The EMBO Journal vol.13 no.1 pp.1-7, 1994

Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning

Jörg W.Riesmeier, Lothar Willmitzer and Wolf B.Frommer[1]

Institut für Genbiologische Forschung, Ihnestr. 63, D-14195 Berlin, Germany
[1]Corresponding author

Communicated by J.Schell

Sucrose is the principal transport form of assimilates in most plants. In many species, translocation of assimilates from the mesophyll into the phloem for long distance transport is assumed to be carrier mediated. A putative sucrose proton cotransporter cDNA has been isolated from potato and shown to be expressed mainly in the phloem of mature exporting leaves. To study the *in vivo* role and function of the protein, potato plants were transformed with an antisense construct of the sucrose transporter cDNA under control of the CaMV 35S promoter. Upon maturation of the leaves, five transformants that expressed reduced levels of sucrose transporter mRNA developed local bleaching and curling of leaves. These leaves contained >20-fold higher concentrations of soluble carbohydrates and showed a 5-fold increase in starch content as compared with wild type plants, as expected from a block in export. Transgenic plants with a reduced amount of sucrose carrier mRNA show a dramatic reduction in root development and tuber yield. Maximal photosynthetic activity was reduced at least in the strongly affected transformants. The effects observed in the antisense plants strongly support an apoplastic model for phloem loading, in which the sucrose transporter located at the phloem plasma membrane represents the primary route for sugar uptake into the long distance distribution network.

Key words: apoplastic loading/phloem loading/proton cotransport/sink-to-source transition/*Solanum tuberosum* L.

Introduction

Carbohydrates deriving from mature leaves are distributed in the vascular system mainly in the form of sucrose through the veinal network for allocating assimilates to support the growth of heterotrophic tissues such as developing leaves, the apex, roots and reproductive organs. Little is known about the molecular processes that are responsible for the loading of the phloem in the leaves, the translocation and the unloading in sink organs. Both active transport by specific carriers across the apoplast, and symplastic transport via plasmodesmata have been discussed as possible mechanisms for loading. Nevertheless, direct demonstration of the actual mechanism in the plant is still missing. This is of special relevance to an unresolved question: whether the major route for phloem loading occurs via carrier mediated processes across the apoplast or is symplastic (Robards and Lucas, 1991).

Sucrose transport activities have been identified in a number of plant species (reviewed in Bush, 1993). The transport is active and has been described as a sucrose-proton cotransport with a 1:1 stochiometry (Bush, 1990). Comparison of the transport activity in developing versus mature leaves has shown that active sucrose transport activity in leaves of sugar beet develops only upon maturation of the leaves (Lemoine *et al.*, 1992). To resolve the question of whether carrier-mediated sucrose transport represents an essential step in phloem loading, the respective genes have been identified.

Complementation of yeast mutants has proven to be an effective method for the isolation of plasma membrane associated metabolite transporter genes (Frommer *et al.*, 1993; Kwart *et al.*, 1993). A strain deficient in secreted invertase, but able to metabolize ingested sucrose due to expression of a sucrose cleaving activity has been successfully used as a complementation system to isolate a sucrose transporter cDNA from spinach and potato (Riesmeier *et al.*, 1992, 1993a). The biochemical properties of the transporter when expressed in yeast are similar to those described in protoplasts or in plasma membrane vesicles from a variety of plant species.

From the structural point of view, the transporters are highly hydrophobic proteins and belong to the class of metabolite transporters consisting of two sets of six membrane spanning regions, separated by a central cytoplasmic loop. The expression profile of the sucrose transporter (SUT1) RNA from *Solanum tuberosum* (StSUT1) follows the sink-to-source transition in leaves, i.e. being low in young importing leaves and high in mature exporting leaves. The association of expression of the carrier gene with the development of active transport activity of maturing leaves suggests a role for SUT1 in phloem loading. SUT1 RNA is also found in stems though at a lower level than in leaves. This is taken as an argument against a sole function of the transporter in retrieval (Maynard and Lucas, 1985). RNA *in situ* hybridization could localize the expression of the carrier to the phloem of minor veins and thus supports the role of the carrier in phloem loading (Riesmeier *et al.*, 1993a). To analyse further the *in vivo* role of the sucrose transporter, transgenic potato plants have been created that show a reduction in the amount of the sucrose carrier mRNA due to antisense inhibition. These plants were analysed for effects on plant development, biochemical changes in the leaves, and effects on photosynthesis.

Results

Antisense repression of the sucrose transporter

To study the effects of a reduction in the activity of the sucrose transporter, the potato sucrose transporter cDNA P62 was cloned into a binary vector in antisense orientation

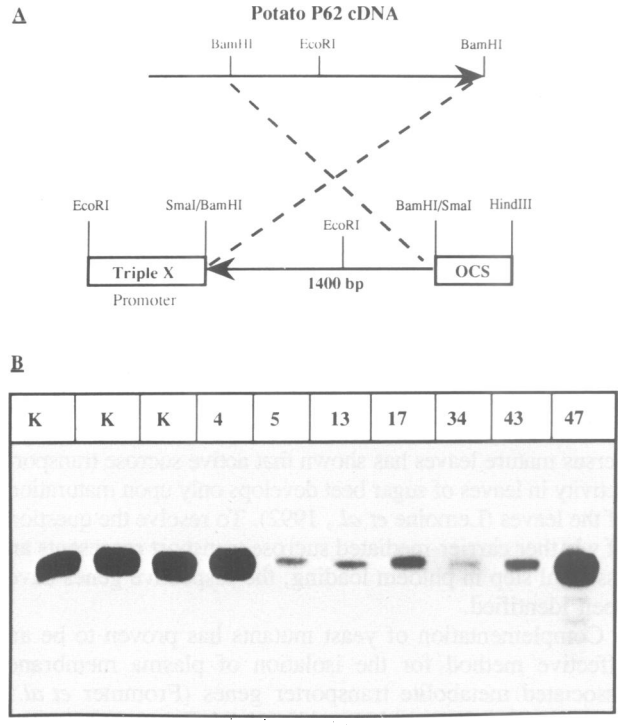

Fig. 1. Structure of the chimeric gene and analysis of transgenic potato plants whose expression of the sucrose transporter SUT1 is inhibited. (**A**) Structure of the chimeric sucrose transporter SUT1 antisense gene used for transformation of potato plants. (**B**) Analysis of sucrose transporter P62 mRNA expression in source leaves of transgenic and control (K) plants. Total RNA (25 μg/lane) was hybridized to a multiprime labelled SUT1 specific probe. The transcript size is ~2 kb. The transformants αSUT_5, αSUT_{13}, αSUT_{17}, αSUT_{34} and αSUT_{43} showed a reduction in sucrose transporter SUT1 mRNA levels.

behind the TripleX promoter, a modified version of the CaMV 35S promoter (Figure 1A; Gatz *et al.*, 1992). Leaf discs of *S.tuberosum* L., cv. Désirée were transformed with the chimeric construct and, after transfer of the plants to the greenhouse, 70 transformants were analysed at the RNA level. Five transformants (αSUT_5, αSUT_{13}, αSUT_{17}, αSUT_{34} and αSUT_{43}) showed a significant reduction in SUT1 mRNA (Figure 1B). These plants and replicas thereof were further analysed. In no case was a complete reduction of the sucrose transporter mRNA observed.

Phenotypic effects of sucrose transporter inhibition

Plants in axenic culture with reduced SUT1 mRNA levels show accumulation of anthocyanins at the lower surface of the rims of the leaves. Growth is retarded after transfer to soil (Figure 2A). The transformants αSUT_5 and αSUT_{34} are most strongly affected. During maturation the leaves begin to curl, the rims and intercostal fields start to bleach and accumulate anthocyanins at the lower epidermis (Figure 2B and D). This phenotype is found in all five of the antisense plants with reduced SUT1 mRNA levels but not in any of the control plants or the other 65 antisense plants that still contain normal SUT1 mRNA levels. The phenotype in the leaves develops only upon maturation and is not visible in young leaves in the sink stage (Figure 2B). Around the time of tuber setting, the plants αSUT_{13} and αSUT_{43} start to grow strongly and become up to 2-fold bigger compared with wild type controls (Figure 2C). The angle of the petioles relative to the stem in these plants is much lower than in wild type plants. The dramatic increase in size of the antisense plants later in development and the decreased angle of the petioles relative to the stem is so far not understood. After 2 months of cultivation in the greenhouse, the antisense plants had developed only a small root system and tuber yield was strongly reduced (Figure 2E and F). In the case of the most strongly affected transformant αSUT_5, not even a stolon develops. These phenotypic changes were reproducibly observed with several sets of transgenic plants obtained by multiplication of the primary transformants in tissue culture. The tuber yield from a second set of four replicas of each antisense plant is given in Table I.

Metabolite accumulation and photosynthesis

To get an insight into the changes at the biochemical level that accompany the altered phenotype of the leaves, the carbohydrate content of the leaves was determined. As shown in Figure 3, the leaves of all five transformants showing a reduction in sucrose transporter mRNA contain 7- to 9-fold higher levels of both sucrose and starch and an up to 100-fold increase in hexose concentration. To determine how far the large increase in sugar concentrations in the leaf affects the capacity of the photosynthetic machinery, the maximal rate of photosynthesis was determined under saturating light and CO_2 conditions in oxygen leaf disc electrodes. The most strongly affected plants αSUT_5 and αSUT_{34} show an almost 2-fold reduction in photosynthesis under saturating conditions, whereas αSUT_{43} is only slightly affected and αSUT_{13} is not significantly reduced compared with the wild type (Figure 4). The large reduction in the growth of sink organs therefore cannot be due to reduced photosynthetic capacity of the system.

Effects on other genes and proteins involved in carbohydrate metabolism

To see how far these alterations also influence the expression of other genes involved in carbohydrate metabolism, a Northern blot of wild type and antisense plants was hybridized to the SUT1 probe (P62), potato sucrose synthase cDNA (Salanoubat and Belliard, 1989), AGPase B cDNA (Müller-Röber *et al.*, 1990), chloroplastidic FBPase cDNA (Kossmann *et al.*, 1992) and potato sucrose phosphate synthase (U.Sonnewald, personal communication), as shown in Figure 5. No significant changes were observed for AGPase, FBPase and SPS, in contrast to sucrose synthase which is increased in all cases but most dramatically in the plants which were most strongly affected (αSUT_5 and αSUT_{34}).

Consistent with the results at the RNA level, the determination of the respective enzyme activities shows no significant changes for AGPase, chloroplastidic FBPase and sucrose phosphate synthase, but a strong increase in the sucrose synthase activity (4.6 ± 0.9 nmol sucrose per mg per min for αSUT_5, 8.3 ± 3.9 nmol sucrose per mg per min for αSUT_5, 1.1 ± 1.0 nmol sucrose per mg per min for wild type and data not shown).

Reduced export of carbohydrates

The increase in accumulation of carbohydrates and the reduction in root and tuber development are consistent with an essential role of the sucrose transporter in phloem loading.

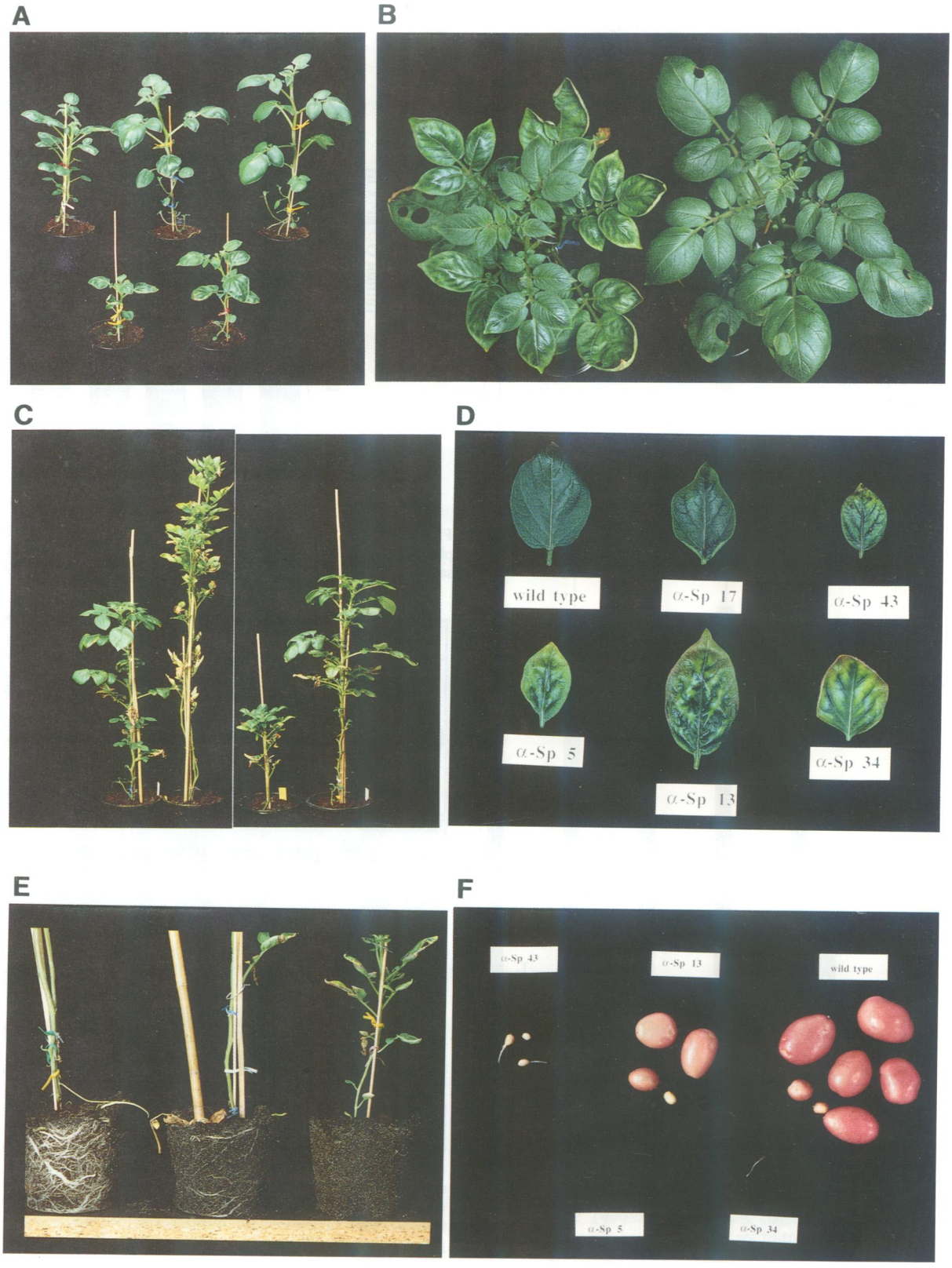

Fig. 2. Phenotypic changes in transgenic potato plants expressing the chimeric sucrose transporter antisense gene. (**A**) Transgenic potato plants after 4 weeks in the greenhouse. Upper row (from right to left): control, αSUT_{13} and αSUT_{43}. Lower row: αSUT_5 (left) and αSUT_{34} (right). (**B**) Top view of a control plant (right) and the transgenic plant αSUT_{43} (left). The plants were kept for 8 weeks in the greenhouse. Phenotypic changes of the leaf shape correspond to the sink-to-source transition of the leaves. (**C**) 16 week old control and antisense plants. From left to the right: control, αSUT_{43}, αSUT_{34} and control. (**D**) Leaves of control and antisense plants. (**E**) The antisense plants after 12 weeks in the greenhouse showed reduced formation of roots. From left to the right: control, αSUT_{13} and αSUT_{34}. (**F**) The inhibition of SUT1 gene expression leads to a drastic reduction of tuber yield (only one stolon with a microtuber for αSUT_{34} and no tubers in the case of αSUT_5).

Table I. Tuber yield of sucrose transporter antisense plants

Plant	Tuber yield (g fresh weight per plant)	% yield
control	158 ± 25	100
αSUT_5	1.0 ± 0.5	0.6
αSUT_{13}	77 ± 31	48
αSUT_{34}	0.3[a]	0.2
αSUT_{43}	14 ± 4	9

[a]Three plants produced no tubers, one plant had one tuber (1.2 g). The transgenic plants were amplified in tissue culture. For each transformant four plants (control eight plants) were transferred to the greenhouse for 20 weeks.

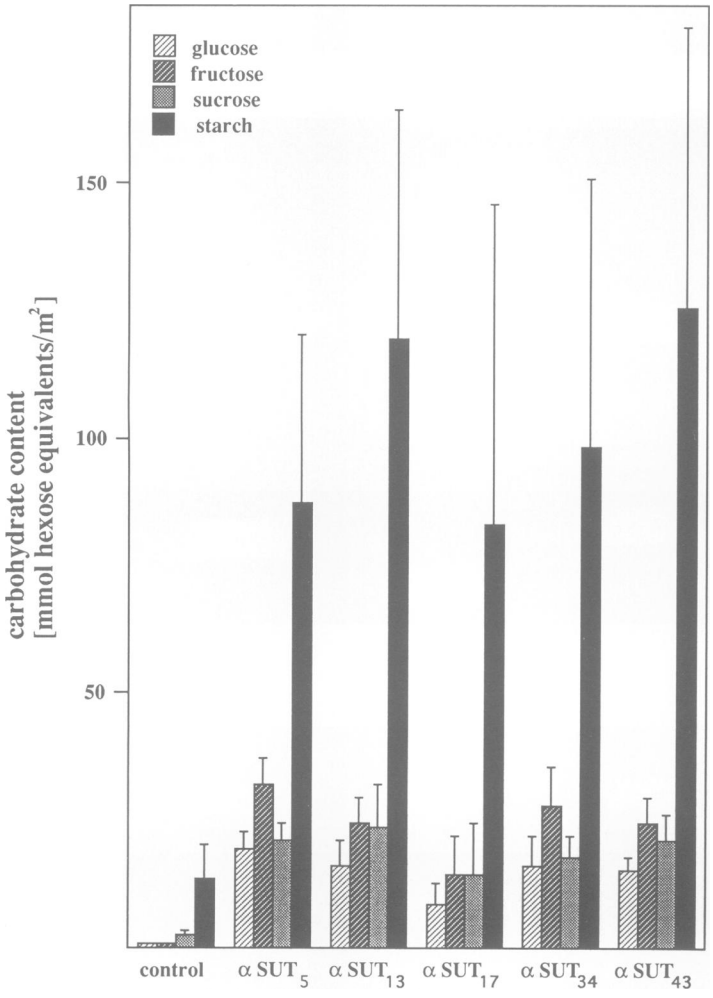

Fig. 3. Carbohydrate content in the leaves of control and transgenic potato plants expressing the chimeric sucrose transporter. (Amounts of carbohydrates ± SD in wild type plants: glucose: 0.5 ± 0.1 mmol/m²; fructose: 0.5 ± 0.1 mmol/m²; sucrose: 2.5 ± 0.5 mmol/m²; starch: 14 ± 6 mmol glucose equivalents/m²). Samples were taken 6 h after the start of the light period.

To study the effect of a reduction in the sucrose transporter mRNA on the export rates directly, leaves were cut from the stem, their petioles transferred to a solution containing EDTA to prevent callose formation and the effluxing sugars were measured as a function of time (King and Zeevaart, 1974). After 8 h of exposure to light the plants αSUT_{13}, αSUT_{34} and αSUT_{43} showed a significant decrease in sugar content in the efflux medium compared with control plants

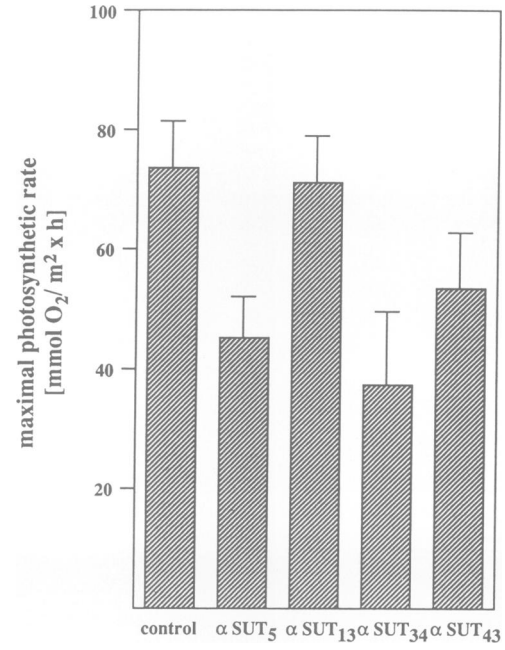

Fig. 4. Maximal rates of photosynthesis of control and transgenic plants. Oxygen development of excised leaf discs was measured under saturating light and CO_2 conditions, and were given as mmol $O_2/m^2/h$.

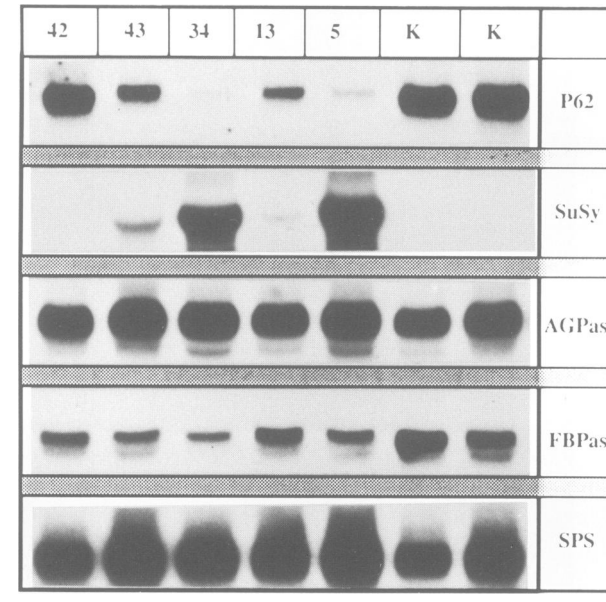

Fig. 5. Northern analysis of SUT1 antisense and wild type plants. Total RNA (25 μg/lane) from two control plants (K), a transformant that is unaffected (42), and from the four antisense plants αSUT_5 (5), αSUT_{13} (13), αSUT_{34} (34) and αSUT_{43} (43) was hybridized to multiprime labelled probes for the sucrose transporter StSUT1 (P62), sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (AGPase), the chloroplastidic fructose 1,6-bisphosphatase (FBPase) and sucrose phosphate synthase (SPS).

(Figure 6). A reduction was also observed in the case of the most strongly affected plant αSUT_5, but was not comparable due to the reduced leaf area and petiole length.

Discussion

Molecular studies of metabolite transport across the plasma membrane in plants have been neglected for many years due

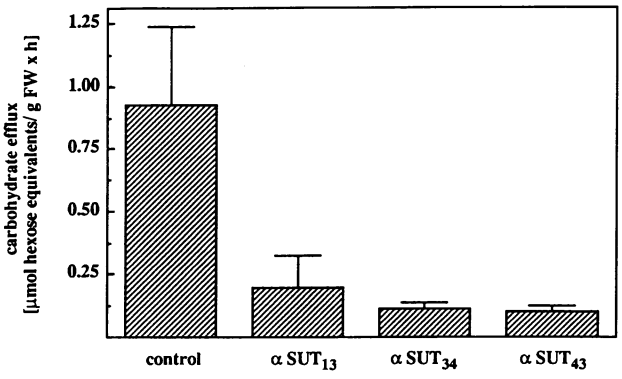

Fig. 6. Efflux of carbohydrates from petioles of control and antisense plants. Pinnules were cut from 10 week old greenhouse plants and incubated in 5 mM EDTA pH 6.0 for 8 h in the light (100 $\mu E/m^2/s$). The amounts of glucose, fructose and sucrose in the incubation solution were determined enzymatically. The sum of hexose equivalents is shown because there were no differences in the relative concentrations in control and antisense plants.

to the problems associated with the identification and purification of the respective proteins. The use of heterologous expression systems has recently allowed access to sucrose and amino acid transporters (Riesmeier *et al.*, 1992, 1993a; Frommer *et al.*, 1993). The tissue specificity, i.e. prevalence in the phloem together with the increase in sucrose transport activity during the sink-to-source transition of sugar beet leaves and the increase of SUT1 mRNA during leaf development from sink-to-source in potato was taken as an indication that the sucrose transporter is involved in phloem loading (Lemoine *et al.*, 1992; Riesmeier *et al.*, 1993a).

Evidence that the sucrose transporter is involved in phloem loading

To analyse directly the function of the transporter, potato plants were transformed with a SUT1 antisense gene under the control of the CaMV 35S promoter. Transformants containing reduced amounts of SUT1 mRNA display several significant phenotypic changes. The leaves of five transformants having reduced levels of SUT1 mRNA accumulate up to 100-fold higher amounts of hexoses, and 5- to 10-fold higher levels of sucrose and starch in mature leaves. Probably due to this dramatic increase in osmotically active carbohydrate content, the leaves develop a curly phenotype with local bleaching and accumulation of anthocyanins. Both the leaf phenotype and the increased accumulation of carbohydrates are comparable in all antisense plants. This is in contrast to gradual effects on the growth of the plant, development of roots and tubers and on photosynthesis, which vary between the different transformants. We therefore have to assume that in the transformant showing the weakest inhibition, the sugar accumulation has already potentially reached its maximum. Based on an estimation of the mesophyll sucrose concentration of ~40 mM in potato (D.Heineke and H.-W.Heldt, personal communication), the concentration of soluble sugars in the leaves of the transformants reaches up to 1500 mM hexoses and 400 mM sucrose. In addition to effects on carbohydrate metabolism, the transformants also show a 1.15- to 1.85-fold increase in the total amino acid content as compared with the wild type (C.Kühn and W.B.Frommer, unpublished results). This increase is in the same range as that found if export is blocked by ectopic expression of an invertase in the cell wall (Heineke *et al.*, 1992).

Cold girdling of the petiole is assumed to block the phloem transport. The increase in carbohydrate content within 4.5 days of cold girdling of potato petioles was up to 10-fold for fructose, glucose and sucrose and 1.5- to 3-fold for starch (Krapp *et al.*, 1993). The similarity of the effects, i.e. the increased accumulation of carbohydrates between SUT1 antisense plants and cold girdled plants supports the model that sucrose export from the leaves is blocked in the transgenic plants. The increase in sugar content in the leaves is not sufficient to explain the reduction in photosynthesis as, despite the high levels of sugars in the leaf, transformant αSUT_{13} seems to be unaffected in terms of the capacity of its photosynthetic machinery. The reduced efflux from the leaves should lead to a feedback inhibition of the cytosolic FBPase via fructose 2,6-bisphosphate and thus an increase in triose phosphates in the cytosol. This in turn restricts orthophosphate recycling to the chloroplast and limits photosynthesis (Stitt, 1990). Under the conditions tested, maximal rates of photosynthesis are only slightly reduced, at least in the transformant αSUT_{13} which is least affected. Thus the existing regulatory systems in the leaves seem to be unable to keep the sugar content at normal levels, indicating that either the regulation capacity of the pathway is overridden or that a signal coming from the sink keeps the carbohydrate production in the leaves at a maximum. Thus the high levels of soluble sugars in the leaves could be a compensatory effect that allows at least a small amount of carbohydrates to enter the phloem, possibly by simple diffusion to supply the vital organs, e.g. the root system. Such sink regulation of source tissues has been observed in many systems (Stitt, 1991).

Measurements of the export of soluble sugars through the petiole provide direct evidence that the flow of sucrose is significantly reduced in the antisense plants. It is therefore not surprising that the root system, which is dependent on the supply of sucrose from the leaves, is strongly reduced. The same also holds true for tuber yield. In the first set of experiments, the transgenic plant αSUT_{34} did not even develop stolons. This is rather surprising, as antisense inhibition of other functions such as triose phosphate translocator or the chloroplastidic FBPase strongly reduce photosynthesis, but only slightly affect tuber yield (Riesmeier *et al.*, 1993b; J.Kossmann, personal communication). These plants, in contrast with the SUT1 antisense plants seem to have a backlog with sufficient capacity to retain the essential function of tuber production for vegetative reproduction. We anticipate that flowering is also severely affected in the antisense plants. However, as under the growth conditions used, no flowering was observed in the control plants, no conclusions are possible. To study the effects of antisense inhibition on flower and seed development, the use of different species in which flower production is more effective, e.g. tobacco, would be advantageous. Furthermore, as the embryo is symplastically isolated from the maternal tissue, sucrose carriers and, potentially, the SUT1 gene have to be involved. Antisense inhibition in this case must also affect the unloading.

Regarding the phenotype, the young developing leaves do not seem to be affected by the antisense inhibition. This is consistent with the finding that sink leaves show only low

levels of sucrose transporter mRNA and sucrose transport activity (Lemoine *et al.*, 1992; Riesmeier *et al.*, 1993a). Nevertheless sink leaves are supposed to be dependent on the sucrose supply from the source leaves. However, the antisense plants seem to be unaffected in the development of sink leaves. The data may be explained if, at least in potato, sink leaves are not dependent on the import of assimilates from source leaves but rather are able to sustain themselves.

Taken together, the biochemical properties, expression pattern and the effects of the antisense inhibition of the sucrose transporter provide strong evidence that the carrier is involved in and responsible for phloem loading. How sucrose enters the phloem in exporting leaves is controversial. The distribution of plasmodesmata and microscopical studies with fluorescent dyes have provided evidence for symplastic transport at least between mesophyll cells (Robards and Lucas, 1990). The data obtained with the antisense inhibition indicate that under the conditions tested, the carrier mediated apoplastic route is the major pathway for phloem loading with sucrose. This agrees with the assumption that the concept of symplastic loading is confined largely to plants displaying a high degree of connectivity between mesophyll and the sieve element companion cell complex (van Bel *et al.*, 1992). Regarding the abundance of plasmodesmata on the pathway from the mesophyll to the sieve elements, potato belongs to the IIa type with a limited number of plasmodesmata between mesophyll and the sieve element companion cell complex (McCauley and Evert, 1989; van Bel *et al.*, 1992). Several species including potato contain ~20–100 mM sucrose in the cytosol of the mesophyll and a 10- to 20-fold higher concentration in the phloem sap (Lohaus *et al.*, 1993; D.Heineke and H.W.Heldt, personal communication). Because of the comparatively low symplastic connectivity, together with the uphill transport of sucrose into the phloem, potato is assumed to be an apoplastic loader. It will therefore be very interesting to extend the antisense studies to species with different symplastic connectivity, or to study the effects of a block in the function of plasmodesmata. To provide direct evidence, another approach could be the cloning of genes involved in plasmodesmatal functions and the modification of their expression in transgenic plants. Overexpression of viral movement proteins has been shown to alter the size exclusion limit of plasmodesmata. The increase in the size exclusion limit in a single transformant of tobacco due to overexpression of a movement protein led to an increase in carbohydrate content in the mature leaves and a decreased efflux of assimilates. As this is opposite to what might have been expected in the case of a major flux through plasmodesmata, the rate limiting step for phloem loading must reside inside the phloem (Lucas *et al.*, 1993).

Further support for the apoplastic hypothesis comes from an analysis of plants that express an invertase from yeast in the apoplast of potato. If sucrose transport is mainly mediated through the plasmodesmata, it should not appear in high concentrations in the cell wall compartment. Thus the presence of an invertase in the apoplast should not lead to major effects. However, if the major route of sucrose is carrier mediated and thus sucrose has to pass the apoplastic compartment, the invertase should dramatically affect assimilate partitioning because hexoses do not seem to be translocated efficiently into the phloem. Strong phenotypic effects such as leaf curling, reduced root growth and tuber yield and significant changes in the concentration of various metabolites occur. It is therefore concluded that at least in the cases tested so far (potato, tomato, tobacco and *Arabidopsis*), apoplastic, carrier mediated loading of the phloem is predominant (von Schaewen *et al.*, 1990; Dickinson *et al.*, 1991; Sonnewald *et al.*, 1991; Heineke *et al.*, 1992). The similarities between the SUT1 antisense plants described here and the transgenic potato plants overexpressing the invertase in the cell wall are striking. Similar to what is described here for the SUT1 antisense plants, the plants expressing invertase in the apoplast do not show apparent effects in the sink leaves. However, direct measurements of the photosynthetic activity in these leaves showed compensatory effects (Sonnewald *et al.*, 1991). Nevertheless the phenotypes are different. The surface of the SUT1 antisense plants is not shiny, and the bleaching and the increase in carbohydrate content is much stronger in the SUT1 antisense plants than with the invertase plants.

Other potential functions of the sucrose transporter

Low levels of expression of the SUT1 gene were also found in stem and sink tissues. Because the promoter that was used for antisense inhibition is expressed in all tissues of the plant, we cannot exclude the possibility that the effects observed are additive from a reduction of the export from the leaves and an inhibition of the unloading in sink tissues. The use of tissue specific promoters for antisense inhibition and grafting of transformed parts of transgenic plants to wild type plants will be used to dissect this complexity.

The antisense plants will provide an excellent tool to study the mechanisms guiding phloem translocation, as, if sucrose loading is the primary driving force for long distance transport, the reduced loading should also affect the translocation of other solutes such as amino acids and ions.

Conclusion

The results described above indicate that the sucrose transporter is responsible for the loading of the phloem with sucrose and is crucial for growth and development of the potato plant. As the RNA level is not reduced to zero, we have to assume that a null mutation in the SUT1 gene would be lethal. Further experiments aim at a determination of the rate of reduction and to see if the carriers may become limiting *in vivo*, as in the case of TPT (Riesmeier *et al.*, 1993b). As a next step, overexpression studies directed towards increasing the transport capacity will be necessary.

Materials and methods

Recombinant DNA

The 1.35 kb *Bam*HI fragment of the potato sucrose transporter cDNA P62 (Riesmeier *et al.*, 1993a) was ligated in reverse orientation into the *Sma*I restriction site of pBinHygTx (a pBin19 based binary vector with a hygromycin selectable marker under control of the 35S promoter and an expression cassette containing the TripleX promoter, a multiple cloning site and an octopine synthase terminator; R.Höfgen, personal communication). The SUT1 antisense gene under control of the Triple X-CaMV 35S promoter was excised and cloned into pBin19 (Bevan, 1984; Gatz *et al.*, 1992). As the TripleX promoter was used in the absence of the tetracyclin repressor, no quantitative or qualitative differences should be expected as compared with the normal CaMV 35S promoter.

Transformation and analysis of transgenic plants

Transfer of the chimeric construct into *Agrobacterium tumefaciens* GV2260 and transformation of *S.tuberosum* cv. Désirée was performed as described

(Rocha-Sosa *et al.*, 1989). Transgenic plants were transferred to soil and analysed under greenhouse conditions. Experiments were repeated independently using *in vitro* propagated clones of the transformants. For Northern blot analysis, RNA was isolated from mature leaves of greenhouse grown transformants and wild type plants after 4–6 h of light as described previously (Riesmeier *et al.*, 1992). To ensure the reproducibility, RNA was isolated from both young and mature plants and from different source leaves. Similar levels of reduction in SUT1 mRNA were observed irrespective of the age of the plant.

Physiological measurements

All physiological measurements were performed with plants grown in the greenhouse under the low light conditions with ~100 PAR (100 $\mu E/m^2/s$) between January and March. Maximal photosynthesis rates were measured from leaf discs of greenhouse grown plants with a Hansatech leaf-disc electrode (Kings Lynn, Norfolk, UK) under saturating light conditions. Each measurement was reproduced independently at least six times (Quick *et al.*, 1989). Starch determination was performed according to Lin *et al.* (1988) and metabolites were analysed (Gerhardt *et al.*, 1987). The data shown for sugar and starch levels are representative of multiple repetitions of the analysis from greenhouse grown plants with samples taken from different leaves. Enzyme activities were determined essentially as described in Lea (1990).

Efflux

Efflux of carbohydrates from petioles of control and antisense plants was determined after petioles from mature leaves were cut from 10 week old greenhouse plants and incubated in 5 mM EDTA pH 6.0 for 8 h in the light (100 $\mu mol/m^2/s$). The amounts of glucose, fructose and sucrose in the incubation solution were determined enzymatically. The sum of hexoses is shown because there were no differences in the relative concentrations in control and antisense plants.

Acknowledgements

We would like to thank R.Höfgen (Schering AG, Berlin), J.Kossmann and B.Müller-Röber (IGF, Berlin) and U.Sonnewald (IPK, Gaterleben) for providing the binary expression cassette pBinHygTx, the FBPase, AGPase and SPS cDNA clones respectively. We are very grateful to J.Dietze for transformation and B.Burose for taking care of the plants in the greenhouse. This work was supported by grants to W.B.F. from BRIDGE Project EC BIOTEC-0175EC and Bundesministerium für Forschung und Technologie (Saccharose Transport).

References

Bevan,M. (1984) *Nucleic Acids Res.*, **12**, 8711–8721.
Bush,D.R. (1990) *Plant Physiol.*, **93**, 1590–1596.
Bush,D.R. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **44**, 513–542.
Dickinson,C.D., Altabella,T. and Chrispeels,M.J. (1991) *Plant Physiol.*, **95**, 420–425.
Frommer,W.B., Hummel,S. and Riesmeier,J.W. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 5944–5948.
Gatz,C., Frohberg,C. and Wendenburg,R. (1992) *Plant J.*, **2**, 397–404.
Gerhardt,R., Stitt,M. and Heldt,H.W. (1987) *Plant Physiol.*, **83**, 399–407.
Heineke,D., Sonnewald,U., Büssis,G., Günter,K., Leidreiter,K., Wilke,I., Raschke,K., Willmitzer,L. and Heldt,H.W. (1992) *Plant Physiol.*, **100**, 301–308.
King,R.W. and Zeevart,J.A.D. (1974) *Plant Physiol.*, **53**, 96–103.
Kossmann,J., Müller-Röber,B.T., Dyer,T., Raines,C.A., Sonnewald,U. and Willmitzer,L. (1992) *Planta*, **188**, 7–12.
Krapp,A., Hofmann,B., Schäfer,C. and Stitt,M. (1993) *Plant J.*, **3**, 817–828.
Kwart,M., Hirner,B., Hummel,S. and Frommer,W.B. (1993) *Plant J.*, **4**.
Lea (1990) In Dey,P.M. and Harbourne,J.B. (eds), *Methods in Plant Biochemistry*. Vol 3. Academic Press, London.
Lemoine,R., Gallet,O., Gaillard,C., Frommer,W.B. and Delrot,S. (1992) *Plant Physiol.*, **100**, 1150–1156.
Lin,T.-P., Caspar,T., Somerville,C. and Preiss,J. (1988) *Plant Physiol.*, **86**, 1131–1135.
Lohaus,G., Winter,H., Riens,B. and Heldt,H.W. (1993) *Planta*, in press.
Lucas,W.J., Olesinski,A., Hull,R.J., Haudenshield,J.S., Deom,C.M., Beachy,R.N. and Wolf,S. (1993) *Planta*, **190**, 88–96.
Maynard,J.W. and Lucas,W.J. (1985) *Plant Physiol.*, **70**, 1436–1443.
McCauley,M.M. and Evert,R.F. (1989) *Bot. Gaz.*, **150**, 351–368.
Müller-Röber,B.T., Kossmann,J., Hannah,L.C., Willmitzer,L. and Sonnewald,U. (1990) *Mol. Gen. Genet.*, **224**, 136–146.
Quick,P., Siegl,G., Neuhaus,E., Feil,R. and Stitt,M. (1989) *Planta*, **177**, 535–546.
Riesmeier,J.W., Willmitzer,L. and Frommer,W.B. (1992) *EMBO J.*, **11**, 4705–4713.
Riesmeier,J.W., Hirner,B. and Frommer,W.B. (1993a) *Plant Cell*, in press.
Riesmeier,J.W., Flügge,U.I., Schulz,B., Heineke,D., Heldt,H.W., Willmitzer,L. and Frommer,W.B. (1993b) *Proc. Natl Acad. Sci. USA*, **90**, 6160–6164.
Robards,A.W. and Lucas,W.J. (1990) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **41**, 369–419.
Rocha-Sosa,M., Sonnewald,U., Frommer,W.B., Stratmann,M., Schell,J. and Willmitzer,L. (1989) *EMBO J.*, **8**, 23–29.
Salanoubat,M. and Belliard,G. (1989) *Gene*, **84**, 181–185.
Sonnewald,U., Brauer,M., von Schaewen,A., Stitt,M. and Willmitzer,L. (1991) *Plant J.*, **1**, 95–106.
Stitt,M. (1990) In Dennis,D.T. and Turpin,D.H. (eds), *Plant Physiology, Biochemistry and Molecular Biology*. Longman Science, UK, pp. 309–326.
Stitt,M. (1991) *Plant Cell Env.*, **14**, 741–762.
van Bel,A.J.E., Gamalei,Y.V., Ammerlaan,A. and Bik,L.P.M. (1992) *Planta*, **186**, 518–525.
von Schaewen,A., Stitt,M., Schmidt,R., Sonnewald,U. and Willmitzer,L. (1990) *EMBO J.*, **9**, 3033–3044.

Received on August 19, 1993; revised on October 7, 1993