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Biochemical, Physiological, and Molecular Characterization of Sucrose Synthase from *Daucus carota*

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Sucrose synthase (EC 2.4.1.13) from carrot (*Daucus carota*) is a tetramer with a molecular mass of 320 kD and subunits of 80 kD. The enzyme has a pH optimum of 7.0 (cleavage direction). Maximal activities were measured at 55°C. The K_m for Suc was estimated as 87 mM and for UDP as 0.39 mM. Fructose acts as a noncompetitive inhibitor with an inhibition constant of 17.2 mM. In contrast, glucose inhibits carrot sucrose synthase uncompetitively with an inhibition constant of 4.3 mM. cDNA clones encoding a single class of sucrose synthase polypeptide were isolated and sequenced. DNA gel blot analysis also indicated the occurrence of only one to two genes. The deduced amino acid sequence of the carrot enzyme is highly homologous to the sucrose synthase sequences of tomato, potato, and bean. A comparison of the cDNA-derived amino acid sequence with the SS1- and SS2-type sucrose synthase sequences of the monocot plants maize, rice, and barley showed that the carrot enzyme is neither of the SS1 nor of the SS2 type. High enzyme activity was found in roots and petioles of developing carrot plants, with maximal activity in roots at the transition of primary roots to tap roots. Enzyme activity was highly correlated with both polypeptide and transcript levels, indicating that gene expression is regulated mainly at the mRNA level in the different tissues and organs of developing carrot plants.

Suc, the major form of translocated carbon in most plants, is a nonreducing disaccharide that consists of α-D-glucopyranose and β-D-fructofuranose joined by an α(1→2)-β linkage. Its synthesis is catalyzed by Suc-P synthase and Suc phosphatase (Kruger, 1990). The first step in the breakdown of Suc in plant tissues is the cleavage of the glycosidic bond by either β-fructofuranosidase (β-D-fructofuranoside fructohydrolase, also called invertase, EC 3.2.1.26) or Suc synthase (UDP-Glc:D-Fru 2-α-D-glycosyl transferase, EC 2.4.1.13) (Kruger, 1990). Cleavage by invertase is irreversible (free energy = -29.3 kJ mol^{-1}) and generates Glc and Fru (Copeland, 1990). Suc synthase catalyzes the transfer of the Glc residue from Suc to UDP, yielding UDP-Glc and Fru (Copeland, 1990). The free energy of the reaction is -3.99 kJ mol^{-1}, which is readily reversible (Geigenberger and Stitt, 1993). Although the enzyme is able to synthesize Suc under appropriate test-tube conditions, there is good evidence that in vivo Suc synthase is involved primarily in its breakdown (Hawker, 1985; Kruger, 1990).

Suc synthase was first described by Cardini et al. (1955). The enzyme is cytosolic (Keller et al., 1988) and has been characterized in various plant species and studied in numerous plant organs, such as roots (Hole and McKee, 1988; Koch et al., 1992), tubers (Keller et al., 1988; Geigenberger and Stitt, 1993), cotyledons (Ross and Davies, 1992), leaves (Nguyen-Quoc et al., 1990; Gupta et al., 1991), fruits (Moriguchi and Yamaki, 1988; Wang et al., 1994b), and seeds (Rowland and Chourey, 1990; Heim et al., 1993).

Suc synthase is a tetramer with a native molecular mass in the range of 280 to 400 kD. It has optimal activity in the cleavage direction between pH 6.0 and 8.5 at 50 to 55°C. In the direction of Suc synthesis, pH 8.5 to 9.5 at 35°C was found to be optimal (Claussen, 1983). The K_m values of Suc synthase differ considerably from plant to plant. They are in the range of 10 to 290 mM for Suc and 0.05 to 6.6 mM for UDP.

Several studies have demonstrated the existence of multiple forms of Suc synthase (Gross and Pharr, 1982; Echt and Chourey, 1985; Nguyen-Quoc et al., 1990; Buczynski et al., 1993), e.g. two isozymes, SS1 and SS2, have been characterized from maize, cucumber, and sugarcane. The two Suc synthase isozymes in maize are encoded by two genes, *Sh* (Shrunken) and *Sus* (Chourey and Nelson, 1976; Chourey, 1981). The genes encoding the two isozymes are differentially expressed. Whereas the gene for SS1 is expressed only in the endosperm, SS2 has been found in many tissues, including endosperm, embryo, roots, and shoots (Chourey et al., 1986). SS1 and SS2 polypeptides have a high overall amino acid identity (Werr et al., 1985; Gupta et al., 1988; Huang et al., 1994) and very similar kinetics parameters. Homologous pairs of Suc synthase genes have also been characterized from the monocot plants barley (Sanchez de la Hoz et al., 1992; Martinez de Ilarduya et al., 1993), wheat (Marana et al., 1988), and rice (Wang et al., 1992; Yu et al., 1992). In contrast, in several dicot plants, such as potato (Salanoubat and Belliard, 1987), tomato (Wang et al., 1993), and two different bean cultivars (Arai et al., 1992; Heim et al., 1993), Suc synthase appears to be encoded by only one gene.

We would like to understand the role(s) of Suc-cleaving enzymes in Suc partitioning in carrot (*Daucus carota*). In general, this process largely determines the yield of crop plants (Gifford et al., 1984) and is, therefore, of great interest for agriculture. The driving force for Suc transport from leaves into sink organs seems to be a turgor pressure gradient caused by a Suc concentration gradient. The generation of such an assimilate concentration gradient is con-

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Abbreviation: TBS, Tris-buffered saline.

trolled, among other factors, by the rate of Suc utilization in sink tissues and by an energy-dependent transport of Suc through membranes. Recent studies have suggested that in rapidly growing sink organs Suc synthase is the main Suc-cleaving activity and, thus, that the enzyme may be used as a biochemical marker of sink strength (Sung et al., 1989; Sun et al., 1992). This suggestion is strongly supported by the fact that the level of Suc synthase activity is generally low in photosynthetic source tissues and high in actively growing sink organs (ap Rees, 1984; Sung et al., 1989). Whether Suc synthase is involved in Suc partitioning in carrot is not known. To provide the tools necessary to investigate this question, we characterized the carrot enzyme at the biochemical, physiological, and molecular levels.

MATERIALS AND METHODS

Plant Material and Tissue Culture

Carrot plants (*Daucus carota* cv Nantaise) were grown near Basel either in a field or in a greenhouse in soil or vermiculite. Fresh plant material was used for the determination of enzyme activity. The isolation of periderm, phloem, cambium, and xylem tissue was performed as described by Sturm et al. (1995).

Cells of *D. carota* cv Queen Anne's lace (wild carrot cell culture line W001C; Sung, 1976) were grown in Murashige-Skoog medium (Murashige and Skoog, 1962), supplemented with 0.1 mg/L 2,4-D at 26°C in the dark. The cell-suspension cultures were transferred at 1-week intervals into fresh Murashige-Skoog medium.

Extraction of Suc Synthase and Enzyme Assay

Plant material (about 2 g) was homogenized four times for 15 s each with a Polytron homogenizer in 10 mL of an ice-cold extraction buffer (20 mM Hepes-KOH, pH 7.5, containing 1% β-mercaptoethanol). The homogenates were centrifuged for 30 min at 48,000*g* at 4°C. The supernatants were desalted on Sephadex G-25 PD-10 columns (Pharmacia) to remove low mol wt compounds. Supernatant (2.5 mL) was applied to the column previously equilibrated with 20 mM Hepes-KOH buffer, pH 7.5, and eluted with 3.5 mL of the same buffer. The protein recovery was greater than 90%.

The cleavage of Suc by Suc synthase was determined by a combination of the methods described by Avigad (1964), Morell and Copeland (1985), and Witt (1989). The assay buffer (20 mM Hepes-KOH, pH 7.5) contained 100 mM Suc and 2 mM UDP. The reaction was carried out at 30°C. After 30 min, the reaction was stopped by boiling the sample for 1 min. The UDP-Glc that was produced was determined by its reduction with 1.5 mM NAD in the presence of an excess of UDP-Glc dehydrogenase (type III, Sigma), resulting in an increase in A_{340}. The formation of 2 nmol of NADH corresponds to 1 nmol of UDP-Glc. Enzyme activity was expressed as the amount (nmol) of UDP-Glc formed in 1 min (units) or as activity per mg protein (units/mg protein).

For the determination of the K_m, the concentration of Suc was varied between 0 and 400 mM and that of UDP was varied between 0 and 4 mM. For the inhibition of Suc synthase activity, 0 to 10 mM Fru, 0 to 10 mM Glc, 0 to 100 mM Tris-HCl, and 0 to 100 μM $HgCl_2$ were used. As possible stimulators of the enzyme activity, 0 to 10 mM $MgCl_2$, $MnCl_2$, and $CaCl_2$ were tested.

Determination of Native Molecular Mass

The molecular mass of carrot Suc synthase was estimated by gel filtration, using a Sephacryl S-300 column (Pharmacia) equilibrated with 100 mM Hepes-KOH, pH 7.5. The standard proteins alcohol dehydrogenase (150 kD), β-amylase (200 kD), apoferritin (443 kD), and thyroglobulin (669 kD) (Sigma) were applied individually, and their elution volumes were determined by absorption at 280 nm. Elution of Suc synthase was followed by the standard assay. The molecular mass was estimated by interpolation.

Protein Assay

Protein content was determined by the Pierce Protein Plus Assay, according to the manufacturer's protocol.

SDS-PAGE and Detection of Proteins on Nitrocellulose Membranes

Proteins were separated by SDS-PAGE as described by Laemmli (1970) using a Bio-Rad Mini Electrophoresis System. Proteins were either stained with Coomassie blue or transferred onto a Transblot nitrocellulose membrane (Bio-Rad) with an Electro Transblot apparatus (Bio-Rad). For the determination of the relative molecular mass, prestained molecular mass markers in the range of 14 to 200 kD (Gibco/BRL) were used. The free polypeptide-binding sites on the nitrocellulose membrane were blocked for 1 h in 5% nonfat milk powder in TBS (20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl). Immunodetection of proteins on nitrocellulose membranes (western blots) was done with an antibody against maize Suc synthase (Chourey et al., 1986) at a dilution of 1:1000. The blots were incubated for 1 h with primary antibody in TBS containing 5% nonfat milk powder and subsequently washed twice for 15 min in TBS containing 0.1% Tween 20 with 5% nonfat milk powder, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Bio-Rad) in TBS with 5% nonfat milk powder at a dilution of 1:1000 for 1 h. After the blot was washed once for 15 min and twice for 5 min each in TBS containing 0.1% Tween 20 without milk powder, the color reaction was performed according to the manufacturer's protocol (Bio-Rad) using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in the buffer containing 0.1 M $NaHCO_3$ and 1 mM $MgCl_2$, pH 9.8.

cDNA Cloning

The total RNA was extracted from 4-week-old carrot roots, according to the method of Prescott and Martin (1987). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (Maniatis et al., 1989). cDNA was synthe-

sized by using the cDNA Synthesis System Plus from Amersham, as described in the manufacturer's protocol. After *Eco*RI linkers were added, the cDNA was size fractionated on an agarose gel. cDNAs of 1500 to 5500 bp were ligated into the λgt11 vector (Stratagene) and packaged into phages (Gigapack II Plus packaging extract, Stratagene), as described in the manufacturer's protocol.

The cDNA library was screened with a 1500-bp-long *Eco*RI/*Hin*dIII fragment of the cDNA for Suc synthase from potato (Salanoubat and Belliard, 1987). After hybridization at 60°C (Maniatis et al., 1989), the filters were washed at 60°C, twice for 30 min with 2× SSC (Maniatis et al., 1989), and once for an additional 30 min with 0.5% SDS and 0.1× SSC. Ten positive clones were isolated and purified. The longest clone contained a 2.8-kb *Eco*RI fragment. Whereas this clone was completely sequenced in both directions by the dideoxy nucleotide chain-termination reaction (Messing, 1983), the sequences of the other clones were only partially determined.

Analysis of DNA Sequences

Computer-assisted analysis of DNA was performed with the Beta version of the Genetics Computer Group Sequence Analysis software package (version 7.3, June 1993, University of Wisconsin, Madison, WI).

Analysis of DNA

For DNA gel blot analysis, carrot genomic DNA (10 μg/lane) digested with *Eco*RI, *Kpn*I, and *Xba*I was separated on 0.7% agarose gels (Maniatis et al., 1989). DNA blots were performed on nylon membranes (Hybond-N, Amersham) with probes that were labeled with ^{32}P by random priming (Maniatis et al., 1989). The central region of the cDNA clone for carrot Suc synthase (*Kpn*I/*Sac*I, 1150 bp) was used as a probe. Prehybridizations were done at 65°C in 6× SSC, 5× Denhardt's solution, 100 mg/mL denatured calf thymus DNA, and 0.5% SDS (Maniatis et al., 1989). Hybridizations were carried out in the same buffer overnight at 65°C. The blots were washed with 0.1× SSC, 0.5% SDS at 65°C for 30 min.

RESULTS

Biochemical Characterization of Carrot Suc Synthase

The cleavage activity of Suc synthase was analyzed in protein extracts of carrot roots. An optimal pH region between 6.5 and 8.0 was found (Fig. 1, top). The velocity of the reaction increased with increasing temperature and was optimal between 50 and 60°C. An equally high temperature optimum was reported for Suc synthase from tomato (Sun et al., 1992), potato, and bean (Xu et al., 1989). Above 60°C the activity of the carrot enzyme rapidly decreased and was abolished above 70°C (Fig. 1, middle). In protein extracts stored at 4°C, Suc synthase activity remained constant for several days, whereas at temperatures above 20°C the activity was labile and slowly decreased (Fig. 1, bottom).

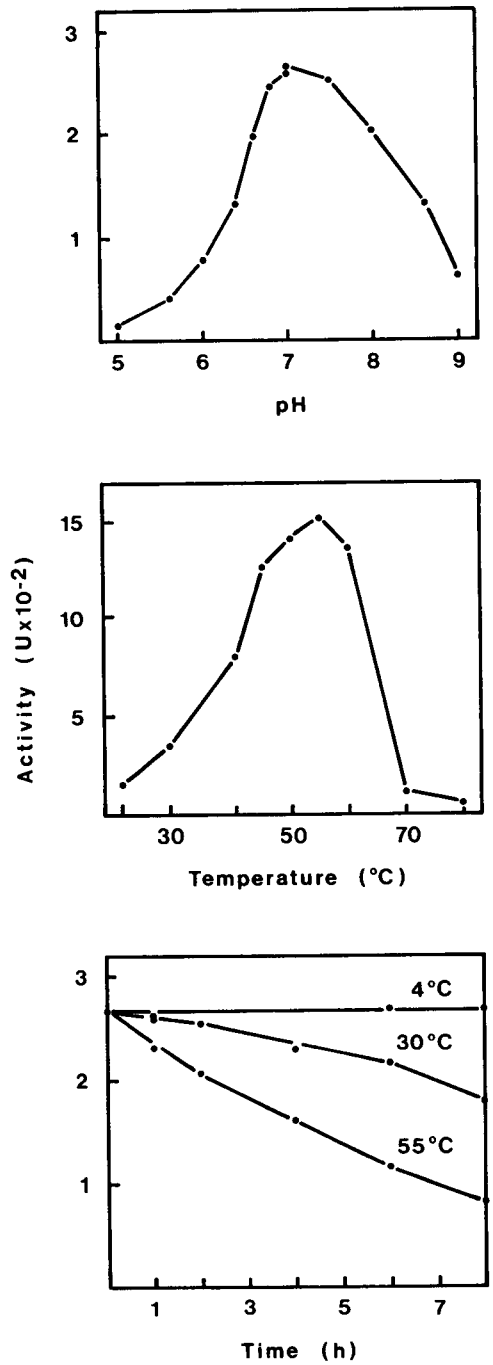

Figure 1. Characterization of carrot Suc synthase. The pH optimum for Suc cleavage (top) was determined to be between pH 5 and 9 (pH 5–7, 20 mM Mes-KOH; pH 7–9, 20 mM Hepes-KOH). The temperature optimum (middle) and temperature stability (bottom) were determined in 20 mM Hepes-KOH buffer at pH 7.5.

The Suc-cleavage activity was inhibited by low concentrations of heavy metal ions such as mercurate, indicating the involvement of sulfhydryl groups in the catalytic process. The Suc-cleavage activity was also inhibited by Tris-HCl and millimolar concentrations of $MgCl_2$ and $MnCl_2$. At low concentrations, the latter two salts as well as $CaCl_2$ had a slight stimulating activity (data not shown).

Among the nucleotide diphosphates tested, only UDP facilitated Suc cleavage; ADP was not significantly effective in replacing UDP under these conditions (data not shown).

The effect of substrate concentration on the rate of Suc synthase cleavage activity is presented in Figure 2. The concentration required for half-maximal activity was 87 mM for Suc (Fig. 2, top) and 0.39 mM for UDP (Fig. 2, bottom). However, the K_m for Suc could not be determined accurately because saturation did not occur, even with 400 mM Suc (Fig. 2, top). Similar kinetics properties for Suc saturation have been reported for Suc synthase from maize (Su and Preiss, 1978) and bean (Ross and Davies, 1992).

Glc and Fru are both inhibitors of the cleavage of Suc by Suc synthase. Dixon plots gave estimated K_i values of 4.3 mM for Glc and 17.2 mM for Fru at Suc concentrations of 25, 50, and 100 mM (Fig. 3). Inhibition by Fru was noncompetitive (Fig. 3, bottom), whereas inhibition by Glc was uncompetitive (Fig. 3, top).

The molecular mass of Suc synthase obtained by gel filtration chromatography was approximately 320 kD. On SDS polyacrylamide gels, Suc synthase migrated as a polypeptide with an M_r of 80,000 (data not shown). The protein, therefore, appears to be a tetramer, as are other plant Suc synthases.

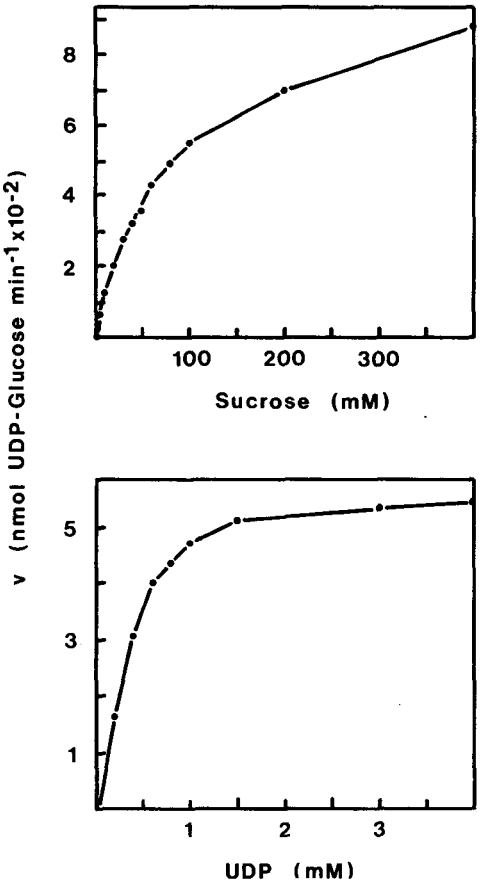

Figure 2. Determination of the K_ms for Suc and UDP. The concentration of Suc was varied between 0 and 400 mM and that of UDP was varied between 0 and 4 mM.

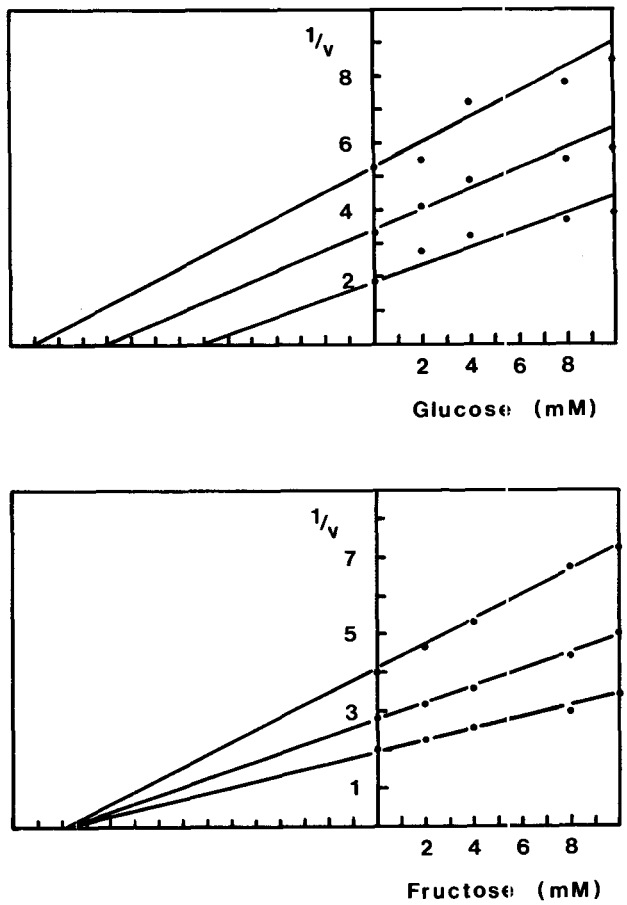

Figure 3. Inhibition of Suc cleavage by Glc (top) and Fru (bottom). Three sets of experiments were performed for each inhibitor containing 25, 50, and 100 mM Suc (from bottom to top). The hexose concentration was varied between 0 and 10 mM.

Molecular Characterization of Suc Synthase

A full-length cDNA clone (2866 bp) for carrot Suc synthase was isolated. It contained one open reading frame, starting at nucleotide 139 with an ATG start codon and ending at nucleotide 2564 before a TAG stop codon. The open reading frame encodes a polypeptide chain of 808 residues with a calculated molecular mass of 92,473 D and a pI of 6.66. In addition to the open reading frame, the cDNA also contains 138 bp of 5′ untranslated and 304 bp of 3′ untranslated sequence. A consensus signal for polyadenylation, AATAAA, is located close to the end of the cDNA.

The deduced amino acid sequence of carrot Suc synthase was compared with known sequences of plant Suc synthases and a phylogenetic tree was generated (Fig. 4). A striking similarity between the various sequences was observed. Carrot Suc synthase is closely related to Suc synthase from tomato (tom, Wang et al., 1994a) and potato (pot, Salanoubat and Belliard, 1987), with 85.1 and 85.6% identity, respectively, and well related to monocot Suc synthases, such as SS1 (sh1, Werr et al., 1985) and SS2 from maize (sus1, Huang et al., 1994), with 73.1 and 68.7% identity, respectively. The lowest but still good homology

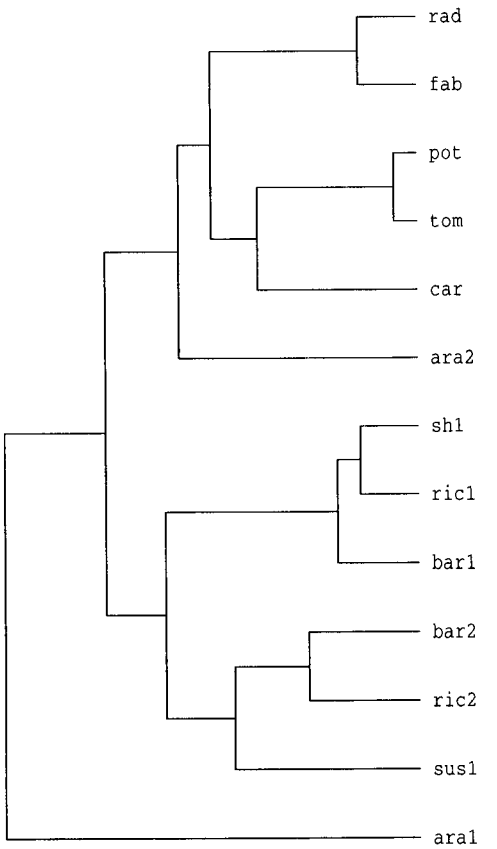

Figure 4. Comparison of amino acid sequences of plant Suc synthases. The dendrogram was generated by the comparison of the known protein sequences of plant Suc synthases by the PileUp program of the Genetics Computer Group Sequence Analysis software package. rad, Bean (*Vigna radiata*) (Arai et al., 1992); fab, bean (*Vicea faba*) (Heim et al., 1993); pot, potato (Salanoubat and Belliard, 1987); tom, tomato (Wang et al., 1994a); car, carrot (see this paper); ara2, Arabidopsis (Martin et al., 1993); sh1, maize (Werr et al., 1985); ric1, rice (Wang et al., 1992); bar1, barley (Sanchez de la Hoz et al., 1992); bar2, barley (Martinez de Ilarduya et al., 1993); ric2, rice (Yu et al., 1992); sus1, maize (Huang et al., 1994); and ara1, Arabidopsis (Chopra et al., 1992).

was found between the carrot sequence and that of Suc synthase of Arabidopsis (ara1, Chopra et al., 1992), with 67.9% identity.

Monocot plants seem to have at least two genes for Suc synthase belonging either to the *Sh* type or the *Sus* type of maize (Gupta et al., 1988). In the dicot plants, with the exception of Arabidopsis, only one gene has been detected. To understand whether the amino acid sequence of the carrot enzyme is related to the SS1- or the SS2-type sequence, SS1- or SS2-specific sequences first had to be identified. For this purpose, the SS1-type sequences of maize (sh1, Werr et al., 1985), rice (ric1, Wang et al., 1992), and barley (bar1, Sanchez de la Hoz et al., 1992) and their SS2 counterparts (ric2, Yu et al., 1992; bar2, Martinez de Ilarduya et al., 1993; sus1, Huang et al., 1994) were compared (Fig. 5). Sixty-four positions were identified at which the amino acids were identical in the SS1-type and in the SS2-type sequences, although the actual amino acid was specific for the type (indicated by asterisks below the six sequences). These 64 positions were compared with their respective positions in the carrot sequence. In 20 cases, the carrot amino acid was of the SS1 type, in 25 cases it was of the SS2 type, and in 19 cases it was unique to carrot. Taken together, these data indicate that carrot Suc synthase is neither of the SS1 nor of the SS2 type. This analysis was also applied to the other dicot enzymes, and the same results were obtained (data not shown). It is interesting that the two Arabidopsis sequences (Chopra et al., 1992; Martin et al., 1993) also could not be assigned to either of the monocot enzyme types.

To determine the copy number of the Suc synthase gene in cv Nantaise, DNA gel blot analyses (Southern, 1975) were performed (Fig. 6). The labeled cDNA fragment hybridized to only a few restriction fragments, indicating the presence of only one or two copies of the gene.

Expression of Suc Synthase in Developing Plants

The steady-state levels of Suc synthase mRNA in different tissues and organs of developing carrot plants have already been determined (Sturm et al., 1995). Elevated levels were found in the leaves of young plants and in roots at the transition of primary roots to developing tap roots. Low levels of transcripts were found in all other tissues analyzed. The analysis of young leaves revealed that high transcript levels were restricted to the petioles. Only low levels were detected in the leaf lamina. In developing tap roots, high levels of Suc synthase transcripts were found in all of the main root tissues (periderm, phloem, cambium, and xylem).

We now compared these steady-state transcript levels with Suc synthase polypeptide levels and enzyme activity (Fig. 7). Our data showed a strong correlation of mRNA levels with polypeptide levels and enzyme activity, suggesting that transcription may be the key regulatory step in the developmental expression of carrot Suc synthase.

DISCUSSION

Carrot Suc synthase is a tetramer with a molecular mass of 320 kD and subunits of 80 kD. The enzyme has a high K_m for Suc and a low K_m for UDP. Optimal activity is at a neutral pH, which is in accordance with its location in the cytoplasm. The cleavage reaction of carrot Suc synthase appears to be specific for UDP, with no appreciable activity in the presence of ADP. A comparison of enzyme activities with polypeptide and transcript levels showed a strong correlation, indicating that the regulation of gene expression is mainly at the mRNA level. Because millimolar concentrations of Glc and Fru inhibit Suc synthase, the levels of these sugars may fine-tune enzyme activity in vivo.

Monocotyledonous plants contain at least two genes for Suc synthase (*Sh* and *Sus*), which are differentially regulated. The polypeptides encoded by these genes (SS1 and SS2) have highly homologous sequences, and their kinetics parameters are fairly similar. A comparison of the SS1- and SS2-type sequences of maize, rice, and barley revealed 64

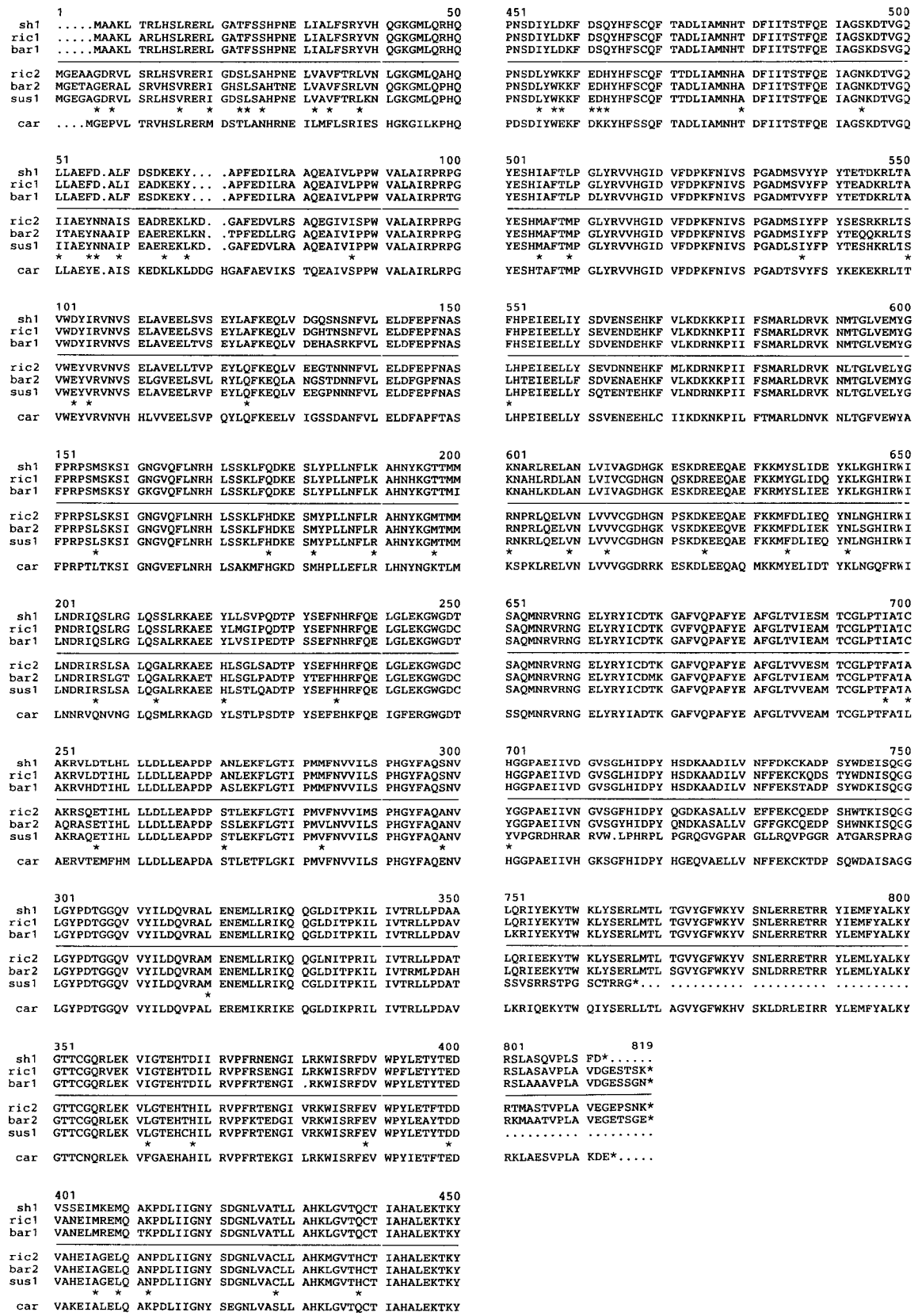

Figure 5. Comparison of the amino acid sequence of carrot Suc synthase (car) with the sequences of the SS1-type and SS2-type Suc synthase of maize (sh1, sus1), rice (ric1, ric2), and barley (bar1, bar2). Positions having SS1- and SS2-specific amino acid residues are marked by asterisks below the six sequences.

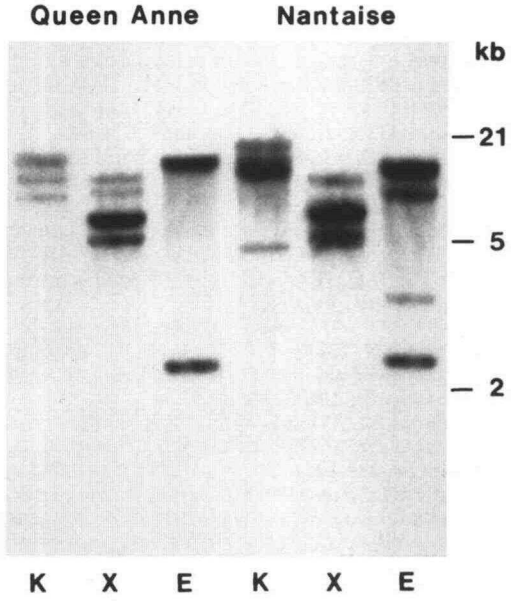

Figure 6. DNA gel blot analysis of Suc synthase sequences in the carrot genome. Genomic DNA (10 μg/lane) from cv Queen Anne's lace and cv Nantaise was digested with *Kpn*I (K), *Xba*I (X), and *Eco*RI (E). The fragments were separated by agarose gel electrophoresis and blotted before hybridization with a ^{32}P-labeled fragment of the cDNA of carrot Suc synthase (*Knp*I/*Sac*I, 1150 bp).

positions at which the amino acids appear to be specific for the two sequence types. In several dicotyledonous plants, only one gene for Suc synthase has been found. Ten different cDNA clones have been isolated with identical partial sequences, which also suggests the existence of only one gene. This finding is supported by the simple hybridization pattern on a DNA gel blot that is hybridized with a fragment of the cDNA, indicating the presence of only one to two genes. A comparison of the cDNA-derived amino acid sequence with the 64 type-specific positions revealed that the carrot enzyme cloned is of neither the SS1 nor the SS2 type. This finding is supported by the position of the carrot sequence in a phylogenetic tree, which suggests a separation of a dicot Suc synthase gene from a monocot ancestor gene before the evolution of the monocot SS1-type and SS2-type sequences.

It has been suggested that Suc synthase plays a key role in Suc partitioning and, therefore, the activity of the enzyme can be directly used as a marker for sink strength (Sung et al., 1989; Sowokinos and Varns, 1992; Sun et al., 1992). The activities of Suc synthase found in different organs and tissues of developing carrot plants support this view. High activity was found in petioles and roots, whereas only low activity was detected in the leaf lamina. Suc synthase activity was highest in roots at the transition from primary to secondary roots, corresponding there with a high requirement for carbon and energy for rapid cell growth.

The view that Suc synthase activity can be directly used as a marker for sink strength is in contrast to the findings of Geigenberger and Stitt (1993). These authors provided evidence that Suc synthase catalyzes a near-equilibrium reaction in tubers of potato, in cotyledons of *Ricinus communis*, and in heterotrophic suspension-cultured cells of *Chenopodium rubrum*. The authors concluded that such kinetics properties enable the enzyme to respond automatically to the supply of Suc and the demand for Suc in the cell. Suc would only be degraded as required. Consequently, in this model, sink strength would be controlled by Suc utilization and not by Suc synthase activity.

Carrots store significant amounts of Suc and hexose in their tap roots in the parenchyma of an outer sheath of phloem and an inner core of xylem (Hole and Dearman, 1994). The relative contribution of symplastic and apoplas-

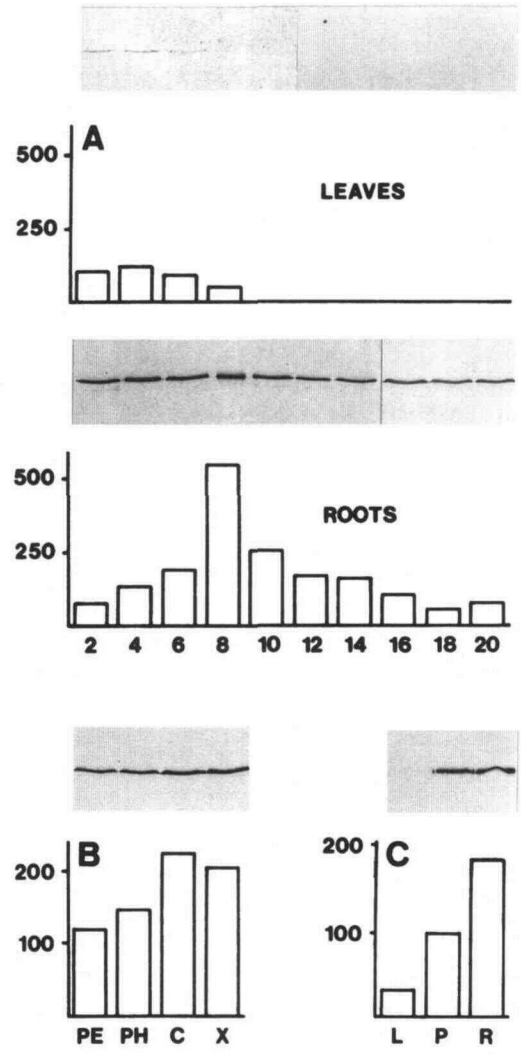

Figure 7. Comparison of Suc synthase polypeptide levels with enzyme activity in different organs and tissues of developing carrot plants. For western blot analysis, equal amounts of protein were loaded (20 μg/lane). A, Polypeptide level and enzyme activity (units/g fresh weight) in developing leaves (top) and roots (bottom). The numbers on the *x* axis indicate the age of the plant organs analyzed in weeks postgermination. B, Polypeptide level and enzyme activity (units/g fresh weight) in periderm (PE), phloem (PH), cambium (C), and xylem (X) of 12-week-old carrot tap roots. C, Polypeptide level and enzyme activity (units/mg protein) in leaf lamina (L), petioles (P), and roots (R) of 6-week-old carrot plants.

tic transport to movement of assimilate within the carrot storage root is not known. Our finding that Suc synthase activity is not restricted to the sites of phloem unloading but is evenly distributed throughout the storage root (Fig. 7) favors the view of apoplastic Suc transport and uptake by each individual storage cell.

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LITERATURE CITED

ap Rees T (1984) Sucrose metabolism. *In* DH Lewis, ed, Storage Carbohydrates in Vascular Plants. Cambridge University Press, Cambridge, UK, pp 53–73

Arai M, Mori H, Imaseki H (1992) Expression of the gene for sucrose synthase during growth of mung bean seedlings. Plant Cell Physiol **33:** 503–506

Avigad G (1964) Sucrose-uridine diphosphate glucosyltransferase from Jerusalem artichoke tubers. J Biol Chem **239:** 3613–3618

Buczynski SR, Thom M, Chourey PS, Maretzki A (1993) Tissue distribution and characterization of sucrose synthase isozymes in sugarcane. J Plant Physiol **142:** 641–646

Cardini CE, Leloir LF, Chiriboga JJ (1955) The biosynthesis of sucrose. J Biol Chem **214:** 149–155

Chopra S, Del-favero J, Dolferus R, Jacobs M (1992) Sucrose synthase of Arabidopsis: genomic cloning and sequence characterization. Plant Mol Biol **18:** 131–134

Chourey PS (1981) Genetic control of sucrose synthetase in maize endosperm. Mol Gen Genet **184:** 372–376

Chourey PS, Latham MD, Still PE (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

Chourey PS, Nelson OE (1976) The enzymatic deficiency conditioned by the shrunken-1 mutations in maize. Biochem Genet **14:** 1041–1055

Claussen W (1983) Investigations on the relationship between the distribution of assimilates and sucrose synthase activity in *Solanum melongena* L. 1. Characterization and distribution of sucrose synthetase. Z Pflanzenphysiol **110:** 165–173

Copeland L (1990) Enzymes of sucrose metabolism. Methods Plant Biochem **3:** 73–85

Echt CS, Chourey PS (1985) A comparison of two sucrose synthetase isozymes from normal and shrunken-1 maize. Plant Physiol **79:** 530–536

Geigenberger P, Stitt M (1993) Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. Planta **189:** 329–339

Gifford RM, Thorne JH, Hitz WD, Giaquinta RT (1984) Crop productivity and photoassimilate partitioning. Science **225:** 801–808

Gross KC, Pharr DM (1982) Cucumber fruit sucrose synthase isozymes. Phytochemistry **21:** 1241–1244

Gupta AK, Mann P, Kaur N, Singh R (1991) Profiles of enzymes of sucrose metabolism in the leaves of chicory (*Cichorium intybus*) during development. Plant Sci **77:** 191–196

Gupta M, Chourey PS, Burr B, Still PE (1988) cDNAs of two non-allelic sucrose synthase genes in maize: cloning, expression, characterization and molecular mapping of the sucrose synthase-2 gene. Plant Mol Biol **10:** 215–224

Hawker JS (1985) Sucrose. *In* PM Dey, RA Dixon, eds, Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, London, pp 1–51

Heim U, Weber H, Baumlein H, Wobus U (1993) A sucrose-synthase gene of *Vicia faba* L.: expression pattern in developing seeds in relation to starch synthesis and metabolic regulation. Planta **191:** 394–401

Hole CC, Dearman J (1994) Sucrose uptake by the phloem parenchyma of the carrot storage root. J Exp Bot **45:** 7–15

Hole CC, McKee JMT (1988) Changes in soluble carbohydrate levels and associated enzymes of field-grown carrots. J Hortic Sci **63:** 87–93

Huang XF, Quoc BN, Chourey PS, Yelle S (1994) Complete nucleotide sequence of the maize (*Zea mays* L.) sucrose synthase 2 cDNA. Plant Physiol **104:** 293–294

Keller F, Frehner M, Wiemken A (1988) Sucrose synthase, a cytosolic enzyme in protoplasts of Jerusalem artichoke tubers. Plant Physiol **88:** 239–241

Koch KE, Nolte KD, Duke ER, McCarty DR, Avigne WT (1992) Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell **4:** 59–69

Kruger JN (1990) Carbohydrate synthesis and degradation. *In* DT Dennis, DH Turpin, eds, Plant Physiology, Biochemistry, and Molecular Biology. Longman Scientific & Technical Publishers, Harlow, UK, pp 59–76

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

Maniatis T, Frisch E, Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Marana C, Garcia-Olmedo F, Carbonero P (1988) Linked sucrose synthase genes in group-7 chromosomes in hexaploid wheat (*Triticum aestivum* L.). Gene **63:** 253–260

Martin T, Frommer WB, Salanoubat M, Willmitzer L (1993) Expression of an Arabidopsis sucrose synthase gene indicates a role in metabolization of sucrose both during phloem loading and in sink organs. Plant J **4:** 367–377

Martinez de Ilarduya O, Vicente-Carbajosa J, Sanchez de la Hoz P, Carbonero P (1993) Sucrose synthase genes in barley. cDNA cloning of the Ss2 type and tissue-specific expression of Ss1 and Ss2. FEBS Lett **320:** 177–181

Messing J (1983) New M13 vectors for cloning. Methods Enzymol **101:** 20–78

Morell M, Copeland L (1985) Sucrose synthase of soybean nodules. Plant Physiol **78:** 149–154

Moriguchi T, Yamaki S (1988) Purification and characterization of sucrose synthase from peach (*Prunus persica*) fruit. Plant Cell Physiol **28:** 1361–1366

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant **15:** 473–497

Nguyen-Quoc B, Krivitzky M, Huber SC, Lecharny A (1990) Sucrose synthase in developing maize leaves. Plant Physiol **94:** 516–523

Prescott A, Martin C (1987) A rapid method for the quantitative assessment of levels of specific mRNAs in plants. Plant Mol Biol Rep **4:** 219–224

Ross HA, Davies HV (1992) Purification and characterization of sucrose synthase from the cotyledons of *Vicia faba* L. Plant Physiol **100:** 1008–1013

Rowland LJ, Chourey PS (1990) *In situ* hybridization analysis of sucrose synthase expression in developing kernels of maize. Maydica **35:** 373–383

Salanoubat M, Belliard G (1987) Molecular cloning and sequencing of sucrose synthase cDNA from potato (*Solanum tuberosum* L.): preliminary characterization of sucrose synthase mRNA distribution. Gene **60:** 47–56

Sanchez de la Hoz P, Vicente-Carbajosa J, Mena M, Carbonero P (1992) Homologous sucrose synthase genes in barley (*Hordeum vulgare*) are located in chromosomes 7H (syn. 1) and 2H. FEBS Lett **310:** 46–50

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol **98:** 503–517

Sowokinos JR, Varns JL (1992) Induction of sucrose synthase in potato tissue culture: effect of carbon source and metabolic regulators on sink strength. J Plant Physiol **139:** 672–679

Sturm A, Šebková V, Lorenz K, Hardegger M, Lienhard S, Unger C (1995) Developmental- and organ-specific expression of the genes for sucrose synthase and three isoenzymes of acid β-fructofuranosidase in carrot. Planta **195:** 601–610

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

Sun J, Loboda T, Sung SJS, Black CC (1992) Sucrose synthase in wild tomato, *Lycopersicon chmielewskii*, and tomato fruit sink strength. Plant Physiol **98:** 1163–1169

Sung SJS, Xu DP, Black CC (1989) Identification of actively filling sucrose sinks. Plant Physiol **89:** 1117–1121

Sung ZR (1976) Turbidimetric measurements of plant cell culture growth. Plant Physiol **57:** 460–462

Wang F, Sanz A, Brenner ML, Smith A (1993) Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol **101:** 321–327

Wang F, Smith AG, Brenner ML (1994a) Isolation and sequencing of tomato fruit sucrose synthase cDNA. Plant Physiol **103:** 1463–1464

Wang F, Smith AG, Brenner ML (1994b) Temporal and spatial expression pattern of sucrose synthase during tomato fruit development. Plant Physiol **104:** 535–540

Wang MB, Boulter D, Gatehouse JA (1992) The complete sequence of the rice sucrose synthase-1 (RSs1) gene. Plant Mol Biol **19:** 881–885

Werr W, Frommer WB, Maas C, Starlinger P (1985) Structure of the sucrose synthase gene on chromosome 9 of *Zea mays* L. EMBO J **4:** 1373–1380

Witt H-J (1989) Activities of enzymes involved in sucrose and starch metabolism in *Riella helicophylla* (Bory et. Mont.) Mont. during differentiation. J Plant Physiol **135:** 99–104

Xu DP, Sung SJS, Loboda T, Kormanik PP, Black CC (1989) Characterization of sucrolysis via the uridine diphosphate and pyrophosphate-dependent sucrose synthase pathway. Plant Physiol **90:** 635–642

Yu WP, Wang AY, Juang RH, Sung HY, Su JC (1992) Isolation and sequences of rice sucrose synthase cDNA and genomic DNA. Plant Mol Biol **18:** 139–142