIVL          (77)
BoSuS2  -----MAAKLTRLHSLRERLSATFSSHPNELIALFSRYVHQCGKGMLQRHQLLAEFDALI---AADKEKYAPFEDILRAAQEAIVL (77)
BoSuS4  -----MAAKLTRLHSLRERLGATFSSHPNELIALFSRYVNQGKGMLQRHQLLAEFDALM---DADKEKYAPFEDILRAAQEAIVL  (77)

BoSuS1  PPWVALAIRPRPGVWEYVRVNVSELAVEELRVPEYLQFKEQLVEGSTNNNFVLELDFEPFNASFPRPSLSKSIGNGVQFLNRHLS  (170)
BoSuS3  PPWVALAIRPRPGVWEYVRVNVSELAVEELRVPEYLQFKEQLVEGSTNNNFVLELDFEPFNASFPRPSLSKSIGNGVQFLNRHLS  (170)
RSuS1   SPWVALAIRPRPGVWEYVRVNVSELAVEELRVPEYLQFKEQTLWKKANNNFVLELDFEPFNASFPRPSLSKSIGNGVQFLNRHLS  (170)
RSuS3   PPWIALAIRPRPGVWEYLRINVSQLGVEELSVPEYLQFKEQLVDGSTQNNFVLELDFEPFNASFPRPSLSKSIGNGVQFLNRHLS  (170)
RSuS2   PPWVALAIRPRPGVWDYIRVNVSELAVEELSVSEYLAFKEQLVDGHTNSNFVLELDFEPFNASFPRPSMSKSIGNGVQFLNRHLS  (162)
BoSuS2  PPWVALAIRPRPGVWDYIRVNVSELAVEELSVSEYLEFKEQLVDGHTNSNFVLELDFEPFNASFPRPSMSKSIGNGVQFLNRHLS  (162)
BoSuS4  PPWVALAIRPRPGVWDYIRVNVSELAVEELSVSEYLAFKEQLVDGHTNSNFVLELDFEPFNASFPRPSMSKSIGNGVQFLNRHLS  (162)

BoSuS1  SKLFHDKESMYPLLNFLRAHNYKGMTMMLNDRIRSISALQGALRKAEEHLSGLSADTSYSDFHHRFQELGLEKGWGDCAKRAQET  (255)
BoSuS3  SKLFHDKESMYPLLNFLRAHNYKGMTMMLNDRIRSISALQGALRKAEEHLSGLSADTPYSDFHHRFQELGLEKGWGDCAKRAQET  (255)
RSuS1   SKLFHDKESMYPLLNFLRAHNYKGMTMMLNDRIRSISALQGALRKAEEHLSGLSADTPYSEFHHRFQELGLEKGWGDCAKRSQET  (255)
RSuS3   SKLFHDKESMYPLLNFLRAHNYKGMTMMLNDRIRSISALQGALRKAEKHLAGITADTPYSEFHHRFQELGLEKGWGDCAQRVRET  (255)
RSuS2   SKLFQDKESLYPLLNFLKAHNHKGTMMMLNDRIQSIRGLQSSLRKAEEYLMGIPQDTPYSEFNHRFQELGLEKGWGDCAKRVLDT  (247)
BoSuS2  SKLFQDKESLYPLLNFLKAHNHKGTMMMLNDRIQSIRGLQSALRKAEEYLMSFPQDTPYSEBNHRFQELGLEKGWGDTAKRVLDT  (247)
BoSuS4  SKLFQDKESLYPLLNFLKAHNPKGKTMMLNDRIQSIRGLQSALRKAEEYLISIPQDTPCSEFNHRFQELGLEKGWGDTAKRVLDT  (247)

BoSuS1  IHLLLDLLEAPDPSTLEKFLGTIPMVFNVVILSPHGYFAQANVLGYPDTGGQVVYILDQVRAMENEMLLRIKQQGLNITPRILIV  (340)
BoSuS3  IHLLLDLLEAPDPSTLEKFLGTIPMVFNVVILSPHGYFAQANVLGYPDTGGQVVYILDQVRAMENEMLLRIKQQGLNITPRILIV  (340)
RSuS1   IHLLLDLLEAPDPSTLEKFLGTIPMVFNVVISPHGYFAQRNVLGYPDTGGQVVYILDQVRAMENEMLLRIKQQGLNITPRILIV   (340)
RSuS3   IHLLLDLLEAPEPSALEKFLGTIPMVFNVVILSPHGYFAQANVLGYPDTGGQVVYILDQVRAMENEMLLRIKQQGLNITPRILIV  (340)
RSuS2   IHLLLDLLEAPDPANLEKFLGTIPMMFNVVILSPHGYFAQSNVLGYPDTGGQVVYILDQVRALENEMLLRIKQQGLDITPKILIV  (332)
BoSuS2  IHLLLDLLEAPDPANLEKFLGTIPMTFNVVILSPHGYFAQSNVLGYPDTGGQVVYILDQVRALENEMLLRIKQQGLDITPKILIV  (332)
BoSuS4  IHLLLDLLEAPDPANLEKFLGTIPMTFNVVILSPHGYFAQSNVLGYPDTGGQVVYILDQVRALENEMLLRIKQQGLDITPKILIV  (332)

BoSuS1  TRLLPDATGTTCGQRLEKVLGTEHTHILRVPFRTENGIVRKWISRFEVWPYLETFTDDVAHEIAGELQANPDLIIGNYSDGNLVA  (425)
BoSuS3  TRLLPDATGTTCGQRLEKVLGTEHTHILRVPFRTENGIVRKWISRFEVWPYLETFTDDVAHEIAGELQANPDLIIGNYSDGNLVA  (425)
RSuS1   TRLLPDATGTTCGQRLEKVLGTEHTHILRVPFRTENGIVRKWISRFEVWPYLETFTDDVAHEIAGELQANPDLIIGNYSDGNLVA  (425)
RSuS3   TRLLPDAHGTTCGQRLEKVLGTEHTHILRVPFRTENGTVRKWISRFEVWPYLETYTDDVAHEISGELQATPDLIIGNYSDGNLVR  (425)
RSuS2   TRLLPDAVGTTCGQRVEKVIGTEHTDILRVPFRSENGILRKWISRFDVWPFLETYTEDVANEIMREMQAKPDLIIGNYSDGNLVA  (417)
BoSuS2  TRLLPDAVGTTCGQRLEKVLGTEHTDILRVPFRTENGILRKWISRFDVWPFLETYTEDVANEIMREMQAKPDLIIGNYSDGNLVA  (417)
BoSuS4  TRLLPDAVGTTCGQRLEKVLGTEHTDILRVPFRTENGILRKWISRFDVWPFLETYTEDVANEIMREMQAKPDLIIGNYSDGNLVA  (417)

BoSuS1  CLLAHKMGVTHCTIAHALEKTKYPNSDLYWKKFEDHYHFSCQFTTDLIAMNHADFIITSTFQEIAGNKDTVGQYESHMAFTMPGL  (510)
BoSuS3  CLLAHKMGVTHCTIAHALEKTKYPNSDLYWKKFEDHYHFSCQFTTDLIAMNHADFIITSTFQEIAGNKDTVGQYESHMAFTMPGL  (510)
RSuS1   CLLAHKMGVTHCTIAHALEKTKYPNSDLYWKKFEDHYHFSCQFTTDLIAMNHADFIITSTFQEIAGNKDTVGQYESHMAFTMPGL  (510)
RSuS3   CLLAHKLGVTHCTIAHALEKTKYPNSDLYWKKFEDHYHFSCQFTADLIAMNHADFIITSTFQEIAGNKETVGQYESHMAFTMPGL  (510)
RSuS2   TLLAHKLGVTQCTIAHALEKTKYPNSDIYLDKFDSQYHFSCQFTADLIAMNHTDFIITSTFQEIAGSKDTVGQYESHIAFTLPGL  (502)
BoSuS2  TLLAHKLGVTQCTIAHALEKTKYPNSDIYLDKFDSQYHFSCQFTADLIAMNHTDFIITSTFQEIAGSKDTVGQYESHIAFTLPGL  (502)
BoSuS4  TLLAHKLGVTQCTIAHALEKTKYPNSDIYLDKFDSQYHFSCQFTADLIAMNHTDFIITSTFQEIAGSKDTVGQYESHIAFTLSGL  (502)

BoSuS1  YRVVHGIDVFDPKFNIVSPGADLSIYFPYTESHKRLTSLHPEIEELLYSDVDNHEHKFVLKDRNKPIIFSMARLDRVKTLTGLVE  (595)
BoSuS3  YRVVHGIDVFDPKFNIVSPGADMSIYFPYSESHKRLTSLHPEIEELLYSDVDNNEHKFVLKDRNKPIIFSMARLDRVKNLIGLVE  (595)
RSuS1   YRVVHGIDVFDPKFNIVSPGADMSIYFPYSESRKRLTSLHPEIEELLYSEVDNNEHKFRLKDRNKPIIFSMARLDRVKNLTGLVE  (595)
RSuS3   YRVVHGIDVFDPKFNIVSPGADMSIYFPFTESQKRLTSLFLEIEBELLFSDVENTEHKFVLKDKKKPIIFSMARLDHVKNLTGLVE (595)
RSuS2   YRVVHGIDVFDPKFNIVSPGADMSVYFPYTEADKRLTAFHPEIEELLYSEVENDEHKFVLKDKNKPIIFSMARLDRVKNMTGLVE  (587)
BoSuS2  YRVVHGIDVFDPKFNIVSPGADMSVYFPYTETDKRLTAFHPEIEELIYSDVENSEHKFVLKDKNKPIIFSMARLDRVKNMTGLVE  (587)
BoSuS4  YRVVHGIDVFDPKFNIVSPGADMSVYFPYTETDKRLTAFHPEIEELIYSDVENSEHQFVLKDKNKPIIFSMARLDRVKNMTGLVE  (587)

BoSuS1  LYGRNPRLQELVNLVVVCGDHGNPSKDKEEQAEFQKMFDLIEQYNLNGHIRWISAQMNRVRNGELYRYICDTKGAFVQPAFYEAF  (680)
BoSuS3  LYGRNPRLQELVNLVVVCGDHGNPSKDKEEQAEFKKMFDHIEQYNLNGHIRWISAQMNRVRNGELYRYICDTRGAFVQPAFYEAF  (680)
RSuS1   LYGRNPRLQELVNLVVVCGDHGKESKDKEEQAEFKKMFDLIEQYNLNGHIRWISAQMNRVRNGELYRYICDTKGAFVQPAFYEAF  (680)
RSuS3   LYGRNPRLQELVNLVVVCGDHGKESKDKEEQAEFKKMFNLIEQYNLNGHIRWISAQMNRVRNGELYRYICDMRGAFVQPALYEAF  (680)
RSuS2   MYGKNAHLRDLANLVIVCGDHGNQSKDREEQAEFKKMYGLIDQYKLKGHIRWISAQMNRVRNGELYRYICDTKGVFVQPAFYEAF  (672)
BoSuS2  MYGKNAHLRDLANLVIVAGDHGKESKDREEQAEFKRMYSLIDEYKLKGHIRWISAQMNRVRNGELYRYICDTKGVFVQPAFYEAF  (672)
BoSuS4  MYGKNAHLRDLANLVVAGDHGKESKDREEQAEFKRMYSLIEEYKLKGHIRWISAQMNRVRNGELYRYICDTRGVFVQPAFYEAF  (672)

BoSuS1  GLTVVESMYCGLPTFATAYGGPAEIIVHGVSGFHIDPYQGDKASALLVEFFEKCQQDHSHWTKISQGGLQRIEEKYTWKLYSERL  (765)
BoSuS3  GLTVVESMSCGLPTFATAYGGPAEIIVHGVSGFHIDPYQGDKASALLVEFFEKCQQDPAHWTKISQGGLQRIEEKYTWKLYSERL  (765)
RSuS1   GLTVVESMTCGLPTFATAYGGPAEIIVNGVSGFHIDPYQGDKASALLVEFFEKCQEDPSHWTKISQGGLQRIEEKYTWKLYSERL  (765)
RSuS3   GLTVIEAMTCGLPTFATAYGGPAEIIHGVSGYHIDPYQNDKASALLVNFFEKCKQDPNHWIKISQGGLQRIEEKYTWKLYSERL  (765)
RSuS2   GLTVIEAMTCGLPTIATCHGGPAEIIVDGVSGLHIDPYHSDKAADILVNFFEKCKQDSTYWDNISQGGLQRIYEKYTWKLYSERL  (757)
BoSuS2  GLTVIESMTCGLPTIATCHGGPAEIIVDGVSGLHIDPYHSNKAADILVNFFEKCKEDPTYWDKISQGGLKRIYEKYTWKLYSERL  (757)
BoSuS4  GLTVIESMTCGLPTIATCHGGPAEIIVDGVSGLHIDPYHSDKAADILVNFFEKCKEDPTYWDKISLGGLKRIYEKYTWKLYSERL  (757)

BoSuS1  MTLTGVYGFWKYVSNLERRETRRYLEMLYALKYRTMASTVPLAVDGEPSSK  (816)
BoSuS3  MTLTGVYGFWKYVSNLERRETRRYLEMLYALKYRKMASTVPLAVEGEPSNK  (816)
RSuS1   MTLTGVYGFWKYVSNLERRETRRYLEMLYALKYRTMASTVPLAVEGEPSNK  (816)
RSuS3   MTLSGVYGFWKYVTNLDRRETRRYLEMLYALKYRKMATTVPLAIEGEASTK  (816)
RSuS2   MTLTGVYGFWKYVSNLERRETRRYIEMFYALKYRSLASAVPLAVDGESTSK  (808)
BoSuS2  MTLTGVYGFWKYVSNLERRETRRYLEMFYALKYRSLASAVPLAVDGDSAAN  (808)
BoSuS4  MTLTGVYGFWKYVSNLERRETRRYLEMFYALKYRSLASAVPLAVDGDSVAK  (808)
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Fig. 1 Alignment of the deduced amino acid sequences derived from bamboo (*Bambusa oldhamii*) sucrose synthase (*BoSus*) and rice (*Oryza sativa*) *RSus* cDNAs. Identical residues are shown on a black background. The dots above the sequences indicate the variant residues among the amino acid sequences of *BoSus1*, *BoSus3* and *RSus1*. The asterisks below the sequences indicate the variant residues among *BoSus2*, *BoSus4* and *RSus2*. The accession numbers for *RSus1*, *RSus2* and *RSus3* are X64770, X59046 and L003366, respectively.
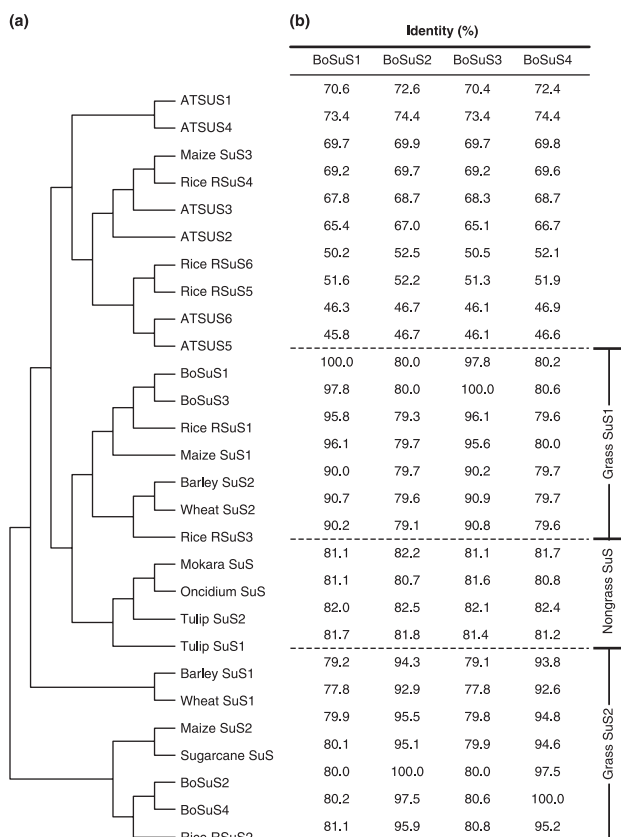
(a)

(b)

	Identity (%)			
	BoSuS1	BoSuS2	BoSuS3	BoSuS4
ATSUS1	70.6	72.6	70.4	72.4
ATSUS4	73.4	74.4	73.4	74.4
Maize SuS3	69.7	69.9	69.7	69.8
Rice RSuS4	69.2	69.7	69.2	69.6
ATSUS3	67.8	68.7	68.3	68.7
ATSUS2	65.4	67.0	65.1	66.7
Rice RSuS6	50.2	52.5	50.5	52.1
Rice RSuS5	51.6	52.2	51.3	51.9
ATSUS6	46.3	46.7	46.1	46.9
ATSUS5	45.8	46.7	46.1	46.6
BoSuS1	100.0	80.0	97.8	80.2
BoSuS3	97.8	80.0	100.0	80.6
Rice RSuS1	95.8	79.3	96.1	79.6
Maize SuS1	96.1	79.7	95.6	80.0
Barley SuS2	90.0	79.7	90.2	79.7
Wheat SuS2	90.7	79.6	90.9	79.7
Rice RSuS3	90.2	79.1	90.8	79.6
Mokara SuS	81.1	82.2	81.1	81.7
Oncidium SuS	81.1	80.7	81.6	80.8
Tulip SuS2	82.0	82.5	82.1	82.4
Tulip SuS1	81.7	81.8	81.4	81.2
Barley SuS1	79.2	94.3	79.1	93.8
Wheat SuS1	77.8	92.9	77.8	92.6
Maize SuS2	79.9	95.5	79.8	94.8
Sugarcane SuS	80.1	95.1	79.9	94.6
BoSuS2	80.0	100.0	80.0	97.5
BoSuS4	80.2	97.5	80.6	100.0
Rice RSuS2	81.1	95.9	80.8	95.2

Groupings: Grass SuS1, Nongrass SuS, Grass SuS2.

Fig. 2 Comparison of the amino acid sequences of sucrose synthase (SuS) from monocot plants and *Arabidopsis thaliana*. (a) Phylogram showing the evolutionary relationships of the sequences. (b) Percentage identity between the four *Bambusa oldhamii* sucrose synthase (BoSuS) isoforms and other sequences. The sequences compared were from barley (*Hordeum vulgare*) SuS1 (X65871) and SuS2 (X69931); maize (*Zea mays*) SuS1 (L22296), SuS2(Sh1) (X02400) and SuS3 (AY124703); mokara (*Mokara Yellow*) SuS (AF530568); oncidium SuS (AF530567); sugarcane (*Saccharum officinarum*) SuS (AF263384); tulip (*Tulipa gesneriana*) SuS1(X96938) and SuS2 (X96939); wheat (*Triticum aestivum*) SuS1 (AJ001117) and SuS2 (AJ000153); and *A. thaliana* AtSUS1 (CAA50317), AtSUS2 (CAA43303), AtSUS3 (CAB80721), AtSUS4 (CAB89040), AtSUS5 (BAB11375) and AtSUS6 (AAG30975). The accession numbers for rice (*Oryza sativa*) SuS are X64770 (RSuS1), X59046 (RSuS2), L003366 (RSuS3), AK102158, CAE03896 and BAD23005 (designated RSuS4, RSuS5 and RSuS6, respectively, for convenience).

Fig. 3 Southern analysis of genomic DNA from *Bambusa oldhamii*. DNA was digested with *Eco*RI (E), *Hin*dIII (H) and *Xba*I (X) and resolved on a 0.8% agarose gel, after which it was transferred to a nylon membrane. Identical blots were hybridized with gene-specific probes for *Bambusa oldhamii* sucrose synthase genes *BoSus1*, *BoSus2*, *BoSus3* and *BoSus4*.
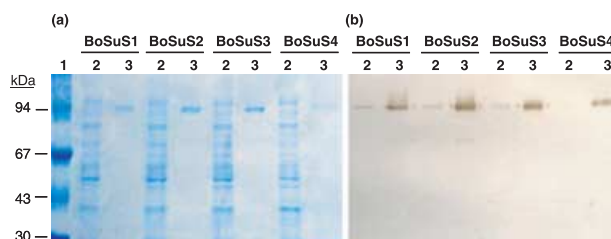
Fig. 4 Purification of recombinant *Bambusa oldhamii* sucrose synthase (BoSuS) proteins. Protein samples collected at various stages of the purification were separated on 10% sodium dodecyl sulphate (SDS) polyacrylamide gels. The resolved proteins were then stained with Coomassie blue (a) or transferred to a PVDF membrane (Millipore) and immunodetected with anti-RSuS antibody (b). Lane 1, molecular mass markers; lane 2, crude extract of Isopropylthio-β-D-galactoside (IPTC) induced *Escherichia coli* TOP10 harbouring pHisBoSus1, pHisBoSus2, pHisBoSus3 or pHisBoSus4; lane 3, proteins purified by cobalt-based immobilized metal affinity chromatography (IMAC).

examine the expression of the four *BoSus* genes in the various bamboo tissues. Sequencing of the amplified DNA fragments showed that each pair of primers (Table 1) specifically annealed to their corresponding gene (data not shown).

As shown in Figs 5b and c, mRNAs from all four genes were detected in the various tissues, but at different relative abundances. The highest abundances of *BoSus1* and *BoSus3* mRNA were found in the middle region of the 100-cm green shoot, while the highest abundances of *BoSus2* and *BoSus4* mRNA were in the leaf sheath. For purposes of comparison among tissues, the expression level of each *BoSus* gene in the
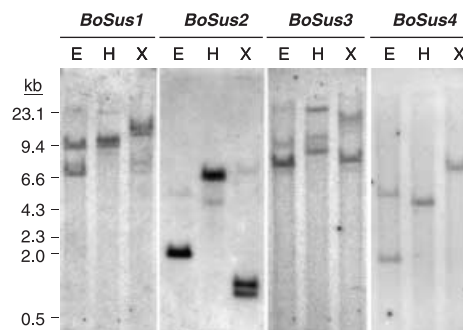
leaf sheath was taken as 100%. In the etiolated shoots, the abundance of *BoSus1* mRNA was low in the base and middle regions but high in the top region. As the shoots grew, the abundance of *BoSus1* mRNA increased in each region, except in the top region of the 30-cm shoot. The pattern of growth-dependent changes in the abundance of *BoSus3* mRNA in each shoot region was similar to that of *BoSus1*. However, abundances of *BoSus3* mRNA in shoot samples were higher than in leaf sheath, whereas the opposite was generally found for the *BoSus1* transcript. The abundance of *BoSus2* mRNA in different regions of the same shoot did not show the distinct differences seen with *BoSus1* and *BoSus3*, and the abundance of *BoSus4* mRNA in shoots was low. The highest abundances of *BoSus2* and *BoSus4* mRNA were seen in the 100-cm green shoots. In leaves, the abundances of *BoSus1* and *BoSus3* mRNA were higher in sink leaves than in source leaves. In sink leaves, the abundance of *BoSus1* mRNA was similar to that in the leaf sheath, while the abundance of *BoSus3* mRNA was 2.2-fold
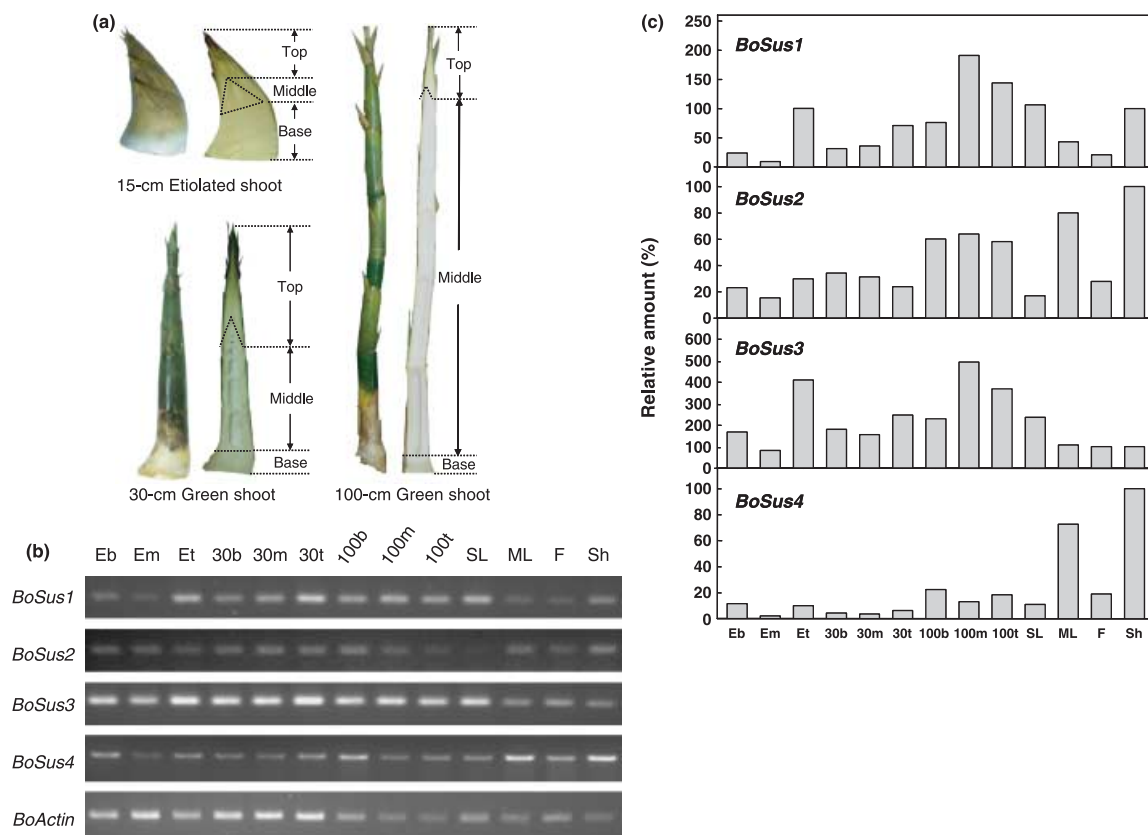
Fig. 5 Expression of *Bambusa oldhamii* sucrose synthase (*BoSus*) genes. Total RNA was isolated from 15-cm etiolated shoots (E), 30-cm green shoots (30), 100-cm green shoots (100), sink leaves (unexpanded leaves, SL), source leaves (mature leaves, ML), flowers (F) and leaf sheaths (Sh). Each shoot was divided into three parts: the base (b), middle (m) and top (t). The relative abundance of mRNA was analysed by reverse transcriptase–polymerase chain reaction (RT-PCR) using specific primers for each gene. Amplification of actin transcript (*BoActin*) was carried out in parallel as an internal control. (a) Bamboo shoot samples. (b) RT-PCR products analysed by agarose gel electrophoresis. (c) Relative expression profile. The densities of the cDNA bands in (b) were quantified using an image analyser and normalized to the actin cDNA level. The transcript abundance of each *BoSus* gene in the leaf sheath was taken as 100%. The figures show a representative example from five independent experiments giving similar results.

higher than in the leaf sheath. In contrast to *BoSus1* and *BoSus3*, the abundances of *BoSus2* and *BoSus4* mRNA were high in mature leaves but low in sink leaves. The transcript abundances of the *BoSus1*, *BoSus2* and *BoSus4* were all lower in flowers than in the leaf sheath, while the abundances of *BoSus3* mRNA were similar in the two organs.

Discussion

We have isolated four *BoSus* cDNAs from bamboo shoots. Their identity as *Sus* genes was confirmed by their close homology to known SuS sequences from other monocot plants, and also by the heterologous expression and characterization of their encoded proteins in *E. coli*. Based on phylogenetic analysis, *Sus* genes in plants have been classified into four distinct groups: the dicot SUS1 group, the monocot SUS group, the dicot SUSA group and a new group that includes only *A. thaliana AtSUS5* and *AtSUS6* (Fu & Park, 1995; Sturm *et al.*, 1999; Komatsu *et al.*, 2002; Baud *et al.*, 2004). Except

for maize *Sus3* and three newly cloned rice *Sus* genes (accession numbers AK102158, CAE03896, and BAD23005, designated *RSus4*, *RSus5* and *RSus6*, respectively, for convenience; Feng *et al.*, 2002; Kikuchi *et al.*, 2003), the other known monocot *Sus* sequences, including the four *BoSus* cDNAs cloned from bamboo shoots, belong to the monocot SUS group (Fig. 2). Maize *Sus3* and *RSus4* are more similar to members of the dicot SUSA group than to the monocot *Sus* group, and *RSus5* and *RSus6* are closely related to *A. thaliana AtSUS5* and *AtSUS6*. *Sus* genes in the monocot *Sus* group can be further divided into three types: grass *Sus1*, grass *Sus2* (*Sh1*) and nongrass *Sus* (Fig. 2). Both *BoSus1* and *BoSus3* are of the grass *Sus1* type, while both *BoSus2* and *BoSus4* are of the grass *Sus2* (*Sh1*) type. Although the two variants of each *Sus* type showed very high identity (> 97% at the amino acid level) to one another, their different hybridization patterns in Southern analysis (Fig. 3) and their different expression patterns (Fig. 5) indicate them to be derived from nonallelic genes, perhaps resulting from gene duplication during evolution. Given that

bamboos are polyploidal (Soderstorm & Ellis, 1986), it is also plausible that the two *BoSus* variants of each *Sus* type were homeologous genes originating from two different diploid species. High sequence identities between the same *Sus*-type genes were also found between bamboo, rice and maize (Fig. 2). The deduced amino acid sequences of the two *Sus1*-type *BoSus* cDNAs exhibited about 96% identity with those of maize *Sus1* and rice *RSus1*, as did *Sus2*-type *BoSus* cDNAs with maize *Sus2* (*Sh1*) and rice *RSus2*, indicating that *Sus* genes are highly conserved in these three species. We predict that other *BoSus* genes that are orthologues of *RSus3*, *RSus4*/maize *Sus3*, *RSus5* and *RSus6* also exist in green bamboo.

By expression of the four *BoSus* cDNAs in *E. coli*, the recombinant BoSuS proteins could be purified to near homogeneity. Proteins expressed from the same *Sus*-type genes had similar affinity for sucrose but different affinity for UDP, which suggests that the two isoforms do not share all the same functions. Notably, proteins from the same *Sus*-type genes differed from one another by only 17 or 18 amino acid residues scattered along their entire length. Site-directed mutagenesis will be carried out to determine which amino acid residues are involved in nucleotide binding.

In an earlier study, Su *et al.* (1977) purified and characterized a SuS from bamboo shoots and found the substrate saturation curve for sucrose to be sigmoidal, which suggests that the enzyme was allosteric. In the present study, by contrast, no sucrose concentration-dependent modulation was observed for any of the recombinant enzymes; their substrate saturation curves were all hyperbolic in shape. This could be because the enzyme purified from bamboo shoots contained multiple SuS isoforms. It could also reflect a change in the folded structure of the recombinant enzyme caused by addition of the N-terminal His-tag, or a difference in posttranslational modification. It is known that SuS can be phosphorylated on a Ser residue at the amino terminus by protein kinases. Such phosphorylation has been suggested to play a role in regulating the activity of the enzyme (Zhang & Chollet, 1997; Nakai *et al.*, 1998; Anguenot *et al.*, 1999; Zhang *et al.*, 1999; Haigler *et al.*, 2001; Tanase *et al.*, 2002), in determining the distribution of the enzyme among the cytosol, plasma membrane and actin cytoskeleton (Amor *et al.*, 1995; Winter *et al.*, 1997; Winter & Huber, 2000) and in determining the response of cells to environmental and developmental signals (Subbaiah & Sachs, 2001). In addition to the major phosphorylation site (Ser15), a second phosphorylation site (Ser170) was found both in maize SUS1 (Hardin *et al.*, 2003) and in rice RSuS1 (Tsai & Wang, 2003). Phosphorylation at Ser170 may play a role in regulating the turnover of the enzyme (Hardin *et al.*, 2003; Hardin & Huber, 2004). The four BoSuS proteins also contain the conserved serine residues (Ser15 and Ser170 in BoSuS1 and BoSuS3, and Ser10 and Ser162 in BoSuS2 and BoSuS4). Whether phosphorylation of BoSuS affects its kinetic properties remains to be determined, and this will be investigated in future work.

The four *BoSus* mRNAs were widely distributed at different abundances among the bamboo organs (Fig. 5). The absolute abundances of each transcript in the same tissue or organ could not be determined and compared using the results of our RT-PCR analysis. Nevertheless, based on our evaluation of the expression pattern and relative abundances of each transcript among tissues, we are able to conclude that *BoSus3* was predominantly expressed in sink organs, including growing shoots and developing leaves, while *BoSus4* was predominantly expressed in source organs. Preferential expression in sink or source organs was less obvious for *BoSus1* and *BoSus2*, but their expression patterns in leaves did appear complementary. These differences in the patterns of gene expression, together with the differences in the affinity of the expressed enzymes for UDP, suggest that each BoSuS isoform has a specific set of functions *in vivo*.

Bamboo shoots are very active sink organs that require abundant translocated carbon to support their rapid growth. It is noteworthy that we obtained 650 positive clones among 165 000 recombinant plaques when we screened an unamplified cDNA library constructed from etiolated shoots. Such a high frequency (0.39%) of positive clones means that *BoSus* genes are strongly expressed in the bamboo shoot. Furthermore, the overall expression level of all four *BoSus* genes was even higher in 100-cm green shoots than in etiolated shoots (Fig. 5b,c). The rate of culm elongation in the former is higher than that in the latter (Lin, 1958). Thus, the increase in mRNA abundance paralleled the growth of the bamboo, which underscores the importance of *BoSus* expression to bamboo growth. Indeed, we suggest that the abundant and multiple SuS isoforms play a variety of roles in bamboo shoots, directing translocated carbon towards both the polysaccharide biosynthesis and energy production necessary to sustain the remarkably rapid growth of bamboo.

Acknowledgements

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