The Sucrose Transporter *StSUT1* Localizes to Sieve Elements in Potato Tuber Phloem and Influences Tuber Physiology and Development[1[w]]

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The sucrose (Suc) H^+-cotransporter *StSUT1* from potato (*Solanum tuberosum*), which is essential for long-distance transport of Suc and assumed to play a role in phloem loading in mature leaves, was found to be expressed in sink tubers. To answer the question of whether SUT1 serves a function in phloem unloading in tubers, the promoter was fused to *gusA* and expression was analyzed in transgenic potato. SUT1 expression was unexpectedly detected not in tuber parenchyma but in the phloem of sink tubers. Immunolocalization demonstrated that StSUT1 protein was present only in sieve elements of sink tubers, cells normally involved in export of Suc from the phloem to supply developing tubers, raising the question of the role of SUT1 in tubers. *SUT1* expression was inhibited by antisense in transgenic potato plants using a class I patatin promoter B33, which is primarily expressed in the phloem of developing tubers. Reduced *SUT1* expression in tubers did not affect aboveground organs but led to reduced fresh weight accumulation during early stages of tuber development, indicating that in this phase SUT1 plays an important role for sugar transport. Changes in Suc- and starch-modifying enzyme activities and metabolite profiles are consistent with the developmental switch in unloading mechanisms. Altogether, the findings may suggest a role of SUT1 in retrieval of Suc from the apoplasm, thereby regulating the osmotic potential in the extracellular space, or a direct role in phloem unloading acting as a phloem exporter transferring Suc from the sieve elements into the apoplasm.

In Solanaceae, phloem loading has been extensively investigated, whereas relatively few studies focused on the molecular processes responsible for phloem unloading in sink organs (Lalonde et al., 1999). Several possible routes exist for Suc exit from the phloem, i.e. apoplasmic or symplasmic routes (Oparka et al., 1992). In the case of apoplasmic unloading, the first step must be the export of Suc from the sieve element companion cell complex (SECCC) by mechanisms that are currently not understood. Three different routes can be envisaged for the subsequent uptake into storage parenchyma: (a) import by Suc uptake carriers, (b) cleavage of Suc by an apoplasmic invertase and subsequent uptake by hexose transporters (Frommer and Sonnewald, 1995; Giaquinta, 1977) or (c) uptake by endocytosis as indicated by studies using fluorescent dyes (Oparka and Prior, 1988).

The efficiency of phloem unloading, whether it occurs symplasmically or apoplasmically, is strongly related to the sink strength of an organ. The sink strength of an organ is defined as the ability of this organ to attract photoassimilates (Ho, 1988), and the sink strength of growing potato (*Solanum tuberosum*) tubers is believed to be limited by Suc metabolism and/or starch synthesis (Zrenner et al., 1995). Thus, the removal of Suc from the equilibrium by sucrolytic enzymes is an important factor in determining the sink strength.

Symplasmic connectivity between the conducting phloem cells and the storage parenchyma cells of potato tubers indicates that, in principle, symplasmic phloem unloading is also possible (Van Bel, 1992; Frommer and Sonnewald, 1995). Confocal imaging of fluorescent dye movement in Arabidopsis root tips (Oparka et al., 1994) and unloading of a green fluorescent protein in tobacco (*Nicotiana tabacum*) plants (Imlau et al., 1999; Oparka et al., 1999) provided compelling evidence for symplasmic unloading pathways also into other heterotrophic tissues.

In stolons showing the first detectable subapical swelling, the membrane impermeant tracer carboxyfluorescein remains confined to the phloem strands,

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whereas ^{14}C unloading was detected on autoradiographs of the swelling stolons, indicating that apoplasmic phloem unloading takes place. In visibly swollen stolons, the phloem unloading occurs symplasmically as shown by dye movement from the phloem into parenchyma tissues (Viola et al., 2001).

Thus, in very early stages of tuber development, i.e. during the elongation phase of stolon growth, apoplasmic Suc unloading predominates. The occurrence of a switch from apoplasmic to symplasmic phloem unloading in tuberizing stolons is supported by two very strong arguments. In swelling stolons, a marked decline in invertase activity indicates that a switch from the invertase-sucrolytic pathway to a Suc synthase-sucrolytic pathway occurs in parallel (Ross et al., 1994; Appeldorn et al., 1997). This metabolic switch would be compatible to apoplasmic phloem unloading in swelling stolons, which can be followed by Suc transporter-mediated, hexose transporter-mediated, or endocytotic import into the parenchyma cells. The important contribution of apoplasmic unloading is underlined by expression of a yeast invertase in the apoplasm of potato tubers, which led to an increase in tuber size and a decrease in tuber number (Heineke et al., 1992; Sonnewald et al., 1997), indicating that during unloading, significant amounts of Suc are released into the apoplasmic space.

Additional support comes from biphasic Suc uptake kinetics, the saturable component of which is sensitive to para-chloromercuribenzenesulfonic acid, an effective inhibitor of protein-mediated membrane transport that has been detected in potato tubers (Wright and Oparka, 1989), arguing for two different and independent Suc uptake mechanisms in tubers.

The cleavage of Suc in sink tissues not only creates a steeper chemical gradient but also helps to maintain the difference of the osmotic potential between the SECCC and the storage parenchyma cells. In sink organs, such as developing seeds, Suc uptake is controlled by a turgor homeostat (Patrick, 1997). Turgor-dependent efflux is part of the homeostat mechanism regulating seed coat turgor. This mechanism is able to integrate assimilate demand by the cotyledons with assimilate import into and unloading from the coat. Turgor has also been suggested to be important as a regulator of plasmodesmal function, thereby indirectly influencing symplasmic phloem unloading processes (Oparka and Prior, 1992).

To address the relative contribution of the apoplasmic pathway to phloem unloading in developing potato tubers, localization and the physiological role of the Suc proton symporter SUT1 were investigated in potato tubers. Besides being expressed in mature exporting leaves, low mRNA levels of the Suc transporter *StSUT1* were found in sink tissues such as potato tubers, roots, or young sink leaves. Using promoter reporter gene fusions and immunolocalization, SUT1 is shown to be present in sieve elements of sink tubers. The function in tubers was analyzed by antisense repression of *StSUT1* using the patatin class I promoter B33, which is highly expressed in vascular tissue at early stages of tuber development (diameter less than 5 mm) and in both vascular and parenchyma tissue during later stages of development (Liu et al., 1991). Reduced tuber yield and alterations in metabolite content indicate that during early stages of tuber development, SUT1 plays a crucial role in the unloading process, consistent with the suggestion that an impact of SUT1 on tuber initiation or early tuber development, but not later during tuber growth, when unloading is thought to occur predominantly symplasmically (Viola et al., 2001).

RESULTS

In solanaceous species, i.e. potato, tomato (*Lycopersicon esculentum*), and tobacco, four Suc transporter genes, SUT1 to -4, have thus far been identified. These transporters fall into three phylogenetically defined groups: SUT1 and SUT3 (which are 64.9% identical) and SUT2 and SUT4 forming individual subgroups. *NtSUT3* was expressed exclusively in pollen (Lemoine et al., 1999), whereas all of the other solanaceous Suc transporter-like proteins were immunolocalized to the plasma membrane of sieve elements (Kühn et al., 1997; Barker et al., 2000; Reinders et al., 2002a). These four genes seem to be the only SUT genes present in the genomes, because public databases contain 18 expressed sequence tags (ESTs) from potato (from EST libraries derived from leaves, petioles, *Phytophtera infestans*-challenged leaves, roots, stolons, and tubers) and 22 ESTs from tomato and *Lycopersicon pennelli* (from EST libraries derived from radicles, flower buds, ovaries, seedlings, trichomes, callus, and elicitor-treated leaves), when SUT1 was entered as query (BLAST search of GenBank from March 28, 2002). All 18 ESTs from potato were at least 99% identical to *StSUT1*, and all 22 ESTs from tomato corresponded to LeSUT1 (GenBank accession no. AF176638). Furthermore, using various methods including low stringency hybridization, we were not able to identify additional Suc transporter genes (data not shown).

Expression of SUT1 in Leaves and Tubers

RNA gel-blot analysis revealed low levels of SUT1 expression of SUT1 in sink tubers, potentially indicating a role in the unloading process (Fig. 1). To address the question of whether SUT1 is localized in storage parenchyma or in the phloem, the cellular expression pattern of SUT1 was analyzed using a SUT1-promoter-β-glucuronidase (GUS) fusion. The attempt to isolate the promoter from potato failed because of the potential toxicity of the Suc transporter to *Escherichia coli* (B. Hirner and W.B. From-

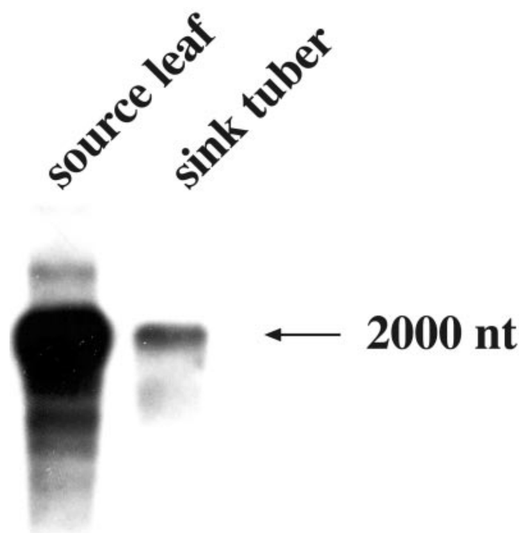

Figure 1. RNA gel-blot analysis of the tissue-specific expression of the Suc transporter *StSUT1* in wild-type potato plants. RNA from mature leaves and from sink tubers (12 μg lane^{-1}) was hybridized under highly stringent conditions to the 1.3-kb fragment of the *StSUT1* cDNA.

mer, unpublished data). The high similarity of SUT1 proteins from potato and tomato (94.3% identity) together with the identical localization and regulation indicate that the genes are orthologous (Riesmeier et al., 1993; Kühn et al., 1997). Therefore, a genomic SUT1 clone was isolated from a tomato library. The promoter region was sequenced, and a 1.7-kb fragment was fused with the GUS reporter gene. Ten of 54 transgenic potato lines showed GUS activity in the veins, although the promoter seemed to lack enhancer sequences because only two plants showed high GUS activity, whereas eight lines had low GUS expression levels. All other regulatory elements seem to be present, because as expected from RNA gel-blot experiments, GUS expression was low in sink leaves, induced in the veins of source leaves, and present in stem phloem (Fig. 2). The same expression pattern was observed using a transcriptional GUS fusion, but again only 20% of the plants were GUS positive (B. Hirner, unpublished data). The same observation was made in transgenic tomato plants where 21% of the transformed lines showed staining of leaf veins (B. Hirner, unpublished data). In developing potato tubers, GUS expression was found exclusively in the vasculature, indicating that SUT1 is not involved in loading of the parenchyma cells but rather plays a role in the phloem (Fig. 2, E and F). The GUS analysis was verified by tissue prints using affinity-purified StSUT1-specific antibodies (Kühn et al., 1997; Fig. 3A).

Localization of SUT1 in Sink Tubers

To analyze whether SUT1 is expressed in sieve elements or in other cells of the vasculature, the protein was immunolocalized on semithin sections of embedded material (Fig. 3, B–D). Similar to what had been described for source leaves, StSUT1 was found in sieve elements of sink tubers (Kühn et al., 1997). Thus, in sink tubers, SUT1 is not present in storage parenchyma or in other importing cells, but in the actual conduits for Suc transport, the sieve tubes. Because sieve elements at their mature state are enucleate, the protein probably derives from mRNA produced in tuber companion cells.

Tuber-Specific Antisense Inhibition of *StSUT1*

To analyze the potential role of SUT1 in sink tuber phloem, plants were transformed with an *StSUT1* antisense construct under control of the tuber-specific B33 patatin promoter (αSUT1-T). Seven of 60 transformants showed reduced levels of *StSUT1* mRNA in tubers (Fig. 4A). In addition, smaller RNA species were detected that were not present in wild-type tubers. These RNAs may be attributable to the presence of antisense transcripts using alternative termination signals or may represent degradation products as found in cosuppression (Metzlaff et al., 2000). Because antisense repression of SUT1 from tobacco was not possible with a construct using an 85% identical potato gene, it is highly improbable that SUT1 antisense in potato tubers affects expression of StSUT2 or StSUT4 that is less than 50% identical to StSUT1 directly (Bürkle et al., 1998).

Phenotype of SUT1 Antisense Plants

In five independent experiments, none of the seven transformants showed any detectable changes in the phenotype of the aerial parts of the plant. Because inhibition of Suc transporter expression in leaves would lead to a clearly visible phenotype (Riesmeier et al., 1994; Kühn et al., 1996; Bürkle et al., 1998), Suc export from leaves seems unaffected in the case of tuber-specific antisense (Fig. 4C). However, in all five experiments, tuber yield was significantly reduced when tubers were harvested after 2 months in the greenhouse (Table I), suggesting that SUT1 expression in tuber veins is relevant for tuber development.

Because the tuber-specific patatin B33 promoter is inducible by Suc in leaves (Rocha-Sosa et al., 1989), additional experiments were carried out to exclude effects of B33-mediated inhibition of SUT1 activity in leaves. However, SUT1 transcript levels were not changed significantly in leaves from antisense plants (Fig. 4B; differences in signal intensity correspond to differences in loading). Furthermore, no patatin mRNA was detectable in leaves of transgenic plants (Fig. 4B). Hence, at the stages analyzed and under the conditions used, the activity of the endogenous patatin promoter was not induced in the transgenic plants, allowing the conclusion that antisense inhibition is tuber specific.

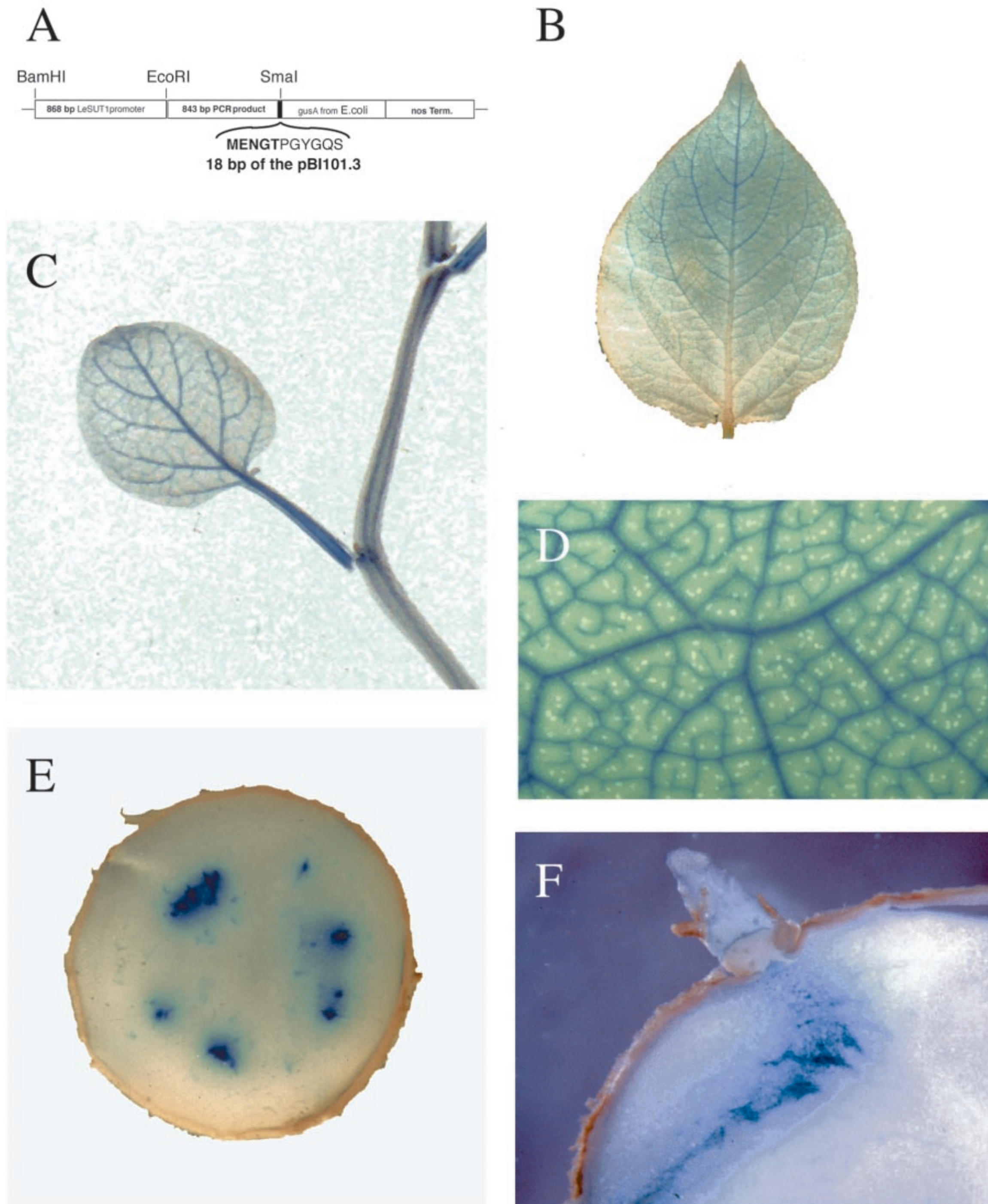

Figure 2. The GUS reporter gene expressed under control of the *LeSUT1* promoter fragment in transgenic potato plants. A, Translational GUS fusion construct used for transformation. B, *LeSUT1* promoter activity follows the sink to source transition in leaves. C, *LeSUT1* promoter activity is restricted to the vasculature of mature leaves and petioles. GUS expression is detectable in major and minor veins of source leaves (D), sink tubers (E), and sprouting source tubers (F).

Effects of SUT1 Inhibition on Tuber Metabolism

More detailed yield analyses demonstrated that tuber yield of antisense plants depends on the developmental stage. Because tubers are supposed to switch between apoplasmic and symplasmic unloading, distinct stages of tuber development were defined. The switch from apoplasmic to symplasmic phloem unloading occurs during stolon-to-tuber-transition of potato (Viola et al., 2001), and, thus,

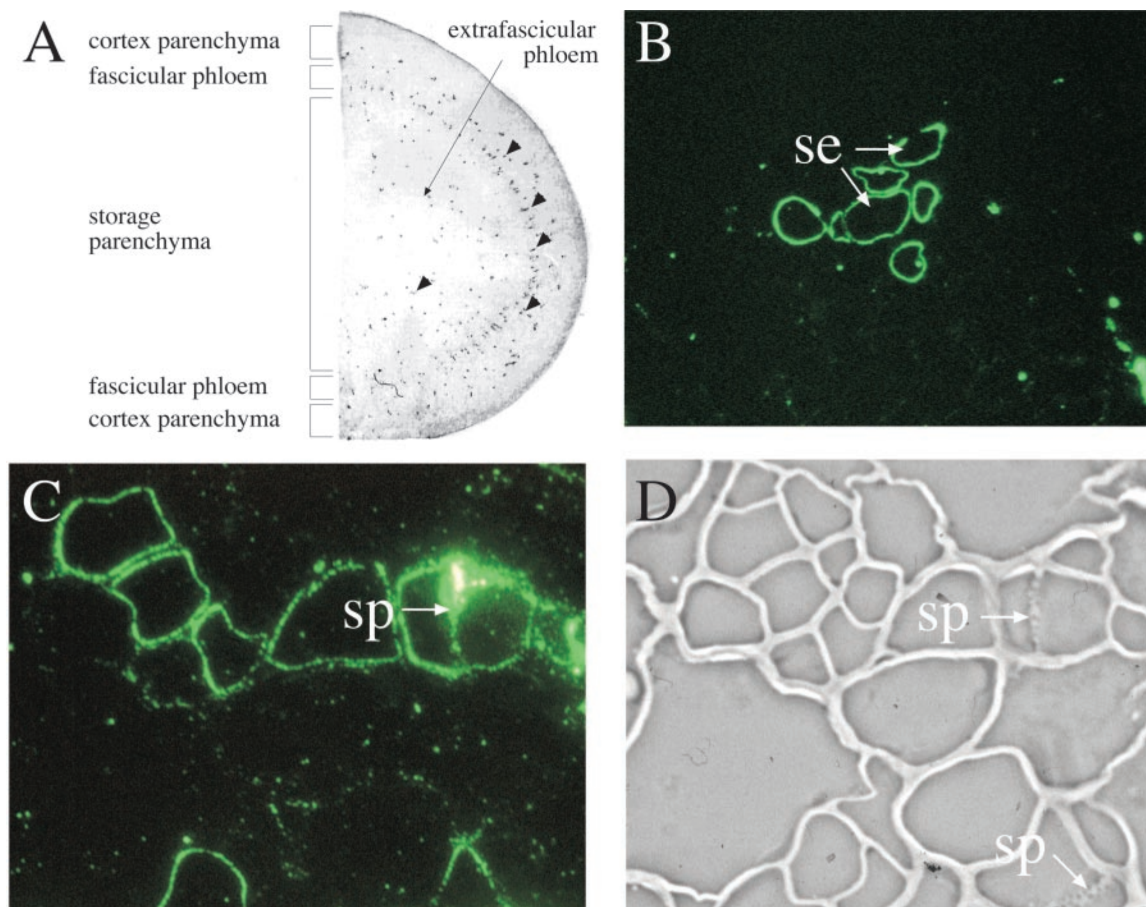

Figure 3. A, Tissue print of a potato sink tuber incubated with *StSUT1* antisera. *StSUT1* detection was visualized via alkaline phosphatase reaction directly on the nitrocellulose membrane. Arrowheads indicate labeling of phloem cells, whereas no label was found on tissue prints where the first antibody was omitted. B and C, Immunolocalization experiments were performed with affinity-purified *StSUT1*-specific peptide antibodies and visualized using a secondary fluorescein isothiocyanate-labeled anti-rabbit antibody, showing *StSUT1* protein localized in the sieve elements of the phloem tissue of sink tubers. Magnification in B is 1,000× and in C is 2,500×. D, Transmission micrograph of the same sink tuber cross-section as shown in C. Arrow, Sieve plates are visible in the sieve elements of the phloem. sp, Sieve plates; se, sieve elements.

soon after tuber initiation. Therefore, the time before tuberization was further subdivided into developmental stages, defined as stages I and II.

For metabolic analysis a discrimination was done to differentiate between swelling stolons and small tubers 3 weeks after transfer into the greenhouse: This developmental stage was dissected into small swollen stolons of 0.01 g (stage I) and small tubers of 0.02 to 0.06 g (stage II). Stage III tubers (1.5–2.5 g) were harvested 6 weeks and stage IV tubers (4–8 g) 9 weeks after transfer into the greenhouse.

For yield analysis, tubers were harvested at different time points after the beginning of tuberization beginning with stage III (6 weeks after transfer into the greenhouse) and going up to stage VII (15 weeks after transfer). The different developmental stages are summarized in Figure 5.

At a very early developmental stage (stage I), the starch content in antisense tubers was significantly lower in three of five antisense lines and increased in the following developmental stages to end up with a significantly higher amount of starch per gram fresh weight than in wild-type tubers (Table II). However, the ratio between tuber fresh weight and tuber dry weight was the same in wild-type and αSUT1-T plants (not shown). Determination of the starch content at different developmental stages was repeated twice using different methods.

Invertase activities decreased, whereas Suc synthase activity dramatically increased during the development of potato wild-type tubers (Table III; Appeldorn et al., 1997; Hajirezaei et al., 2000). The increase of Suc synthase activity parallels the increase in starch content, because Suc synthase is the main sucrolytic activity in vivo in the developing tuber (Zrenner et al., 1995). The activity of Suc synthase in antisense tubers, which at stage I was significantly lower in four of five antisense lines than in wild-type tubers, increased with time, a finding that may explain increased starch content. As shown in

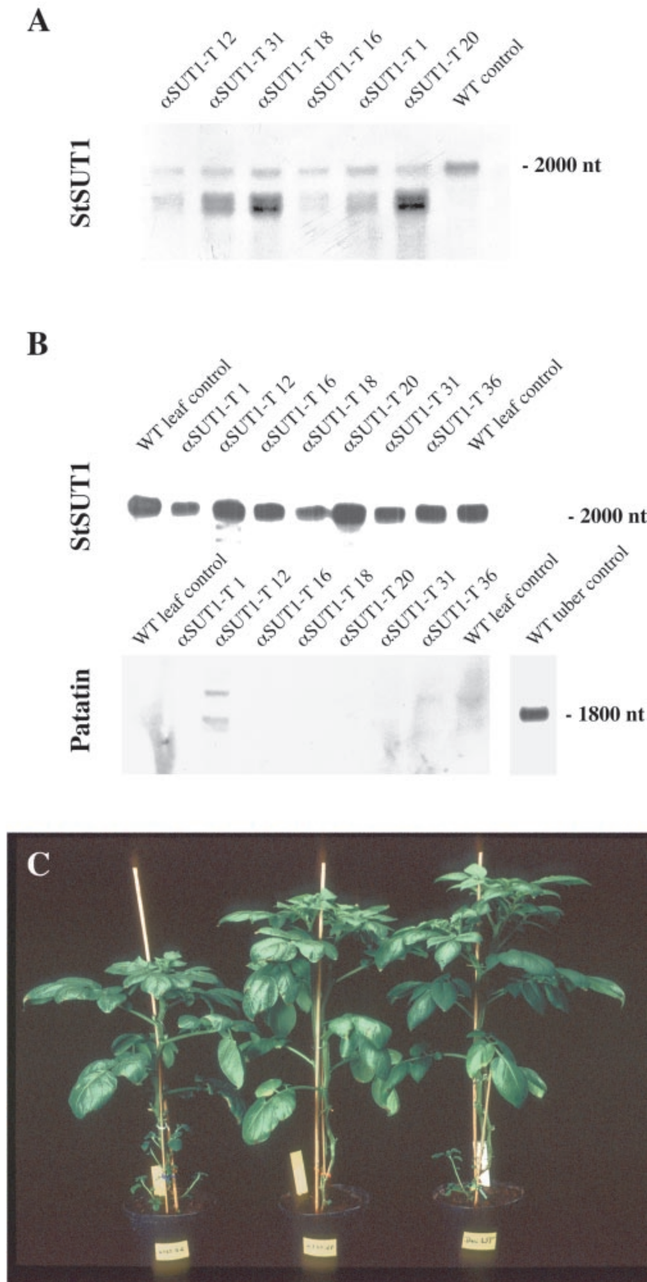

Figure 4. A, RNA gel-blot analysis of *StSUT1* mRNA expression in potato tubers of control and antisense plants. Total tuber RNA (12 μg lane^{-1}) was separated on a 1.2% (w/v) formaldehyde gel, transferred to a nylon membrane, and hybridized with the radiolabeled 1.3-kb *Bam*HI fragment of the *StSUT1* cDNA. The transcript size is approximately 2 kb. The transformants showed a reduction in Suc transporter *SUT1* mRNA levels. In addition, shorter antisense RNA molecules can be detected in antisense plants. B, RNA gel-blot analysis of potato leaf RNA of control and antisense plants hybridized with a StSUT1 or a patatin probe. StSUT1 expression in leaves is not altered in transgenic plants, neither is patatin expression induced in the leaves of transgenic plants. Therefore, inhibition of StSUT1 expression is tuber specific. C, Phenotype of αSUT1-T plants. From left to right, αSUT1-T$_{12}$, αSUT1-T$_{1}$, potato cv Désirée and wild type. Leaf morphology is unchanged in transgenic compared with control plants.

Suc synthase antisense plants, a strong correlation exists between the amount of Suc synthase transcripts and the starch content of potato tubers (Zrenner et al., 1995). The analysis of the *Shrunken1* maize mutant showed that a reduction in Suc synthase activity leads to an even stronger reduction of starch accumulation (Chourey and Nelson, 1976). However, it is worth noting that the total starch content per plant was not altered, because tuber yield was still notably reduced in the transgenic lines at this stage of development (Table I).

To test whether reduced SUT1 levels in antisense plants are accompanied by changes in the concentration of other metabolites, a broad metabolic screen was carried out for lines 12, 18, and 20 using gas chromatography-mass spectrometry (GC-MS) at developmental stage IV, when tuber yield was reduced (Table I), and when plants started compensating (Fig. 5). Glc, Fru, or Suc levels of the transgenic tubers were not significantly different from wild-type levels, independent of whether detection was by GC-MS or with conventional enzymatic methods (data added as supplementary file, which can be viewed at www.plantphysiol.org; Table IV). The levels of direct precursors for plastidial starch synthesis within tubers, Glc-6-phosphate and Fru-6-phosphate (Tauberger et al., 1999) were only slightly decreased. Thus, the increase in the maximal catalytic activity of Suc synthase did not lead to a corresponding increase in in vivo sucrolytic activity. Concentrations of sugars, sugar phosphates, and organic acids (with the exception of fumarate and succinate, which were significantly decreased) showed only minor alterations.

In contrast to most of the carbohydrate metabolites, the majority of the soluble free amino acid pools varied more widely: with up to 60% decrease in the levels of a broad variety of amino acids, whereas the acidic amino acids Asp and Glu were the only amino acids with unchanged levels in antisense tubers (Table IV).

Time Dependence of Tuber Yield

Figure 5 shows that tuber yield of antisense plants was reduced at early developmental stages (stages III–V), whereas at later stages, tuber yield transiently exceeded that of wild type (stage VI). This transient yield surplus was observed in several independent experiments. However, tuber yield of antisense plants was never above the final tuber yield of wild-type plants; the final tuber yield is reached later in wild type and earlier in antisense tubers. A similar phenomenon was observed in StSUT1 overexpressing pea (*Pisum sativum*) seeds with increased seed biomass compared with wild-type seeds. However, the increase in seed growth rate of pea seeds overexpressing StSUT1 in storage parenchyma was not accompanied by detectable changes in final seed dry weight. The increase in seed biomass is obviously

Table I. *Tuber yield of aSUT-T antisense plants*

Plants were harvested at stage IV after 9 weeks in the greenhouse (13-cm pots). The SD is given (n = 4). This experiment was repeated four times independently showing comparable results. Representative values are given here.

Plant No.	Average Tuber Yield	Yield (n = 4)
	g fresh wt	%
Potato cv Désirée wild type	30.6 ± 5.0	100.0
αSUT1-T1	20.3 ± 4.5	66.1
αSUT1-T12	18.8 ± 2.5	61.4
αSUT1-T16	20.8 ± 8.2	68.0
αSUT1-T18	17.2 ± 2.2	56.3
αSUT1-T20	12.1 ± 9.3	39.4
αSUT1-T31	23.2 ± 4.5	75.8
αSUT1-T36	22.7 ± 4.0	74.0

related to an increase in water content (Rosche et al., 2002). Therefore, this transient yield surplus was not analyzed further.

This experiment was repeated five times independently with similar results. One to 2 weeks after tuber induction, the development of potato tubers generally follows a linear growth rate (Marschner et al., 1984), as was the case for the control plants in this experiment. The relationship between tuber weight from the antisense plants and the growth time was, however, sigmoidal (Fig. 5). The transient reduction of the tuber yield at stages III to V might be explained by a delayed response to SUT1 inhibition at earlier stages.

DISCUSSION

StSUT1 is essential for long-distance transport of Suc in potato plants (Riesmeier et al., 1994; Kühn et al., 1996) and SUT1 protein has been localized in sieve elements of the collection and the transport phloem (Kühn et al., 1997). Here, it is shown that SUT1 also localizes to the sieve elements of the delivery phloem of potato tubers. To address the question of SUT1 function in tubers, SUT1 expression was down-regulated in transgenic potato antisense plants using the tuber-specific patatin promoter B33 (Liu et al., 1991). At early stages of tuber development, a remarkable reduction in tuber yield was observed in antisense plants, which was attributable to a reduction in tuber size rather than tuber number (Table I). Moreover, starch and amino acid content were altered in antisense tubers at these early developmental stages. After successful induction of tuber initiation, tuber development did not appear to be disturbed, and antisense plants were able to produce the same amount of tuber fresh weight compared with wild type.

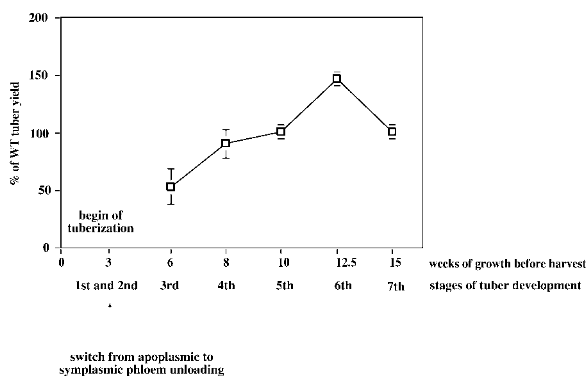

Figure 5. Time dependence of tuber yield. Tuber yield is given as percentage of the corresponding wild-type yield (100%) at the time of tuber harvest. Error bars indicate the SE (n = 10). Lines αSUT1-T_1, αSUT1-T_{12}, αSUT1-T_{16}, αSUT1-T_{18}, and αSUT1-T_{20} are represented. The experiment was repeated five times (the number of plants harvested for each time point was between 28 and 72). Representative data from one experiment are given.

The effects of antisense repression may be explained by the following hypotheses: SUT1 (a) plays a role in Suc retrieval in potato tubers along the stolon-apex axis, (b) influences the apoplasmic Suc concentration and thereby affects sink strength of tubers, (c) regulates plasmodesmal opening and closure, or (iv) is directly involved in phloem unloading.

Unloading of Suc in potato tubers includes an apoplasmic step, as already shown with transgenic potatoes expressing yeast-derived apoplasmic invertase in tubers affecting yield (Sonnewald et al., 1997). Therefore, Suc must be present in the extracellular space having an effect on sink strength of potato tubers.

The changes in metabolite levels of Suc transporter antisense lines approximate those previously observed in plants expressing yeast invertase in the apoplasm (Roessner et al., 2000, 2001). These results add to the growing body of correlative evidence that the Suc level can influence the amino acid levels within the tuber (Trethewey et al., 1999; Roessner et al., 2000; Fernie et al., 2001). In potato antisense plants with reduced expression of the amino acid permease StAAP1, reduced levels of amino acids were paralleled with unaffected or even increased starch content relative to wild type (Koch et al., 2002). The mechanism by which this metabolic cross-talk is regulated is at present unknown. Secondary effects resulting from changes of the energy status or osmotic changes within the tissue could have an impact

Table II. *Influence of tuber-specific SUT1 antisense inhibition on starch accumulation at different developmental stages in potato tubers*

The results are means of four independent samples ± SE. First (<0.01 g) and second stage tubers (0.02–0.06 g) were harvested 3 weeks after transfer into the greenhouse, third stage tubers (1.5–2.5 g) 6 weeks after transfer, and fourth stage tubers (4–8 g) 9 weeks after transfer. *, Values that were determined by the Student's *t* test to be significantly different from the wild type ($P < 0.05$).

Plant No.	Starch Content			
	First stage	Second stage	Third stage	Fourth stage
	mmol g^{-1} fresh wt			
Potato cv Désirée wild type	71.5 ± 10	233 ± 26	440 ± 21	450 ± 29
αSUT1-T1	39.2 ± 6.0*	272 ± 25	840 ± 97*	572 ± 71
αSUT1-T12	30.6 ± 4.6*	282 ± 25	394 ± 14	510 ± 49
αSUT1-T16	46.4 ± 11	189 ± 34	690 ± 60*	599 ± 22*
αSUT1-T20	41.9 ± 9.3	257 ± 87	570 ± 30*	582 ± 35
αSUT1-T31	45.4 ± 9.3	354 ± 59	551 ± 90	518 ± 52

on the metabolic fluxes and thereby on the ratio of C- and N-compounds. Recent reports indicate the presence of a plasma membrane factor affecting cellular metabolism by sensing external concentrations of metabolites (Lalonde et al., 1999; Fernie et al., 2000, 2001).

Sugars are considered to play a role as signaling molecules coordinating a variety of metabolic processes in the plant, with Suc transporter-like proteins potentially acting as corresponding sensors (for review, see Hellmann et al., 2000). Suc, for example, was described to be an effective inducer of the patatin gene (Martin et al., 1997) and a signal regulating expression of the Suc transporter in sugar beet (*Beta vulgaris*; Smeekens and Rook, 1997; Chiou and Bush, 1998; Rook et al., 1998). High Suc concentrations are known to induce the transcription of several genes involved in tuber storage metabolism (Müller-Röber et al., 1990). It has been proposed that a defined level of apoplasmic sugars could, in addition to phytohormones, be required to induce tuberization (Tauberger et al., 1999). Inhibition of *StSUT1* expression at this stage of development could thus reduce apoplasmic sugar concentration below a threshold level necessary for efficient tuber initiation.

Plasmodesmata function as pressure-sensitive valves (Oparka et al., 1991). Tauberger et al. (1999) proposed that sink strength of potato tubers is directly related to the osmotic potential of the apoplasm. High osmolarity in the apoplasmic space would result in closure of plasmodesmata, thus inhibiting symplasmic phloem unloading. According to Oparka et al. (1992), Suc is unloaded symplasmically in potato tubers via plasmodesmata, followed by Suc storage in the vacuole and starch synthesis in amyloplasts, thus maintaining a Suc gradient be-

Table III. *Influence of tuber specific antisense inhibition of StSUT1 expression on starch- and Suc-metabolizing enzyme activities*

The results are means of four independent samples ± SE. Tuber stages are the same as in Table II. *, Values that were determined by the Student's *t* test to be significantly different from the wild type ($P < 0.05$).

Plant No.	First Stage	Second Stage	Third Stage	Fourth Stage
	nmol g^{-1} fresh wt min^{-1}			
Sucrose synthase activity				
Potato cv Désirée wild type	54.4 ± 5.6	968 ± 124	2,144 ± 99	1,518 ± 150
αSUT1-T1	15.5 ± 1.7*	926 ± 270	2,042 ± 207	1,859 ± 321
αSUT1-T12	42.2 ± 4.9	1,017 ± 214	2,288 ± 254	1,709 ± 285
αSUT1-T16	15.8 ± 2.0*	881 ± 194	1,932 ± 342	1,547 ± 213
αSUT1-T20	28.8 ± 3.1*	1,383 ± 89*	2,873 ± 372	1,403 ± 86
αSUT1-T31	31.5 ± 6.0*	1,479 ± 255	2,859 ± 117*	1,877 ± 76
Alkaline invertase activity				
Potato cv Désirée wild type	752 ± 145	119 ± 28	19.3 ± 5.7	31.2 ± 4.1
αSUT1-T1	409 ± 122	104 ± 16	33.9 ± 2.7	21.1 ± 4.1
αSUT1-T12	402 ± 161	224 ± 57	22.1 ± 1.3	27.7 ± 5.3
αSUT1-T16	676 ± 200	244 ± 66	33.8 ± 7.3	23.0 ± 1.3
αSUT1-T20	389 ± 96	233 ± 58	22.4 ± 5.5	32.1 ± 3.1
αSUT1-T31	371 ± 94	381 ± 101	30.8 ± 6.2	21.9 ± 1.1
Acid invertase activity				
Potato cv Désirée wild type	2,842 ± 480	117 ± 21	21.1 ± 4.2	15.7 ± 2.1
αSUT1-T1	3,780 ± 607	230 ± 70	6.2 ± 1.1	25.6 ± 6.3
αSUT1-T12	2,928 ± 681	263 ± 75	9.5 ± 0.9	44.8 ± 1.6
αSUT1-T16	3,501 ± 235	308 ± 54	7.7 ± 1.2	35.5 ± 4.1
αSUT1-T20	2,358 ± 480	186 ± 22	11.3 ± 2.5	16.2 ± 1.3
αSUT1-T31	2,013 ± 376	387 ± 75	20.5 ± 4.5	25.1 ± 6.9

Table IV. *Metabolite content of antisense tubers (α SUT1-T12, α SUT1-T18, and α SUT1-T_{20}) as determined by gas chromatography mass spectrometry*

Wild type content was set to 1.00. Plants were harvested at stage IV (see above).

	Wild Type (±SE)	No. 12 (±SE)	No. 18 (±SE)	No. 20 (±SE)
Sugars and sugar alcohols				
Fru	1.00 ± 0.16	1.30 ± 0.28	2.56 ± 0.63	1.20 ± 0.44
Glc	1.00 ± 0.32	1.12 ± 0.48	0.86 ± 0.20	0.61 ± 0.41
Glycerol	1.00 ± 0.08	1.06 ± 0.16	1.23 ± 0.17	1.15 ± 0.17
Inositol	1.00 ± 0.13	0.65 ± 0.10	0.78 ± 0.08	0.68 ± 0.08
Mannitol	1.00 ± 0.08	0.80 ± 0.05	0.94 ± 0.06	0.80 ± 0.06
Mannose	1.00 ± 0.18	0.87 ± 0.13	0.97 ± 0.11	0.87 ± 0.21
Suc	1.00 ± 0.13	0.90 ± 0.21	0.98 ± 0.14	0.77 ± 0.13
Raffinose	1.00 ± 0.17	0.74 ± 0.19	1.07 ± 0.19	0.62 ± 0.19
Phosphorylated metabolites				
Fru-6-P	1.00 ± 0.07	0.76 ± 0.06	0.84 ± 0.08	0.82 ± 0.08
Glc-6-P	1.00 ± 0.07	0.85 ± 0.09	0.86 ± 0.07	0.84 ± 0.08
3-Phosphoglyceraldehyde	1.00 ± 0.31	0.66 ± 0.24	0.43 ± 0.16	0.59 ± 0.14
Phosphate	1.00 ± 0.06	1.10 ± 0.07	1.15 ± 0.08	1.04 ± 0.12
Organic acids				
Ascorbate	1.00 ± 0.22	0.86 ± 0.25	0.99 ± 0.13	1.58 ± 0.63
Citrate	1.00 ± 0.07	0.92 ± 0.02	0.98 ± 0.04	0.85 ± 0.06
Fumarate	1.00 ± 0.11	0.88 ± 0.05	0.87 ± 0.07	0.68 ± 0.04
Glucuronate	1.00 ± 0.11	0.89 ± 0.03	0.87 ± 0.07	0.73 ± 0.05
Glycerate	1.00 ± 0.12	0.88 ± 0.12	0.90 ± 0.11	0.87 ± 0.10
Isocitrate	1.00 ± 0.13	0.82 ± 0.25	0.79 ± 0.13	0.74 ± 0.06
Malate	1.00 ± 0.13	1.09 ± 0.26	1.77 ± 0.23	1.01 ± 0.24
Oxalate	1.00 ± 0.19	1.19 ± 0.25	0.96 ± 0.25	0.55 ± 0.09
Quinate	1.00 ± 0.11	0.89 ± 0.26	0.98 ± 0.18	0.74 ± 0.08
Shikimate	1.00 ± 0.17	0.99 ± 0.11	1.13 ± 0.11	1.00 ± 0.14
Succinate	1.00 ± 0.08	0.88 ± 0.09	0.68 ± 0.07	0.56 ± 0.09
Amino acids				
Ala	1.00 ± 0.12	0.76 ± 0.14	0.53 ± 0.11	0.56 ± 0.10
Arg	1.00 ± 0.10	0.86 ± 0.15	0.71 ± 0.14	0.77 ± 0.12
Asn	1.00 ± 0.18	0.89 ± 0.23	0.58 ± 0.19	0.90 ± 0.18
Aspartate	1.00 ± 0.11	0.85 ± 0.07	0.95 ± 0.08	1.19 ± 0.04
β-Ala	1.00 ± 0.14	0.90 ± 0.10	0.73 ± 0.12	0.62 ± 0.07
Cys	1.00 ± 0.07	0.61 ± 0.12	0.74 ± 0.18	0.47 ± 0.07
γ-Aminobutyrate	1.00 ± 0.11	0.82 ± 0.11	0.74 ± 0.07	0.72 ± 0.07
Glu	1.00 ± 0.12	0.80 ± 0.10	0.77 ± 0.06	0.97 ± 0.06
Gln	1.00 ± 0.07	0.87 ± 0.16	0.77 ± 0.12	0.81 ± 0.14
Gly	1.00 ± 0.08	0.84 ± 0.07	0.78 ± 0.10	0.74 ± 0.07
Homo-Ser	1.00 ± 0.20	0.80 ± 0.15	0.75 ± 0.07	0.73 ± 0.10
Ile	1.00 ± 0.07	0.65 ± 0.11	0.67 ± 0.12	0.51 ± 0.08
Leu	1.00 ± 0.17	0.54 ± 0.14	0.67 ± 0.19	0.44 ± 0.08
Lys	1.00 ± 0.09	0.64 ± 0.10	0.61 ± 0.11	0.46 ± 0.08
Met	1.00 ± 0.08	0.75 ± 0.14	0.65 ± 0.10	0.66 ± 0.11
Orn	1.00 ± 0.20	0.60 ± 0.10	0.44 ± 0.06	0.43 ± 0.09
5-Oxo-Pro	1.00 ± 0.08	0.71 ± 0.17	0.88 ± 0.17	0.92 ± 0.13
Phe	1.00 ± 0.07	0.73 ± 0.13	0.58 ± 0.09	0.55 ± 0.09
Pro	1.00 ± 0.37	0.41 ± 0.23	0.14 ± 0.03	1.25 ± 0.58
Ser	1.00 ± 0.12	0.73 ± 0.10	0.70 ± 0.10	1.09 ± 0.12
Thr	1.00 ± 0.08	0.72 ± 0.10	0.67 ± 0.10	0.72 ± 0.12
Trp	1.00 ± 0.27	0.59 ± 0.16	0.42 ± 0.14	0.42 ± 0.11
Tyr	1.00 ± 0.13	0.69 ± 0.15	0.63 ± 0.21	0.45 ± 0.06
Val	1.00 ± 0.05	0.75 ± 0.10	0.79 ± 0.11	0.73 ± 0.06

tween the SECCC and storage parenchyma. An active and turgor-sensitive Suc uptake system is responsible for retrieval of Suc escaping into the apoplasm. Our findings support the essential aspects of Oparka's model (Oparka et al., 1992). SUT1 might control the osmolarity of the apoplasm and thus regulate plasmodesmal opening by affecting the apoplasmic Suc concentration.

StSUT1 expression data and antisense inhibition argue for a role of *SUT1* in phloem unloading in potato tubers during early stages of tuber development. Apoplasmic phloem unloading is in agree-

ment with the increase in tuber yield in transgenic potato plants expressing a yeast-derived invertase apoplasmically (Heineke et al., 1992; Sonnewald et al., 1997).

As mentioned above, Viola and co-workers (2001) have demonstrated by using a phloem mobile tracer that a switch occurs from apoplasmic to symplasmic phloem unloading during the transition from stolon to tuber development. This switch is accompanied by an increase of Suc metabolizing enzyme activities and a switch from the invertase-sucrolytic pathway to the Suc synthase-sucrolytic pathway as postulated by Appeldorn et al. (1997).

StSUT1 might be the carrier responsible for phloem unloading at early stages of tuberization, followed either by Suc cleavage by an apoplasmic invertase and subsequent uptake by hexose transporters, or by endocytosis, as was shown for the fluorescent dye LYCH microinjected into the apoplasm of the stolon cortex (Oparka and Prior, 1988).

The involvement of SUT1 in phloem unloading would implicate that not only can it act as a Suc importer, but also in the inverse orientation, facilitating Suc export from the SECCC following the Suc gradient. SUT1-mediated Suc transport would then be reversible. Suc efflux activities have already been described (e.g. Laloi et al., 1993). Suc efflux was measured in castor bean (*Ricinus communis*) leaf discs (Russel et al., 1999) and with mesophyll protoplasts of pea (Opaskornul et al., 1999), and in potato plasma membrane vesicles, Suc transport can occur in the absence of a proton gradient (Lemoine et al., 1996). The presence of a uniporter system allowing Suc efflux down its gradient has been postulated by Winter et al. (1994), with Suc being unloaded without a proton gradient because it can follow its own concentration gradient. It could be shown for the hexose transporter, CkHUP1 from *Chlorella* spp. that, depending on the external pH, the transporter can act as a Glc uniporter catalyzing Glc transport in both directions depending on concentration differences (Komor and Tanner, 1974). The bacterial lacS lactose transporter can exist in two states: as a uniporter when present as a monomer or as a proton-coupled system when present as an oligomer (Veenhof et al., 2001). Because SUT1 is distantly related to lacS and can also exist as a homo-oligomer, it is conceivable that also SUT1 can exist in different states (Reinders et al., 2002a, 2002b). Impaired apoplasmic phloem unloading in young potato tubers with reduced *StSUT1* transcript levels can be explained by StSUT1-mediated phloem unloading directly at the plasma membrane of sieve elements, SUT1 acting here as a Suc efflux carrier. Electrophysiological analyses together with a determination of the actual gradients for Suc, protons, and membrane potential are needed to evaluate this possibility.

CONCLUSIONS

Antisense inhibition of the Suc transporter SUT1 in potato tubers impairs early tuber development. This is in agreement with observations in transgenic potato plants expressing yeast-derived invertase either in the apoplasm or in the cytosol of storage parenchyma cells (Sonnewald et al., 1997; Tauberger et al., 1999). In both sets of plants, the tuber yield (and the turgor pressure) is affected in opposite directions, indicating that Suc passes through the apoplasm. We propose that SUT1 is either directly involved in phloem unloading in potato tubers or indirectly by regulating the apoplasmic osmolarity via its retrieval function. Another possibility is a combination of both: SUT1 being responsible for the osmolarity of the apoplasm, which in turn is important for the opening and closure of plasmodesmata. Inhibition of SUT1 could thus inhibit the retrieval of Suc from the apoplasm, leading to an increase in osmolarity and therefore the closure of plasmodesmata, making symplasmic phloem unloading impossible. The switch from apoplasmic to symplasmic phloem unloading as shown by Viola et al. (2001) could help the plants compensate for their reduced sink strength. The latter would explain why antisense plants, at later stages of development, are able to reach the same final tuber yield than wild-type plants.

MATERIALS AND METHODS

Isolation of the *LeSUT1* promoter

A genomic library of tomato (*Lycopersicon esculentum* cv VFN8) in EMBL-3 (BD Biosciences Clontech, Palo Alto, CA) was screened using potato (*Solanum tuberosum*) SUT1 cDNA as a probe. Seven λ-clones were characterized by restriction analysis that fell into five different groups. Two of the λ-clones, showing the strongest hybridization signals, were investigated in more detail by DNA gel-blot analysis. A 2.1-kb *Bam*HI fragment was found to contain 1.7 kb of the *LeSUT1* promoter region and the 5′ end of the *LeSUT1* coding region.

Recombinant DNA

The 1.35-kb *Bam*HI fragment of the potato Suc transporter cDNA *StSUT1* (Riesmeier et al., 1993) was ligated in reverse orientation into the *Sma*I restriction site of pBinB33 (Rocha-Sosa et al., 1989). pBinB33 is a derivative of pBinAR (Höfgen and Willmitzer, 1990), in which the 35S promoter cassette has been replaced with the 1.5-kb *Dra*I-fragment of the patatin class I B33 promoter (Romanov et al., 1998).

GUS Staining

Plant material (tuber sections) was infiltrated under vacuum with 2 mM 5-bromo-4-chloro-3-indolyl-glucuronide, 50 mM sodium phosphate buffer, pH 7.2, and 0.5% (v/v) Triton X-100 and incubated overnight at 37°C. Destaining was performed in ethanol.

Plant Transformation

Transfer of the chimeric construct into *Agrobacterium tumefaciens* GV2260 and transformation of potato cv Désirée was performed as described (Riesmeier et al., 1994). Transgenic plants were amplified in tissue culture, and 60 plants were transferred to soil and grown in a cycle of 16 h of light (22°C) and 8 h of darkness (15°C) in 60% humidity. The light conditions were

approximately 150 μE m^{-2} s^{-1} (8 kLux). Experiments were repeated independently using in vitro propagated clones of the transformants. Starch determination was performed according to Lin et al. (1988), and metabolites were analyzed as described by Gerhardt et al. (1987).

RNA Gel-Blot Analysis

Hybridization with the 1.3-kb *Bam*HI-fragment of the *StSUT1* cDNA gave rise to a weak signal of 2 kb on tuber RNA. Hybridization was performed under high stringency to prevent cross hybridization with transcripts of other Suc transporters. As a patatin probe, a 590-bp *Hin*fI/*Bam*HI-fragment from the coding region of class I patatin clone pcT58 was isolated from the vector M13 mp8-58.

Immunolocalization

For StSUT1 antisera, rabbits were immunized with synthetic peptides. Immunodetection was performed as described previously (Kühn et al., 1997). Tuber material was embedded in methacrylate, semithin sections of 1-μm thickness were cut with an ultramicrotome, and StSUT1 was detected using an fluorescein isothiocyanate-coupled secondary antibody.

Tissue prints were performed on nitrocellulose (Schleicher & Schüll, Dassel, Germany), dried, and blocked with 5% (w/v) milk powder in phosphate-buffered saline (PBS; 100 mM $NaPO_4$ buffer, pH 7.5, and 100 mM NaCl). After 1 h of incubation with affinity-purified antibodies against StSUT1, blots were washed twice in PBS-T (PBS with 0.1% [v/v] Tween) and once with PBS, followed by a 1-h incubation with anti-rabbit IgG-AP conjugate (Roche Diagnostics, Mannheim, Germany). After three final washes with PBS-T and PBS, the color reaction was developed by adding nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution (Roche Diagnostics) and stopped by rinsing in distilled water. Tissue prints were analyzed with a binocular microscope (Leica, Wetzlar, Germany). A control membrane incubated without StSUT1 antibody but with the secondary antibody did not show any signal (not shown).

Analysis of Enzyme Activities, Determination of Soluble Sugars and Starch

All enzyme activities were determined as described previously (Hajirezaei et al., 1994). Soluble sugars and starch were quantified in tuber samples extracted with 80% (v/v) ethanol and 20 mM HEPES-KOH, pH 7.5, as described previously (Sonnewald, 1992).

Extraction, Derivatization, and Analysis of Potato Tuber Metabolites Using GC-MS

Measurement of metabolites in potato tuber tissue was carried out exactly as described by Roessner et al. (2000). Normalized data was calculated as detailed by Roessner et al. (2001).

Statistical Analysis

Differences described as significant were analyzed using the *t* test algorithm incorporated into Microsoft Excel (v9.0, Microsoft, Redmond, WA) that yielded a value below 5% ($P < 0.05$).

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