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Sugar Levels Modulate Differential Expression of Maize Sucrose Synthase Genes

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The two genes encoding sucrose synthase in maize (*Sh1* and *Sus1*) show markedly different responses to changes in tissue carbohydrate status. This enzyme is widely regarded as pivotal to sucrose partitioning, import, and/or metabolism by developing plant organs. Excised maize root tips were incubated for varying periods in different sugars and a range of concentrations. The *Sh1* mRNA was maximally expressed under conditions of limited carbohydrate supply (~0.2% glucose). In contrast, *Sus1* transcript levels were low or nondetectable under sugar-depleted conditions and peaked at 10-fold greater glucose concentrations (2.0%). Responses to other metabolizable sugars were similar, but L-glucose and elevation of osmolarity with mannitol had little effect. Plentiful sugar supplies thus increased expression of *Sus1*, whereas reduced sugar availability enhanced *Sh1*. At the protein level, shifts in abundance of subunits encoded by *Sh1* and *Sus1* were much less pronounced but corresponded to changes in respective mRNA levels. Although total enzyme activity did not show net change, cellular localization of sucrose synthase protein was markedly altered. In intact roots, sucrose synthase was most prevalent in the stele and apex. In contrast, sugar depletion favored accumulation in peripheral cells, whereas high sugar levels resulted in elevated expression in all cell types. The differential response of the two sucrose synthase genes to sugars provides a potential mechanism for altering the pattern of enzyme distribution in response to changing carbohydrate status and also for adjusting the sucrose-metabolizing capacity of importing cells relative to levels of available photosynthetic products.

INTRODUCTION

The role of sugars in gene regulation is well established in microbial systems (e.g., control of the lactose operon and catabolite repression in yeasts), and glucose-regulated genes have been described in animal cells (Lin and Lee, 1984). Specific gene responses to sugars in higher plants have also been found but primarily in connection with storage processes in potato (Rocha-Sosa et al., 1989; Salanoubat and Belliard, 1989; Wenzler et al. 1989; Müller-Röber et al., 1990) and sweet potato (Hattori et al., 1990). Sugar effects have also been observed for identified mRNAs associated with cell wall regeneration by protoplasts (Maas et al., 1990) and down regulation of photosynthesis in maize mesophyll protoplasts (Sheen, 1990). For bacteria and yeasts, sugar-modulated gene expression is an essential mechanism for adjustment to changes in nutrient availability (Carlson, 1987; Vyas et al., 1988; Schuster, 1989). This may also be true for higher plants in which their multicellular structure places more complex demands on adaptation and resource allocation. Sugar effects on gene expression have the potential to profoundly influence carbohydrate distribution and utilization within and among plant parts.

For this reason, genes encoding sucrose synthase (EC 2.4.1.13) may have a role in key whole plant adjustments to altered sugar supplies. This enzyme is considered pivotal to sugar distribution and metabolism in many plant species in which developing structures depend on imported sucrose (Claussen 1983; Claussen et al., 1985; Sung et al., 1988; Nguyen-Quoc et al., 1990). In these tissues, sucrose synthase is often the primary enzyme responsible for sucrose cleavage. The reversible conversion of sucrose+UDP to fructose + UDP-glucose is one of only two known pathways for metabolic utilization of sucrose in plant cells (Hawker, 1985; Huber and Akazawa, 1986). The sucrose synthase route is apparently favored in many instances and requires half the net energy of the alternate pathway through invertase (Black et al., 1987). The extent of sugar import into many plant organs has been correlated with sucrose synthase activity (Claussen, 1983; Sung et al., 1988; Nguyen-Quoc et al., 1990). Elevated activity has also been found in association with starch storage (Chourey and Nelson, 1976; Claussen et al., 1985, 1986; Doehlert, 1990), rapid cell wall synthesis (Hendrix, 1990), and increased carbon flow through the respiratory path (Black et al., 1987; Farrar and Williams, 1990).

Two genes in maize (*Sh1* and *Sus1*) encode sucrose synthase isozymes with very similar characteristics (SS1 and

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SS2, respectively; Echt and Chourey, 1985). These proteins are expressed in different tissues and under different conditions, however (Roland et al., 1988; Nguyen-Quoc et al., 1990). The *Sh1* gene product was initially considered an "endosperm form" of sucrose synthase, and its gene name arose from the shrunken phenotype of kernels lacking a functional *Shrunken-1* (*Sh1*) gene (Chourey and Nelson, 1976). Expression of this sucrose synthase-encoding gene (*Sh1*) has since been demonstrated in other maize tissues (Chourey et al., 1986; Springer et al., 1986; Heinlein and Starlinger, 1989) in which stress conditions further enhance transcript abundance (McCarty et al., 1986; Springer et al., 1986) and alter protein distribution (Roland et al., 1988). The *Sus1* gene, named for its product sucrose synthase, encodes the SS2 protein of Echt and Chourey (1985). This isozyme is more widely distributed among plant parts than is the *Sh1* form (Chen and Chourey, 1988) and is present at higher levels in young leaves (Nguyen-Quoc et al., 1990).

The possibility that higher plant genes could respond to sugar levels was initially indicated by apparent effects of carbohydrate supplies on enzyme activities in a range of tissues. Particularly evident were changes in activities of enzymes involved in sugar metabolism. Rises in sugar concentrations were implicated in the elevation of fructan-synthesizing enzyme activity in Lolium (Housley and Pollock, 1985) and sucrose synthase activity in excised leaves (Claussen et al., 1985) and detached potato tubers (Ross and Davies, 1992). Increased supplies of sugars also were found to enhance activity of invertase in Avena stem segments (Kaufman et al., 1973) and in cell cultures of sugar cane (Maretzki et al., 1974) and sugar beet (Masuda et al., 1988). In contrast, exogenous sugars (glucose) partially repressed invertase activity in elongating sugar cane internodes (Glasziou, 1969) and inhibited the increase in phleinase (a fructan hydrolase) that otherwise accompanies a depletion of tissue hexoses in orchard grass (Yamomoto and Mino, 1987).

The present research was motivated by sugar starvation studies in which unidentified proteins appeared in response to sugar depletion. A starvation "stress protein" was reported in pea root tips (Webster, 1980; Webster and Henry, 1987), and "carbohydrate responsive proteins" in pearl millet (Baysdorfer and VanDerWoude, 1988). Our initial work indicated that carbohydrate deprivation could up regulate the *Sh1* gene in maize root tips and that sucrose addition would decrease levels of its message (Koch and McCarty, 1988). Consistent with this observation was the recent report that the *Sh1* promoter could be repressed by sucrose in transient expression assays (Maas et al., 1990). In contrast, other work has indicated that sugar is a positive regulator of genes encoding sucrose synthase (Salanoubat and Belliard, 1989) as well as storage proteins in potato (Rocha-Sosa et al., 1989; Wenzler et al., 1989) and sweet potato (Hattori et al., 1990).

In this article, we demonstrate that in maize root tips, sugar-modulated gene expression includes an unexpected, differential responsiveness of an isozyme gene system encoding sucrose synthases. The identity of the genes involved underscores the importance of their sugar responsiveness as a potential means of adjusting form, function, and sucrose partitioning within a multicellular organism.

RESULTS

Figure 1 shows that the level of *Sh1* mRNA increased relative to total RNA in excised root tips incubated 24 hr without sugars. In contrast, the relative *Sh1* transcript level was markedly

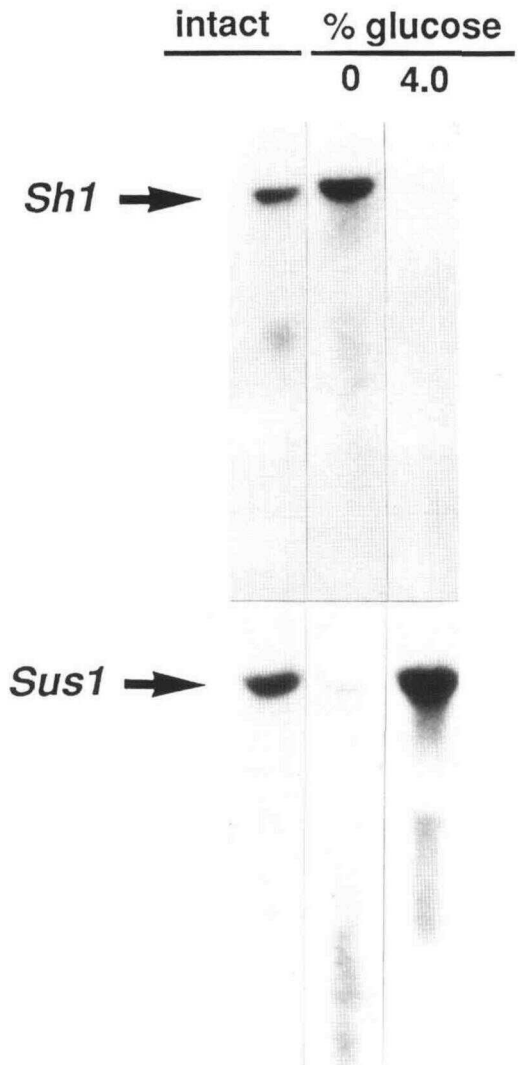

Figure 1. RNA Gel Blot Analysis of *Sh1* and *Sus1* Expression in Root Tips Incubated with or without 4% Glucose.

RNA gel blots with equal aliquots (10 μg) of total RNA isolated from maize root tips before (intact) or after a 24-hr incubation in solution culture (with 0 or 4.0% glucose) were probed with ^{32}P-labeled *Sh1* cDNA or *Sus1* genomic clones for sucrose synthase. The film was exposed for 24 hr.

lower when 4% glucose was included in the medium. An opposite response to sugars was observed for the *Sus1* gene (Figure 1). Whereas *Sus1* transcript levels were low or undetectable in excised root tips starved for 24 hr, substantially more message was present when 4% glucose was added to the incubation medium. Relative amounts of *Sus1* mRNA in sugar-supplemented root tips were as great or greater than those of intact root tips. Both gene message levels in intact root tips tended to vary with growing conditions (data not shown). Soluble sugars were depleted to minimal levels in excised root tips within 24 hr unless exogenous carbohydrate was provided (data not shown; Saglio and Pradet, 1980).

The sugar-depletion response of the *Sh1* gene was distinct from its previously characterized anaerobic induction (Springer et al., 1986). Both *alcohol dehydrogenase-1* (*Adh 1*) and *Sh1* are up regulated under low oxygen; however, *Sh1* alone responded to low sugar supplies (data not shown). To ensure aerobic conditions, an airflow of 40% oxygen was maintained during root tip incubations. In addition, all experiments were routinely monitored for expression of *Adh1* as a further test for aerobic metabolism.

The differential responses of the *Sh1* and *Sus1* genes to sugars were further apparent when gradations of glucose concentrations were supplied to excised root tips. RNA gel blots in Figure 2A and quantifications of replicate experiments in Figure 2B show that *Sh1* mRNA was most prevalent at low glucose concentrations, whereas relative abundance of *Sus1* mRNA was greatest when exogenous glucose levels were high. *Sh1* message typically peaked when ~0.2% glucose was added to media. *Sus1* mRNA levels were maximal when 10-fold more glucose was present (2.0%). Overall, changes in the mRNA level showed an optimal rather than an "on-off" type of response.

To examine the effect of sugars on sucrose synthase expression at the protein level, native protein gel blots that resolved the tetrameric forms of sucrose synthase were probed with a polyclonal antiserum cross-reactive with both sucrose synthase gene products. Figure 3 shows the resulting comparison between treatments with varying levels of exogenous glucose. Shifts are evident in band density of the tetrameric holoenzymes enriched in subunits encoded by *Sh1* versus *Sus1* (compare data from 0 and 2.0% glucose). These correspond to relative changes observed at the RNA level in the same experiment.

Table 1 shows that the reciprocal changes in *Sh1* and *Sus1* mRNA level did not result in a significant net change in total enzyme activity extracted from whole root tips after 24-hr sugar treatments. The lack of such difference at the organ level is not surprising considering the involvement of both gene products and several tissue types.

The fact that the altered expression of *Sh1* and *Sus1* did not result in net changes in enzyme activity raised the question of physiological significance of the response. The possibility remained that reciprocal alterations in gene expression might be associated with physiologically significant changes at the cellular level that would be masked by whole organ measurements. Relative expression of given isozyme genes could be

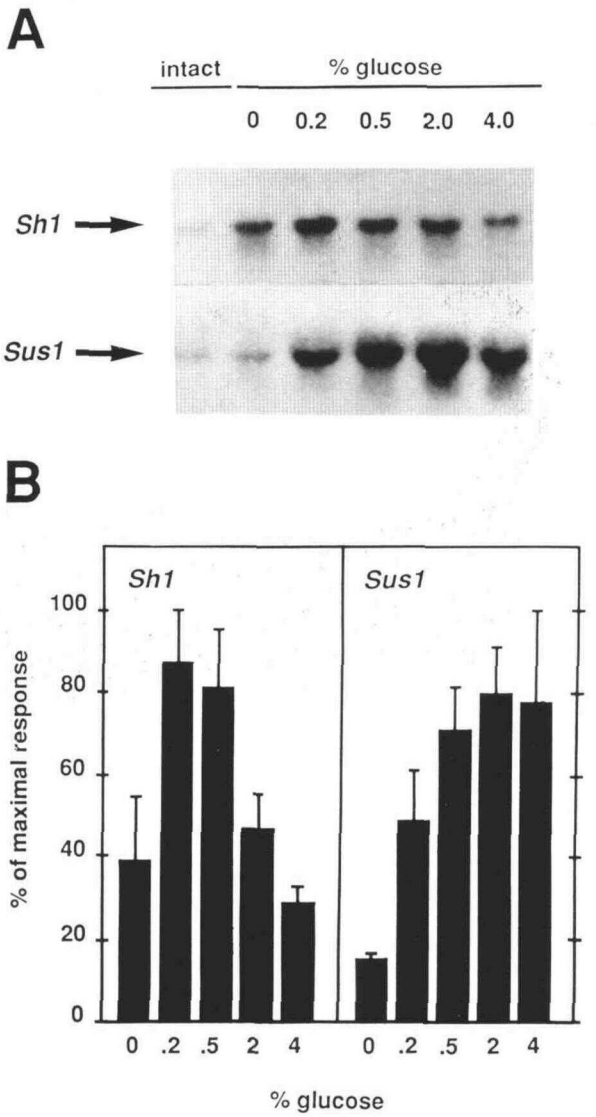

Figure 2. RNA Gel Blot Analysis of *Sh1* and *Sus1* Expression in Root Tips Incubated with a Range of Glucose Concentrations.

(A) RNA gel blots with equal aliquots (10 μg) of total RNA isolated from maize root tips before (intact) or after a 24-hr incubation in solution culture (supplemented with a range of glucose concentrations). Blots were probed with ^{32}P-labeled *Sh1* cDNA or *Sus1* genomic clones for sucrose synthase. The film was exposed for 24 hr.
(B) Quantification of RNA gel blots from three replicate experiments conducted as in **(A)**. Scanning densitometry (Molecular Dynamics) was used to quantify relative RNA levels.

enhanced in specific tissues and favor optimization of different functions.

To address the question of altered cellular localization, the sucrose synthase antibody was used to localize the enzyme in thin sections of excised root tips incubated at various glucose levels. Figure 4 shows that in intact root tips, sucrose synthase protein was most abundant in and near the stele,

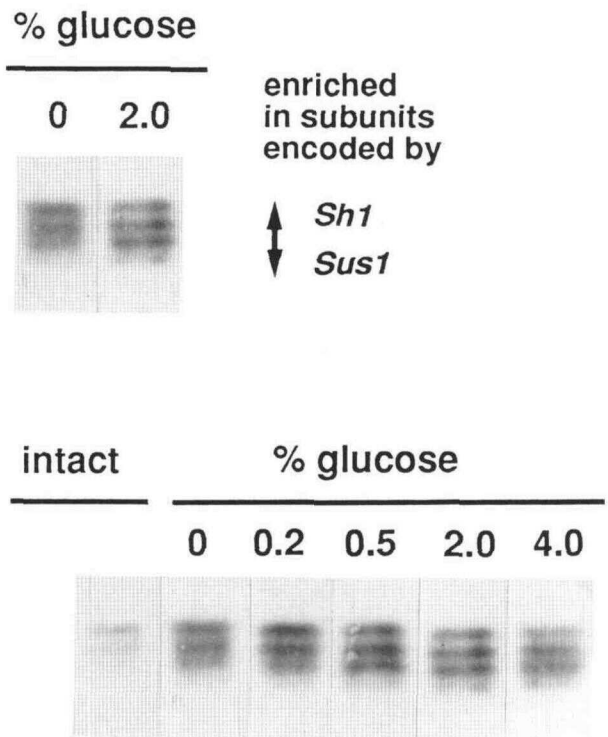

Figure 3. Native Protein Gel Blot Analysis of Sucrose Synthase Tetramers from Root Tips Incubated with a Range of Glucose Concentrations.

Protein gel blot with equal aliquots (0.5 μL) of total protein extracted from maize root tips before (intact) or after a 24-hr incubation in solution culture supplemented with a range of glucose concentrations. The blot was probed with polyclonal antibodies raised against purified sucrose synthase. Bands represent homotetramers and heterotetramers of sucrose synthases with varying proportions of *Sh1* (upper bands) or *Sus1* (lower bands) gene products. The upper panel shows a side-by-side comparison of the lanes from the 0 and 2% glucose treatments. Results pictured were obtained from the same set of root tips used for the RNA gel blots in Figure 2A.

particularly in association with vascular tissues (Figures 4B and 4F). In sugar-depleted root tips (0.2% glucose), the distribution showed a marked shift to peripheral cells in the root, with the greatest concentration occurring in the epidermis (Figures 4C and 4G). At greater glucose concentrations, sucrose synthase protein was more uniformly distributed throughout the root profile (Figures 4D and 4H). In each treatment, the region sectioned appeared to be composed of tissues that were newly formed during the incubation period. Sections were obtained from the apical 3.0 to 3.3 mm of roots (1.0 to 1.5 mm for transverse sections), whereas mean length of new growth in 24 hr was 4.7 and 7.2 mm for 0.2 and 2.0% glucose treatments, respectively.

Table 2 shows that growth rates of root tips increased with the concentration of exogenous glucose until a maximum was reached at 2.0%. These elongation rates corresponded to levels of *Sus1* mRNA.

Time course experiments in Figure 5 showed that differences in relative abundance of the *Sh1* and *Sus1* messages were detectable after 16 hr. The treatment regimes shown in Figures 5A and 5C were chosen on the basis of the optimal response shown in Figure 2B. Changes were maximally expressed after 24 hr under these conditions. Longer periods of time were needed before detectable differences were apparent in *Sus1* mRNA when sugar levels favored less marked changes in expression (low sugar treatments in Figure 5B were 0.2% glucose compared to 0% in Figure 5C). Treatment reversals at 24 hr showed that root tips remained sensitive to changes in sugar levels after excision (Figure 5A), unless deprived entirely of exogenous sugars during this period (Figure 5C). Levels of *Sus1* message from root tips exposed to 2% exogenous glucose for 24 hr followed by an additional 24-hr period of deprivation did not show a low sugar response. The extent of carbohydrate accumulated during the high-sugar treatment may have abbreviated and/or minimized the subsequent stress.

Other metabolizable carbohydrate sources also affected the differential expression of the two genes encoding sucrose synthase. Figure 6 shows that sucrose, despite its limited capacity to enter cells of maize roots (Robbins, 1958; Giaquinta et al., 1983; Lin et al., 1984), down regulated the *Sh1* message to an equal or greater extent than did glucose. In contrast, sucrose consistently had a minimal effect on *Sus1* mRNA. Fructose effects were generally similar to those of glucose but tended to be less pronounced. Incubations in media with osmolarity elevated by mannitol addition or with nonmetabolizable sugars (L-glucose) were ineffective in

Table 1. Total Sucrose Synthase Activity in Whole Maize Root Tips from Three Replicate Experiments[a]

		Glucose, %				
Experiment	Intact	0	0.2	0.5	2.0	4.0
		(μmol sucrose cleaved mg^{-1} protein h^{-1})				
1	1.0	0.7	0.8	1.3	0.6	0.7
2	0.5	0.5	0.4	0.6	0.6	0.6
3	0.9	1.2	0.9	1.6	0.9	1.5
Mean ± SE	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	1.1 ± 0.3	0.7 ± 0.1	0.9 ± 0.3

No statistically significant differences were observed between treatments based on a two-way analysis of variance.
[a] As shown in Figure 2B.

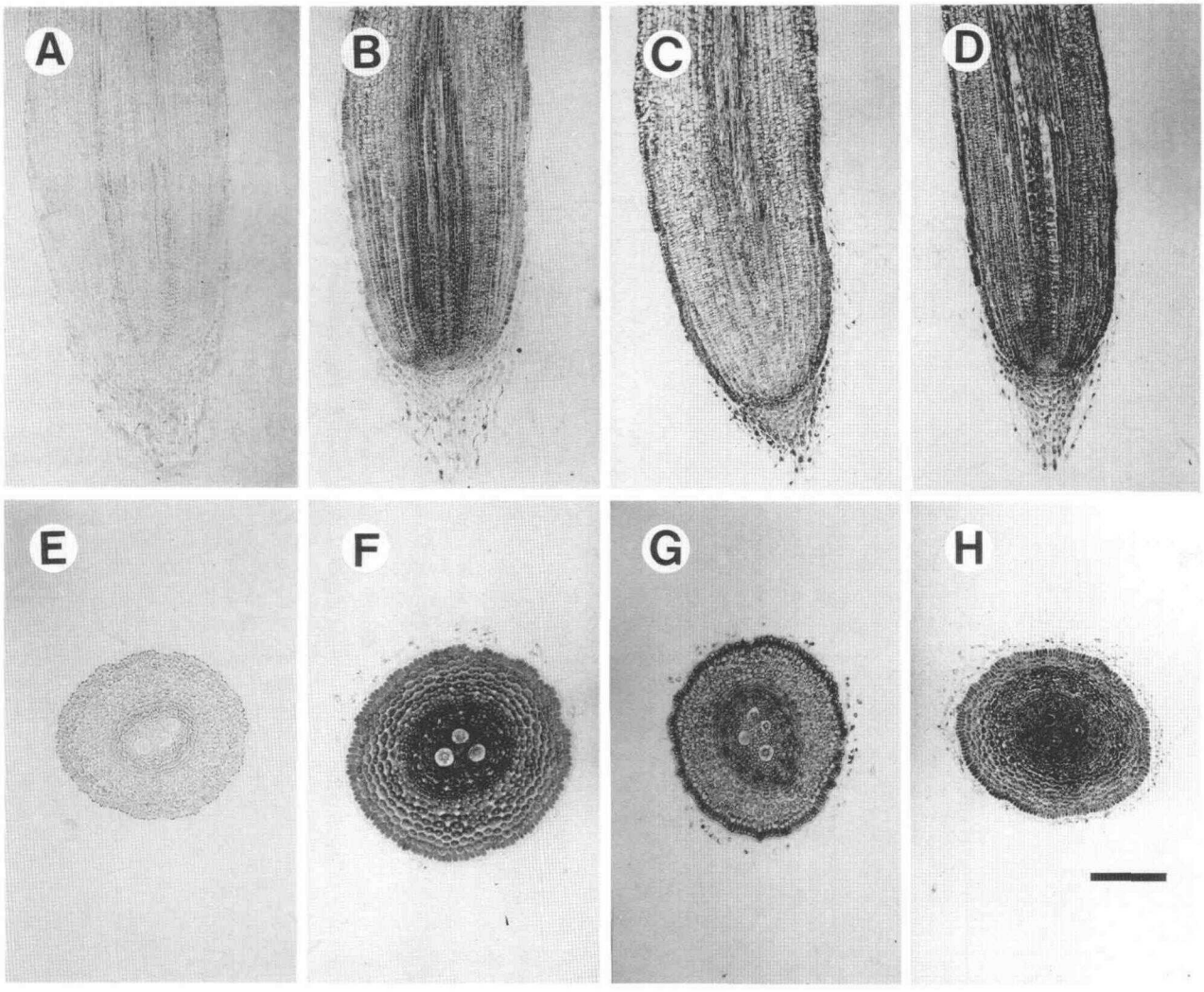

Figure 4. Localization of Sucrose Synthase Protein in Root Tips Incubated with a Range of Glucose Concentrations.

Root tips were fixed, embedded in paraffin, sliced into 8-μm sections, and probed with polyclonal sucrose synthase antisera as outlined in Methods. The apical 3.0 to 3.3 mm of each longitudinal section is pictured, and transverse sections are from a zone 1.0 to 1.5 mm behind the tip of the root cap. These appear to be comprised of tissues newly formed during the preceding 24 hr (see text). Bar = 50 μm.

(A) Longitudinal section of the root tip probed with preimmune serum (control).

(B) to **(D)** Longitudinal sections of an intact root **(B)**, a root tip incubated for 24 hr with a limited supply of exogenous sugars (0.2% glucose) **(C)**, or a root tip incubated for 24 hr in 2.0% glucose **(D)** probed with antisucrose synthase antisera.

(E) Transverse section of a root tip probed with preimmune serum (control).

(F) to **(H)** Transverse sections through the tip of an intact root **(F)**, a root tip incubated for 24 hr with a limited supply of exogenous sugar (0.2% glucose) **(G)**, or a root tip incubated for 24 hr in 2.0% glucose **(H)** probed with antisucrose synthase antisera.

bringing about the high and low sugar responses described above.

DISCUSSION

The findings revealed here have a threefold significance. First, sugar-responsive gene expression in plant tissues was shown to have an unexpected level of complexity involving differential regulation of isozyme genes within a plant organ. Second, this process was associated with changes in the pattern of sucrose synthase localization within root tips. Third, this response has potential physiological implications as a mechanism by which importing cells and organs may adjust their sucrose-metabolizing capacity to changes in available photosynthate supply.

Our results indicated that metabolizable sugars have both positive and negative effects on the sucrose synthase gene

Table 2. Length of Root Tips before and after 24-Hr Incubations in Media with Varying Levels of Glucose (± SE)

	Glucose, %				
Intact	0	0.2	0.5	2.0	4.0
			(cm)		
1.18 ± 0.2	1.30 ± 0.2	1.65 ± 0.4	1.77 ± 0.4	1.90 ± 0.4	1.59 ± 0.2

system and that the *Sh1* and *Sus1* genes of maize respond in a reciprocal manner. It is possible that the positive (Salanoubat and Belliard, 1989) and negative modes of regulation (Maas et al., 1990) that have been described in other systems are manifestations of a common regulatory mechanism. It has not been determined whether the changes in relative mRNA levels result from transcriptional regulation or altered mRNA stability. Changes in rate of transcription were not resolved in standard nuclear run-on experiments (K.E. Koch, unpublished data). Nonetheless, the overall effect was a change in the relative abundance of the *Sh1* and *Sus1* gene products. The in situ localization results suggested that significant changes in expression occur in cells that are newly formed during the course of the experiment.

Results indicated that changes in RNA are reflected at the protein level within 24 hr, particularly in tetramers enriched in subunits encoded by the *Sh1* or *Sus1* genes. These differences (Figure 3) were less pronounced than changes in message levels. This may be due in part to the time course of the response, and we do not rule out the possibility of additional translational regulation. Although the reciprocal regulation of *Sh1* and *Sus1* by sugars did not cause a net change in whole organ enzyme activity (Table 1), immunohistochemical analyses revealed altered distribution of sucrose synthase protein at the cellular level (Figure 4). The response may therefore have important physiological consequences. The change in localization probably does not stem from strict cell-specific expression of *Sh1* and *Sus1* genes. Heterotetramers were evident at all sugar concentrations, indicating substantial overlap in expression of the two isozymes. We suggest that sugar levels modify relative expression of the two genes in root cells, giving rise to altered patterns of localization.

These shifts in localization (Figure 4) may help to optimize the physiological role of sucrose synthase isozymes under conditions of altered carbohydrate availability. The enhanced expression of *Sus1* and widespread distribution of sucrose synthase protein when sugars were plentiful is consistent with evidence of increased respiratory activity in root tips under these conditions (Saglio and Pradet, 1980; Farrar and Williams, 1990; Brouquisse et al., 1991) and enhanced demand for cell wall synthesis by rapidly expanding cells (Hendrix, 1990). It is noteworthy that root tip growth rate appeared to correlate well with *Sus1* expression. In contrast, rises in levels of *Sh1* message under carbohydrate depletion coincided with development of a more limited pattern of sucrose synthase localization in epidermal cells. These results are consistent with the possibility that a starving organ might prioritize retention of the capacity for sucrose import, and hence sucrose synthase activity, in areas of sugar entry. Typically, this would be at the site of phloem unloading or, in the present system, in epidermal cells that are in contact with the external medium. Overall, the distribution of sucrose synthase in sugar-limited root tips may reflect a survival response similar to that proposed at the whole plant and organ levels by Baysdorfer et al. (1988). These authors suggested that vital components for recovery of a starved system are preserved at the expense of others.

Elevation of *Sh1* message has been well documented in flooded roots (McCarty et al., 1986; Springer et al., 1986), although data vary in regard to efficiency of subsequent translation under these conditions (McElfresh and Chourey, 1988; Talercio and Chourey, 1989; Ricard et al., 1991). The pattern of sucrose synthase distribution in root tips under low sugar conditions (Figure 4) differed, however, from that reported for low oxygen (Roland et al., 1988). Low sugar levels (Figures 4C and 4G) did not induce the extensive vascular or root cap distribution reported under low oxygen conditions (Roland et al., 1988). The *Sus1* gene is much less responsive to low O_2 (McCarty et al., 1986).

The time course of the sugar response (Figure 5) indicated, first, that most pronounced changes in message levels

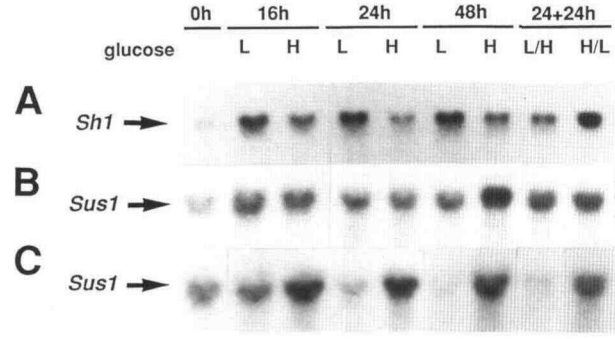

Figure 5. RNA Gel Blot Analysis of *Sh1* and *Sus1* Expression in Root Tips during a Time Course of Incubation with Exogenous Glucose and after Treatment Reversals.

RNA gel blots with equal aliquots (10 μg) of total RNA isolated from root tips previously incubated for varying periods in solution culture were probed with ^{32}P-labeled *Sh1* cDNA or *Sus1* genomic clones for sucrose synthase.

(A) and **(B)** Root tips were incubated for varying periods in solution cultures in which exogenous sugar supplies were either lower (L) (0.2% glucose) or higher (H) (2.0% glucose). Levels were selected to provide contrast in expression of the *Sh1* gene for sucrose synthase based on data shown in Figure 2B.

(C) Root tips were incubated for varying periods in solution cultures in which exogenous sugar supplies were either lower (L) (0%) or higher (H) (2.0% glucose). Levels were selected to provide contrast in expression of the *Sus1* gene for sucrose synthase.

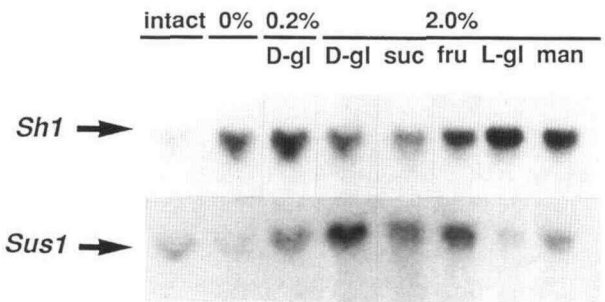

Figure 6. RNA Gel Blot Analysis of *Sh1* and *Sus1* Expression in Root Tips Incubated with Various Sources of Exogenous Sugars.

RNA gel blots with equal aliquots (10 μg) of total RNA isolated from root tips previously incubated for 24 hr in various sources of exogenous sugars were probed with ^{32}P-labeled *Sh1* cDNA or *Sus1* genomic clones for sucrose synthase. Root tips were harvested from intact seedlings and after incubation in White's basal salts medium either without exogenous sugars (0%) or with D-glucose (D-gl) at 0.2 or 2.0%, 2% sucrose (suc), 2% fructose (fru), 2% L-glucose (L-gl), or 2% mannitol (man). The film was exposed for 9 and 12 hr, respectively, for *Sh1* and *Sus1*.

occurred sometime between 16 and 24 hr at sugar concentrations favoring differential effects on a given gene and, second, that root tips remained responsive to changes in carbohydrate availability for 24 hr when at least some sugar was included in the media. This is consistent with the work of Brouquisse et al. (1991), who reported respiratory increases with glucose addition after 24 hr of carbohydrate depletion in excised maize root tips. However, sugar levels in root tips completely without exogenous carbohydrate may have dropped too low to permit subsequent up regulation of the *Sus1* gene. Overall, the duration of time required for *Sh1* and *Sus1* responses to altered carbohydrate status was consistent with the proposed physiological function of sugars in coarse control of metabolism (Farrar and Williams, 1990). Changes in carbohydrate availability may be integrated over a moderately extended period.

Glucose per se does not appear to be a direct effector of this process because responses were also elicited by other sugars subject to import and metabolism (Figure 6). The effect of sucrose on *Sus1* was consistent with the limited capacity of maize root tips to import this sugar from extracellular space (Robbins, 1958; Giaquinta et al., 1983; Lin et al., 1984). Interestingly, *Sh1* appeared to be more sensitive to sucrose addition. Some exogenous sucrose may have entered root cells by way of the phloem if loading occurred in these excised roots. However, import as hexoses following hydrolysis by extracellular invertase is also likely to occur (Duke et al., 1991). The ineffectiveness of L-glucose and mannitol indicated that osmotic changes alone had little influence on expression of sucrose synthase genes. The effect of L-glucose also implied that metabolism was necessary. Sugars may act indirectly, as suggested by Farrar and Williams (1990). The response could be mediated through sensitivity to tightly controlled levels of specific metabolic intermediates (UDP and/or alternate adenylates; Farrar and Williams, 1990) or other metabolites having regulatory functions at the enzyme level (e.g., F2, 6BP; Huber and Akazawa, 1986; Black et al., 1987).

The sugar-responsive gene expression described here occurred in growing, rapidly metabolizing structures. In contrast, previous studies have generally focused on storage tissues, in which physiological processes associated with starch and protein accumulation predominate. Sucrose synthase appears to be closely tied to starch synthesis in such tissues and is most prevalent when storage programs are most active (Chen and Chourey, 1988). Loss of function by the *Sh1* gene in maize results in a *shrunken-1* phenotype having a collapsed, starch-poor endosperm (Chourey and Nelson, 1976). Starch accumulation accompanies that of storage protein synthesis in developing potato tubers when storage programs have been initiated, so it is reasonable to expect levels of mRNA encoding sucrose synthase to rise (Salanoubat and Belliard, 1989) with those of storage proteins such as patatin (Rocha-Sosa et al., 1989). Both are elevated when potato tuber formation is induced by application of exogenous sucrose to vegetative tissues (Rocha-Sosa et al., 1989; Salanoubat and Belliard, 1989; Wenzler et al., 1989). Similarly, a rise in sugar availability can result in the appearance of mRNA for storage proteins in atypical tissues of sweet potatoes (Hattori et al., 1990) and for sucrose synthase in potato leaves where it is otherwise not observed (Salanoubat and Belliard, 1989). A similar effect has been reported in eggplants at the level of protein synthesis (and enzyme activity) in excised leaf sections (Claussen et al., 1986), leaf midribs (Claussen et al., 1985), and detached leaves (Claussen et al., 1986) as photosynthetic products build up and storage processes are initiated in severed green tissues.

The diversity of metabolic processes operating in the system examined here suggested that sugar-responsive gene expression, particularly that of sucrose synthase, may have broader implications in the formation and function of nonstorage plant tissues. Sucrose synthase may contribute to a variety of other metabolic processes. First, the demand for nucleotide sugars for cellulose biosynthesis has been implicated in a need for greater activity of this enzyme (Hendrix, 1990; Maas et al., 1990). Studies with isolated protoplasts also indicated that the process of cell wall regeneration could gain a singular, and perhaps overriding, importance in metabolic events regulating expression of the *Sh1* gene for sucrose synthase (Maas et al., 1990). Sugar modulation of sucrose synthase genes could effectively integrate production of cell wall materials with other aspects of increased growth (Farrar and Williams, 1990) most likely to accompany an enhanced supply of sugars.

Second, another potentially important aspect of sugar-modulated gene expression may be that of adjustment in respiratory rate in response to sugar availability. Evidence indicates that this may occur over time in root tips of both barley (Farrar and Williams, 1990) and maize (Saglio and Pradet,

1980). Sucrose synthase activity could clearly be key to the rate of carbon entry into the respiratory path (Huber and Akazawa, 1986; Black et al., 1987); however, any coarse control of respiratory activity by sugar effects on transcription probably includes a broad spectrum of genes (as in yeast; Schuster, 1989). Farrar and Williams (1990) note that (in a complex multicellular organism) sucrose would be an excellent messenger enabling sink metabolism to adjust to rates of supply from source leaves while minimizing cellular investments in expensive metabolic machinery.

Third, another implication of differentially sugar-responsive sucrose synthase-encoding genes is the possible role of *Sh1* in low sugar tolerance/recovery. Its involvement in survival may lie in its capacity to preserve (Baysdorfer et al., 1988) or possibly enhance (Huber and Akazawa, 1986) the capability for import or utilization of low levels of sugars.

Fourth, and finally, sucrose synthase may have an as yet undefined role in phloem transport. Claussen et al. (1985) proposed that sucrose synthase may in some way control sucrose levels in phloem. The suggestion is not surprising given the reversible nature of the sucrose synthase reaction (Hawker, 1985) and increasing evidence for vascular localization of sucrose synthase (Chen and Chourey, 1988; Lowell et al., 1989; Koch and Avigne, 1990; Yang and Russell, 1990). The contrasting expression of the two maize sucrose synthase genes and the different tissue localizations of their products indicate a complex and carefully controlled regulation of these isozymes.

METHODS

Plant Material

Hybrid *Zea mays* (NK508) was used for all experiments unless designated otherwise (initial studies were conducted with a wild-type inbred [W22]). Seeds were primed for 6 days at 10°C in polyethylene glycol 8,000 adjusted to −1.0 megapascals, with 2 g/liter captan (Bodsworth and Bewley, 1981). Subsequent transfer to germination trays was preceded by 20 min in 1.05% (v/v) NaClO and 20 min of continuous rinsing with water. Germination took place in the dark at 18°C on two layers of moist 3MM paper (Whatman, Inc., Clifton, NJ) in 17 × 26 cm glass pans. A continuous airflow of 1 liter/min was provided for each pan throughout the 7-day period, with 40% O_2 supplied during the final 48 hr before root tip excision. The moisture level was adjusted daily by applying mist and draining excess water. Root tips (terminal 1 cm) were excised from adventitious roots under a sterile transfer hood.

Experimental Conditions

Approximately 175 root tips (~750 mg) were used for each experimental treatment. These were incubated in the dark for 8 to 48 hr in Whites' basal salts medium (mineral nutrients alone), either with or without an array of supplemental sugars. Each group of root tips was agitated at 130 cycles per minute in a 125-mL side-arm Erlenmeyer flask with 50 mL of sterile media. Airflow (40% O_2) through airstones in each flask was maintained at 250 mL min^{-1} throughout the incubations.

RNA Extraction and Hybridization

Root tip samples were rinsed twice in sterile water, blotted dry, weighed, and frozen in liquid N_2. Samples were ground into a fine powder in liquid N_2, and RNA was extracted according to the method of McCarty (1986). RNA was quantified spectrophotometrically by absorbance at 260 nm.

Total RNA was separated by electrophoresis in 1% agarose gels containing formaldehyde (Thomas, 1980), blotted to a nylon membrane, and probed according to the method of Church and Gilbert (1984). *Sh1* sucrose synthase cDNA (Sheldon et al., 1983) and genomic clones of *Sus1* (McCarty et al., 1986), and *Adh1* (Dennis et al., 1984) were radiolabeled by random primer. No cross-reactivity between *Sh1* and *Sus1* probes was observed when hybridizations were conducted at high stringency. Blots were washed as described by Church and Gilbert (1984), followed by detection on x-ray film at −80 C. Scanning densitometry (Molecular Dynamics, Sunnyvale, CA) was used to quantify relative RNA levels.

Enzyme Assay

Activity of total sucrose synthase (encoded by both the *Sh1* and *Sus1* genes) was assayed using a rapid radiometric procedure developed to circumvent the instability of activity observed when this enzyme is extracted from vegetative tissues of maize (E.R. Duke, D.R. McCarty, L.C. Hannah, and K.E. Koch, unpublished data). The assay involved recovery of radiolabeled reaction product on DEAE ion exchange paper, an approach initially described by Delmer (1972) and Su and Preiss (1978). Samples were weighed (~0.25 g), frozen, and ground to a fine powder in liquid N_2. A chilled mortar and pestle were used for subsequent grinding in 2.5 mL of extraction media (a buffer/tissue ratio of 10:1) containing 200 mM Hepes buffer (pH 7.5), 1 mM DTT, 5 mM MgCl, 1 mM phenylmethylsulfonyl fluoride, and 10% (w/v) polyvinylpolypyrrolidone. Aliquots (200 μL) of each extract were passed through individual 1-mL Sephadex G 50-80 columns preequilibrated with extraction buffer and spun at 800 *g* for 1 min to remove sugars and other low molecular weight solutes. Eluate, obtained within 3.5 min after the start of extraction, was assayed for 5 min at 30°C in a 50-μL volume containing 20 μL of extract, 80 mM morpholinoethanesulfonic acid (pH 5.5), 5 mM NaF, 100 mM ^{14}C-sucrose, and 5 mM UDP (omitted in controls). Reactions were terminated by the addition of 50 μL of Tris (pH 8.7), followed by boiling for 1 min. Radiolabel in ^{14}C-UDP-glucose was quantified after the entire reaction volume was blotted onto disks of DEAE ion exchange paper, dried, and rinsed three times in deionized water (once for 2 hr in 40 mL of water shaking at 175 rpm, again for a second 2-hr period, then rinsed with a stream of deionized water for 30 sec). Accuracy depended on previous removal of trace DEAE-binding contaminants (such as phosphorylated sugars) from the ^{14}C-sucrose substrate. Concentrated anion-free ^{14}C-sucrose was obtained by collecting eluate (the first two to three drops) from the base of a V-tipped strip of DEAE paper (2.5 × 23 cm) after descending chromatography of commercially obtained ^{14}C-sucrose (Du Pont–New England Nuclear, Boston, MA) in water.

Protein Gel Blots

Proteins extracted as described above were resolved by native PAGE in a vertical gel apparatus (Hoeffer Scientific Instruments, San Francisco, CA) using the discontinuous Tris-glycine buffer system of Laemmli (1970) without SDS. Polyacrylamide in the gels (1.0 mm × 14 cm × 16 cm) was 5% (w/v) for the separating gel and 2.5% (w/v) for the stacking gel. Electrophoresis proceeded at 4°C for 9 hr at 15 V and 11 hr at 125 V (constant current) with a bromphenol blue tracking dye.

Proteins were transferred to nitrocellulose and probed with antibody essentially as described by Towbin et al. (1979). Following transfer, membranes were incubated in 3% (w/v) BSA in PBS plus 0.05% Tween 20. The BSA-coated membranes were carefully washed 3 × 20 min in PBS-Tween and reacted with rabbit antisucrose synthase antisera diluted 1:1000 with PBS-Tween containing 0.2% (w/v) BSA. Polyclonal antibodies had been raised against a combination of *Sh1* and *Sus1* gene products extracted from whole maize kernels (W64A × 182E) 22 days after pollination. After probing, membranes were washed 3 × 20 min in PBS-Tween and incubated with secondary antibodies (alkaline phosphatase-conjugated goat anti-rabbit IgG [Bio-Rad, Richmond, CA]) diluted 1:2000 in PBS-Tween containing 0.2% (w/v) BSA. Incubation with antisera was carried out on a shaker for 1 hr at 24°C. Membranes were again rinsed in PBS-Tween 2 × 20 min, and then in PBS alone (20 min). Antibody hybridizations were subsequently visualized for 10 min in a reaction medium of 0.3 mg mL^{-1} 5-bromo-4-chloro-3-indolyl phosphate, 0.6 mg mL^{-1} nitro blue tetrazolium chloride (both from Sigma, St. Louis, MO), in 100 mM Tris-HCl (pH 9.5), containing 100 mM NaCl and 5 mM $MgCl_2$.

Immunohistochemistry

Root tips excised from intact plants or harvested after experimental treatments were fixed in formalin acetic acid, dehydrated through a tertiary butyl alcohol series, and embedded in paraffin. Microscopic slides were acid washed and silinized according to Uhl (1986). Sections (8 μm) were cut using a rotary microtome and floated on water-flooded slides. Paraffin ribbons were spread using an alcohol flame and dried onto slides by incubating at 45°C overnight.

Antibody labeling of sucrose synthase protein was carried out at room temperature in a humid atmosphere using a procedure adapted from manufacturer's recommendations (Janssen Biotech N.V., Olen, Belgium). Briefly, sections were deparaffinized, rehydrated, and washed in PBS (pH 7.2). Nonspecific binding groups in the tissue were blocked by incubating in 5% heat-inactivated normal goat serum in PBS for 20 min. Sections were then treated with 500 μL of 1:250 diluted primary antiserum (polyclonal antisucrose synthase [described above]) for 60 min and washed in PBS (3 × 10 min). The 1:100 diluted secondary antibody, goat anti-rabbit IgG linked to 5-nm-diameter colloidal gold particles (Zymed Laboratories, Inc., South San Francisco, CA), was allowed to react for 60 min, and the sections were washed in PBS (3 × 10 min), followed by deionized water (3 × 5 min). The sections were treated with freshly prepared silver enhancement reagents (Janssen) for 3 min, washed with excess distilled water, counterstained with Fast green, and permanently mounted for microscopic evaluation. The immunolabeled tissue was examined using a light microscope (Optiphot model; Nikon Inc., Melville, NY), and bright-field photomicrographs were taken using color film (Ektachrome Professional, tungsten, 160 ASA; Kodak).

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REFERENCES

Baysdorfer, C., and VanDerWoude, W.J. (1988). Carbohydrate responsive proteins in the roots of *Pennisetum americanum*. Plant Physiol. **87,** 566–570.

Baysdorfer, C., Warmbrodt, R.D., and VanDerWoude, W.J. (1988). Mechanisms of starvation tolerance in pearl millet. Plant Physiol. **88,** 1381–1387.

Black, C.C., Mustardy, L., Sung, S.S., Kormanik, P.P., Xu, D.-P., and Pas, N. (1987). Regulation and roles for alternative pathways of hexose metabolism in plants. Physiol. Plant. **69,** 387–394.

Bodsworth, S., and Bewley, J.D. (1981). Osmotic priming of seeds of crop species with polyethylene glycol as a means of enhancing early and synchronous germination at cool temperatures. Can. J. Bot. **59,** 672–676.

Brouquisse, R., James, F., Raymond, P., and Pradet, A. (1991). Study of glucose starvation in excised maize root tips. Plant Physiol. **96,** 619–623.

Carlson, M. (1987). Regulation of sugar utilization in *Saccharomyces* species. J. Bacteriol. **169,** 4873–4877.

Chen, Y.-C., and Chourey, P.S. (1988). Spatial and temporal expression of the two sucrose synthase genes in maize: Immunohistochemical evidence. Theor. Appl. Genet. **78,** 553–559.

Chourey, P.S., and Nelson, O.E. (1976). The enzymatic deficiency conditioned by the *shrunken-1* mutations in maize. Biochem. Genet. **14,** 1041–1055.

Chourey, P.S., Latham, M.D., and Still, P.E. (1986). Expression of two sucrose synthetase genes in endosperm and seedling cells of maize: Evidence of tissue specific polymerization of protomers. Mol. Gen. Genet. **203,** 251–255.

Church, G.M., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA **81,** 1991–1995.

Claussen, W. (1983). Investigations on the relationship between the distribution of assimilates and sucrose synthetase activity in *Solanum melongena* L. 2. Distribution of assimilates and sucrose synthetase activity. Z. Pflanzenphysiol. **110,** 175–182.

Claussen, W., Hawker, J.S., and Loveys, B.R. (1985). Comparative investigations on the distribution of sucrose synthase activity and invertase activity within growing mature and old leaves of some 3-carbon photosynthetic pathway and 4-carbon photosynthetic pathway plant species. Physiol. Plant. **65,** 275–280.

Claussen, W., Hawker, J.S., and Loveys, B.R. (1986). Influence of sucrose and hormones on the activity of sucrose synthase and invertase in detached leaves and leaf sections of eggplants *Solanum melongena*. J. Plant Physiol. **124,** 345–358.

Delmer, D.P. (1972). The regulatory properties of purified *Phaseolus aureus* sucrose synthetase. Plant Physiol. **50,** 469–472.

Dennis, E., Gerlach, W., Proyor, A., Bennetson, J., Inglis, A., Llewellyn, D., Sachs, M., Ferl, R., and Peacock, W. (1984). Molecular analysis of the alcohol dehydrogenase gene of maize. Nucl. Acids Res. **12,** 3983–3990.

Doehlert, D. (1990). Distribution of enzyme activities within the developing maize (*Zea mays*) kernel in relation to starch, oil, and protein accumulation. Physiol. Plant. **78,** 560–576.

Duke, E., McCarty, D., and Koch, K. (1991). Organ-specific invertase deficiency in the primary root of an inbred maize line. Plant Physiol. **97,** 523–527.

Echt, C.S., and Chourey, P.S. (1985). A comparison of two sucrose synthase isozymes from normal and *shrunken-1* maize. Plant Physiol. **79,** 530–536.

Farrar, J.F., and Williams, J.H.H. (1990). Control of the rate of respiration in roots: Compartmentation, demand and the supply of substrate. In Compartmentation of Metabolism, M. Emes, ed (London: Butterworths), pp. 167–188.

Giaquinta, R.T., Lin, W., Sadler, N.L., and Franceschi, V.R. (1983). Pathway of phloem unloading of sucrose in corn roots. Plant Physiol. *72,* 362–367.

Glasziou, K.T. (1969). Control of enzyme formation and inactivation in plants. Annu. Rev. Plant Physiol. **20,** 62–88.

Hattori, T., Nakagawa, S., and Nakamura, K. (1990). High-level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown *in vitro* on sucrose medium. Plant Mol. Biol. **14,** 595–604.

Hawker, J.S. (1985). Sucrose. In Biochemistry of Storage Carbohydrates in Green Plants, P.M. Dey and R.A. Dixon, eds (London: Academic Press), pp. 1–51.

Heinlein, M., and Starlinger, P. (1989). Tissue- and cell-specific expression of the two sucrose synthase isoenzymes in developing maize kernels. Mol. Gen. Genet. **215,** 441–446.

Hendrix, D.L. (1990). Carbohydrates and carbohydrate enzymes in developing cotton ovules. Physiol Plant. **78,** 85–92.

Housley, T.L., and Pollock, C.J. (1985). Photosynthesis and carbohydrate metabolism in detached leaves of *Lolium temulentum* L. New Phytol. **99,** 499–507.

Huber, S.C., and Akazawa, T. (1986). A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. Plant Physiol. **81,** 1008–1013.

Kaufman, P.B., Ghosheh, N.S., LaCroix, J.D., Sarvjit, L.S., and Ikuma, H. (1973). Regulation of invertase levels in *Avena* stem segments of gibberellic acid, sucrose, glucose, and fructose. Plant Physiol. **52,** 221–228.

Koch, K.E., and Avigne, W. (1990). Postphloem, nonvascular transfer in citrus: Kinetics, metabolism, and sugar gradients. Plant Physiol. **93,** 1405–1416.

Koch, K.E., and McCarty, D.R. (1988). Induction of sucrose synthase by sucrose depletion in maize root tips. Plant Physiol. **86,** 35.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature **227,** 680–685.

Lin, A.Y., and Lee, A.S. (1984). Induction of two genes by glucose starvation in hamster fibroblasts. Proc. Natl. Acad Sci. USA **81,** 988–992.

Lin, W., Schmitt, M.R., Hitz, W.D., and Giaquinta, R.T. (1984). Sugar transport in isolated corn root protoplasts. Plant Physiol. **76,** 894–897.

Lowell, C.A., Tomlinson, P.T., and Koch, K.E. (1989). Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. Plant Physiol. **90,** 1394–1402.

Maas, C., Schaal, S., and Werr, W. (1990). A feedback control element near the transcription start site of the maize *Shrunken* gene determines promoter activity. EMBO J. **9,** 3447–3452.

Maretzki, A., Thom, M., and Nickell, L.G. (1974). Utilization and metabolism of carbohydrates in cell and callus cultures. In Tissue Culture and Plant Science, H.E. Street, ed (London: Academic Press), pp. 329–361.

Masuda, H., Takahashi, T., and Sugawara, S. (1988). Acid and alkaline invertases in suspension cultures of sugar beet cells. Plant Physiol. **86,** 312–317.

McCarty, D.R. (1986). A rapid and simple method for extracting RNA from maize tissues. Maize Gen. Coop. News Lett. **60,** 61.

McCarty, D.R., Shaw, J.R., and Hannah, C.L. (1986). The cloning, genetic mapping, and expression of the constitutive sucrose synthase locus of maize. Proc. Natl. Acad. Sci. USA **83,** 9099–9103.

McElfresh, K.C., and Chourey, P.S. (1988). Anaerobiosis induces transcription but not translation of sucrose synthase in maize. Plant Physiol. **87,** 542–546.

Müller-Röber, B.T., Kossmann, J., Hannah, L.C., Willmitzer, L., and Sonnewald, U. (1990). One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. Mol. Gen. Genet. **224,** 136–146.

Nguyen-Quoc, B., Krivitzky, M., Huber, S.C., and Lecharny, A. (1990). Sucrose synthase in developing maize leaves: Regulation of activity by protein level during the import to export transition. Plant Physiol. **94,** 516–523.

Ricard, B., Rivoal, J., Spiteri, A., and Pradet, A. (1991). Anaerobic stress induces the transcription and translation of sucrose synthase in rice. Plant Physiol. **95,** 669–674.

Robbins, W.J. (1958). Sucrose and growth of excised roots of an inbred *Zea mays*. Proc. Natl. Acad. Sci. USA **44,** 1210–1212.

Rocha-Sosa, M., Sonnewald, U., Frommer, W., Stratmann, M., Schell, J., and Willmitzer, L. (1989). Both developmental and metabolic signals activate the promoter of a class I patatin gene. EMBO J. **8,** 23–29.

Roland, L.J., Chen, Y.-C., and Chourey, P.S. (1988). Anaerobic treatment alters the cell specific expression of *Adh-1*, *Sh*, and *Sus* genes in roots of maize seedlings. Mol. Gen. Genet. **218,** 33–40.

Ross, H.A., and Davies, H.V. (1992). Sucrose metabolism in tubers of potato (*Solanum tuberosum* L.). Effects of sink removal and sucrose flux on sucrose-degrading enzymes. Plant Physiol. **98,** in press.

Saglio, P.H., and Pradet, A. (1980). Soluble sugars, respiration, and energy charge during aging of excised maize root tips. Plant Physiol. **66,** 516–519.

Salanoubat, M., and Belliard, G. (1989). The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis, and sucrose concentration. Gene **84,** 181–185.

Schuster, J.R. (1989). Regulated transcriptional systems for the production of proteins in yeast: Regulation by carbon source. In

Yeast Genetic Engineering, P.J. Barr, A.J. Brike, and P. Valenzuela, eds (London: Butterworths), pp. 83–108.

Sheen, J. (1990). Metabolic repression of transcription in higher plants. Plant Cell **2,** 1027–1038.

Sheldon, E., Ferl, R., Fedoroff, N., and Hannah, L.C. (1983). Isolation and analysis of a genomic clone encoding sucrose synthase in maize: Evidence for 2 introns in *Sh*. Mol. Gen. Genet. **190,** 421–426.

Springer, B., Werr, W., Starlinger, P., Bennett, D.C., Zokolica, M., and Freeling, M. (1986). The *shrunken* gene on chromosome 9 of *Zea mays* L. is expressed in various plant tissues and encodes an anaerobic protein. Mol. Gen. Genet. **205,** 461–468.

Su, J.-C., and Preiss, J. (1978). Purification and properties of sucrose synthase from maize kernels. Plant Physiol. **61,** 389–393.

Sung, S.-J., Xu, D.-P., and Black, C.C. (1988). Identification of actively filling sucrose sinks. Plant Physiol. **89,** 1117–1121.

Talercio, E.W., and Chourey, P.S. (1989). Post-transcriptional control of sucrose synthase expression in anaerobic seedlings of maize. Plant Physiol. **90,** 1359–1364.

Thomas, P.S. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose paper. Proc. Natl. Acad. Sci. USA **77,** 5201–5205.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA **76,** 4350–4354.

Uhl, G. (1986). Sectioning and slide treatment. In *In Situ* Hybridization in Brain, G. Uhl, ed (New York: Plenum Press), pp. 266–267.

Vyas, N.K., Vyas, M.N., and Quiocho, F.A. (1988). Sugar and signal-transducer binding sites of the *Escherichia coli* galactose chemoreceptor protein. Science **242,** 1290–1295.

Webster, P.L. (1980). "Stress" protein synthesis in pea root meristem cells? Plant Sci. Lett. **20,** 141–145.

Webster, P.L., and Henry, M. (1987). Sucrose regulation of protein synthesis in pea root meristem cells. Env. Exp. Bot. **27,** 255–262.

Wenzler, H.C., Mignery, G.A., Fisher, L.M., and Park, W.D. (1989). Analysis of a chimeric class-I patatin-GUS gene in transgenic potato plants: High-level expression in tubers and sucrose inducible expression in cultured leaf and stem explants. Plant Mol. Biol. **12,** 41–50.

Yamamoto, S., and Mino, Y. (1987). Effect of sugar level on phleinase induction in stem base of orchardgrass after defoliation. Physiol. Plant. **69,** 456–460.

Yang, N.S., and Russell, D. (1990). Maize sucrose synthase-1 promoter directs phloem cell-specific expression of *gus* gene in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA **87,** 4144–4148.