

Characterization of Source- and Sink-Specific Sucrose/H⁺ Symporters from Carrot

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To understand how sucrose (Suc) is transported from source leaves to developing tap roots of carrot (*Daucus carota* L.), we cloned two cDNAs (*DcSUT1* and *DcSUT2*) for proteins with homologies to plant Suc/H⁺ symporters. The deduced polypeptide sequences are 52% identical and have 12 predicted membrane-spanning domains each. Transport activities were confirmed by expression of the clones in yeast cells. Both transporters had optimal activity below pH 5.0 and Michaelis constant values of 0.5 mM. Suc uptake was inhibited by protonophores, suggesting that Suc transport is linked to the proton electrochemical potential across the plasma membrane. *DcSUT1* and *DcSUT2* had markedly different expression patterns. Transcripts of *DcSUT1* were found only in the green parts of plants, with highest levels in the lamina of source leaves, indicating that *DcSUT1* is required for the loading of Suc into the phloem. In leaf lamina expression was diurnally regulated, suggesting that Suc export from the leaves is higher during the day than during the night. The mRNA of *DcSUT2* was found mainly in sink organs, and no diurnal expression pattern was detected in the storage root. Here, expression was not restricted to the phloem but was much higher in storage parenchyma tissues of phloem and xylem. The close relationship of *DcSUT2* with a Suc/H⁺ symporter from fava bean, which facilitates Suc uptake into the cotyledons of developing seeds, indicates that this carrot Suc transporter may be involved in loading Suc into storage parenchyma cells.

Although higher plants are photoautotrophic, some tissues are dependent on the import of photoassimilates. In most plants, these heterotrophic tissues (sink tissues) are supplied with carbon in the form of Suc (Avidad, 1982). Long-distance transport of the disaccharide from source leaves to sink tissues takes place in the sieve tube/companion cell complex (phloem) and may require apoplastic loading and unloading steps (Ho, 1988). Passage of Suc through membranes is facilitated by energy-dependent, cargo-specific transport proteins (Giaquinta, 1977; Bush, 1993; Sauer et al., 1994; Bush et al., 1996; Rentsch et al., 1998).

A few years ago, the first plant sugar transporters were cloned by using engineered yeast cells, which can only live on Suc when they express a functional Suc transport protein. With this method, cDNA clones for Suc/H⁺ symporters were isolated from spinach (Riesmeier et al., 1992) and potato (Riesmeier et al., 1993). Subsequently, these clones were used as probes for the heterologous cloning of Suc transporters from other plant species such as *Arabidopsis*

(Sauer and Stolz, 1994), *Plantago major* (Gahrtz et al., 1994), and castor bean (Weig and Komor, 1996). Most of these clones were predominantly expressed in the vascular system (Truernit and Sauer, 1995) and seem to be involved in loading Suc into the phloem (Riesmeier et al., 1994; Stadler and Sauer, 1996). Only two cDNA clones were isolated that were preferentially expressed in sink organs: a flower-specific transporter of *P. major* (Gahrtz et al., 1996), and a seed-specific transporter of fava beans (Weber et al., 1997). Characterization of these clones in yeast cells showed that the two transport proteins had different biochemical characteristics: the transporter from *P. major* was fairly insensitive to pH changes (neutral Suc transporter), whereas the Suc transporter from fava beans had an acidic pH optimum.

The transporters involved in the import of Suc into storage cells of roots and tubers are not known. Therefore, we set out to identify a tap-root-specific Suc transporter in carrot (*Daucus carota*), a plant that we use as a model for studying Suc metabolism and partitioning (Sturm, 1996). Our cloning approach was based on conserved peptide domains of plant Suc/H⁺ symporters. Here we describe two cDNA clones and demonstrate their functions in cells of *Saccharomyces cerevisiae*. *DcSUT1* encodes a Suc transporter for the loading of Suc into the phloem of source leaves, whereas *DcSUT2* is highly expressed in phloem and xylem parenchyma tissues of developing tap roots.

MATERIALS AND METHODS

Plant Material

Carrot plants (*Daucus carota* L. cv Nantaise) were grown in a garden near Basel. After harvest, plant organs were directly frozen in liquid nitrogen and stored at -80°C .

Isolation of cDNA Clones for Suc/H⁺ Symporters

Total RNA was isolated from leaves and roots of carrot plants with developing tap roots. For this purpose, the method described by Prescott and Martin (1987) was modified by adding 20 mg of Polyclar AT (Serva, Heidelberg, Germany) per gram of tissue before grinding in liquid nitrogen. Poly(A⁺) RNA was purified according to the batch protocol of the Oligotex kit (Qiagen, Chatsworth, CA). First-strand cDNA was obtained by reverse transcription of poly(A⁺) RNA with an oligo(dT25) primer (Amer-

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Abbreviation: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

sham). A DNA fragment corresponding to a 330-bp-long domain of plant Suc/H⁺ symporters was amplified by PCR with the degenerated primers 5'-CA(AG)TT(CT)-GG(GT)TGGGC(CT)(CT)T(AGT)CA-3' and 5'-GC(AC)AC-(AG)TC(AG)AG(AG)ATCCA(GT)AA-3'. Amplification was achieved in a DNA thermal cycler (Perkin-Elmer Cetus) with the following conditions: 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 2 min, and elongation at 72°C for 2 min. Finally, the reaction mixture was kept at 72°C for 10 min. Reverse-transcription PCR products were analyzed by agarose-gel electrophoresis. Amplified DNA bands of the appropriate length were cloned into the pCR2.1 vector (Invitrogen, San Diego, CA). The plasmids generated were transformed into the *Escherichia coli* strain InVαF' (Invitrogen).

For the isolation of full-length cDNAs, we generated cDNA libraries from poly(A⁺) RNA isolated from roots and leaves of carrot plants with developing tap roots (Amersham). The double-stranded cDNA was ligated to *Eco*RI adaptors. Excess adaptors and cDNA smaller than 1 kb were removed by a spin column. cDNA was ligated into λ MOSSlox vector arms and packed into phage particles (Amersham). The libraries were screened with the corresponding ³²P-labeled leaf- or root-specific PCR fragments.

Analysis of DNA and Protein Sequences

Both strands of the PCR fragments and the cDNA clones were automatically sequenced by the dideoxynucleotide chain-termination method (Messing, 1983). Computer-assisted analysis of DNA and protein sequences was performed using the Wisconsin package (version 9.1, Genetics Computer Group, Madison, WI).

Analysis of RNA and DNA

For RNA gel-blot analysis, total RNA (10 μg/lane) was separated on a 1.2% agarose gel containing 6% formaldehyde (Sambrook et al., 1989). For DNA gel-blot analysis, restricted DNA was separated on a 0.8% agarose gel (10 μg/lane). RNA and DNA were transferred to nylon membranes (Hybond N, Amersham). The gene-specific PCR fragments (approximately 330 bp) of *DcSUT1* and *DcSUT2* were labeled with ³²P by random priming (Sambrook et al., 1989). As a probe for a constitutively expressed gene, a 1700-bp-long fragment (*Eco*RI/*Eco*RI) of the 18S rRNA gene from tomato was used (Schmidt-Puchta et al., 1989). The blots were prehybridized at 65°C for 5 h in 6× SSC, 5× Denhardt's solution, 100 mg/mL denatured calf thymus DNA, and 0.5% SDS (Sambrook et al., 1989). Hybridization was carried out in the same buffer overnight at 65°C. Blots were washed at 65°C with 1× SSC and 0.1% SDS, and 0.1× SSC and 0.1% SDS for 30 min each.

Expression of *DcSUT1* and *DcSUT2* in *Saccharomyces cerevisiae*

For the expression of *DcSUT1* and *DcSUT2* cDNAs in yeast cells, DNA fragments corresponding to the open reading frames of the carrot cDNA clones were isolated by

PCR. PCR primers for the *DcSUT1a* sense construct were designed by introducing a *Pst*I site upstream of the translation initiation codon ATG and a *Bam*HI site beyond the stop codon, a *Bam*HI site and a *Pst*I site for the antisense constructs for *DcSUT1a*, an *Eco*RI site and a *Not*I site for the sense construct for *DcSUT2*, and a *Sma*I site and an *Eco*RI site for the antisense construct for *DcSUT2*. The following primers were used for the sense construct for *DcSUT1a*, 5'-CAAGCTGCAGATAGCAGCGATAACAG-3' and 5'-GTCTTGGATCCTAACCAAGTCTGACAG-3'; for the antisense construct for *DcSUT1a*, 5'-CAAGGGATCCATAGCAGCCATAACAG-3' and 5'-GTCTTCTGCAGTAACCAAGTCTGACAG-3'; for the sense construct for *DcSUT2*, 5'-CACAGAATTCACCATAAACAGATAAATC-3' and 5'-CTAAAATGCGGCCGCTAACCACTTCACAG-3'; and for the antisense construct for *DcSUT2*, 5'-CACACCCGGGACCATAAACAGATAAATC-3' and 5'-CTAAATGAATCTAACCACTTCACAG-3'. Amplification was achieved with the following conditions: 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and elongation at 72°C for 2 min. Finally, the reaction mixture was kept at 72°C for 10 min. The PCR fragments obtained were cleaved and ligated into the corresponding restriction sites of the *E. coli*/*S. cerevisiae* shuttle vector 112 A1NE (Riesmeier et al., 1992).

The *S. saccharomyces* strain SUSY7 (Riesmeier et al., 1992), which lacks the *Trp* gene for selection of recombinant cells, was transformed with the cDNAs in sense or antisense orientation according to the method of Gietz et al. (1992). As an additional control, the empty vector 112 A1NE was used. Cells were grown in 300 mL of yeast extract, peptone, and dextrose to an A₆₀₀ of 0.5, washed in sterile water, and resuspended in 1.5 mL of freshly prepared lithium acetate buffer (1 volume of 10× Tris-EDTA, 1 volume of 1 M lithium acetate, and 8 volumes of sterile water). Ethanol-precipitated plasmid DNA (0.6–0.8 μg) was mixed with 200 μg of carrier DNA in a total volume of 20 μL of water. Yeast cells (200 μL) were added to each DNA sample, followed by 1.2 mL of 50% PEG 600 in water. Samples were incubated on a shaker for 30 min at 30°C, and then for an additional 15 min at 42°C. Finally, the cells were harvested by centrifugation and resuspended in 1 mL of 1× Tris-EDTA buffer. Different volumes of cells were plated on 2% agar in minimal medium (2% Glc, 0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, and 0.13% Trp minus dropout powder). After 4 d of incubation at 30°C, large colonies were picked and replated on solidified minimal medium with 2% Suc (pH 5.5). Fast-growing colonies from sense constructs were picked after incubation for 4 d at 30°C.

[¹⁴C]Suc Uptake Studies

Yeast cells were grown in 20 to 30 mL of minimal medium up to an A₆₀₀ of 0.9 to 1.0, harvested by centrifugation, washed with 25 mM sodium phosphate buffer, pH 5.5, and resuspended in 1 mL of the same buffer. Suc uptake was determined as described by Cirillo (1989). For each assay, 1 mg of cells in 200 μL of buffer was used. The assays were started by the addition of 0.5 μCi of [¹⁴C]Suc (final

concentration, 0.2 mM). Cells were incubated for 0, 2, 4, 6, and 8 min at 30°C in a shaking water bath. Sugar uptake was stopped by transfer of the cells into 10 mL of ice-cold water over a Whatman glass microfiber filter (0.7 μm) in a filtration apparatus. Cells were rapidly washed three times with 5 mL of ice-cold water. Finally, filters were transferred to liquid-scintillation vials and counted. In some experiments, D-Glc (10 mM) was added 5 min before the addition of labeled Suc.

To determine the pH dependence of Suc uptake, cells were treated as described above but were washed and resuspended in 25 mM sodium phosphate buffer of pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0. To disrupt the proton gradient across membranes, CCCP was added 30 s before the addition of Suc at a final concentration of 5, 50, or 500 μM. To measure the K_m values of DcSUT1 and DcSUT2, Suc concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mM were used.

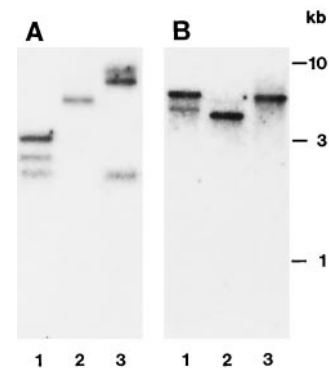


Figure 2. DNA gel-blot analysis of Suc/H⁺ symporter sequences in the carrot genome. Genomic DNA (10 μg/lane) was digested with *Hind*III (lanes 1), *Hind*III (lanes 2), or *Pst*I (lanes 3). The blot was hybridized with a ³²P-labeled PCR fragment of *DcSUT1* (A) or *DcSUT2* (B), neither of which contained cleavage sites for the restriction enzymes used.

RESULTS

Isolation of cDNA Clones for Suc/H⁺ Symporters from Carrot

A comparison of the amino acid sequences deduced from the sequences of cDNA clones for plant Suc/H⁺ symporters (the sequences used are listed in the legend to Fig. 3) identified two highly conserved heptapeptides, QFGWALQ and FWILDVA, located in the first and fourth membrane-spanning domains, respectively (marked by asterisks in Fig. 1). Degenerated primers generated from these peptide sequences and first-strand cDNA made from mRNA from leaves or roots of carrot plants with developing tap roots were used to amplify DNA fragments of about 330 bp, which were subcloned and sequenced. Two types of sequences were obtained with clear homologies to the sequences of plant Suc/H⁺ symporters and that share about 58% sequence identity at the DNA level. Most of the fragments amplified from leaf cDNA were of one type (leaf-specific PCR fragment), whereas the majority of fragments generated from root cDNA were of the other type (root-specific fragment). Subsequently, the two PCR fragments were used to isolate full-length cDNA clones from a leaf- and a root-specific cDNA library.

Screening of the leaf library resulted in the isolation of two clones of different lengths (*DcSUT1a*, 1861 bp; *DcSUT1b*, 2132 bp). No differences were found when their 1503-bp-long open reading frames were compared. However, the two clones showed sequence deviations in the 5' upstream noncoding sequences and were completely different in their 3' noncoding domains. The two open reading frames code for a polypeptide of 54,066 D with a pI of 8.33. Only one type of cDNA clone was isolated from the root library (*DcSUT2*, 1858 bp). Its open reading frame of 1545 bp codes for a protein of 54,424 D with a pI of 8.85. The two polypeptide sequences are 52% identical (62.0% similar) and have 12 predicted membrane-spanning domains each (Fig. 1).

The identification of two closely related but different leaf-specific cDNA clones and only one root-specific clone

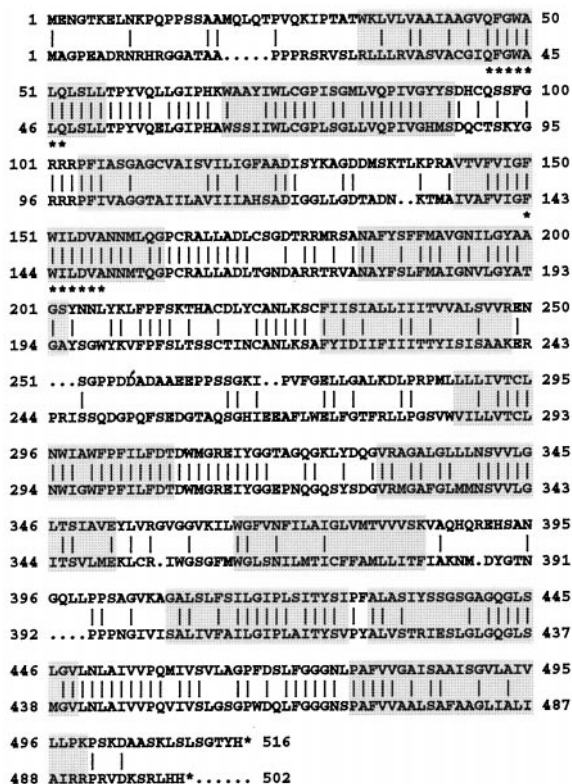


Figure 1. Comparison of the amino acid sequences derived from the cDNA clones *DcSUT1* (bottom line) and *DcSUT2* (top line). The amino acid sequences are in the one-letter code and have been aligned by introducing gaps (...) to maximize identity. Vertical bars indicate identical amino acid residues, and the highlighted regions represent putative membrane-spanning domains. The two peptide sequences marked with asterisks were used for the generation of degenerated primers for the isolation of the cDNA clones.

fits very well with the results of DNA gel-blot analysis of the respective sequences in the carrot genome. The number of hybridization signals obtained with the leaf-specific PCR fragment as a probe (Fig. 2A) suggests the presence of at least two genes per carrot genome, whereas hybridization of a similar DNA gel blot with the root-specific fragment indicates the presence of only one gene.

The amino acid sequences deduced from the leaf- and root-specific cDNA clones and the published amino acid sequences of Suc/H⁺ symporters from dicotyledonous plants were used to generate the phylogenetic tree shown in Figure 3. The 13 sequences appear to be divided into several subclasses with a very close relationship between DcSUT2 (*car2*) and the Suc/H⁺ symporter from fava bean (*vif*). In contrast, the sequence of DcSUT1 (*car1*) appears to be fairly different from the sequences of plant Suc/H⁺ symporters.

Characterization of *DcSUT1* and *DcSUT2* in *S. cerevisiae*

To determine whether the carrot cDNA clones code for Suc/H⁺ symporters, they were expressed in the *S. cerevisiae*

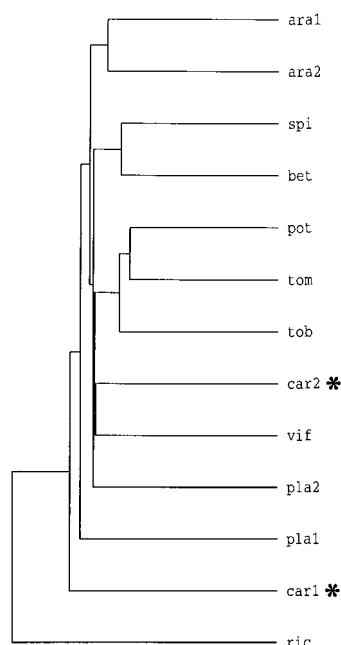


Figure 3. Comparison of amino acid sequences of Suc/H⁺ symporters from dicotyledonous plants. The dendrogram was generated by comparison of the sequences by the PileUp program of the Genetics Computer Group sequence analysis software package. *ara1*, Arabidopsis (accession no. X75365); *ara2*, Arabidopsis (accession no. X75382); *spi*, spinach (accession no. X67125); *bet*, *Beta vulgaris* (accession no. X83850); *pot*, potato (accession no. X69165); *tom*, tomato (accession no. X82275); *tob*, tobacco (accession no. X82276); *car2*, DcSUT2 (accession no. Y16768); *vif*, fava bean (accession no. Z93774); *pla2*, *P. major* (accession no. X75764); *pla1*, *P. major* (accession no. X84379); *car1*, DcSUT1 (accession no. Y16766); and *ric*, castor bean (accession no. Z31561). The two carrot polypeptides are marked by asterisks. *ara1* and *pla1* have neutral pH optima, whereas all other transporters have maximal activities below pH 5.0.

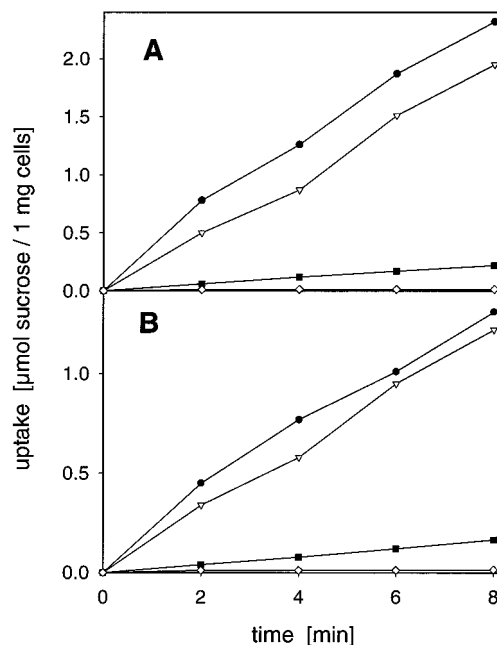


Figure 4. Uptake of Suc in transgenic *S. cerevisiae* cells. Uptake of [¹⁴C]Suc (0.2 mM final concentration) in yeast cells transformed with *DcSUT1* (A) or *DcSUT2* (B) in the presence (●) or absence (▼) of 10 mM D-Glc, or the presence of 5 μM of the protonophore CCCP (■). ◇, Suc uptake into control cells transformed with *DcSUT1* or *DcSUT2* in the antisense orientation.

siae strain SUSY7 (Riesmeier et al., 1992), which lacks the invertase gene (*SUC2*) and contains a functional plant gene for Suc synthase for its expression in the cytoplasm. These yeast cells can only live on Suc as the sole carbon source when they also express a functional Suc transporter gene.

DNA fragments containing the open reading frames of *DcSUT1* or *DcSUT2* were cloned into the *E. coli/S. cerevisiae* shuttle vector 112 A1NE (Riesmeier et al., 1992), containing the *ADH* promoter in front of a multiple cloning site and the *ADH* terminator. Both carrot cDNA clones allowed the growth of SUSY7 cells on Suc-containing agar plates. Uptake studies with radiolabeled Suc showed up to a 200-fold accumulation of the disaccharide compared with yeast cells containing only the vector or the clones in the antisense orientation (Fig. 4). Suc uptake catalyzed by both carrot transporters was optimal below pH 5.0 and was less than 10% of optimal activity at pH 7.0 (Fig. 5). Low concentrations of the proton uncoupler CCCP markedly inhibited Suc uptake (Fig. 4), indicating that Suc transport is linked to the proton electrochemical potential across the plasma membrane. The *K_m* of DcSUT1 for Suc at pH 5.5 was 0.5 mM and that of DcSUT2 was 0.7 mM (data not shown). Both transporter activities were stimulated by Glc, which is in agreement with the results obtained by Riesmeier et al. (1992) and Sauer and Stolz (1994).

Tissue-Specific Expression of *DcSUT1* and *DcSUT2* mRNA

The development of leaves from leaf primordia to the mature organ is characterized by a gradual transition from

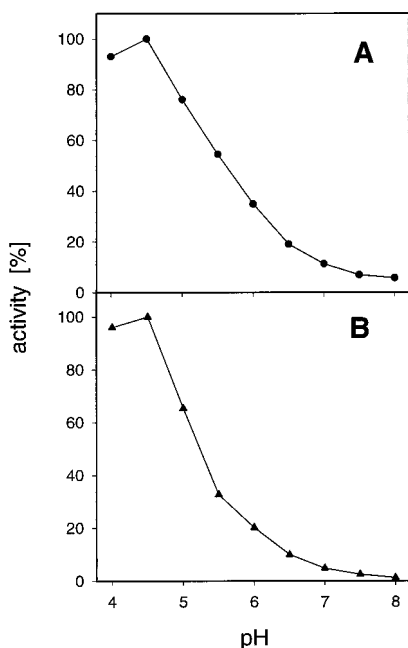


Figure 5. pH dependence of Suc transport in transgenic *S. cerevisiae* cells expressing either *DcSUT1* (●, A) or *DcSUT2* (▲, B). Measurements were performed at 0.2 mM Suc and 10 mM Glc in 25 mM sodium phosphate buffer between pH 4.0 and 8.0.

net importers of photoassimilates (sink leaves) to net exporters (source leaves) (Turgeon, 1989). To determine when *DcSUT1* and *DcSUT2* are expressed during carrot leaf development, leaf lamina from approximately 12-week-old carrot plants were harvested and separated according to their size into five classes (Fig. 6, top). Total RNA was isolated and analyzed on RNA gel blots with the leaf- or root-specific PCR fragment (Fig. 6A; only the blot probed with the fragment of *DcSUT1* is shown). Because the probes are fragments of the open reading frames, a distinction between transcripts of the two highly related leaf clones *DcSUT1a* and *DcSUT1b* was not possible. Instead, the common expression pattern of *DcSUT1* was detected. The highest transcript levels for *DcSUT1* were found in the lamina size classes 4 and 5, which are thought to be source leaves. *DcSUT2* mRNA levels were very low but detectable in all five tissue samples (data not shown).

Source leaves, which had not yet reached their final size (between size classes 4 and 5), were dissected into lamina and six consecutive stem segments (Fig. 7, top). Total RNA from these tissues was analyzed on RNA gel blots. The highest levels of *DcSUT1* transcripts were found in the leaf lamina (Fig. 7A), indicating a function of the Suc/H⁺ symporter in loading of Suc into the phloem. Lower transcript levels were found in the stem, with a decreasing gradient toward the stem base. In contrast, only very low mRNA levels of *DcSUT2* were found in the leaf lamina, indicating that the encoded polypeptide is functionally different from *DcSUT1*. High transcript levels were found in the central stem segments, and lower levels at the two stem ends (Fig. 7B).

Developing tap roots were also divided into zones with different biological activities. The root elongated and thickened at the tip (Fig. 8, top, segment 6) (utilization sink for Suc) and storage of sugars occurred in the upper part (segments 1–5) (storage sink for Suc). These upper segments also increased in diameter (secondary root growth) but the increase was small compared with the rate of root elongation at the root tip. Transcripts for *DcSUT1* were barely detectable (data not shown in this figure, but the lack of expression of *DcSUT1* in roots can be seen in Fig. 11D). High and approximately equal levels of mRNA for *DcSUT2* were found in all root sections (Fig. 8A).

To determine whether *DcSUT1* and *DcSUT2* are tissue-specifically expressed in tap roots, 12-week-old roots were separated into xylem (a), cambium plus a few millimeters of phloem tissue (the phloem-containing tissue; Sturm et al., 1998) (b), phloem (c), and periderm (d) (Fig. 9, top). Transcripts for *DcSUT1* were barely detectable, whereas mRNA for *DcSUT2* was found in all four tissues, with the highest levels in phloem and xylem parenchyma tissues (Fig. 9A).

At different developmental stages of carrot reproductive organs only low levels of *DcSUT1* transcripts were detected (data not shown). In contrast, *DcSUT2* mRNA was found at all times during organ development, with the highest levels in flowers and developing seeds (Fig. 10A).

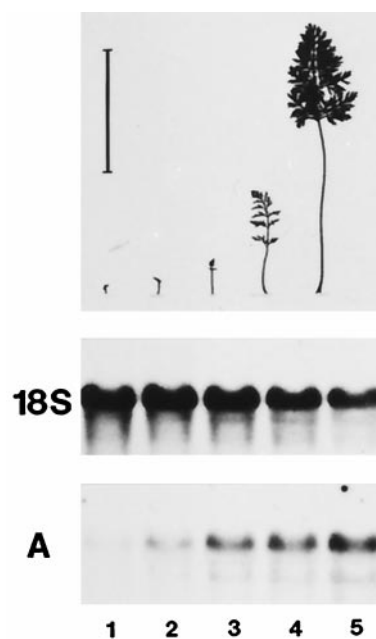


Figure 6. Steady-state mRNA levels of *DcSUT1* in lamina of different size classes of carrot leaves. Leaf lamina from 12-week-old carrot plants were separated into five size classes as shown in the schematic representation (upper panel). The bar above the smallest leaf (class 1) indicates a length of 10 cm. A northern blot with total RNA (10 μg/lane) was hybridized with the ³²P-labeled probe for *DcSUT1* (A). As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used (middle).

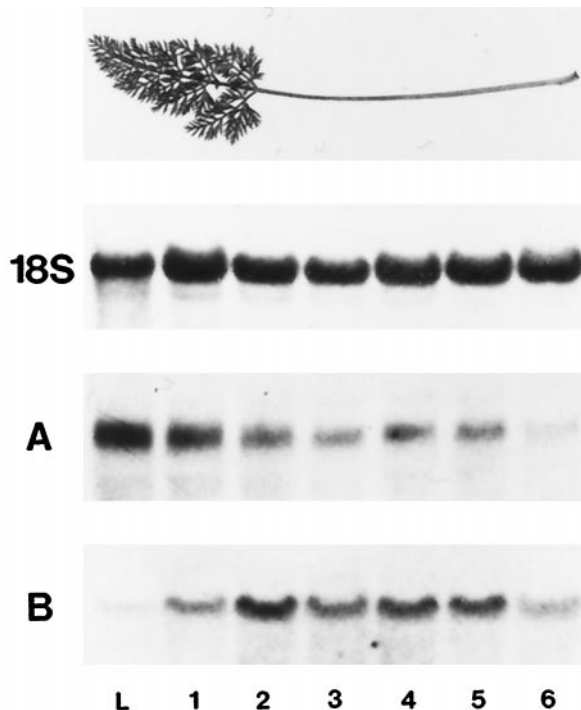


Figure 7. Steady-state mRNA levels of *DcSUT1* and *DcSUT2* in leaf lamina and sections of the stem. Top, Leaves of about 12-week-old plants that had not yet reached their mature size were dissected into lamina and six consecutive stem sections. A, A northern blot with total RNA (10 μ g/lane) from leaf lamina (L) and stem sections (sections 1–6 from top to bottom; section 1 was a highly branched stem segment connecting the numerous leaf lamina) was hybridized with a 32 P-labeled probe for *DcSUT1*. B, A blot identical to that in A was hybridized with a 32 P-labeled probe for *DcSUT2*. As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used.

Light-Dependent Expression of *DcSUT1* and *DcSUT2* mRNA

To determine whether the expression of the carrot Suc/H⁺ symporters is coordinated with the diurnal changes in photosynthesis, steady-state mRNA levels for *DcSUT1* and *DcSUT2* were analyzed at 3-h intervals for 24 h (Fig. 11). As a positive control, RNA isolated from entire leaves (leaf lamina and stems) was probed with a PCR fragment of the cDNA for the chlorophyll *a/b*-binding (*cab*) protein (approximately 80% identical with the petunia gene; accession no. X04966). As expected, high transcript levels were found during the day and only low levels were found during the night (A). Low expression of the *cab* gene was also detected in roots but no diurnal regulation was seen (B). Like the *cab* gene in leaves, the levels of *DcSUT1* transcripts were light dependent, with higher levels during the day and low levels during the night (C). In contrast, roots had only barely detectable levels of *DcSUT1* mRNA and no diurnal concentration alterations (D). In leaves (in stems and not in lamina; see Fig. 7), *DcSUT2* transcript levels were highest at midday and lower during the night (E). High, constant levels were found in roots throughout the 24-h time course (F).

DISCUSSION

The deposition of storage reserves in plant storage organs is intimately linked to photosynthesis and long-distance transport of photoassimilates such as Suc. In most plants, Suc transport from its place of biosynthesis in source leaves to the sink organs is accompanied by the passage of the disaccharide through several membranes. In general, it has been shown that this passage is facilitated by cargo-specific, energy-dependent transport proteins (Giacinta, 1977; Bush, 1993; Sauer et al., 1994). In the present study, two Suc transporters were isolated from carrot, a plant that accumulates high levels of soluble sugars in parenchyma cells of a tap root. The two polypeptides have significant homology to previously described plant Suc carriers (Riesmeier et al., 1992, 1993; Sauer and Stolz, 1994). All belong to the large family of major facilitator proteins (Marger and Saier, 1993), with 12 membrane-spanning domains and a molecular mass of about 54 kD. Yeast cells expressing the carrot cDNA clones imported Suc efficiently and accumulated it at least 200-fold more than cells containing only the vector or the clones in the antisense orientation. The yeast-expressed transporters had optimal activities in the acidic pH range and K_m values for Suc of about 0.5 mM. Protonophores such as CCCP inhibited Suc completely, strongly suggesting that the two polypeptides transport the disaccharide together with protons.

The amino acid sequences of *DcSUT1* and *DcSUT2* were only 52% identical and most sequence conservation was found in the transmembrane domains. In a phylogenetic tree generated from the deduced sequences of the two carrot proteins and other plant Suc/H⁺ symporters, transporters with similar biochemical characteristics were not grouped together (e.g. transporters with a neutral pH optimum). Because of this lack of correlation between amino acid sequences and transporter properties, it is not possible

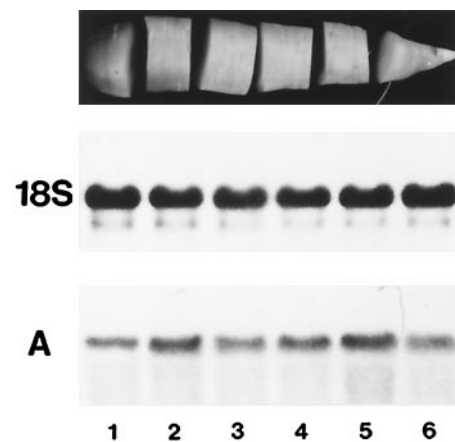


Figure 8. Steady-state mRNA levels of *DcSUT2* in developing tap roots. Twelve-week-old carrot plants were harvested in the middle of a sunny, warm day. The tap roots were washed and divided into six equal, consecutive sections (top). Bottom, A northern blot with total RNA (10 μ g/lane) from the six root sections (1–6) was hybridized with the 32 P-labeled probe for *DcSUT2* (A). As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used (middle).

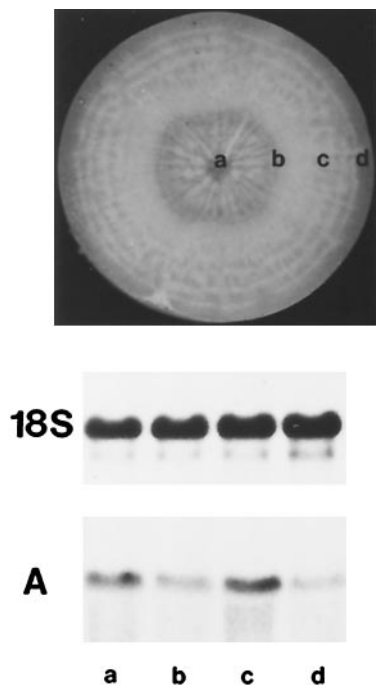


Figure 9. Steady-state mRNA levels of *DcSUT2* in developing tap roots. Top, The middle third sections of tap roots of 12-week-old carrot plants (excluding crown and tip) were dissected into xylem (a), cambium plus a few millimeters of adjacent phloem (b), the remaining phloem (c), and periderm (d). Bottom, A northern blot with total RNA (10 μ g/lane) was hybridized with the ³²P-labeled probe for *DcSUT2* (A). As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used (middle).

to make property predictions based on the position in the dendrogram.

One of the carrot transporters was predominantly expressed in source cells (*DcSUT1*) and the other in different sink tissues (*DcSUT2*), suggesting different biological functions. Analysis of steady-state transcript levels indicated that the expression of *DcSUT1* is linked to photosynthesis and the provision of the plant with assimilated carbon. Like the Suc/H⁺ symporters of *Arabidopsis* (*AtSUC2*) (Sauer and Stolz, 1994; Truernit and Sauer, 1995) and po-

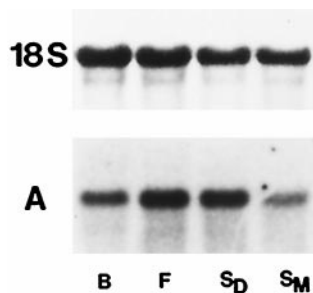


Figure 10. Steady-state mRNA levels of *DcSUT2* in reproductive organs of carrot plants. A northern blot with total RNA (10 μ g/lane) from flower buds (lane B), flowers (lane F), developing seeds (lane S_D), and mature seeds (lane S_M) was hybridized with the ³²P-labeled probe for *DcSUT2* (A). As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used (top).

tato (*StSUT1*) (Riesmeier et al., 1993), *DcSUT1* may be involved in loading of Suc into the phloem of source leaves and retrieval of escaped Suc in the stem. Transport activity appears to be regulated in a diurnal fashion (Kühn et al., 1997) and, as a consequence, we speculate that transport rates may be high during the day and low during the night. This hypothesis fits very well with our recent finding of transient accumulation of starch in tap roots (Sturm et al., 1998). Our data suggest that during the day some of the Suc made in leaves is directly transported into vacuoles of storage parenchyma cells without intermediate cleavage by a cell wall invertase. Any excess is converted into transitory starch in tap root cells surrounding the phloem. During the night, the flow of Suc into storage cells is kept constant by the reconversion of starch into Suc.

High steady-state transcript levels of *DcSUT2* were found only in sink organs such as the growing stems, developing tap roots, flowers, and developing seeds. In tap

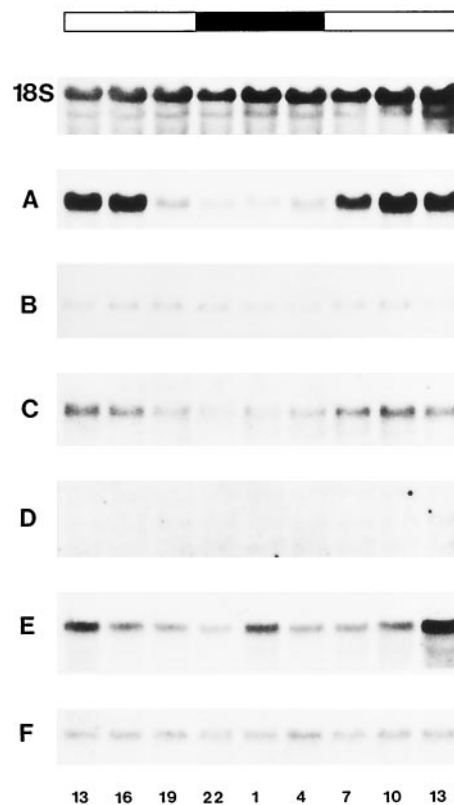


Figure 11. Light-dependent expression of *DcSUT1* and *DcSUT2* mRNA. Carrot plants (12 weeks old) were harvested at 3-h intervals between 1 PM of d 1 and 1 PM of d 2. Total RNA (10 μ g/lane) from entire leaves (leaf lamina and stems) (A, C, and E) and the middle third of tap roots (excluding crown and tip) (B, D, and F) were hybridized on northern blots with a ³²P-labeled probe for the carrot chlorophyll *a/b*-binding protein (A and B), *DcSUT1* (C and D), and *DcSUT2* (E and F). As a probe for a constitutively expressed gene, a fragment of the 18S rRNA gene from tomato was used (only the blot for leaf RNA is shown). The bar at the top shows the light conditions at the time of organ harvest (white bar, daylight; black bar, darkness). The numbers below the RNA gel blots indicate time of organ harvest (13 = 1 PM; 1 = 1 AM).

roots expression was clearly not restricted to the phloem. However, higher mRNA levels were found in phloem and xylem storage parenchyma tissues (Fig. 9). The amino acid sequence of DcSUT2 is closely related to that of a Suc/H⁺ symporter of fava bean (Weber et al., 1997), suggesting that these polypeptides may have a common function. *VfSUT1* is highly expressed in transfer cells of maturing seeds, providing the developing cotyledons with a source of carbon and energy. Likewise, DcSUT2 may import Suc into storage parenchyma cells.

One of the questions still completely open is how Suc is unloaded from the phloem in carrot sink organs. Simple thermodynamics and knowledge of plant cell bioenergetics suggest that the proton-motive-force-driven symporters are never involved in efflux reactions. Thus, either an as-yet-unidentified transporter facilitates the exit of Suc from the phloem or the disaccharide may passively leak out into the apoplast because of the high concentration of Suc.

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The accession numbers for the sequences reported in this article are Y16766 (*DeSUT1a*), Y16767 (*DeSUT1b*), and Y16768 (*DcSUT2*).

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