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Potato Sucrose Transporter Expression in Minor Veins Indicates a Role in Phloem Loading

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The major transport form of assimilates in most plants is sucrose. Translocation from the mesophyll into the phloem for long-distance transport is assumed to be carrier mediated in many species. A sucrose transporter cDNA was isolated from potato by complementation of a yeast strain that is unable to grow on sucrose because of the absence of an endogenous sucrose uptake system and the lack of a secreted invertase. The deduced amino acid sequence of the potato sucrose transporter gene *StSUT1* is highly hydrophobic and is 68% identical to the spinach sucrose transporter *SoSUT1* (pS21). In yeast, the sensitivity of sucrose transport to protonophores and to an increase in pH is consistent with an active proton cotransport mechanism. Substrate specificity and inhibition by protein modifiers are similar to results obtained for sucrose transport into protoplasts and plasma membrane vesicles and for the spinach transporter, with the exception of a reduction in maltose affinity. RNA gel blot analysis shows that the *StSUT1* gene is highly expressed in mature leaves, whereas stem and sink tissues, such as developing leaves, show only low expression. RNA in situ hybridization studies show that the transporter gene is expressed specifically in the phloem. Both the properties and the expression pattern are consistent with a function of the sucrose transporter protein in phloem loading.

INTRODUCTION

Molecular studies of metabolite transport across the plasma membrane of plants have been neglected for many years because of the problems associated with the identification and purification of the respective proteins. Transport processes are central for assimilate allocation and the partitioning of sucrose between different organs of a plant (Gifford et al., 1984). A controversy exists as to how sucrose enters the phloem in exporting leaves. The distribution of plasmodesmata and microscopical studies with fluorescent dyes have provided evidence for symplastic transport (Robards and Lucas, 1990). This concept has been confined to plants displaying a high degree of connectivity between mesophyll and the sieve element companion cell complex (van Bel et al., 1992).

Transport studies with isolated cells and plasma membrane vesicles indicate the presence of carrier-mediated apoplastic transport processes. Sucrose transport activities have been identified in a number of plant species (for review, see Bush, 1993). Transport is active and has been described as a sucrose proton symport. The activity is sensitive to thiol group modifying agents and to diethylpyrocarbonate. Comparison of the transport activity in developing versus mature leaves has shown that the sucrose proton cotransport develops during maturation and gain of export capacity in leaves (Lemoine et al., 1992). So far, a putative candidate for the sucrose transporter protein has been partially purified (Li et al., 1992). In several species, phloem loading occurs against a concentration gradient that is energized by H^+-ATPases that are localized at the phloem plasma membrane (DeWitt et al., 1991). Sucrose synthase, which is present in the phloem, might be involved in catabolism of sucrose to provide ATP as substrate for the H^+-ATPase (Martin et al., 1993). As a first step toward resolving the question of whether protein-mediated sucrose transport represents an essential step in phloem loading, it would be useful to identify the respective genes and proteins.

Complementation of yeast mutants has proven to be an effective tool for the isolation of K^+ channels and amino acid permease genes from plants (Anderson et al., 1992; Sentenac et al., 1992; Frommer et al., 1993). Because of the capability of budding yeast to metabolize sucrose extracellularly, initially, complementation does not seem to be suitable for the isolation of sucrose transporters. A strain that is deficient in secreted invertase but is able to metabolize ingested sucrose due to expression of a sucrose cleaving activity has successfully been used as a complementation system to isolate a sucrose transporter cDNA from spinach (Riesmeier et al., 1992). Moreover, the expression in yeast has allowed us to demonstrate that the characteristics of the transporter are similar to the sucrose transport activity present in protoplasts or plasma membrane vesicles. To understand the role and function of transporters in assimilate partitioning, we have used transgenic plants with altered expression of carrier genes (Riesmeier et al., 1993). To analyze the expression of the carrier and to be able to create transgenic plants with altered transport activity, it is

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necessary to isolate the respective genes from a species accessible for transformation, i.e., from potato. Here, we describe the isolation of a cDNA encoding a sucrose transporter from potato and the biochemical properties of the carrier. To gain insight into the mechanisms of phloem loading in potato, we have studied the tissue-specific expression of the sucrose transporter by RNA gel blot analysis and in situ hybridization.

RESULTS

Construction of a Yeast Expression cDNA Library from Potato

To isolate sucrose carrier–encoding cDNAs from potato, a yeast expression cDNA library from leaves of potato (cv Désirée) was constructed. For this purpose, the inserts of a cDNA library from potato source leaves first established in λ ZAPII (Koßmann et al., 1992) were excised and separated on a preparative agarose gel; the fraction larger than 1 kb was cloned into YEP112A1NE (Riesmeier et al., 1992). The library was amplified in *Escherichia coli* and shown to contain a high proportion of recombinant clones (data not shown).

Transformation of SUSY7 and Selection for Growth on Sucrose

The yeast strain SUSY7 is unable to grow efficiently on sucrose as the sole carbon source because of the lack of secreted invertase and the absence of endogenous sucrose uptake systems. The presence of cytoplasmic sucrose synthase, however, enables the strain to metabolize sucrose intracellularly (Riesmeier et al., 1992). After transformation of SUSY7 with the expression cDNA library, transformants were selected on glucose containing minimal medium yielding ~10^5 primary transformants. The cells were washed from the plates and selected on minimal medium containing 0.5% sucrose as the sole carbon source. Faster growing colonies were isolated, and plasmid DNA was extracted, amplified in *E. coli*, and analyzed. One clone encoding the sucrose transporter from potato *StSUT1* (pP62) with an insert size of ~1.8 kb was further characterized. Transformation of SUSY7 with pP62 showed that growth on sucrose was dependent on the presence of the recombinant plasmid.

DNA Sequence of the Sucrose Transporter from Potato

DNA sequence analysis of *StSUT1* revealed that the cDNA encodes a predicted polypeptide of 516 amino acids, as shown in Figure 1 (55 kD). The cDNA clone contained no poly(A) tail, an artifact previously observed for other cDNAs from this library (Schulz et al., 1993). The large open reading frame starts

```
                                                  I
1    MENGTKREGLG KLTVSSSLQVEQ     PLAPSKLWKIIVVASIAAGVQFGWALQLSLLTPYVQL  StSUT1
1    .AGRNI:N.ENN.: AG...H:.KNPTTP.E.EAT.K.:G:...:.....................  SoSUT1

                        II                                  III
61       LGIPHKFASFIWLCGPISGMIVQPVVGYYSDNCSSRFGRRRPFIAAGAALVMIAVFLIGF  StSUT1
66       .....TW.A:.....:.....R.:.....A:..G....                        SoSUT1

                                IV
121      AADLGHASGDTLGKGFKPRAIAVFVVGFWILDVANNMLQGPCRALLADLSGGKSGRMRTA  StSUT1
126      ...:.A....PT.NVA.....T.....MA:.SQT:T.Y.                       SoSUT1

                    V                                   VI
181      NAFFSFFMAVGNILGYAAGSYSHLFKVFPFSKTKACDMYCANLKSCFFIAIFLLLSLTTI  StSUT1
186      .........:...G.....R.:T....:..A...V.....S.T..:V..I:           SoSUT1

                                                    VII
241  ALTLVRENELP  EKDEQEIDEK  LAGAGKSKVPFFGEIFGALKELPRPMWILLLVTCLNWI  StSUT1
246  ..::.:.RQ:TID.IQ.E. .L.NRNNSS.CA:....Q:I....:..:..L.....A....    SoSUT1

                                        VIII
301      AWFPFFLYDTDWMAKEVFGGQVGDARLYDLGVRAGAMGLLLQSVVLGFMSLGVEFLGKKI  StSUT1
309      .....L.:.....:...:..T..:::...Q..H...L..M:N.....V...S:.G.::M:  SoSUT1

                    IX                                  X
361  GGAKRLWGILNFVLAICLAMTILVTKMAEKSRQ HDPAGTLMGPTP  GVKIGALLLFAALGI  StSUT1
369  .........:.I:..:.....:....S..HF.DS.HIM.:AVP.P.PA...G...A:..V...  SoSUT1

                        XI                                  XII
421      PLAATFSIPFALASIFSSNRGSGQ GLSLGVLNLAIVVPQMLVSLVGGPWDDLFGGGNLPG  StSUT1
429      ...I.............ASS.....................F..:TS....AM.......:  SoSUT1
258      VQTLIPFFA...:NTIDLSV I..T.. .... .:.S.I I:TG:PLIVA.K:L...DGT:  KGP

                                XIII
481      FVVGAVAAAASAVLALTMLPSPPADAKPAVAMGLSIK*                         StSUT1
489      ........T.....SF.L.....P:..IGGS..GH*                          SoSUT1
315      G:A:SSS.:.AVATP:LIAEMV..F .. ..P:A:TLVATSVIVTSVLVPIIT         KGP
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Figure 1. Comparative Sequence Data.

Comparison of the amino acid sequences of the sucrose transporters StSUT1 (potato), SoSUT1 (spinach; Riesmeier et al., 1992), and the 2-keto-3-deoxygluconate permease (KGP; *Erwinia*, Allen et al., 1989). The translation stop is marked by asterisks, and putative membrane-spanning regions are overscored. Dots indicate identical amino acids, and colons stand for similar amino acids (L≅I≅V, S≅T, R≅K, G≅A, F≅Y, E≅D, N≅Q).

three nucleotides in front of the first ATG, which is located at the position corresponding to the presumed translation start of the spinach sucrose transporter cDNA *SoSUT1* (Riesmeier et al., 1992). Thus, pP62 (*StSUT1*) contains the entire coding region, but both the 5′ and 3′ ends of the transcript are missing. No extensive homologies were found to other proteins in the data base. *StSUT1* is 60% homologous on the DNA and 68% (83%) identical (similar) on the protein level to the spinach sucrose transporter. The sequence homology is in a similar range as found for the triose phosphate translocators from spinach and potato (Schulz et al., 1993). The regions of highest conservation are the membrane-spanning domains, whereas major differences are located in the N-terminal sequence preceding the first potential membrane-spanning domain and in the large hydrophilic loop in the center of the protein. Potential N-linked glycosylation sites are located at positions 3 and 92 in hydrophilic regions in front of the first and the third putative membrane-spanning domain. A third potential N-linked glycosylation site located in SoSUT1 (position 272) is not present in StSUT1. At position 167, the potato protein displays homologies to the nucleotide binding motif characteristic for ATP binding proteins involved in active transport (Higgins et al., 1990). However, no such homology could be found in SoSUT1. The hydropathy plot of the predicted protein reveals the presence of 12 hydrophobic segments. A weak homology was found

around the putative membrane-spanning region XI to the 2-keto-3-deoxygluconate permease from *Erwinia* (Allen et al., 1989). The deduced protein sequences of the complete polypeptides are 53% similar and 22% identical according to a BESTFIT analysis (Figure 1; Devereux et al., 1984). The bacterial transporter might therefore represent a distant relative of the plant sucrose transporter.

StSUT1 Mediates Sucrose Transport Activity

Uptake experiments with yeast cells expressing the StSUT1 protein demonstrate that ^{14}C-sucrose is transported by the protein with a K_m for sucrose of ~1 mM. In contrast to α-phenylglucoside, the sugars palatinose, raffinose, trehalose, and lactose do not significantly compete for sucrose uptake at a 10-fold excess, as shown in Table 1 (and data not shown). Maltose shows only marginal inhibition in the case of StSUT1 as opposed to SoSUT1 (Riesmeier et al., 1992). Direct uptake measurements demonstrate that StSUT1 still mediates maltose uptake, although with a K_m value of 10 mM, and has an approximately twofold lower affinity for maltose as compared to SoSUT1. Protonophores, such as carbonyl cyanide *m*-chlorophenylhydrazone and 2,4-dinitrophenol, strongly inhibit sucrose transport, and sucrose uptake is stimulated by decreasing the pH. This argues for active transport and is indicative of a proton symport mechanism (Table 1). StSUT1 is highly sensitive to thiol modifying agents, such as *p*-chloromercuribenzenesulfonic acid and *N*-ethylmaleimide, and to diethylpyrocarbonate (Table 1). Thus, when expressed in yeast, the transport characteristics of the potato sucrose carrier are very similar to those of sucrose/proton cotransporters described in protoplasts and vesicles of a variety of plant species and the spinach sucrose carrier (Riesmeier et al., 1992; Bush, 1993).

Table 1. Specificity of the Sucrose Carrier StSUT1 and Sensitivity of Sucrose Transport to Inhibitors

Inhibitor	Concentration	% Activity
Sucrose	0.2 mM	100
Sucrose	2 mM	40
Maltose	2 mM	90
α-Phenylglucoside	2 mM	8
Phloridzin	2 mM	13
Carbonyl cyanide *m*-chlorophenylhydrazone	10 μM	9
2,4-Dinitrophenol	100 μM	3
p-Chloromercuribenzenesulfonic acid	100 μM	20
N-Ethylmaleimide	1000 μM	22
Diethylpyrocarbonate	500 μM	6

Inhibition of sucrose uptake of the yeast strain SUSY7-StSUT1 by different substances, which were added to activated cells 30 sec prior to the addition of labeled sucrose. The 100% value corresponds to 45 pmol of sucrose per min per mg yeast cells.

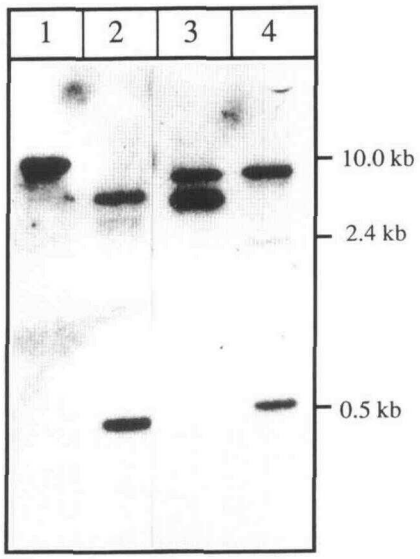

Figure 2. DNA Gel Blot Analysis of Genomic Potato DNA.

Ten micrograms of genomic DNA, which were isolated from the dihaploid potato cultivar 22/14, were digested with different restriction enzymes (lane 1, Asp718; lane 2, BamHI; lane 3, XbaI; lane 4, EcoRI), separated by gel electrophoresis, transferred to a nylon membrane, and hybridized to the 1.8-kb potato sucrose transporter cDNA *StSUT1*. The lengths are given in kilobases to the right of the autoradiograph.

Sucrose Transporter Genes

Gel blot analysis of genomic DNA of the dihaploid potato line 22-14 under stringent conditions shows that the *StSUT1* cDNA hybridizes to two sets of bands. The major bands probably derive from a single copy of the *StSUT1* gene, whereas the weak bands might be due to a second related gene, as shown in Figure 2. A single major band of 10 kb and a minor band of 9 kb were found in the case of restriction with Asp718. For BamHI, for which one internal site is present in the cDNA, two strong bands and one weak band are detectable. For EcoRI, for which two sites are located in the cDNA, two major bands (0.5 and 9 kb) and one minor band (2.4 kb) were found. Therefore, we concluded that under stringent conditions, two related loci are detectable in potato. We have isolated genomic clones corresponding to the second weaker hybridizing locus. Sequence analysis shows that this clone probably encodes a pseudogene. A stretch of high homology starts at amino acid 12 in relation to the start codon in *StSUT1* but stops nine amino acids further downstream. Four frameshifts then follow, and the adjacent homologous region contains several stop codons (J.W. Riesmeier and W.B. Frommer, unpublished observation).

Transporter Is Expressed in an Organ-Specific Manner

Gel blot analysis of total RNA from mature leaves indicates a single 2000-nucleotide transcript hybridizing with *StSUT1* cDNA, as shown in Figure 3. The gene is highly expressed in mature source leaves, whereas only low amounts of transcript were found in developing sink leaves or in leaves of plants grown in tissue culture (Figure 3A and data not shown). The expression in stems and in sink tissues, such as roots and tubers, was always significantly lower as compared to source leaves (Figure 3B). Nevertheless, the expression in sink organs is variable (data not shown). Both tubers and roots of potato develop photosynthetic capacity when exposed to light. Light penetrating the soil has been shown to affect gene regulation and might, therefore, be responsible for the variability observed (Mandoli et al., 1990).

Tissue Specificity of the Sucrose Transporter

To analyze the tissue specificity of the sucrose transporter in potato leaves, leaf material around the third-order veins was fixed, embedded in paraffin, and sectioned. The sections were hybridized to ^{35}S-labeled sense and antisense transcripts of the sucrose transporter cDNA *StSUT1*. Microautoradiographs were developed after 3 to 10 days of exposure. In the case of the antisense probe, a high number of silver grains accumulated at the minor abaxial phloem strands located underneath the xylem vessels, as shown in Figure 4. The expansion of label predicts that several cells in the sieve element companion cell complex express the carrier; however, the resolution is not sufficient to determine exactly which cell types in the phloem are involved. No significant accumulation of silver grains was found in xylem, parenchyma, or epidermis. Controls hybridized with the sense probe show only a weak background similar to that for the antisense probes, but no specific staining in the vascular bundles (data not shown). Therefore, we concluded that the sucrose transporter *StSUT1* is expressed to high levels specifically in the phloem.

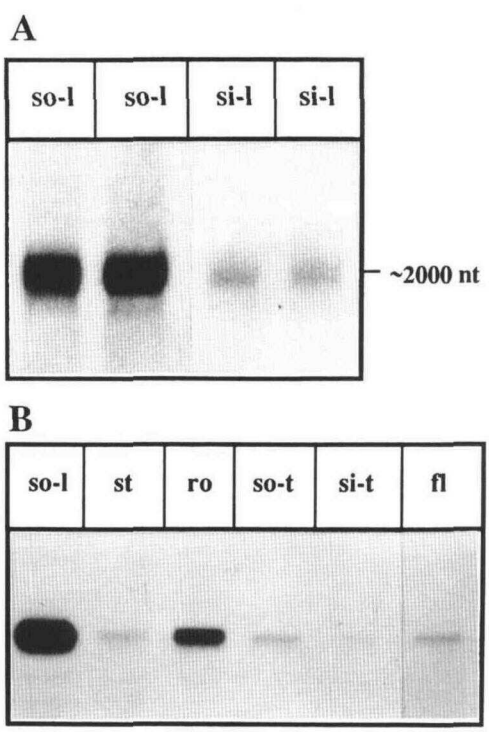

Figure 3. RNA Gel Blot Analysis of the Expression of the Sucrose Transporter Gene *StSUT1* in Different Organs of Potato.

RNA was separated on a 1.2% formaldehyde gel, blotted to a nylon membrane, and hybridized to the radiolabeled *StSUT1* cDNA.
(A) RNA from developing (sink; si-l) and mature (source; so-l) leaves of two independent potato plants. nt, nucleotides.
(B) RNA from mature leaves (so-l), stems (st), roots (ro), sink (si-t) and source (so-t) tubers, and flowers (fl).

DISCUSSION

Comparison of the Sucrose Transporters from Spinach and Potato

Yeast complementation has allowed us to identify sucrose transporters from both spinach and potato (this study; Riesmeier et al., 1992). The similarity of primary structure and biochemical properties might indicate that the proteins serve related functions in the two species. The difference in sequence must therefore represent the evolutionary distance between spinach and potato and does not seem to be relevant for the overall function. The areas with the highest variability are the N- and C-terminal extensions and the large central loop, whereas the putative membrane-spanning regions are highly conserved. Although no sequence homologies were found to the prototype of sugar transporting proteins, the lactose permease from *E. coli*, the carriers seem to be related not only in being disaccharide transporters but also in their structure, with 12 membrane-spanning regions separated by a large central loop (Kaback, 1992). Interestingly, RXGRR motifs are located in the hydrophilic loops 3 (RFGRR) and 9 (FLGKK) of the potato sucrose transporter, and, thus, they are in a similar position as in lactose permease and several other monosaccharide transporters (Henderson, 1990). A low homology was also found to the bacterial ketogluconate transporter (Figure 1; Allen et al., 1989).

The biochemical properties of the sucrose transporters from potato and spinach are very similar with respect to pH dependence and inhibition of transport by protonophores, thiol modifying agents, and to diethylpyrocarbonate. The K_m value of the potato carrier for sucrose is estimated to be ~1.0 mM at pH 4.5, and the specificity toward other derivatives of sucrose strongly reflects the data described for sucrose carriers of other plant systems, as determined in protoplasts or membrane vesicles (for review, see Bush 1993).

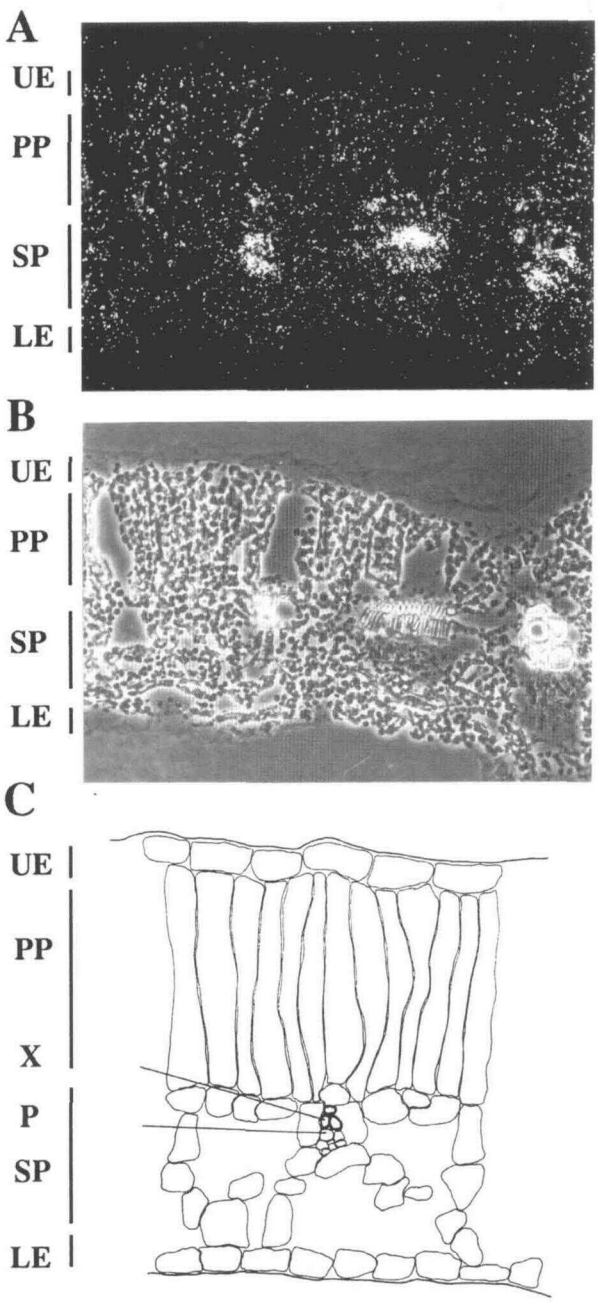

Figure 4. In Situ Localization of the Sucrose Transporter mRNA in Potato Leaves.

Hand-cut sections of material flanking third-order veins were fixed and embedded in paraffin; 16-μm sections were hybridized to ^{35}S-labeled *StSUT1* antisense RNA, washed, and microautoradiographed. UE, upper epidermis; PP, palisade parenchyma; SP, spongy parenchyma; LE, lower epidermis; X, xylem; P, abaxial sieve element companion cell complex.

(A) Dark-field microscopy of a cross-section (225-fold magnification).

(B) Phase contrast microscopy of the identical cross-section as shown in **(A)**.

(C) Schematic drawing (not to scale) of a cross-section through a potato leaf around minor veins.

Regarding substrate specificity, the sucrose transporters from spinach and potato also mediate maltose uptake, although with substantially different affinity. Interestingly, lactose permease can be mutated into a maltose or sucrose transporter (Markgraf et al., 1985; King and Wilson, 1990). Sucrose and maltose are both α-glycosides that share glucose as a common structure. For the sucrose carrier, the glucose moiety seems to be essential for function, whereas the fructose moiety can be exchanged by a variety of different residues, e.g., phenyl moieties (Maynard and Lucas, 1982; Hitz et al., 1986; Hecht et al., 1992). Also in this respect, similar structural requirements were found for lactose permease (Sanderman, 1977). For further studies, the yeast expression system provides a simple system to determine structure–function relationships.

In plants, the proton gradient is the major driving force for secondary transport across the plasma membrane and is generated mainly by H^+-ATPases (Humphreys, 1988). Proton substrate symport has been described as a mechanism for secondary active transport of sucrose, glucose, and amino acids (Komor et al., 1978; Sauer et al., 1990; Bush, 1993; Frommer et al., 1993). The inverse correlation of the pH of the medium and the transport activity in conjunction with the sensitivity to protonophores and metabolic inhibitors are taken as indications that SoSUT1 and StSUT1 are symporting protons. This suggests that the sucrose transporters are involved in *active transport and, thus, in phloem loading.*

Evidence for a Role in Phloem Loading

Several lines of evidence indicate that the sucrose transporter is involved in phloem loading. Both the sucrose gradient between mesophyll cells and phloem and the low symplastic connectivity in potato leaves argue that active loading of the phloem is carrier mediated (McCauley and Evert, 1989; van Bel et al., 1992). Strong support for the involvement of an apoplastic step in phloem loading is the effect of ectopic expression of an invertase activity in the cell wall of potato, tobacco, and Arabidopsis (von Schaewen et al., 1990; Sonnewald et al., 1991; Heineke et al., 1992). The strong effects on the phenotype and carbon partitioning can only be explained if the main route for phloem loading is apoplastic. The presence of the putative sucrose proton symporter at the phloem membrane responsible for import of sucrose from the cell wall into the sieve element companion cell complex has been proposed previously (Riesmeier et al., 1992). Evidence that this activity is identical to StSUT1 comes from the biochemical properties of the carrier, as described above, and from an analysis of the expression.

The sucrose transporter is present to high levels only in mature, exporting leaves. The expression follows the sink-to-source transition of leaves, and, thus, it correlates with the observation that active sucrose transport activity in leaves develops upon maturation (Lemoine et al., 1992). A number of polypeptides similar in size and pI range, as calculated for

sucrose carriers, are specifically expressed in source leaves (W. B. Frommer and S. Delrot, unpublished data). Further support for a role of StSUT1 in phloem loading comes from RNA in situ hybridizations that could localize the expression of the sucrose carrier to the minor veins of the phloem (Figure 4). Further experiments will be necessary to define which cell types in the phloem express the carrier protein in their membranes. A new question arises with respect to how sucrose, in a first step, enters the apoplast. Four models are conceivable: export from the mesophyll occurs along the sucrose concentration gradient by passive leakage across the membrane, by facilitated diffusion, by sucrose proton antiport, or by sucrose symport against the proton gradient. The proton–symport model is also conceivable, because the concentration of sucrose in the apoplast is extremely low (G. Lohaus, H. Winter, B. Riens, and H. W. Heldt, unpublished data). Alternatively, the export from the mesophyll could be mediated by a sucrose proton antiporter. The use of the *StSUT1* cDNA as a probe or the complementation system might be tools to identify this proposed second transport system.

Potential Function of the Carrier in Other Tissues

In contrast, sucrose proton symporters have been proposed to be responsible for retrieval of sucrose leaking from the phloem along the path from source to sink (Maynard and Lucas, 1982). The fact that *StSUT1* expression is lower in stems as compared to mature leaves argues against a sole function in retrieval.

Apart from this, the carrier might also play a role in unloading in sink tissues. In sink tissues, such as roots and tubers, the expression of *StSUT1* is reduced. The route of unloading is different between different species or even in organs within one plant. Developing leaves are supposed to unload symplastically, as may be the case for developing tubers. Nevertheless, sucrose transport activity that is sensitive to *p*-chloromercuribenzenesulfonic acid was found in tubers (Wright and Oparka, 1989). The low but significant expression levels in sink tissues are taken as an indication that the transporter is also present and possibly active during unloading. A more detailed expression analysis of both *StSUT1* RNA and protein at the cellular level, e.g., by immunolocalization, should give further clues on this issue. However, the current data do not allow us to conclude that StSUT1 is involved in unloading, because symplastic transport coexists in sink tubers (Oparka et al., 1993). The complementation system might be a tool to search for other sucrose transporters present in sink tissues.

A sink organ in which apoplastic transport is highly probable is the seed, in which at least the embryo is symplastically isolated from the maternal tissue and assimilates have to pass the apoplastic space to enter the developing seeds (for review, see Thorne, 1985). A detailed analysis of the expression might give hints as to the role of the sucrose carrier in this process. Finally, both seeds and tubers undergo a sink-to-source transition that is accompanied by a reversion in the orientation of transport and the accumulation of a new set of proteins (Borgmann et al., 1991). Amino acid transporters that are expressed specifically during both the import and export phase have been identified (Frommer et al., 1993; Kwart et al., 1993). Nitrogen accumulation in seeds seems to be closely linked to amino acid and sucrose export from the leaves (Barneix et al., 1992). A reduction in sucrose import might, therefore, affect the supply of sink organs with amino acids. Parallel studies of the expression of sucrose and amino acid transporters should enable better understanding of assimilate partitioning in higher plants.

Together, the indications for H^+ symport, the correlation of transcript accumulation during the development of leaves, and the phloem-associated expression strongly argue for a role of the sucrose transporter primarily in phloem loading, but they do not exclude that the protein is also involved in sucrose retrieval on the translocation path and in unloading in the sink. Antisense repression of carrier genes has proven to be an excellent tool to study the in vivo role and function of transporters (Riesmeier et al., 1993). The availability of sucrose and amino acid carrier genes together with the possibility to create transgenic plants in which the expression of these carriers is modulated by expression of antisense RNA or by ectopic overexpression will undoubtedly result in new insights into the role that the sucrose carriers play for assimilate allocation and partitioning in higher plants. Transgenic potato plants with reduced *SUT1* mRNA levels due to expression of an antisense gene show a dramatic increase in leaf carbohydrate content and reduced development of roots and tubers, which is consistent with the assumption that the sucrose transporter StSUT1 is essential for phloem loading (J.W. Riesmeier, L. Willmitzer and W.B. Frommer, unpublished data).

METHODS

Bacteria and Yeast

The following bacterial and yeast strains were used: DH5α (*supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) and SUSY7, a derivative of YSH 2.64-1A (Gozalbo and Hohmann, 1990) expressing sucrose synthase under the control of the alcohol dehydrogenase-1 promoter. The uptake rates of yeast strains for ^{14}C-sucrose and the sensitivity of transport to inhibitors were measured as described by Riesmeier et al. (1992).

Plant Material

Solanum tuberosum cv Désirée was obtained through Mamerow (Berlin), and the cultivar 22/14 came from the Max Planck Institut für Züchtungsforschung (Cologne). Plants were grown in soil in the greenhouse under a 16-hr-light (20°C)/8-hr-dark (15°C) regime. Material was harvested after plants had been exposed to light for 4 to 6 hr. Sink leaves were defined as top leaflets with a size smaller than 1 cm; source leaves were defined as top leaflets from mature pinnules. Tubers were harvested in the sink stage with a diameter of ~2 cm, as defined by Borgmann et al. (1991).

Recombinant DNA Technology

The source leaf cDNA library in λ ZAPII was a gift from J. Koßmann (Koßmann et al., 1992). Recombinant phages (1.5×10^6) were plated and washed from the plates; DNA was prepared on CsCl–sarcosyl gradients (Buckley and Goding, 1988). The inserts were excised with NotI and separated on a 1% agarose gel. Inserts longer than 1.0 kb were excised and eluted. Inserts were cloned into the yeast expression plasmid YEP112A1NE (Riesmeier et al., 1992), which were previously digested with NotI and treated with alkaline phosphatase. Approximately 40,000 clones were obtained (>90% inserts). Colonies were washed from the plates, and plasmids were isolated. SUSY7 was transformed with the yeast expression cDNA library from potato (Dohmen et al., 1991) and plated on standard medium (SD) containing 2% glucose; $\sim 10^4$ transformants per μg of DNA were obtained. The cells were washed in 10 mL of SD from plates, and 10 μL of the transformed cells were plated on solid SD supplemented with 0.5% sucrose. Faster growing colonies were isolated after incubation for 4 days at 30°C. DNA sequence analysis was performed using ExoIII deletions and subclones in conjunction with T7 polymerase (Pharmacia, Sweden). The reported DNA sequence will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession number X69165. All other procedures were performed according to the methods of Sambrook et al. (1989). Computer analysis was performed using the UWGCG programs (Devereux et al., 1984).

Expression Analysis

RNA was isolated according to the method of Logemann et al. (1987), and RNA filters were hybridized to the 1.8-kb *StSUT1* cDNA. In situ hybridization was conducted essentially as described by Cox and Goldberg (1988). Mature leaves were harvested from greenhouse-grown potato plants. The area around third-order veins was excised using a razor blade. The best results for in situ hybridization signals were obtained when material was fixed in 3.7% formaldehyde, 5% acetic acid, and 50% ethanol, as compared to fixation in 1% glutaraldehyde or 4% formaldehyde, 0.25% glutaraldehyde. After fixation, the tissue was embedded in Paraplast, cut into 10- to 16-μm sections, and mounted on poly-L-lysine–coated slides. Prior to hybridization, the sections were soaked in xylene to remove the Paraplast and hydrated with an ethanol series. To reduce background, the sections were incubated in 1% BSA. To increase the permeability of the tissue, the slides were incubated for 30 min in 1 μg/mL proteinase K at 37°C. For further reduction of background, the sections were acetylated by incubation in 0.25% acetic anhydride.

Subsequently, the sections were hybridized to ^{35}S-labeled antisense RNA of *StSUT1*. Sense transcripts were used as controls. Radiolabeled RNA of *StSUT1* was produced with T3 or T7 polymerase according to the instructions for the RNA labeling kit (Stratagene) using linearized pBluescript SK– containing the complete cDNAs of *StSUT1*. The length of the transcripts was determined after separation on agarose gels and transfer of the RNA to nylon membranes by autoradiography. The RNAs were hydrolyzed to an average length of 0.2 kb and hybridized to the sections for 12 to 16 hr at 50°C in a solution containing 50% formamide, 300 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 mM DTT, 25 units per mL of RNasin, 500 μg/mL poly(A) RNA, and 150 μg/mL tRNA, with ~200 ng/mL of probe per kilobase probe complexity. Following hybridization, the slides were incubated in 4 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 5 mM DTT, and single-stranded RNA was digested for 30 min at 37°C with 50 μg/mL RNase A and washed with 0.1 × SSC, 1 mM DTT at 48°C for 1 hr. The material was dehydrated and dipped in *NTB*-2 track emulsion (Kodak). After 3 to 10 days of exposure, the slides were developed and analyzed microscopically. No preferential accumulation of silver grains was found when sense probes were used. A slightly higher background was observed on the tissue sections as compared to neighboring regions of the slide. The results shown have been reproduced several times independently.

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