Plant Physiol. (1991) 95, 420–425
0032-0889/91/95/0420/06/$01.00/0

Received for publication August 22, 1990
Accepted October 6, 1990

Slow-Growth Phenotype of Transgenic Tomato Expressing Apoplastic Invertase[1]

Craig D. Dickinson*[2], Teresa Altabella[3], and Maarten J. Chrispeels
Department of Biology, University of California, San Diego, La Jolla, California 92093–0116

ABSTRACT

The growth of transgenic tomato (*Lycopersicon esculentum*) plants that express in their apoplast yeast invertase under the control of the cauliflower mosaic virus 35S promoter is severely inhibited. The higher the level of invertase, the greater the inhibition of growth. A second phenotypic characteristic of these transgenic plants is the development of yellow and necrotic spots on the leaves, and leaf curling. Again the severity of the symptoms is correlated with the level of invertase. These symptoms do not develop in shaded leaves indicating the need for photosynthesis. Keeping the plants in the dark for a prolonged period (24 hours) results in the disappearance of leaf starch from the control plants, but not from the plants with apoplastic invertase. These results are consistent with the interpretation that apoplastic invertase prevents photosynthate export from source leaves and that phloem loading includes an apoplastic step.

Assimilate partitioning, the allocation of assimilates to various sinks, is one of the most intriguing processes in whole-plant physiology. Central to this process is the translocation in the phloem of the products of photosynthesis, typically sucrose, from the site of synthesis (source tissues) to the site of utilization or accumulation (sink tissues). To be transported in the phloem, sucrose must first be transported from one mesophyll cell to the next and then enter the phloem cells (sieve elements/companion cells). The most likely pathway for the movement of sucrose between mesophyll cells is generally accepted to be symplastic: through the plasmodesmata that connect these cells (15). Much evidence favors the presence of an apoplastic transport step just prior to entry of sucrose into the minor phloem veins (phloem loading) (15). This would presumably involve facilitated efflux of sucrose from mesophyll cells into the apoplast and active transport of sucrose into the phloem. A sucrose-proton cotransporter located at the plasma membrane of phloem cells is thought to be responsible for generating high concentrations of sucrose at source tissues which drives long-distance transport to sinks (8). Another possibility is that sucrose follows an entirely symplastic pathway from mesophyll cells to the phloem (10, 26), or that both symplastic and apoplastic mechanisms operate simultaneously.

Four lines of evidence support the hypothesis for phloem loading of sucrose from the apoplast. First, the sucrose concentration in minor veins is usually at least three times higher than that of the neighboring cells indicating active transport against a concentration gradient (15). This is inconsistent with the passive diffusion which is expected of plasmodesmatal transport. Second, in experiments by Geiger *et al.* (12), high levels of sucrose were trapped in the free space after labeling with $^{14}CO_2$. Third, the nonpermeable sulfhydryl group modifier *p*-chloromercuribenzene sulfonic acid supplied to the apoplast of sugar beet leaves was found by Giaquinta (14) to inhibit assimilate translocation during photosynthesis in $^{14}CO_2$. This has been taken as evidence for an extracellular step in sucrose transport since only extracellular or membrane proteins would be expected to be inactivated by the treatment. Fourth, sucrose dependent membrane depolarization has been observed in leaves (13) and isolated phloem from celery (7). These results are consistent with a sucrose-H^+ cotransporter at the plasma membrane.

The main evidence for the symplastic path for phloem loading of sucrose is the presence of plasmodesmatal connections between mesophyll and phloem cells in mature leaves (3). However, these plasmodesmata may not be functional and could be vestiges of the developmental stage of the leaf in which it was a net importer of carbon (15). The sink-to-source transition in leaf development is an excellent system for studying mechanisms of photoassimilate partitioning. The change is developmentally controlled and proceeds basipetally when the leaf is 30 to 60% expanded (25). Unloading in sink leaves appears to occur symplastically through plasmodesmatal connections (25) although as with loading, hard proof is lacking.

As part of our work on sorting of vacuolar and extracellular proteins, we introduced into tomato plants a chimeric gene, the expression of which would result in the presence of invertase in the apoplast. Although cells secrete invertase under certain conditions (*e.g.* in certain sink tissues or as a result of wounding), apoplastic invertase in source tissues is thought to be quite low (14). We report that expression of secreted yeast invertase from the cauliflower mosaic virus 35S promoter inhibits growth substantially and causes deformation of mature (source) leaves. The phenotype appears to be caused by sucrose hydrolysis in the apoplast which results in an inhibition of photoassimilate export from source leaves. At a recent meeting, Willmitzer *et al.* (28) reported similar results with transgenic tobacco plants.

[1] Supported by grants from the National Science Foundation (Cell Biology) and the United States Department of Energy (Basic Energy Sciences).

[2] Recipient of a US Public Health Service postdoctoral fellowship.

[3] Present address: Laboratorio de Fisiologia Vegetal, Facultad de Farmacia, 08028 Barcelona, Spain.

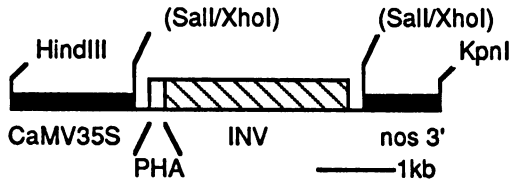

Figure 1. Diagram of the 35sINV44 construct used to transform plants. Relevant restriction sites used in the cloning are shown. Parentheses indicate sites destroyed by the cloning. The cauliflower mosaic virus 35S promoter (CaMV35S) drives expression of a PHA-invertase (INV) hybrid with the nopaline synthase polyadenylation sequences (nos3'). The flanking sites were used to place the chimeric gene into the binary *Agrobacterium* vector BIN19 (2) for transformation of plants to kanamycin resistance.

MATERIALS AND METHODS

Construction of p35PHA44

A map of p35PHA44 is shown in Figure 1. This construct contains a dlec2 (encodes PHA[4]-L)—*SUC2* (encodes yeast invertase) gene fusion from plasmid PHA44 (23). For expression of this fusion protein in plants, a cauliflower mosaic virus 35S promoter-nopaline synthase terminator cassette called p35nos was first constructed. Plasmid p35nos was derived from plasmid pDO432 (21). A *Hin*dIII-*Kpn*I fragment containing the 35S-luciferase gene from pDO432 was cloned into the corresponding sites of BIN19 (2). The luciferase coding region was removed from the resulting plasmid by restricting with *Bam*HI and *Sal*I, filling in the ends with Klenow polymerase, and introducing a *Sal*I linker (5'-CGTCGACG-3') in place of the luciferase sequence. A *Xho*I fragment containing the PHA-invertase fusion was isolated from PHA44 and cloned into the *Sal*I site of p35nos to create plasmid p35PHA44.

Agrobacterium-Mediated Transformations

The invertase expression-plasmid p35PHA44 and a control plasmid BIN19 in *Escherichia coli* strain DH5α were transferred into *Agrobacterium tumefaciens* strain LBA4404 by triparental mating as described by Voelker *et al.* (27). Plant transformations with the tomato hybrid *Lycopersicon esculentum* (cv VF36) × *L. pennellii* (LA716) were performed according to schedule 4 of Chyi and Phillips (6) with minor changes. Stem segments freshly innoculated with *Agrobacterium* were placed on shooting medium with 500 μg/mL Claforan (cefotaxime sodium, Hoechst-Roussel Pharmaceutical Inc.), and after 3 weeks the concentration was reduced to 250 μg/mL. The shoots from the control transformation had to be subcultured several times because of the slower growth of invertase-expressing shoots. Transformants were selected by ability to root in medium containing 100 μg/mL kanamycin. Similar-sized plants about 3 cm in height were transferred to soil for growth comparisons and after 3 weeks in a growth room at 25°C, 5000 lux they were transferred to the greenhouse.

[4] Abbreviations: PHA, phytohemagglutinin; sINV, secreted invertase; wt, wild type; CaMV, cauliflower mosaic virus.

Invertase Enzyme Assays and Activity Gels

To compare levels of invertase activity in different transformants petiole tissue from mature leaves were assayed. Tissue samples were weighed and homogenized in a glass homogenizer with 5 μL/mg tissue of homogenization buffer which contains 25 mM Tris (pH 7.5), 10% glycerol, 5 mM EDTA, 5% β-mercaptoethanol, 0.1% SDS, 2 μM pepstatin, and 1 μg/mL leupeptin. Homogenates were centrifuged for 10 min at 14,000*g*, and the supernatant was stored on ice or at −80°C. Petiolar extracts were adjusted to contain equal concentrations of protein. Protein concentrations were determined by the Bradford reagent method (Bio-Rad). For comparisons of invertase activities of leaves, 5 mm diameter leaf discs were homogenized in 300 μL of homogenization buffer. Invertase was extracted from protoplasts by resuspending 10^6 cells in 100 μL homogenization buffer without SDS and vortexing the cells in microfuge tubes with acid-washed glass beads at 4°C for 5 min. Enzymatic assays were performed with 1 to 5 μL of cleared extract in 100 μL of 0.1 M sodium acetate, 0.1 M sucrose for 30 min at 30°C. Reactions were stopped by boiling for 3 min and 100 μL of 0.5 M potasium phospate (pH 7.0) was added. Glucose liberated by the reactions was detected by a coupled glucose oxidase/horse radish peroxidase procedure using 1 mg/mL 4-chloro-1-napthol as the color reagent as described previously (23). One unit is defined as the amount of glucose (nanomoles) liberated per min at 30°C. Invertase activity gels were performed as described previously (23). For comparisons of the mobility with that of yeast invertase, extracts were made from *Saccharomyces cerevisiae* strain 20B12 (19) grown in YEPD medium with 0.1% glucose in place of sucrose (23).

Protoplast Preparation and Culture

Protoplasts were prepared from leaves of *in vitro* grown shoot cultures of transformed and control plants by the method of Damm and Willmitzer (9). Protoplasts were cultured for 48 h in Murashige and Skoog medium modified to contain 0.4 M glucose, 0.1 mg/L 2,4-dichlorophenoxyacetic acid, 1 mg/L naphtalene acetic acid, and 0.2 mg/L benzyladenine. Fractionation was performed by centrifugation at 100*g* in a swinging bucket rotor. The supernatant or medium fractions were further cleared by consecutive centrifugations at 500*g* and 2000*g*. Protoplast fractions were frozen at −80°C and medium fractions were concentrated with Centricon 30 microconcentrators (Amicon), mixed with an equal volume of 2× homogenization buffer, and saved at −80°C for analysis. The α-mannosidase assay to test for cell disruption was as previously described (5).

Starch Assay

Leaves were qualitatively tested for the presence of starch by iodine staining for 30 min in Lugol's solution (Sigma). Leaves were decolorized by boiling for 10 min in 95% ethanol and rinsed in water before staining.

RESULTS

Construction of fusions between the seed lectin gene encoding PHA-L and the yeast invertase gene have been described

previously (23). One of these fusions, called PHA44, resulted in efficient secretion of invertase to the yeast cell wall (23). The protein produced by this plasmid contained the first 44 amino acids of PHA including a 20 amino acid signal peptide fused to a truncated invertase in which sequences for its own signal peptide were removed. To see if this protein would also be secreted in plants, we placed the PHA-invertase coding sequence into a cauliflower mosaic virus 35S (CaMV35S) promoter-terminator cassette to create 35sINV44 (Fig. 1).

The 35sINV44 construct and BIN19 (as a vector-only control) were introduced into the interspecific tomato hybrid *Lycopersicon esculentum* × *L. pennellii* by *A. tumefaciens* mediated transformation of stem segments (6). Ten transgenic plants were obtained from the 35sINV44 construct (sINV plants) by selection on kanamycin. While selecting for transformants in culture, phenotypic differences between the wt (transformed with BIN19) and the sINV shoots became apparent. Many of the sINV shoots grew slower and produced smaller leaves than the wt. The slower growth was not obvious until the shoots were separated from the parent (untransformed) tissue. To compare growth under greenhouse conditions, sINV and wt plants were transferred to soil coincidentally as plants 2 to 3 cm tall. The wt and some faster growing sINV plantlets had to be subcultured to obtain plants of approximately the same size. Four of the sINV plants died within a week after transfer to soil while this occurred with none of the wt plants. This raised the possibility that yeast invertase expressed from the CaMV35S promoter could in some cases be lethal.

Invertase Activity in Transformed Plants

The sINV plants which survived in soil were shown to contain high levels of invertase in their leaves. Figure 2A shows a result obtained from three of these plants compared with wt. Extracts were made from petioles of mature leaves, fractionated on a native polyacrylamide gel, and stained for invertase activity. With the conditions used for the extraction and assay, no activity was detected in wt extracts. A quantitative assay of the extracts gave values of 0.28, 0.63, and 1.3 units/μg protein for sINV plants a, b, and c, respectively. The invertase activity gel provided information on the assembly and glycosylation state the the invertase produced by the plants. As a mobility standard, an extract of yeast grown under derepressed conditions (low glucose) was included and is shown in the first lane. Two molecular forms of invertase are apparent. The faster migrating discrete band resulted from cytosolic invertase while the slower migrating and more heterogeneous band resulted from secreted invertase which is glycosylated and highly assembled (4). The bands from the sINV extracts more closely resemble the secreted form of invertase. The result suggests that invertase from the sINV plants was glycosylated and assembled as a result of entering the secretory pathway. A slower mobility of the yeast protein was expected because in yeast the core glycans are modified extensively by the addition of outer chain mannose residues and this contributes to a higher mol wt (1). Plant glycoproteins have high mannose glycans with a maximum of nine mannose residues or smaller complex glycans (11). Qualitative invertase assays revealed the presence of high levels of invertase activity

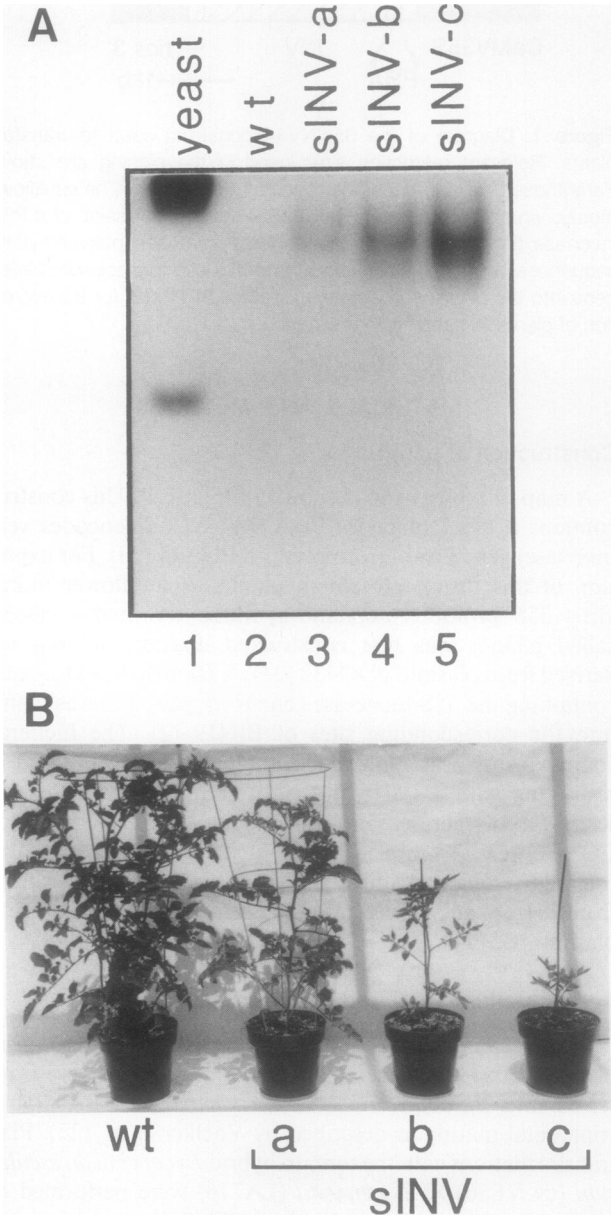

Figure 2. Transgenic plants expressing yeast invertase are inhibited in their growth. Panel A shows invertase activity in transformed plants. Extracts from petioles of fully expanded leaves were prepared from tomato plants that were selected on kanamycin. Aliquots of 700 ng of protein from each sample were loaded onto a native acrylamide gel and stained for invertase activity with triphenyltetrazolium chloride. Lane 1 contains an extract from yeast cultured in 0.1% glucose as a standard, and lane 2 contains an extract from a wt plant (transformed with BIN19 as a vector-only control). Extracts from plants transformed with 35sINV44 were loaded in lanes 3, 4, and 5. Panel B shows the effect of yeast invertase expression on growth of the transformed plants. The same plants as in A are shown after 3 months of growth in the greenhouse.

in roots, stems, and leaves of sINV plants. Mature leaves contained up to threefold more invertase activity than immature leaves on a leaf area basis of comparison. To determine the cellular location of the invertase, leaf protoplasts of transformants were prepared and cultured. After 48 h of incubation 95% of the sINV activity was found in the culture medium (Fig. 3) indicating that the invertase was secreted from these cells.

Invertase Causes Growth Reduction and Altered Leaves

Figure 2B shows a wt plant with three representative sINV plants after 3 months of growth in the greenhouse. The sINV plants grew dramatically slower and the degree to which the plants were growth-inhibited correlated with the level of invertase expression in petioles of mature leaves as can be inferred by comparing sizes of the plants with the intensity of signals obtained from the activity gel shown in the same figure.

The severity of other abnormal characteristics associated with the leaves of sINV plants also correlated with levels of invertase expression. Immature sINV leaves were mophologically indistinguishable from wt immature leaves (Fig. 4A). Mature sINV leaves, however, were smaller, chlorotic, and showed a characteristic curling of the leaflets (Fig. 4, B and C). Leaflet curling was the first abnormality that was observed as the leaf matured. The terminal leaflet at the tip of a leaf was the first to curl and curling spread to other leaflets basipetally during maturation. As leaves of the sINV plants became older they became progressively chlorotic. Yellowing began in the interveinal regions and progressed throughout the leaf, but often the larger veins remained green. In the case of the more severe phenotypes (*i.e.* sINVb and c), occasional necrosis developed in the mature leaves (Fig. 4C). To test if light was required for the development of symptoms in the sINV leaves, the upper surface of an sINVb leaf was shaded with foil at an immature stage and throughout its growth while the lower surface was left unshaded. The shaded leaf was larger, and did not show the full blown symptoms of the neighboring unshaded leaves (Fig. 4D). The sINV plants flowered and set seed while sINVb and c plants flowered yet produced no fruit or seed.

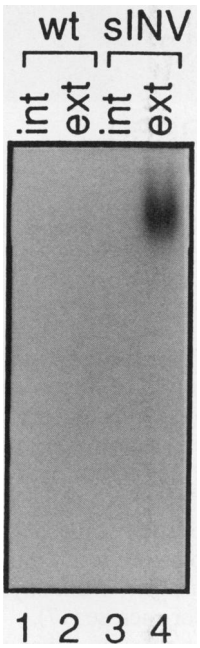

Figure 3. Secretion of invertase from leaf protoplasts. Protoplasts from wt (lanes 1 and 2) and sINV-a (lanes 3 and 4) plants were cultivated for 48 h and fractionated. Internal fractions (lanes 1 and 3) contained cells while extracellular fractions (lanes 2 and 4) contained concentrated media. Samples were loaded onto a native acrylamide gel and stained for invertase activity. Part of the α-mannosidase activity (94%) was found in the internal fractions indicating that the cells were not broken. The integrity of the cells was also monitored by visual inspection under the microscope.

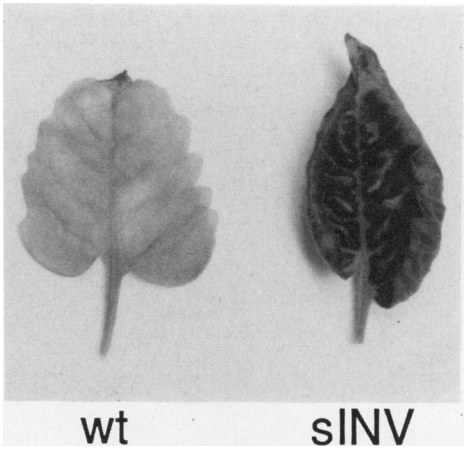

Figure 4. Phenotypes of leaves from plants expressing yeast invertase. A, Immature leaves of a wt plant and an invertase (sINV-b) plant of similar size; B, mature leaves (the second leaf which was fully expanded) from wt and an invertase (sINV-b) plant of similar size (bars represent 1 cm); C, curling, chlorosis, and necrosis of an older leaf from an invertase (sINV-b) plant; D, effect of light on leaf morphology of an invertase (sINV-b) plant. The leaf in the middle was shaded as an immature leaf. Leaves in the flanking positions received full sun. Basipetial direction is from left to right.

Apoplastic Invertase Prevents the Utilization of Stored Leaf Starch

To test if carbohydrate metabolism were altered by the presence of elevated levels of apoplastic invertase, we stained the leaves for starch both at the end of a normal light period and again after keeping the plants in the dark for 24 h. At the end of the normal light period both wt and sINV leaves stained heavily for starch. At the end of the prolonged dark period, the wt leaves had lost all their starch, but the sINV leaves still stained heavily for starch. A typical staining reaction at the end of the dark period is shown in Figure 5. Other leaves of wt and sINV plants showed similar staining. These results show that the plants with apoplastic invertase were unable to catabolize the starch stored in the leaves, or did so at a slower rate. We cannot exclude the possibility that the sINV leaves contained more starch than the wt leaves at the beginning of the dark period.

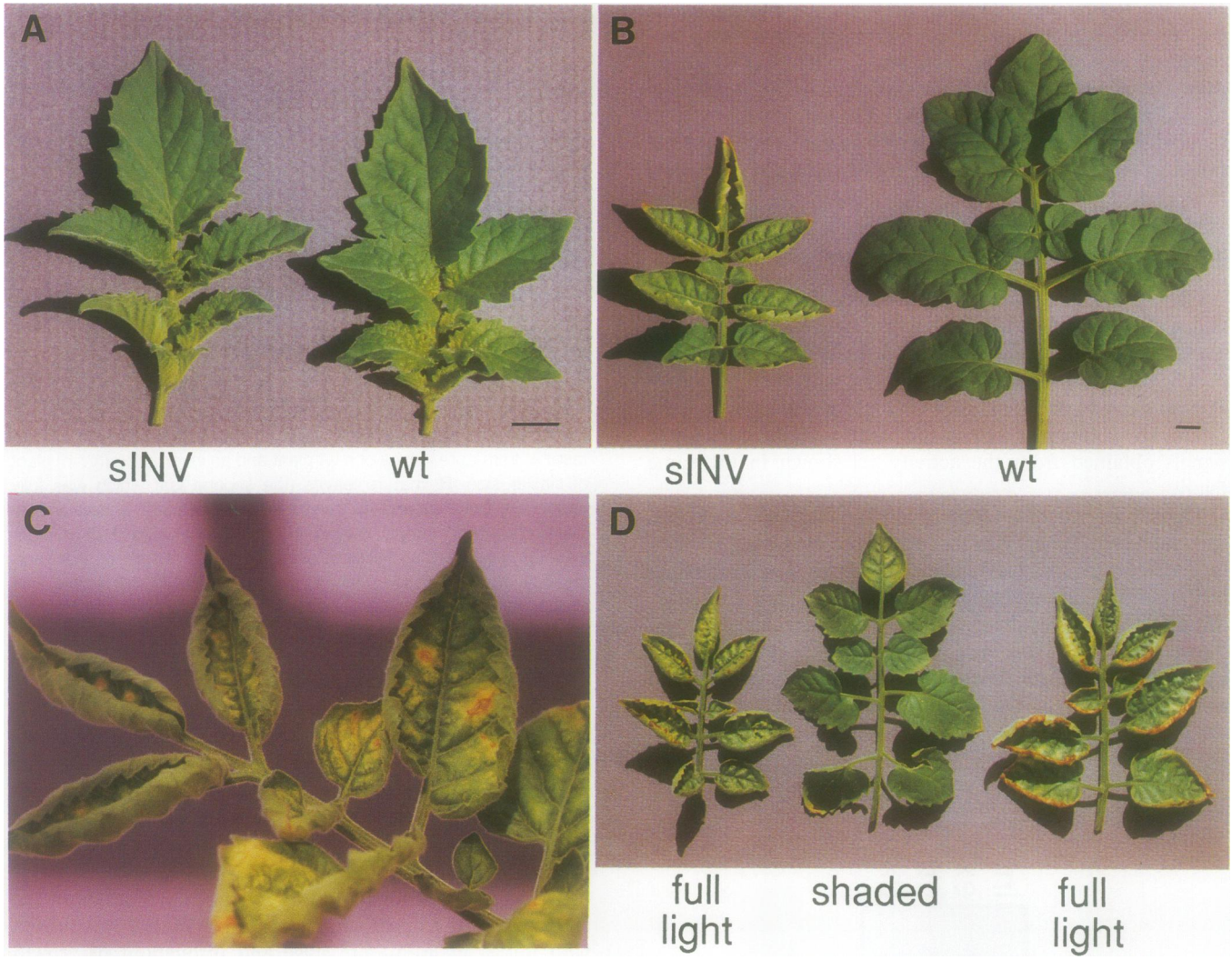

Figure 5. Starch in leaflets of invertase plants after dark treatment. Wt and invertase producing (sINV-b) plants were placed in the dark for 24 h and stained for starch. The central leaflets of the second fully expanded leaf from similarly sized plants were stained with iodine/potasium iodide solution. Strong staining of both wt and sINV leaves was observed at the end of a 12 h period in the light.

DISCUSSION

We have obtained transgenic tomato plants that accumulated high levels of yeast invertase in leaves, stems, and roots. Invertase activity was shown to be efficiently secreted by protoplasts derived from leaves. The product of this PHA-invertase gene fusion has also been shown to be secreted efficiently from yeast (23) and from tobacco protoplasts (our unpublished observations). Its mobility in native acylamide gels suggested that the invertase was glycosylated and therefore traversed the secretory pathway. Transformants were found to contain different amounts of invertase activity. This was probably the result of chromosomal position effects on the transgene which are common with *A. tumifaciens* transformed plants (20).

Transgenic plants producing a secreted form of yeast invertase displayed a dramatic phenotype. The most obvious feature of the phenotype was reduction in overall growth which correlated with the levels of invertase expression from different transformants. The most likely explanation for the effect of invertase on growth is an inhibition of transport of photoassimilate after hydrolysis of sucrose in the apoplast. As we have mentioned, there is compelling evidence in several plant species for an apoplastic step which precedes loading of sucrose into the phloem. If sucrose hydrolysis in the apoplast prevents phloem loading of sugars due to specificity in the transport machinery for sucrose then slower overall growth of the plant may be an expected result. Studies performed on a presumed sucrose transporter from soybean cotyledons suggest a high specificity for sucrose (17).

Although we cannot rule out other mechanisms such as altered gene regulation or changes in hormonal control of the sINV plants, four lines of evidence suggest that the phenotype is caused by a block in sucrose transport from the source leaf. First, the sINV plants grew more slowly and the growth reduction became more severe in plants secreting more invertase. Growing tissues rely on carbon transported from source leaves and if this supply were reduced then slower growth would be expected. It is reasonable to assume that the extracellular invertase would hydrolyze sucrose in the apoplast of

the source leaf, the likely route taken by sucrose for export from the leaf. The second result implicating an inhibition of sucrose transport is that in mature leaves that produce apoplastic invertase starch was not completely mobilized after prolonged periods in the dark. In mature leaves of normal plants, this starch is converted to sucrose for transport during the dark period (22). If carbon transport were blocked, then less starch would be mobilized as appears to be the case for the sINV plants.

A third line of evidence favoring the view that apoplastic invertase inhibits sucrose translocation is the similarity of the sINV phenotype with symptoms displayed by tomato plants grown in high CO_2 (24). Like the sINV plants, conditions of high CO_2 result in an increase in the level of starch, curling of the leaves, and chlorotic leaves. The basis of the leaf deformity is not known, but it appears to be rooted in an oversupply of carbon in the leaves. The similarity of the phenotype of the sINV plants suggests that an excess of carbon occurs in source leaves which may be due to a block in sucrose translocation.

A fourth argument for the sINV phenotype resulting from reduced sucrose translocation is also based on the phenotypic character of the leaves. The sINV immature leaves are mophologically normal and only begin to show symptoms when immature sink leaves develop into source leaves. Furthermore, development of symptoms which appear first at the tip of a developing sINV leaf and proceeds basipetally, parallels the sink to source transition of a leaf which also occurs basipetally (25). The importance of this point is that symptom development appears to coincide with development of phloem loading capacity and, presumably, the presence of extracellular sucrose. Nevertheless, development of phloem loading capacity appears not to be sufficient for symptom development. Shading an sINV leaf alleviates symptoms suggesting that photosynthesis was required to produce the sINV phenotype.

It is clear that extracellular invertase can have a profound effect on carbon metabolism in tomato plants. To the extent the above arguments for a block in sucrose transport are convincing, the results support a model for phloem loading which involves uptake of sucrose from the apoplast. The dramatic effect of secreted invertase on overall growth indicates that an apoplastic step is extremely important for carbon transport in tomato and that the translocation machinery exhibits specificity for sucrose.

There is great potential for improving crop yield through altering patterns of photoassimilate partitioning (16, 18). The results presented here demonstrate that sucrose metabolism and overall growth are amenable to modification by the genetic manipulation of extracellular invertase. Related approaches may be useful in future efforts to improve crop productivity.

LITERATURE CITED

1. **Ballou C** (1982) Yeast cell wall and cell surface. *In* J Strathern, E Jones, J Broach, eds, The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 335–360
2. **Bevan M** (1984) Binary Agrobacterium vectors for plant transformation. Nucleic Acids Res **12:** 8711–8721
3. **Botha CEJ, Evert RF** (1988) Plasmodesmatal distribution and frequency in vascular bundles and contiguous tissues of the leaf of *Themeda triandra*. Planta **173:** 433–441
4. **Carlson M, Botstein D** (1982) Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:** 145–154
5. **Chrispeels MJ, Boulter D** (1975) Control of storage protein metabolism in the cotyledons of germinating mung beans: role of endopeptidase. Plant Physiol **55:** 1031–1037
6. **Chyi Y-S, Philips GC** (1987) High efficiency *Agrobacterium*-mediated transformation of *Lycopersicon* based on conditions favorable for regeneration. Plant Cell Rep **6:** 105–108
7. **Daie J** (1987) Sucrose uptake in isolated phloem of celery is a single saturable system. Planta **171:** 474–482
8. **Daie J** (1989) Phloem loading of sucrose: update and opportunities in molecular biology. Plant Mol Biol Rep **7:** 106–115
9. **Damm B, Willmitzer L** (1988) Regeneration of fertile plants from protoplasts of different *Arabidopsis thaliana* genotypes. Mol Gen Genet **213:** 15–20
10. **Delrot S, Bonnemain JL** (1981) Involvement of protons as a substrate for sucrose carrier during phloem loading in *Vicia faba* leaves. Plant Physiol **67:** 560–564
11. **Faye L, Johnson KD, Sturm A, Chrispeels MJ** (1989) Structure, biosynthesis, and function of asparagine-linked glycans on plant glycoproteins. Physiol Plant **75:** 309–314
12. **Geiger DR, Sovonick SA, Shock TL, Fellows RJ** (1974) Role of free space in translocation in sugar beet. Plant Physiol **54:** 892–898
13. **Giaquinta RT** (1977) Possible role of pH gradient and membrane ATPase in the loading of sucrose into the sieve tubes. Nature **267:** 369–370
14. **Giaquinta RT** (1980) Translocation of sucrose and oligosaccharides. *In* J Preiss, ed, The Biochemistry of Plants. Carbohydrates: Structure and Function. Academic Press, New York, pp 271–320
15. **Giaquinta RT** (1983) Phloem loading of sucrose. Annu Rev Plant Physiol **34:** 347–387
16. **Gifford RM, Thorne JH, Hitz WD, Giaquinta RT** (1984) Crop productivity and photoassimilate partitioning. Science **225:** 801–808
17. **Hitz WD, Card PJ, Ripp KG** (1986) Substrate recognition by a sucrose transporting protein. J Biol Chem **261:** 11986–11991
18. **Ho LC** (1988) Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annu Rev Plant Physiol Plant Mol Biol **39:** 355–378
19. **Jones EW** (1977) Proteinase mutants of *Saccharomyces cerevisiae*. Genetics **85:** 23–33
20. **Kuhlemeier C, Green PJ, Chua N-H** (1987) Regulation of gene expression in higher plants. Annu Rev Plant Physiol **38:** 221–257
21. **Ow DW, Wood KV, DeLuca M, de Wet JR, Helinski DR, Howell SH** (1986) Transient and stable expression on the firefly luciferase gene in plant cells and transgenic plants. Science **234:** 856–859
22. **Preiss J, Levi C** (1980) Starch biosynthesis and degradation. *In* J Preiss, eds, The Biochemistry of Plants. Academic Press, New York, pp 371–423
23. **Tague BW, Dickinson CD, Chrispeels MJ** (1990) A short domain of the plant vacuolar protein phytohemagglutinin targets invertase to the yeast vacuole. Plant Cell **2:** 533–546
24. **Tripp K, Peet M, Pharr DM, Willits D** (1990) CO_2 enrichment of tomatoes: relationship of foliar stress symptoms to starch concentrations and carbon exchange rates (abstract No. 320). Plant Physiol. **93,** S56
25. **Turgeon R** (1989) The sink-source transition in leaves. Annu Rev Plant Physiol Plant Mol Biol **40:** 119–138
26. **Van Bel AJE, Koops AJ** (1985) Uptake of ^{14}C-sucrose in isolated minor vein network of *Commelina benghalensis*. Planta **164:** 362–369
27. **Voelker T, Sturm A, Chrispeels MJ** (1987) Differences in expression between two seed lectin alleles obtained from normal and lectin-deficient beans are maintained in transgenic tobacco. EMBO J **6:** 3571–3577
28. **Willmitzer L, von Schaewen A, Sonnewald U** (1990) Apoplastic expression of a yeast-derived invertase in transgenic plants lead to dramatic changes in habitus and growth. J Cell Biol **14E:** 360, Suppl