Investigation into the Expression of Sucrose Transporters and Sucrose Phosphate Synthase mRNA in Different Plant Species

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Abstract Assimilate partitioning is an important and highly integrated process in higher plants which involves not only transport of sugars from source to sink organs but also the regulation of genes triggered by sugars. In this study, we investigated the transcripts level of sucrose transporters (ShSUT1A and ShSUT4) and sucrose phosphate synthase (SPS) mRNA expression in different plant species. RT-PCR analyses showed that ShSUT1A and ShSUT4 accumulated abundantly in sugarcane tissues. In other plant species, ShSUT1A expressed predominantly in maize (*Zea mays* L.) leaves with low expression in *Arabidopsis thaliana* and *Arabidopsis hallari* shoots and roots. ShSUT4 expressed strongly in sugarcane tissues, but, it was not detected in the other plant species. SPS, considered a key enzyme in regulation of sucrose biosynthesis in plants, was highly expressed in sugarcane as compared with maize, but it was not expressed or detected in *Arabidopsis*. Phylogenetic analysis of 51 plant sucrose transporters fall into two clearly separable groups: Group I and Group II. ShSUT1A shared 67 % identity with ZmSUT1 and was still able to show high SUT expression in maize. The similarity between the cereal SUT1 group and the dicot SUT2 group appears also to extend to gene structure. This high level of conservation of gene structure is intriguing, considering the diverse functions for these proteins. The phylogenetic analysis of SPS revealed that sugarcane and maize assembled into unique subgroup within group III with 95 % identity, while *Arabidopsis* was in a separate small subgroup, group I with 70 % identity.

Keywords Sucrose transporters · ShSUT1A · ShSUT4 · *Arabidopsis thaliana* · *Saccharum* spp. · *Zea mays* L. · Sucrose phosphate synthase

Abbreviations

SUT: Sucrose uptake transporter; SPS: Sucrose phosphate synthase; ShSUT: Sugarcane hybrid sucrose uptake transporter; HvSUT: *Hordeum vulgare* sucrose uptake transporter; ZmSUT: *Zea mays* sucrose uptake transporter; OsSUT: *Oryza sativa* sucrose uptake transporter; AtSUC: *Arabidopsis thaliana* sucrose uptake carrier;

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Introduction

In plants, sucrose transport activity is essential for the distribution of photoassimilates between source and sink tissues. Members of the proton-coupled sucrose uptake transporter (SUT) family within the major facilitator super family play essential roles in long-distance transport of sucrose within the vascular tissue of plants. In solanaceous

plants, high-affinity sucrose transporter SUT1 is required for phloem loading [31]. Transport of sucrose between source and sink is mediated by a specific part of the vasculature, the phloem. There are two principal pathways for the delivery of sucrose into the minor vein sieve element–companion cell complex, firstly, symplastic loading, in which sucrose passes the entire route from the leaf mesophyll cells to the sieve element–companion cell complex in the so-called “symplast,” moving from cell to cell via plasmodesmata; second, via apoplastic loading, where sucrose is released from the mesophyll cells and then is actively taken up by sucrose transporters located in the sieve element–companion cell complex [23]. Kühn and Grof [18] reported that sucrose transporters play a key role not only in phloem loading and unloading processes but also in the exchange of sucrose between beneficial symbionts (mycorrhiza and *Rhizobium*) as well as pathogens such as nematodes and parasitic fungi. They are not only key regulators of transport processes but also integral components of signal transduction between sink and source metabolism.

Plant sucrose transporters (SUTs) belong to the glycoside–pentoside–hexuronide (GPH) cation symporter family (TC2.A.2) that is part of the major facilitator superfamily (MFS) [7]. Transporters in the GPH family have the basic characteristics of MFS proteins: 12 transmembrane domains with N- and C-terminus in the cytoplasm. The first six transmembrane domains display degrees of sequence similarities with the last six, supporting the idea that these transporters arose from at least one ancient gene duplication event [28]. This idea is corroborated by Henderson [13] and Kaback [16] who described the hydrophobicity profile and structure of an integral membrane protein with 12 putative transmembrane domains and a central hydrophilic loop. The GPH family also contains members from bacteria, archaea, and eukaryotes. These include melibiose permease from *Escherichia coli* [21], the α -glucoside transporter SUT1p from *Schizosaccharomyces pombe* [26] and plant SUTs such as SUC2 from *Arabidopsis* [6, 31]. Transporters within the GPH family that have been characterized so far transport glycosides by symport with a cation (H^+ or Na^+). Plant sucrose transporters were mainly associated with phloem loading [18]. Previously, ShSUT1 was the only known sucrose transporter from sugarcane, isolated from an Australian cultivar [5]. Cloning of the sucrose transporter from a Hawaiian cultivar resulted in a slightly different transporter sequence, named ShSUT1A still belonging to the SUT1 group, but with an additional 7 amino acid stretch in the loop between transmembrane helices 5 and 6 [9]. It is expressed in both leaves and stems, but most abundantly in the stem tissue where sucrose is accumulated [5, 9]. In addition, the ShSUT1A protein was mostly localized to the layer of cells surrounding the bundle sheath, but was absent from the phloem itself.

Based on these findings, the ShSUT1 and ShSUT1A may play a role in retrieval of sucrose leaking from the storage parenchyma cells in the stem or alternatively in sucrose export into the storage parenchyma rather than in phloem loading [9, 23]. Sucrose phosphate synthase (SPS) is considered to be one of the key enzymes that regulate the sucrose synthesis pathway. Due to its importance, leaf SPS has been exhaustively studied [8, 39]. Many experiments provided strong evidence for the involvement of SPS in the regulation of carbohydrate partitioning and sugar metabolism [12, 39]. A key aim in sugarcane improvement programs is to increase the concentration of sucrose in the plants. Thus, the objective of the present study was to investigate the expression profiles of SUTs and SPS in different plant species by examining the transcripts level of SUTs and SPS mRNA in these plants.

Materials and Methods

Plants

Saccharum spp. hybrids H73-6110, H87-4319, H78-4153, H65-7052, H78-7750, and H87-4094 were obtained from the Hawaii Agriculture Research Center, Aiea, Hawaii, USA. In addition, cultivar C1051-73 was obtained from Cuba through Medina Borges, Habana, while the Egyptian cultivar GT54-9 was obtained from the Sugar Crops Research Institute, Agriculture Research Centre, Giza, Egypt. *Zea mays* L.; Gemiza 1004 (sweet corn) and Giza 2 (white corn) were obtained from Agriculture Research Centre, Giza, Egypt. *Arabidopsis halleri* and *Arabidopsis thaliana* were provided by Dr. Michael Weber (The University of Bayreuth, Germany). The leaves and internodes were numbered according to breeders' practice, identifying the uppermost leaf with fully developed dewlap and the attached internode beneath as #1.

Isolation of RNA from Plant Tissues

Total RNA was extracted and purified from plant tissues (leaf for sugarcane and maize; shoot and root for *Arabidopsis* and internode (# 7) for sugarcane) according to a modified method of Sambrook and Russell [29]. Approximately 200 mg of the frozen sample was ground to a fine powder in liquid nitrogen and transferred to a snap-cap tube. RNA extraction buffer (Triton X-100, 100 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, and 1 %SDS) and phenol–chloroform were used for the extraction. The supernatant was treated with 5 μ L of DNase buffer and 5 μ L of DNase I (1 U/ μ L) was added and incubated at room temperature for 15 min. The washing and centrifugations were done according to Sambrook and Russell [29]. The

pellet was air dried and resuspended in 50 μ L of DEPC-treated water. The concentration of RNA was determined by Nanophotometer (IMPLEN, Munich, Germany). The preparation was stored at -80°C .

RT-PCR for expression of ShSUT1A, ShSUT4, and SPS in different plant species

RT-PCR was used to determine the expression of ShSUT1A, ShSUT4, and SPS in different plant tissues. RT-PCR was performed with primers according to Table 1. The RNA from all samples was reverse transcribed using RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, USA), primed with 50 pmol of reverse primer (Oligo dT) by following the manufacturer's protocol and carried out in a PTC 100 Peltier Thermal Cycler (MJ Research, Ramsey, MN, USA). The PCR reaction was performed in a volume of 25 μ L containing 1 μ L cDNA, 2.5 μ L of $10\times$ PCR buffer (containing 15 mM MgCl_2 , 0.5 μ L of 10 mM dNTP mix), 10 pmol each of forward and reverse primers, 1 unit of polymerase with proofreading activity (*Pfu*):*Taq* polymerase (5:1) (Stratagene, Waldbronn, Germany), and sterile milliQ water added up to the final volume. This PCR program was performed with initial denaturation at 94°C for 4 min, ten cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min, and 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min with a final 72°C extension for 7 min.

The RT-PCR was performed with internal control, 25S ribosomal RNA (25S rRNA), as a reference gene to normalize gene expression level and to evaluate the integrity of cDNA. Furthermore, the primer sets were optimized using semi-quantitative PCR with different numbers of PCR-cycles. A 10- μ L aliquot of each amplified product was analyzed by electrophoresis on 1 % (w/v) agarose gels stained with ethidium bromide.

Northern Blot Analysis

Ten micrograms of RNA isolated from sugarcane leaves, seedling, and internodes were separated on a 1.2 %

formaldehyde–agarose gel in MOPS buffer run at 80 V for 2 h. Transfer blotting was set up to transfer mRNA to a positively charged nylon membrane (0.45- μm pore size, Hybond N+, Amersham GE Healthcare Amersham Place, UK) by capillary transfer using $20\times$ SSC for 16 h. Thereafter, the membrane was UV cross-linked for 1 min and heat treated at 80°C for 2 h. The membrane was briefly washed in $5\times$ SSC for 1 min at room temperature. The membrane was transferred to hybridization tube with 30 mL of pre-hybridization buffer and incubated for 1 h at 68°C under gentle agitation. The RNA probe of ShSUT1A was produced using PCR-generated templates for in vitro transcription. The DNA fragment was amplified by RT-PCR and cloned into pGEM®-T (Promega, Mannheim, Germany). The orientation of the insert fragment was determined by sequencing. The transcription of RNA antisense probe and the hybridization were performed as described in the DIG System Users Guide (Roche Diagnostics GmbH, Mannheim, Germany). The hybridization was detected and visualized with labelled anti-digoxigenin-AP and chemiluminescence (CDP-Star ready-to-use) and images captured using a chemilux CCD camera (Intas, Göttingen-Germany).

Alignment of Sequences and Construction of Phylogenetic Trees

The nucleotide sequences of sucrose transporter and SPS II provided were added to those retrieved from GenBank and aligned using CLUSTALW 2.1, CLUSTALX 2.0.9 [33] software with default settings. Their phylogenetic relationships were determined by Maximum Likelihood (ML) algorithm incorporated in MEGA version 5 program [36]. Based on the evaluation of best fit substitution model executed in MEGA5, ML tree were reconstructed under the assumption of the substitution model T92 coupled to a discrete Gamma distribution (+G) with five rate categories [33]. The substitution model parameters estimated for SPS II were (i) base frequencies: $f(\text{A}) = f(\text{T}) = 0.280$; $f(\text{C}) = f(\text{G}) = 0.220$, (ii) substitution rates: $r(\text{AT}) = 0.052$, $r(\text{AC}) = 0.041$, $r(\text{AG}) = 0.138$,

Table 1 Primers which were used for sucrose transporters and sucrose phosphate synthase genes amplification

Primer names	5'–3' sequence	Amplified fragment (bp)
ShSUT1A-Fwd	GCTATGCGGTCCTATTGCTG	1,319
ShSUT1A-Rev	AGATCTTGGGCAGCAGGAAC	
ShSUT1A-Fwd	TCTCCTCTCTCCCGAATTGA	800
ShSUT1A-Rev	TCCAACCCACAAGAGTTTCC	
SPS-Fwd	TAAGTGGCCATCATTGCGTA	181
SPS-Rev	AATACAAAACCAACAGCGCC	
ShSUT4-Fwd	CTTTTCACTCTTCATGGCCC	1,097
ShSUT4-Rev	AACCCACAAGAGTTTCCACG	
25 S rRNA-Fwd	GCAGCCAAGCGTTCATAGC	108
25 S rRNA-Rev	CCTATTGGTGGGTGAACAATCC	

$r(\text{CG}) = 0.041$, $r(\text{CT}) = 0.176$, $r(\text{GT}) = 0.041$, and (iii) transition/transversion ratios: $R = 1.67$. The Bayesian Information Criterion value ($\text{BIC} = 56257.282$) with T92+G model was the lowest among 24 models tested. Whereas, the substitution model for Sucrose transporter was T92 + G and assuming that a certain fraction of sites were evolutionary invariable ($I = 0.15$). The parameters estimated were: (i) base frequencies: $f(\text{A}) = f(\text{T}) = 0.246$; $f(\text{C}) = f(\text{G}) = 0.254$; (ii) substitution rates: $r(\text{AT}) = 0.053$, $r(\text{AC}) = 0.054$, $r(\text{AG}) = 0.145$, $r(\text{CG}) = 0.055$, $r(\text{CT}) = 0.145$, $r(\text{GT}) = 0.055$; and (iii) transition/transversion ratios: $R = 1.32$. The Bayesian Information Criterion value ($\text{BIC} = 46736.479$) with T92+G+I model was the lowest among 24 models tested. Bootstrap analyses with 500 replicates were performed to assess the robustness of the branches.

Results

Expression of Sucrose Transporters ShSUT1A, ShSUT4 in Different Plant Species

The expression of sucrose transporter ShSUT1A was detected by RT-PCR and Northern blot in source leaves of different sugarcane cultivars. The result of RT-PCR showed that ShSUT1A is expressed slightly more abundantly in the leaves of Hawaiian cultivars; H65-7052, H78-4153, and H87-4319 than H78-7750, H87-4094, and H73-6110 (Fig. 1a). The Cuban cultivar, C1051-73 also showed strong expression of ShSUT1A. The hybridization of RNA with specific probe to ShSUT1A showed that ShSUT1A was highly accumulated in all sugarcane cultivars (Fig. 1b).

RT-PCR analyses of ShSUT1A expression was also done in maize together with different sugarcane cultivars for comparison (Fig. 2). The ShSUT1A transcript appeared more abundant in sweet corn as compared with white corn. The expression in sweet corn in turn was similar to the sugarcane cultivars most likely due to maize and sugarcane belonging to the same family (*Poaceae*) (Fig. 2).

The ShSUT1A was expressed in both the shoot and root tissue of *Arabidopsis thaliana* and *Arabidopsis halleri*, respectively (Fig. 3). In both *Arabidopsis* species, there appeared to be stronger expression of ShSUT1A in the shoots as compared with the roots.

ShSUT4 was expressed in the leaves of all the sugarcane cultivars leaves (Fig. 4a), while for reasons unknown it was not expressed in maize and *Arabidopsis* (Fig. 4b). Both sucrose transporters, ShSUT1A (Fig. 5a) and ShSUT4 (Fig. 5b), accumulated in the internodes of all the Hawaiian sugarcane cultivars.

Fig. 1 Expression patterns of ShSUT1A in sugarcane hybrids. **a** RT-PCR for sucrose transporter ShSUT1A in sugarcane plants. *M*: DNA molecular size markers 50 bp and 1 kb (Fermentas, St. Leon Rot, Germany). 25S rRNA was amplified from the same samples to serve as loading controls. **b** Northern hybridization of RNA isolated from source leaves of different sugarcane cultivars, probed with a ShSUT1A cDNA. The lower panel shows the same membrane probed for ribosomal RNA as loading control

Fig. 2 Transcript abundance of ShSUT1A in sugarcane and maize (*Zea mays* L., white corn “Giza2”; sweet corn “Gemaza 1004”) leaves. RNA from maize and sugarcane leaves were extracted, transcribed into cDNA, and amplified with ShSUT1A-specific primers (amplifies a 800-bp product) by RT-PCR. *M*: DNA molecular size marker 1 kb (Fermentas, St. Leon Rot, Germany)

Relative Expression of SPS mRNA in Different Plant Species

Sucrose phosphate synthase (SPS) is considered a key enzyme that regulates the sucrose synthesis pathway. The expression of SPS was higher in sugarcane as compared with maize, while in contrast no PCR amplification was obtained with the *Arabidopsis* plants (Fig. 6).

Fig. 3 Comparison of ShSUT1A expression patterns in different plant species (*Arabidopsis thaliana*, *Arabidopsis halleri* (shoot and root), maize and sugarcane. RNA from *A. thaliana*, *A. halleri* (shoot and root); maize and sugarcane leaves were extracted, transcribed into cDNA, and amplified with ShSUT1A-specific primers (amplifies a 800 bp product) by RT-PCR. M: DNA molecular size marker 1 kb (Fermentas, St. Leon Rot, Germany)

Fig. 4 Expression patterns of ShSUT4 in different sugarcane (*Saccharum*) cultivars (a) and in different plant species (*Arabidopsis thaliana*, *Arabidopsis halleri*, maize (white corn and sweet corn) (b) using RT-PCR and ShSUT4-specific primers (amplifies a 1097 bp product). M: DNA molecular size marker 1 kb (Fermentas, St. Leon Rot, Germany)

Phylogenetic Inferences

Regarding ShSUT1A and ShSUT4 accessions, they were phylogenetically distant, belonging to two distinct clusters as shown in Fig. 7. It is worth noting that the nucleotide sequences of sucrose transporters (ShSUT1A and ShSUT4) were separated into two different groups. Sequence divergences of group I contained SUT1 accessions (average percentage around 68 %) (Table 2). The ShSUT1A subgroup comprised *Saccharum officinarum*, *Hordeum vulgare*, *Brachypodium distachyon*, and *Lolium perenne*, where the sequence identities among them ranged between 70 and

Fig. 5 Comparison of expression patterns of sucrose transporters (ShSUT1A and ShSUT4) in sugarcane internode # 7 for different sugarcane cultivars using RT-PCR and specific ShSUT1A (a) and ShSUT4 (b) primers, respectively. M: DNA molecular size marker 1 kb (Fermentas, St. Leon Rot, Germany)

100 %. The second subgroup that was relatively closely related to the ShSUT1A subgroup comprised *Zea mays*, *Sorghum bicolor*, *Lolium perenne*, and *Brachypodium distachyon*. SUT4 was exclusively assembled in group II (Fig. 7), including 24 sequences from monocots (*Oryza sativa*, *Zea mays*, *Hordeum vulgare*, *Sorghum bicolor*, and *Saccharum officinarum*) and many dicot species (*Glycine max*, *Phaseolus vulgaris*, and *Arabidopsis thaliana*). The similarities among sequences varied between 55 and 95 %. The *Poaceae* family falls into clearly separable subgroup that contains only sequences from monocot species (*Saccharum officinarum*, *Sorghum bicolor*, *Zea mays*, *Hordeum vulgare*, *Brachypodium distachyon*, and *Oryza sativa*), whereas the average of identity percentage for ShSUT4 is 90 %. By contrast, seven of *Arabidopsis* SUC sequences

Fig. 6 Comparison of expression patterns of sucrose phosphate synthase (SPS) in different plant species (*Arabidopsis thaliana*, *Arabidopsis halleri*, maize (white corn and sweet corn) and sugarcane using RT-PCR and specific primers for SPS (amplifies 181 bp product). M: DNA molecular size marker 1 kb (Fermentas, St. Leon Rot, Germany)

share roughly 55 % identical nucleotide sequences with ShSUT4.

Concerning SPS II, all tested accessions fell into four major groups (Fig. 8). The provided accession EU269038 was in group III and was composed of heterogeneous species. The sequence divergence rates indicated in (Table 3) was reflected in the clustering pattern in that EU26938 was closest to all members of group III by a percentage average of 82 %. Group I contained ten sequences of SPS from different plant species, mostly *Arabidopsis* spp. which clustered into unique subgroup, and *Glycine max*, *Prunus persica*, *Vicia faba*. Fifteen divergent SPS sequences were assembled in group 2 which contained *Solanum lycopersicum*, *Cucumis melo*, *Nicotiana tabacum*, *Coffea canephora*, and *Ricinus communis* with 69 % identity. Group III was the major cluster of SPS from different plant species which contained most of the *Poaceae* family such as *Saccharum* spp., *Zea mays*, *Oryza sativa*, *Triticum aestivum*, *Sorghum bicolor*, and *Bambusa oldhamii*. The SPS nucleotide sequences of thirteen plant species were grouped into group 4, where *Arabidopsis* spp. were assembled under a unique subgroup with 100 % similarity.

Discussion

Our knowledge of sucrose translocation has increased considerably by the biochemical and molecular characterization of sucrose transporter (SUT) family in the last decade [9]. Furthermore, the disaccharide sucrose has a high chemical and biochemical stability due to its acetal-bond which covers the reducing ends of the two monosaccharides. Sucrose transport is essential for the distribution of carbohydrates in

plants. Recent studies have shown that a specific transporter protein plays an essential role in loading sucrose into the phloem component of the plant vasculature [34]. All the transporters (SUT or SUC, for sucrose transporter, or SCR for sucrose carrier) have been found to belong to the same gene family, the members of which encode highly hydrophobic disaccharide transporters with twelve transmembrane-spanning helices.

RT-PCR and northern blotting analyses showed that transcripts of the sucrose transporters (ShSUT1A and ShSUT4) were found in all the sugarcane tissues tested such as the source leaf and maturing internode (internode #7) (Figs. 1a, b, 4a 5a, b). The amount of transcripts seemed to increase with matured internode #7. This is in agreement with the previously reported increase of sucrose transport into the storage parenchyma cells in sugarcane at the expense of hexose transport [9, 17]. However, possibly sucrose transport and storage in maturing internodes become progressively symplastic in parallel with the maturation process, in which a lignified barrier is developed around the bundle sheath, preventing apoplastic transfer from phloem to storage parenchyma [15, 23]. Furthermore, the cytoplasmic membrane seems to lose its barrier function with maturation leading to very high apoplastic sugar concentrations, possibly in equilibrium with the cytosolic and vacuolar sucrose concentration [37]. Sugarcane sucrose transporters, ShSUT1A and ShSUT4, are expressed both in leaves and stems, but most highly in the stem tissues accumulating sucrose. Rae et al. [23, 24] localized ShSUT1 predominantly in the bundle sheath and not in the phloem, but in situ studies localize ShSUT1A in the phloem of leaves and in the phloem of internode bundles [9]. In agreement with these findings, it has been suggested that ShSUT1 may play a role in retrieval of sucrose leading from the storage parenchyma cells in the stem or alternatively in sucrose export in sinks rather than in phloem loading [23]. The role of sucrose transporters in phloem loading are well documented including in retrieval of sucrose along the transport path in the stem. The localization of ShSUT1A in phloem is therefore no exception; however, its role in storing sucrose in the parenchyma is less clear [9]. Interestingly, sugarcane sucrose transporter ShSUT1A was detected in maize and *Arabidopsis* leaves with low transcript levels even in the roots of *Arabidopsis*. These findings suggest that sugarcane sucrose transporter ShSUT1A is homologous to the sucrose transporters from maize and *Arabidopsis*.

In order to provide further support for this observation phylogenetic analysis was done (Fig. 7) which compared 51 plant sucrose transporters, for which full-length protein sequences were available in the public databases. The 51 sequences fall into two clearly separable groups: Group I and Group II contain sequences from both, dicots and

Fig. 7 Dendrogram depicting evolutionary relationships of sucrose transporter sequences among various plant species from publicly accessible databases. Fifty-one accessions were retrieved into two main clusters. The provided accessions ShSUT1A and ShSUT4 were dispatched into two different clusters. The tree was produced by the

ML algorithm under assumption of T92+G+I model executed in MEGA 5.03. The numbers above the branches indicate the bootstrap confidence value. The scale bar shows the number of substitutions per nucleotide

monocots plants. ShSUT1A which belonged to group I and shared 67 % identity with ZmSUT1 (*Zea mays*) was still able to show high SUT expression in maize. The ShSUT1A sequence shared 53 % identity with *Arabidopsis*, and could explain why there appeared low transcripts levels of SUT in *Arabidopsis* using ShSUT1A primers (Fig. 3). The similarity between the cereal SUT1 group and the dicot SUT2 group appears also to extend to gene structure. This high level of conservation of gene structure is intriguing,

considering the diverse functions for these proteins. The evolutionary distance between *Arabidopsis* and the *Gramineae* (*Poaceae*) species would also suggest that some key elements in the sequence of these proteins have precluded radical changes to the gene structure. Such similarities in gene structure between the cereals and *Arabidopsis* have previously been observed for starch synthase [19] and starch branching enzyme [20]. In contrast, ShSUT4 was expressed only in sugarcane and maize,

Table 2 Identity percentage determined by comparison of sucrose transporters (ShSUT1A and ShSUT4) accessions to other plant species

GenBank accession number	Sucrose transporter (ShSUT1A) identity percentage	Sucrose transporter (ShSUT4) identity percentage	Plant source
XM003558661	84.0	51.0	<i>Brachypodium distachyon</i>
EU255258	82.0	51.0	<i>Lolium perenne</i>
AM055812	82.0	52.0	<i>Hordeum vulgare</i> ssp. <i>vulgare</i>
AJ272309	82.0	52.0	<i>Hordeum vulgare</i> ssp. <i>vulgare</i>
XM003573842	72.0	53.0	<i>Brachypodium distachyon</i>
XM003570440	67.0	46.0	<i>Brachypodium distachyon</i>
NM001153179	67.0	46.0	<i>Zea mays</i>
XM003575166	66.0	50.0	<i>Brachypodium distachyon</i>
AY581895	67.0	46.0	<i>Zea mays</i>
GQ396637	68.0	44.0	<i>Lolium perenne</i>
XM002453038	67.0	46.0	<i>Sorghum bicolor</i> (sorghum)
AY098894	66.0	44.0	<i>Citrus sinensis</i>
XM003530644	65.0	45.0	<i>Glycine max</i>
XM003577230	57.0	83.0	<i>Brachypodium distachyon</i>
HQ540307	56.0	84.0	<i>Oryza sativa</i> Japonica group
AM491808	64.0	44.0	<i>Hevea brasiliensis</i> subsp. <i>brasiliensis</i>
XM003553149	65.0	45.0	<i>Glycine max</i>
XM002443632	55.0	97.0	<i>Sorghum bicolor</i>
NM001144014	54.0	95.0	<i>Zea mays</i>
AY639018	55.0	95.0	<i>Zea mays</i>
AY946204	78.0	44.0	<i>Eucommia ulmoides</i>
AJ272308	60.0	79.0	<i>Hordeum vulgare</i> subsp. <i>vulgare</i>
XM002988433	59.0	59.0	<i>Selaginella moellendorffii</i>
DQ138373	65.0	43.0	<i>Manihot esculenta</i>
AY291289	64.0	44.0	<i>Solanum tuberosum</i>
NM001247392	66.0	45.0	<i>Solanum lycopersicum</i> (<i>Lycopersicon esculentum</i>)
AY065840	58.0	58.0	<i>Brassica oleracea</i>
XM002285532	55.0	64.0	<i>Vitis vinifera</i>
DQ221700	57.0	55.0	<i>Phaseolus vulgaris</i>
HQ403679	56.0	55.0	<i>Rosa hybrid cultivar</i>
NM102118	58.0	55.0	<i>Arabidopsis thaliana</i>
BT000684	58.0	55.0	<i>Arabidopsis thaliana</i>
AY113946	58.0	55.0	<i>Arabidopsis thaliana</i>
AY050986	58.0	55.0	<i>Arabidopsis thaliana</i>
AY091774	58.0	55.0	<i>Arabidopsis thaliana</i>
AY088566	58.0	55.0	<i>Arabidopsis thaliana</i>
AY048256	58.0	55.0	<i>Arabidopsis thaliana</i>
XM003519168	56.0	66.0	<i>Glycine max</i> (soybean)
AJ534442	66.0	45.0	<i>Plantago major</i> (common plantain)
XM003527813	52.0	61.0	<i>Glycine max</i> (soybean)
XM003518300	54.0	56.0	<i>Glycine max</i>
AK353329	58.0	43.0	<i>Eutrema halophilum</i>
XM002975767	53.0	56.0	<i>Selaginella moellendorffii</i>
XM002973719	59.0	56.0	<i>Selaginella moellendorffii</i>
XM002520064	65.0	44.0	<i>Ricinus communis</i>
XM002276712	65.0	44.0	<i>Vitis vinifera</i>
GU356640	65.0	44.0	<i>Cucumis sativus</i>
XM002876797	62.0	44.0	<i>Arabidopsis lyrata</i> ssp. <i>lyrata</i>
AF021809	65.0	44.0	<i>Vitis vinifera</i>
ShSUT1A	100	51.0	<i>Saccharum officinarum</i>
ShSUT4	51.0	100	<i>Saccharum officinarum</i>

Fig. 8 Dendrogram depicting evolutionary relationships of sucrose phosphate synthase among various plant species from publicly accessible databases. Fifty-five accessions were split into four main clusters. The provided accession EU269038 was included in group III.

The tree was produced by the ML algorithm under assumption of T92+G model executed in MEGA 5.03. The numbers above the branches indicate the bootstrap confidence value. The scale bar shows the number of substitutions per nucleotide

but not in *Arabidopsis*; however, the average identity percentage for ShSUT4 was 65 %. There is a high degree of conservation of gene structure within the *Poaceae* family (SUT1 cluster; group I). Those transporters identified from various monocot species differ in their tissue

localization and suggested role in the plant. Of the monocot sucrose transporters of group I and group II, so far, five proteins were functionally characterized as sucrose transporters (HvSUT1 and HvSUT2 [25], OsSUT1 [35], ShSUT1 [27], and ZmSUT1 [4]). OsSUT1 was shown to be

Table 3 Identity percentage determined by comparison of EU269038 accession to other plant species

GenBank accession number	EU269038 identity percentage	Plant source
HM854011	99	<i>Saccharum hybrid</i>
XM002441477	97	<i>Sorghum bicolor</i>
AB232784	88	<i>Lolium perenne</i>
AY445835	88	<i>Bambusa oldhamii</i>
JF423929	72	<i>Dendrobium officinale</i>
AY135211	72	<i>Oncidium Goldiana</i>
XM002282772	70	<i>Vitis vinifera</i>
XM002265437	71	<i>Vitis vinifera</i>
AB005023	71	<i>Citrus unshiu</i>
JQ412751	71	<i>Prunus persica</i>
DQ834321	69	<i>Coffea canephora</i>
XM002515441	71	<i>Ricinus communis</i>
HQ117935	71	<i>Saccharum hybrid</i>
XM003549687	69	<i>Glycine max</i>
Y11821	69	<i>Craterostigma plantagineum</i>
DQ364058	69	<i>Cucumis melo</i>
XM002531896	70	<i>Ricinus communis</i>
XM003542601	69	<i>Glycine max</i>
AF322116	69	<i>Medicago sativa</i>
AF194022	70	<i>Nicotiana tabacum</i>
XM003609383	68	<i>Medicago truncatula</i>
S54379	70	<i>Spinacia oleracea</i>
XM003570786	72	<i>Brachypodium distachyon</i>
NM001247910	69	<i>Solanum lycopersicum</i>
X81975	69	<i>Beta vulgaris</i> subsp. <i>vulgaris</i>
Z56278	68	<i>Vicia faba</i> var. <i>minor</i>
NM121149	68	<i>Arabidopsis thaliana</i>
AF347065	82	<i>Triticum aestivum</i>
AY135211	72	<i>Oncidium Goldiana</i>
EF568782	71	<i>Prunus persica</i>
EF568781	71	<i>Prunus persica</i>
HM123763	71	<i>Xerophyta humilis</i>
AF439861	70	<i>Ipomoea batatas</i>
AB334114	70	<i>Pyrus pyrifolia</i>
XM002873942	70	<i>Arabidopsis lyrata</i> spp. <i>lyrata</i>
NM122035	70	<i>Arabidopsis thaliana</i>
AY079334	70	<i>Arabidopsis thaliana</i>
AY039911	70	<i>Arabidopsis thaliana</i>
XM003563255	72	<i>Brachypodium distachyon</i>
L04803	70	<i>Spinacia oleracea</i>
EU684230	69	<i>Solanum tuberosum</i>
DQ521271	69	<i>Cucumis melo</i>
XM002871410	68	<i>Arabidopsis lyrata</i> spp. <i>lyrata</i>
X73477	69	<i>Solanum tuberosum</i>
NM001246991	69	<i>Solanum lycopersicum</i>
AF534907	71	<i>Triticum aestivum</i>
AF071786	69	<i>Solanum lycopersicum</i>
AY331261	70	<i>Viscum album</i> ssp. <i>Album</i>
JQ086765	68	<i>Solanum lycopersicum</i>
AY069868	69	<i>Arabidopsis thaliana</i>
BT002697	69	<i>Arabidopsis thaliana</i>
U85448	69	<i>Actinidia deliciosa</i>
XM002328863	62	<i>Populus trichocarpa</i>
AY109435	89	<i>Zea mays</i>
NM001068030	86	<i>Oryza sativa</i> Japonica group
XM003573633	85	<i>Brachypodium distachyon</i>
NM001176637	95	<i>Zea mays</i>
NM001052643	68	<i>Oryza sativa</i> Japonica group

expressed in companion cells (CCs) [30] and both, ShSUT1 and ZmSUT1 are thought to be responsible for phloem loading [1, 27]. Thus, group I seems to represent a group of plasma membrane-localized monocot sucrose transporters that catalyze primarily the uptake of sucrose into phloem and sink cells [30]. Most monocot species have more than one SUT. Indeed, two SUTs have been described in barley [38], in sugarcane (ElSayed et al. submitted) and in maize [27]. Sucrose transporters of group II (SUT4 or SUC4) were originally characterized or at least described as plasma membrane localized sucrose transporters: AtSUC4 [35] and HvSUT2 [38]. Furthermore, the localization of SUT 4-type proteins in sieve elements (SEs) is intriguing, as SE does not possess vacuoles. The localization of AtSUC4 and HvSUT2 in the tonoplast [10] and the high levels of LeSUT4 mRNA in sink tissues suggest, therefore, that SUT4-type transporters may represent vacuolar sucrose transporters that are primarily expressed in sink tissues. Interestingly, though ShSUT4 was detected in sugarcane leaves and stem it was not detected in maize and *Arabidopsis* for unknown reasons. Despite this, the phylogenetic analysis revealed that the ZmSUT4 shared sequence identity with ShSUT4 at 88 % identity though the gene structures are not similar. These experiments were repeated several times and the same negative result for maize and *Arabidopsis* was obtained (data not shown). Sucrose transporters play an important role in regulating sucrose fluxes within the plant [22]. In our experiments, SUT1 showed higher expression in both the internodes and leaves of sugarcane as compared to the expression in maize leaves and the shoots and roots of *Arabidopsis* species. This is in contrast to the expression of SUT4 (ShSUT4) observed in mature leaves of maize and *Arabidopsis*. Group II SUTs (SUT4) and (SUC2), are known to be located in the tonoplast of *Arabidopsis* [32] and rice [11]. In rice, SUT2 plays a role in facilitating sucrose exchange between vacuoles and the cytoplasm in the source leaves and sink organs [2]. Hence, species-specific variation has been observed in the expression of sucrose transporters.

Sucrose phosphate synthase (SPS; EC 2.4.1.14) catalyzes the rate-limiting step of sucrose synthesis and is considered to be a major target for increasing sucrose biosynthesis in the leaves of crop plants [14]. Furthermore, SPS and vacuolar (acid) invertases have been reported to play an important role in regulating sucrose fluxes in sink tissues and their activities have been correlated to sucrose accumulation in the sugarcane stem [40]. It is noteworthy that SPS was highly expressed in sugarcane as compared with maize, but not expressed or detected in *Arabidopsis*. A positive correlation between SPS activity and sucrose accumulation was reported in sugarcane stems [3]. The phylogenetic analysis revealed that sugarcane and maize were assembled into unique subgroup within group III with

95 % identity, while *Arabidopsis* was in a separate into a small subgroup, group I with 70 % identity.

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