Contribution of Invertase and Sucrose Synthase Isoforms to Sucrose Catabolism in Developing Sugarbeet Roots

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ABSTRACT

Isoforms of the major sucrolytic enzymes in sugarbeet were identified and their developmental expressions of activity were determined with respect to root growth and carbohydrate accumulation. Sugarbeet roots contained at least seven different sucrolytic activities throughout their development. Two soluble acid invertase isoforms, an insoluble acid invertase activity, two alkaline invertase isoforms and two sucrose synthase isoforms were identified. Each enzyme isoform exhibited a unique pattern of developmental expression. Soluble and insoluble acid invertase activities were the predominant sucrolytic activities in young roots and declined rapidly as roots aged. Soluble acid invertase activity was due primarily to the activity of a single isoform, although a second minor isoform was evident in roots of seedlings. High soluble and insoluble acid invertase activities occurred concurrently with a rapid relative growth rate, high glucose concentration and minimal sucrose accumulation. Sucrose synthase was the major sucrolytic activity during most of root development and was the predominant sucrolytic activity during the period in which nearly all sucrose accumulation and enlargement of the taproot occurred. Sucrose synthase activity correlated highly with absolute growth rate of the root. One sucrose synthase isoform was present throughout development. A second isoform became

evident as roots approached maturity. Alkaline invertase activity was present at low, relatively constant activities at all but the earliest stages of development and was due to two isoforms whose contribution to total alkaline invertase activity changed as roots matured. The presence of multiple, differentially regulated, sucrolytic enzymes seemingly allows control of sucrose catabolism to balance the metabolic needs of the growing sugarbeet root with its function as a sucrose storage organ.

Keywords: *Beta vulgaris*, invertase, sucrose synthase, sugarbeet

The enzymes of carbohydrate metabolism are essential for growth, development and carbohydrate partitioning in sink organs. Sucrose catabolism fuels growth and development by providing substrates for cellular metabolism and synthesis of cellular structures (Kruger, 1997). Sucrose cleavage also governs growth and development by affecting cell expansion (Pfeiffer and Kutschera, 1995), mitotic activity (Cheng and Chourey, 1999), osmotic conditions (Gibeaut *et al.*, 1990) and phloem unloading (Eschrich, 1980). In sink organs, carbon partitioning (Tang *et al.*, 1999) and sink strength (Sung *et al.*, 1989) are also influenced by sucrose catabolism.

Three enzymes, acid invertase, alkaline invertase and sucrose synthase are responsible for nearly all sucrose catabolism in plants. Invertases (ß-D-fructofuranosidase, EC 3.2.1.26) catalyze the irreversible hydrolysis of sucrose to glucose and fructose. Invertases are categorized by their pH optimum for activity and cellular location (Tymowska-Lalanne and Kreis, 1998*a*). Acid invertases exhibit optimum activity at pH 4.5 to 5.5 and are located in the vacuole or bound to the cell wall. Alkaline invertases are most active at pH 7.0 to 8.0 and are located in the cytoplasm. Sucrose synthase (UDP-D-Glc: D-Fru 2-α-glucosyltransferase, EC 2.4.1.13) is a cytoplasmic enzyme that catalyzes the reversible cleavage of sucrose with uridine 5'-diphosphate (UDP) to form UDP-glucose and fructose. Although capable of synthesizing sucrose, sucrose synthase functions primarily in the direction of sucrose degradation (Xu *et al.*,1989).

Sugarbeet (*Beta vulgaris* L.) roots contain all of the major sucrose catabolizing enzymes. Several studies have examined the activity of these enzymes during root development and their correlation with sucrose accumulation or degradation (Berghall *et al.*, 1997; Giaquinta, 1979; Masuda *et al.*, 1987; Pavlinova and Prasolova, 1973). These studies have led to the suggestion that sucrose accumulation is related to acid invertase

activity (Berghall *et al.*, 1997; Giaquinta, 1979), alkaline invertase activity (Masuda *et al.*, 1987) or sucrose synthase activity (Pavlinova and Prasolova, 1973). While the conflicting conclusions drawn from these studies reflect the difficulty of interpreting correlative data, these studies were also complicated by the nature of the enzymes involved. Acid invertase, alkaline invertase and sucrose synthase occur not as single enzymes but as families of related isoenzymes and isoforms (Sturm and Tang, 1999; Anguenot *et al.*, 1999). Isoenzymes and isoforms within an enzyme family typically exhibit different patterns of expression and regulation, have different biochemical properties and may have different functions in plants. Presently, the number of isoenzymes and isoforms for the major sucrolytic activities in sugarbeet roots is unknown, although two alkaline invertases have been reported in mature roots (Masuda *et al.*, 1987). In this paper, isoenzymes and/or isoforms for soluble acid invertase, alkaline invertase and sucrose synthase in sugarbeet roots were identified. The identified enzymes were designated isoforms because it is unknown whether they differ in amino acid sequence or posttranslational modification. The contribution of these isoforms to sugarbeet root sucrolytic activity during development and their relation to growth and carbohydrate accumulation were examined. Enzyme activities were used to determine the sucrolytic contribution of each isoform in developing roots since posttranscriptional and posttranslational regulation of acid invertase and sucrose synthase have been observed in other plant species (McElfresh and Chourey, 1988; Pressey, 1967; Winter and Huber, 2000).

MATERIALS AND METHODS

Plant material

Sugarbeet hybrid, VDH66156 (Van der Have, Netherlands) was greenhouse grown with a 16 h light and 8 h dark regimen. Seeds were sown on February 4. Roots were harvested 2, 4, 6, 8, 12 and 16 weeks after planting. For the two-week harvest date, five replicate samples were collected by combining 30 to 40 roots per sample. For all other harvest dates, ten individual roots were collected. Whole roots or representative longitudinal sections of roots were rapidly frozen in $N_{2(liq)}$ and lyophilized prior to use. Care was taken to insure that all sections were representative of whole roots and included crown and tail tissue. The experiment was replicated using sugarbeet planted 16 weeks subsequent to the first set of plants. Seeds were sown on May 24. Similar results were obtained in both experiments suggesting that the data was not significantly influenced by seasonal variance in greenhouse conditions.

Carbohydrate assays

Sucrose, glucose and fructose content were determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) using lactose as an internal standard. Lactose (2.5 µmol) was added to finely ground, lyophilized tissue (50 mg) and the mixture extracted twice with refluxing 80% EtOH (4 ml) for 20 min. Cell debris was removed by centrifugation and EtOH was evaporated from the combined extracts. A 200 µl aliquot was passed over a 300 mg C_{18} Maxi-Clean SPE Cartridge (Alltech Associates, Deerfield, IL, USA) and eluted with 1 ml of H_2O. The eluate was diluted fivefold and filtered through a 0.22 µm filter. Samples were injected onto a 250 x 4 mm Dionex CarboPak PA-10 column (Sunnyvale CA, USA) equipped with a 4 x 50 mm CarboPak PA-10 guard column. Carbohydrates were eluted isocratically with 60 mM NaOH at 1.0 ml/min and detected with an ESA Coulochem II electrochemical detector (Chemsford, MA, USA) equipped with a gold working electrode and operating in pulsed amperometric mode. The pulse potentials (E) and durations used for detection were E_1 = +200 mV (t_1 = 500 msec); E_2 = +700 mV (t_2 = 100 msec); E_3 = -900 mV (t_3 = 100 msec).

Protein extraction

Lyophilized tissue was homogenized in ten volumes (w/v) of extraction buffer (100 mM HEPES-NaOH, pH 7.2, 10 mM Na_2SO_3, 5 mM DTT, 1 mM $MgCl_2$) and passed over a 20µm filter. The filtrate was centrifuged at 17,000g. The combined pellets were washed three times with extraction buffer and used for cell wall acid invertase activity assays. The supernatant from centrifugation was separated from carbohydrates and salts by dialysis or acetone precipitation. Extracts from roots two to six weeks after planting and all extracts used for sucrose synthase activity assays were dialyzed overnight against dialysis buffer (10 mM HEPES, pH 7.2, 1 mM DTT, 1 mM $MgCl_2$). Extracts of roots eight to sixteen weeks after planting used for invertase assays were concentrated and desalted by addition of an equal volume of cold acetone. Precipitated proteins were pelleted by centrifugation at 10,000g for 15 min, washed with 50% cold acetone and resuspended in dialysis buffer. Acetone precipitation had no effect on invertase specific activity and provided a rapid method to concentrate protein extracts. Extraction of cell wall pellets was performed overnight in ten volumes (w/v) of 100 mM HEPES-NaOH, pH 7.2, 10 mM Na_2SO_3, 5 mM DTT, 2 M NaCl and 15 mM EGTA with agitation. Cell wall debris was removed by centrifugation at 17,000g and the supernatant dialyzed overnight as described above. All manipulations were performed at 4°C.

Enzyme activity and total protein assays

Invertase activity was determined by modification of the method of Goldstein and Lampen (1975). For soluble invertase extracts, 20 to 50 µl extract was incubated for 30 min at 37°C with 100 mM buffer and 100 mM sucrose in 100 µl total volume. Buffers were NaOAc, pH 4.7 and HEPES-NaOH, pH 8.0 for acid and alkaline invertase activity assays, respectively. Reactions were stopped by addition of an equal volume of 0.5 M potassium phosphate, pH 7.0 followed by boiling for 3 min. Glucose concentration was determined by addition of 1 ml of a solution containing 14 U glucose oxidase, 1 U peroxidase, 24 µM *o*-dianisidine and 38% glycerol and subsequent incubation for 30 min at 30°C. Reactions were stopped by addition of 1.5 ml 6 N HCl and the absorbance at 540 nm measured. Insoluble acid invertase was measured as described above using 100 mg of the cell wall pellet resuspended in 500 µl total volume. Assay solutions were centrifuged prior to measurement of absorbance. Control reactions were run on all samples by assaying as above in the absence of sucrose. Sucrose synthase activity was measured by incubation of 20 to 50 µl extract with 250 mM sucrose, 2 mM UDP and 100 mM MES, pH 6.5 in a 200 µl total volume at 35°C for 30 min. Fructose was quantified by the method of Nelson (1944) using the alkaline copper reagent to stop the reaction. Control reactions were run on all samples by assaying in the absence of UDP. Total protein was measured by the method of Bradford (1976) using bovine serum albumin as standard.

Isoelectric focusing gel electrophoresis and activity staining

Enzyme isoforms were separated by flat bed isoelectric focusing on 5% polyacrylamide gels with ampholines in the pH range of 3.5 to 9.5 (Amersham Pharmacia Biotech, Sweden). Electrophoresis was conducted for 1.5 h at 0.10 W/cm^2 and 10°C. Gels were incubated for 30 min at 35°C in 100 mM NaOAc, pH 4.7 and 100 mM sucrose for acid invertase activity staining, 100 mM HEPES-NaOH, pH 7.7 and 100 mM sucrose for alkaline invertase activity staining and 100 mM MES-HCl, pH 6.5, 100 mM sucrose and 2 mM UDP for sucrose synthase activity staining. Gels stained for acid and alkaline invertase activity were pre-incubated for 15 min at 4°C in the appropriate buffer to equalize pH throughout the gel prior to incubation with substrate. After incubation with substrate, gels were rinsed with distilled H_2O and stained with 0.1% (w/v) 2,3,5-triphenyltetrazolium chloride and 0.5 N NaOH with warming to 100°C (Gabriel and Wang, 1969). Control gels were run as described above except sucrose was omitted from the incubation solution for invertase stained gels and UDP was omitted from the incubation solution for sucrose synthase stained gels. Densitometry of gels was performed with a ChemiImager 4000

(Alpha Innotech, San Leandro, CA) or Un-Scan-It software (Silk Scientific, Orem, UT). Isoelectric points were determined by comparison of the mobility of the enzymes in an isoelectric focusing gel with standards of known pI (BioRad, Hercules, CA, USA).

RESULTS

Sugarbeet root growth

The increases in root size and fresh mass were determined during the growth of greenhouse-grown sugarbeet over a sixteen-week period (Fig. 1). Sugarbeet roots accumulated the majority of their size and mass between six and sixteen weeks after planting (Fig. 1A). Seventy percent

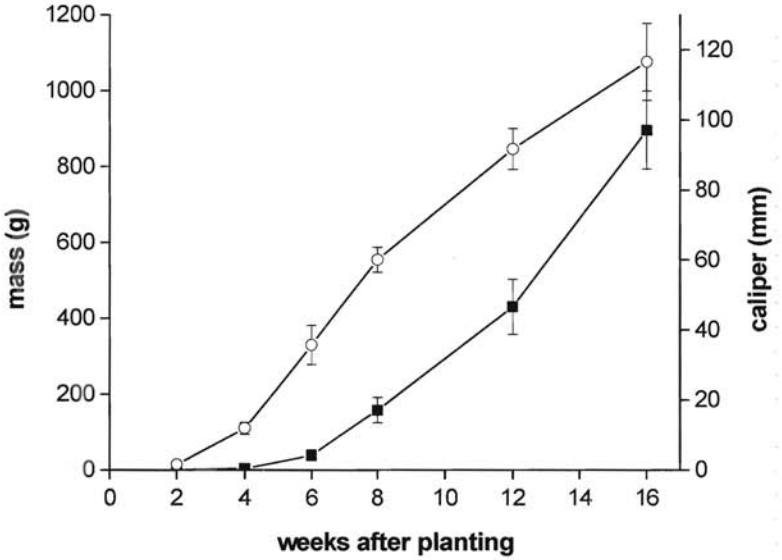

Fig. 1A. Increase in size and fresh mass of sugarbeet roots during growth. Change in (■) total root mass and (○) caliper at widest portion of root. Each data point is the mean of ten values. Error bars equal ± one standard deviation.

of the increase in root size, measured as the caliper at the widest portion of the root, and 96% of the increase in root mass occurred between six and sixteen weeks after planting. The relative rate of root growth, however, was greatest during the first six weeks after planting and decreased with root maturity (Fig. 1B). Relative growth rate was defined as the percent increase in mass or size per week. By the sixth week after planting, the relative growth rate was only a small fraction of the initial relative growth

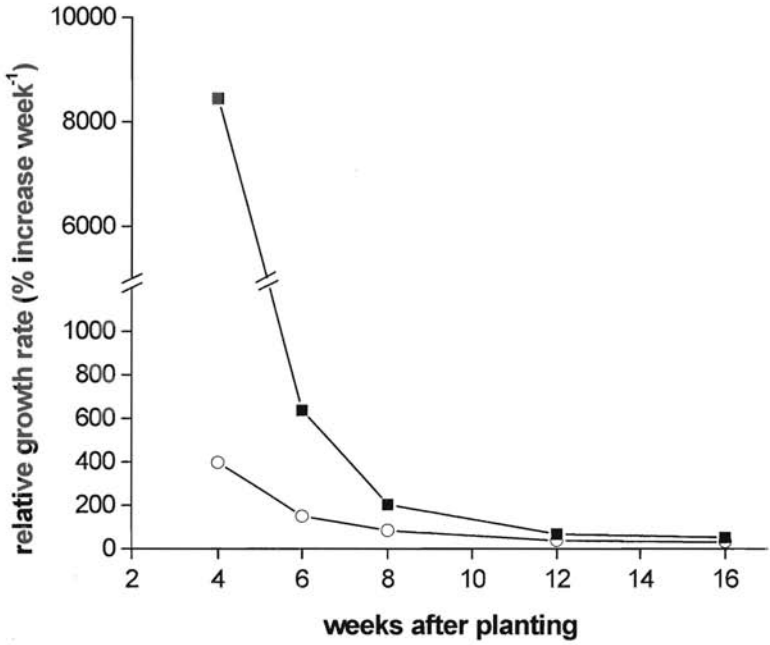

Fig. 1B. Change in the relative rate of growth as the percent increase week^{-1} of (■) total root mass and (○) caliper at widest portion of root. Each data point is the mean of ten values.

rate. Relative rates for mass and size accumulation declined by 97 and 79%, respectively, between two and six weeks after planting. The observed decline in relative growth rate reflected the decline in the proportion of root tissue that was meristematic. Meristematic cells increase in number with root development but become a smaller percentage of the total root. The absolute rate of root growth, defined as the change in mass or caliper per week, generally increased with root age (Fig. 1C). The absolute growth rate, measured by the change in root caliper, increased during the first eight weeks after planting, but declined between eight and sixteen weeks after planting. The absolute growth rate, measured by the change in root mass, exhibited a nearly linear increase with root age.

Carbohydrate content

Sugarbeet roots accumulated sucrose throughout most of their development, although accumulation was greater in the later develop-

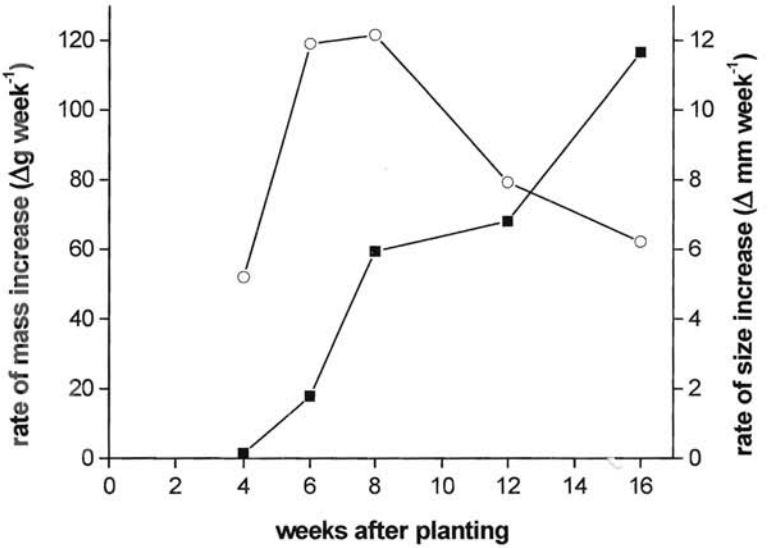

Fig. 1C. Change in the absolute rate of growth for (■) total root mass and (○) caliper at widest portion of root, expressed as the change in mass week^{-1} and the change in caliper week^{-1}. Each data point is the mean of ten values.

mental stages (Fig. 2). Generally, sucrose accumulation increased with root age, increasing in both sucrose concentration and total sucrose content of the roots. Sucrose concentration, expressed as a function of root dry weight, increased rapidly between two and four weeks after planting indicating the ability of roots to store sucrose even when young. Only slight increases in sucrose concentration were evident with subsequent growth. Total sucrose content of the sugarbeet taproot increased rapidly after six weeks. Sucrose accumulation in roots six weeks after planting occurred at a rate sufficient to maintain a nearly constant sucrose concentration despite the large concurrent increase in root mass and size (Fig. 1A).

Glucose and fructose were present in significantly lower concentrations than sucrose at all but the earliest stages of root development. Concentrations of these two sugars were greatest in seedling roots two weeks after planting (Table 1). Glucose was the predominant carbohydrate in roots two weeks after planting and accounted for approximately 60% of the total soluble carbohydrate content. As roots aged, glucose and fructose concentrations declined as did their relative

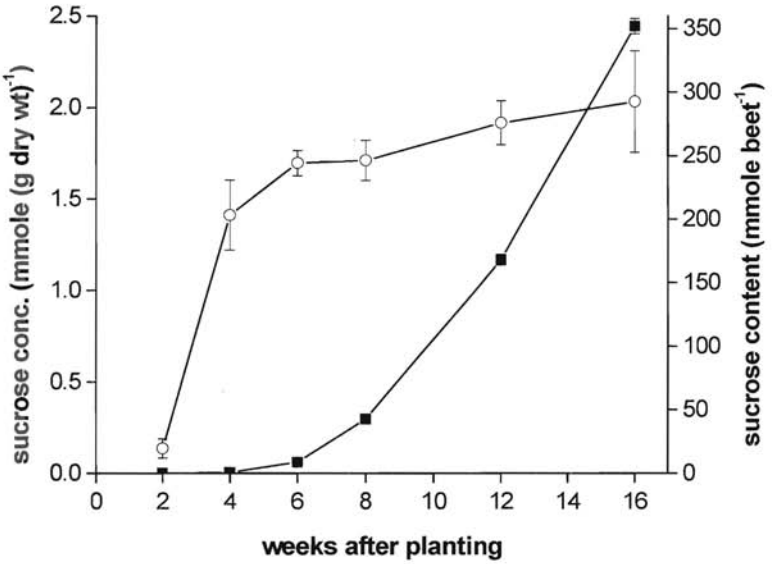

Fig. 2. Sucrose accumulation during root growth. Increase in (○) sucrose concentration and (■) total sucrose content. Each data point is the mean of ten values. Error bars equal ± one standard deviation.

Table 1. Glucose and fructose concentration in sugarbeet roots of different ages. Each value is the mean of ten measurements ± one standard deviation.

Weeks after Planting	Carbohydrate Concentration [mmole (g dry wt)$^{-1}$]	
	Glucose	Fructose
2	0.23 ± 0.12	0.014 ± .008
4	0.030 ± 0.009	0.0020 ± 0.0016
6	0.019 ± 0.006	0.0013 ± 0.0009
8	0.026 ± 0.014	0.0012 ± 0.0006
12	0.019 ± 0.011	0.0019 ± 0.0013
16	0.017 ± 0.011	0.0025 ± 0.0013

contribution to the total soluble carbohydrate content of the root. Combined amounts of glucose and fructose comprised 2.2% and 0.9% of the total soluble carbohydrates by, respectively, four and sixteen weeks after planting.

Soluble and cell wall acid invertase activity

Acid invertase activity in sugarbeet roots was due to the combined action of soluble acid invertase and insoluble cell wall invertase activities. Both soluble and insoluble acid invertase activities were greatest in the roots of seedlings two weeks after planting (Fig. 3A and B). Soluble

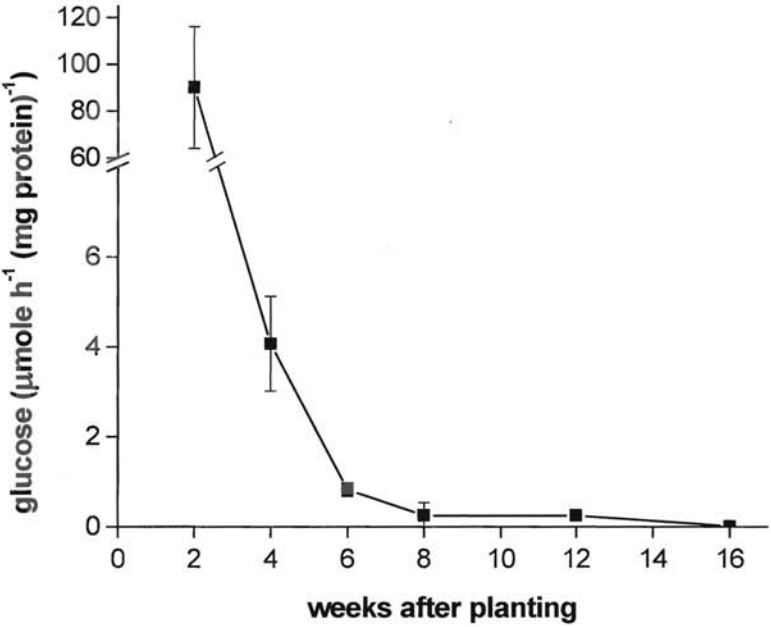

Fig. 3A. Change in soluble acid invertase activity during root growth. Invertase activity was assayed at pH 4.7 and 37°C. Data points are the mean of ten replicates except the data for two week roots in which the mean of five replicates of 30 to 40 roots is shown. Error bars equal ± one standard deviation. Note break in *y-axis*.

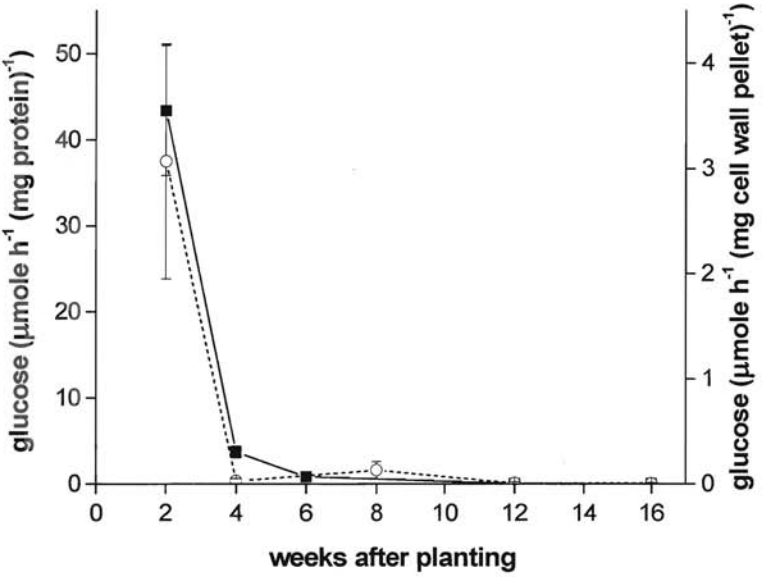

Fig. 3B. Change in cell wall acid invertase activity during root growth. Invertase activities of (○) cell wall pellet or (■) high saline extract of cell wall pellet were assayed at pH 4.7 and 37°C. Data points are the mean of ten replicates except the data for two week roots in which the mean of five replicates of 30 to 40 roots is shown. Error bars equal ± one standard deviation.

acid invertase activity decreased precipitously after the second week after planting and was barely detectable by the sixth week (Fig. 3A). Insoluble acid invertase activity exhibited a similar pattern of decline and was difficult to detect by the fourth week after planting (Fig. 3B). Insoluble acid invertase activity was measured after solubilization of cell wall proteins or directly with the cell wall pellet with similar results. Soluble acid invertase activity was predominantly due to a single acid invertase isoform (AcInvI) as determined by activity stained isoelectric focusing gels (Fig. 3C). A second acid invertase isoform (AcInvII) was evident in roots two weeks after planting, but at a significantly lower activity. Isoelectric points of the two isoforms were 4.7 and 4.8 for the major and minor isoforms, respectively.

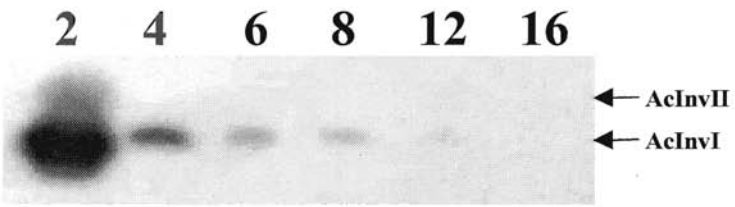

Fig. 3C. Contributions of isoforms to soluble acid invertase activity during root growth. Isoelectric focused (IEF) polyacrylamide gels of soluble proteins extracted from roots 2, 4, 6, 8, 12 and 16 weeks after planting stained for acid invertase activity at pH 4.7 (45μg/lane).

Alkaline invertase activity

Alkaline invertase was a minor sucrolytic activity during sugarbeet root growth (Fig. 3D). Alkaline invertase activity was not detected in roots at two and four weeks after planting, but was evident by six weeks after planting. Low levels of alkaline invertase activity were detected at all later stages of development. Two isoforms, Alkaline Invertase I (AlkInvI) and Alkaline Invertase II (AlkInvII) contributed to alkaline invertase activity (Fig. 3E). AlkInvI, with an isoelectric point of 5.9, was the predominant isoform. AlkInvI comprised 95, 91, 67 and 56% of the total alkaline invertase activity at, respectively, 6, 8, 12 and 16 weeks after planting, as determined by densitometric scanning of activity stained isoelectric focusing gels. AlkInvI activity was greatest in roots eight weeks after planting and declined with subsequent root development. The activity of AlkInvII, with an isoelectric point of 5.3, increased as roots matured.

Initial studies erroneously detected alkaline invertase activity in roots two and four weeks after planting (data not shown). Using an enzyme coupled spectrophotometric assay, glucose formation was detected after incubation of crude protein extract with sucrose at pH 8.0. Glucose formation, however, was caused by a small residual activity of acid invertase at pH 8.0. Activity stained isoelectric focusing gels demonstrated the absence of alkaline invertase activity in roots two and four weeks after planting. By six weeks after planting, acid invertase activity had declined to nearly undetectable levels and did not impact assays for alkaline invertase activity.

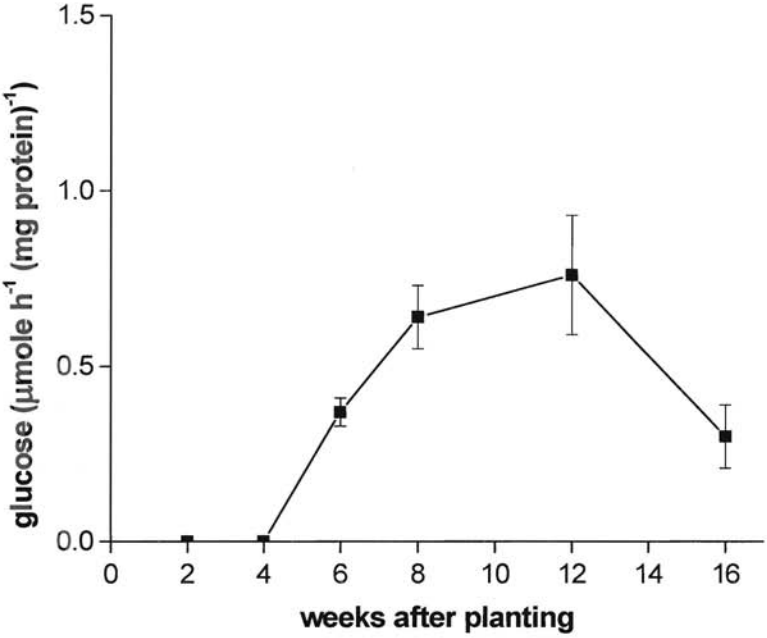

Fig. 3D. Change in alkaline invertase activity during root growth. Invertase activity was assayed at pH 8.0 and 37°C. Data points are the mean of ten replicates except the data for two week roots in which the mean of five replicates of 30 to 40 roots is shown. Error bars equal ± one standard deviation.

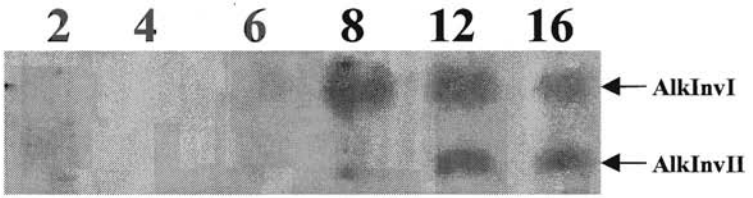

Fig. 3E. Contribution of isoforms to alkaline invertase activity during root growth. Isoelectric focused (IEF) polyacrylamide gels of soluble proteins extracted from roots 2, 4, 6, 8, 12 and 16 weeks after planting stained for alkaline invertase activity at pH 7.7 (90 μg/lane).

Sucrose synthase activity

Sucrose synthase was the major sucrolytic activity at all but the earliest stages of development (Fig. 3F). Sucrose synthase activity increased between two and six weeks after planting and remained at high, relatively constant levels during subsequent growth. Nearly all accumulation of root size, mass and sucrose content occurred concurrently with high sucrose synthase activity. Sucrose synthase activity correlated highly with the rate of increase in root size (Fig. 1C), but was not highly correlated with the rate of increase in root mass. That sucrose synthase activity was highly correlated with the absolute growth rate measured as the increase in caliper but not the absolute growth rate measured as the increase in mass, can be explained by the nature of the measurements. The change in root caliper occurs in one dimension and is a linear measurement; the change in root mass occurs in three dimensions and is a cubic function. Sucrose synthase activity, measured in these experiments as specific activity, is a one-dimensional, linear measurement. No correlation was apparent between sucrose synthase activity and growth (Fig. 1A), relative growth rate (Fig. 1B), sucrose concentration or sucrose content (Fig. 2).

Two sucrose synthase isoforms were identified in developing sugarbeet roots (Fig. 3G). Sucrose Synthase I (SucSynI), with an isoelectric point of 5.7, was the sole isoform during the first twelve weeks after planting. A second isoform, Sucrose Synthase II (SucSynII), with a pI of 6.1, was evident sixteen weeks after planting. SucSynII accounted for 60% of the total sucrose synthase activity at this stage of development.

DISCUSSION

Developing sugarbeet roots contain multiple isoforms of the major sucrolytic enzyme activities. Two soluble acid invertase isoforms, an insoluble acid invertase activity, two alkaline invertase isoforms and two sucrose synthase isoforms were evident in roots at some stage of development. Each isoform exhibited marked changes in activity with respect to root development, growth and sucrose accumulation. Multiple isoenzymes and/or isoforms of the major sucrolytic activities have been observed in many plant species. Multiple isoenzymes of soluble acid invertase, insoluble acid invertase, and sucrose synthase activities have been reported in *Arabidopsis* (Dejardin *et al.*, 1999; Tymowska-Lalanne and Kreis, 1998*b*), carrot (Sturm *et al.*, 1995), maize (Gupta *et al.*, 1988; Taliercia *et al.*, 1999; Xu *et al.*, 1996), and potato (Fu and Park, 1995; Hedley *et al.*, 1994), as well as other plant species. Multiple isoenzymes and/or isoforms for alkaline invertase have also been found in carrot,

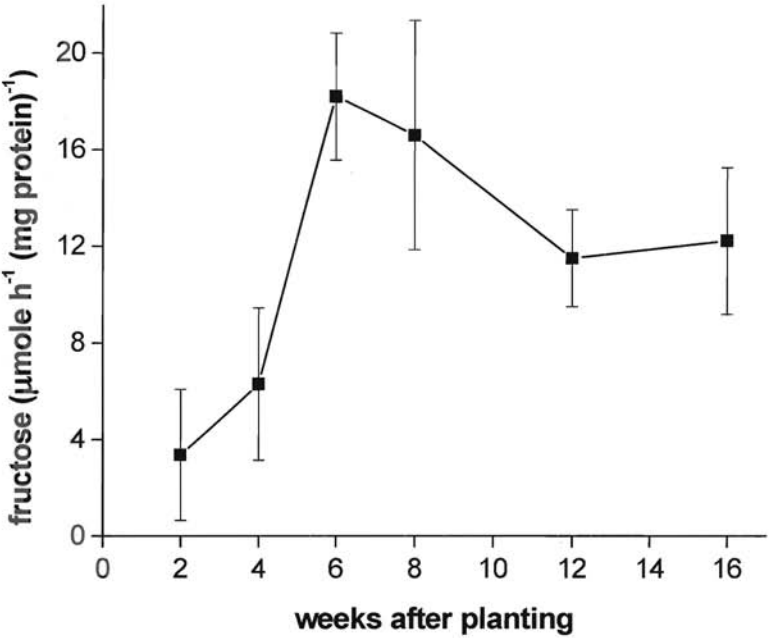

Fig. 3F. Change in sucrose synthase activity during root growth. Sucrose synthase activity was assayed at pH 6.5 and 35°C. Data points are the mean of ten replicates except the data for two week roots in which the mean of five replicates of 30 to 40 roots is shown. Error bars equal ± one standard deviation.

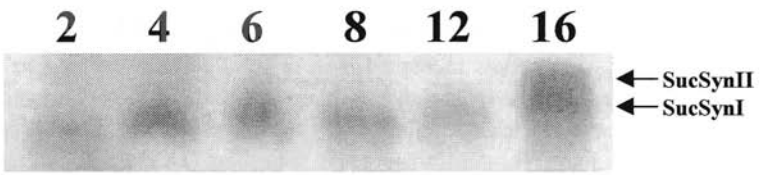

Fig. 3G. Contribution of isoforms to sucrose synthase activity during root growth. Isoelectric focused (IEF) polyacrylamide gels of soluble proteins extracted from roots 2, 4, 6, 8, 12 and 16 weeks after planting stained for sucrose synthase activity at pH 6.5. All lanes contain 25µg protein except last lane which contains 65 µg protein.

faba bean and sugarbeet (Lee and Sturm, 1996; Masuda *et al.*,1987; Ross *et al.*, 1996).

Acid invertase was the predominant sucrolytic activity in young sugarbeet roots. Activity was due to two soluble acid invertase isoforms and a cell wall acid invertase activity. Concurrent with high acid invertase activity, seedling roots grew at a rapid relative rate. The rapid relative growth rate of seedling roots was likely a reflection of the high proportion of meristematic tissue found in young roots. The relative growth rate was most closely correlated with the activity of the major soluble acid invertase isoform. High soluble acid invertase activity has previously been observed in rapidly growing and elongating plant tissues, such as elongating internodes (Morris and Arthur, 1985), hypocotyls (Pfeiffer and Kutschera, 1995), and developing roots (Ricardo and Sovia, 1974) and fruits (Lowell *et al.*, 1989). Insoluble acid invertase activity has also been associated with regions of active growth in tomato roots (Chin and Weston, 1973) and carrot roots (Ricardo and ap Rees, 1970). Authors of these studies suggest that acid invertase activity supports rapid growth by providing hexose substrates for conversion to metabolic energy, the biosynthesis of cellular structures (Morris and Arthur, 1984; Ricardo and ap Rees, 1970) or use as osmolytes for the maintenance of cell osmotic pressure during cell elongation (Kutschera, 1991). Sugarbeet root acid invertase activity is likely to function similarly and probably provides the substrates to support the rapid relative growth observed in young roots.

Sucrose accumulation in sugarbeet roots was inversely proportional to soluble and insoluble acid invertase activities. Sucrose accumulation was not evident in the roots of seedlings when the activity of both soluble acid invertase isoforms and the insoluble acid invertase activity were maximal. Rather, glucose was the major sugar, suggesting that the majority of sucrose transported to the seedling root was hydrolyzed. As sugarbeet plants matured beyond the seedling stage, soluble and insoluble acid invertase activities declined precipitously and sucrose content of the roots increased. An inverse correlation between soluble acid invertase activity and sucrose accumulation has been observed in sugarcane stems (Hatch and Glasziou, 1963), citrus fruits (Lowell *et al.*, 1989), and several root crops including sugarbeet (Giaquinta, 1979), carrot (Ricardo and ap Rees, 1970), radish and turnip (Ricardo and Sovia, 1974). This correlation has lead to the suggestion that soluble acid invertase activity is incompatible with sucrose storage (Berghall *et al.*, 1997; Giaquinta, 1979; Hatch and Glasziou, 1963; Ricardo and ap Rees, 1970). While the data of this study support this theory, the role of the soluble acid invertase isoforms in sucrose storage in sugarbeet roots remains unknown. Although sucrose storage and soluble acid invertase

activity both occur in the cell vacuole (Leigh *et al.*, 1979), whether they occur in the same cells or tissues in sugarbeet roots is unknown.

Young sugarbeet roots contained substantial insoluble acid invertase activity. The function of the insoluble acid invertase in sugarbeet roots is unknown. In other plant species, cell wall acid invertase activity has been implicated in apoplastic phloem unloading involving sucrose hydrolysis (Eschrich, 1980). In sugarbeet, such a role is unlikely, since sucrose is not hydrolyzed during phloem loading, transit or unloading (Giaquinta, 1977). In carrot plants, insoluble acid invertase has been shown to affect sucrose partitioning between source and sink organs (Tang *et al.*, 1999). Taproots were small, underdeveloped and had reduced carbohydrate content in carrot plants with reduced expression of cell wall acid invertase in the root. The observed phenotype was unlikely to involve phloem unloading since carrot plants, like sugarbeet, do not require sucrose hydrolysis for phloem unloading (Sturm *et al.*, 1995). Sugarbeet root insoluble acid invertase may play a similar role in carbohydrate partitioning.

Alkaline invertase activity was minor throughout the development of sugarbeet roots. Alkaline invertase activity was due to two isoforms which exhibited different patterns of developmental expression. No correlation was observed between either isoform and growth, growth rate, carbohydrate composition or accumulation. Masuda *et al.* (1987) observed an increase in total alkaline invertase activity with sugarbeet root development that paralleled sucrose accumulation. In agreement with Masuda *et al.* (1987), no alkaline invertase activity was observed in young sugarbeet roots. In these studies, however, sucrose accumulation was evident prior to the detection of alkaline invertase activity, and total alkaline invertase activity did not mirror sucrose accumulation. The difference in results between this study and that of Masuda *et al.* (1987) may be due to differences in sugarbeet variety or cultural conditions. This study, however, does not support the contention of Masuda *et al.* (1987) that alkaline invertase is involved in the regulation of sucrose accumulation.

The *in planta* function of alkaline invertase activity is unknown. Alkaline invertase was suggested to provide for the metabolic needs of tissues or cells when acid invertase activity is insufficient (Ricardo and ap Rees, 1970). In sugarbeet roots, alkaline invertase and acid invertase activities were present at different stages of development. The relationship between these enzyme activities may be coincidental, however, since tissues containing both acid and alkaline invertases have been identified (Fay and Ghorbel, 1983; Masuda *et al.*, 1988). Lee and Sturm (1996) propose that alkaline invertase is involved in channeling sucrose into

cytoplasmic carbon metabolism. While alkaline invertase may function in this manner in sugarbeet, its role in providing hexose substrates is likely to be minor. Alkaline invertase activity was always found coincident with high sucrose synthase activity.

Sucrose synthase activity was evident at all stages of sugarbeet root development and was the predominant sucrolytic activity at all but the earliest stages of growth. Two sucrose synthase isoforms were identified in this study. One isoform was present throughout development. A second isoform was present only in the late stages of growth. The presence of two or more sucrose synthase isoenzymes with different developmental patterns of expression is typical in higher plants (Sturm and Tang, 1999). The different expression patterns of sucrose synthase isoenzymes suggest that they are likely to have different *in planta* functions. In developing endosperm of maize kernels, different functions have been shown for the two sucrose synthase isoenzymes present; one isoenzyme provides substrate for cellulose biosynthesis, and the second supplies substrate for starch biosynthesis (Chourey *et al.*, 1998). The difference in activities of the two sugarbeet sucrose synthase isoforms during development suggest that they are also likely to have different roles in the developing taproot.

In sugarbeet roots, nearly all accumulation of size, mass and sucrose occurred concurrently with high sucrose synthase activity. Sucrose synthase activity correlated highly with the rate of increase in root size. High sucrose synthase activity has frequently been observed in sink tissues and organs, and a correlation between sucrose synthase activity and sink strength has been shown in potato tubers, bean seeds, and roots of cassava, sugarbeet and sweetgum (Giaquinta, 1979; Sung *et al.*, 1989; Zrenner *et al.*, 1995). Authors of these studies proposed that sucrose synthase has a role in providing substrates for respiration (Sung *et al.*, 1988), synthesis of cell wall carbohydrates (Amor *et al.*, 1995) and starch (Chourey and Nelson, 1976), and may function in regulating sink capacity (D'Aoust *et al.*, 1999; Zrenner *et al.*, 1995). Repression of sucrose synthase expression in potato caused a significant reduction in tuber dry weight and starch content (Zrenner *et al.*, 1995), while in tomato, repression of sucrose synthase activity hindered sucrose unloading in young fruit and led to reduced fruit set (D'Aoust *et al.*, 1999). Although further work is required to determine the function of sucrose synthase in sugarbeet roots, the data of this study suggest that it is important for growth of the taproot and may be a factor controlling root size and ultimately, crop yield.

Sugarbeet roots contain multiple sucrolytic enzymes, with different sucrolytic activities and enzyme isoforms present at different stages of root development. The various sucrolytic activities almost

certainly perform different metabolic functions in the sugarbeet root with different isoforms of these activities functioning at different stages of growth. The presence of multiple, differentially regulated sucrolytic enzymes is likely to be essential for growth and development of sugarbeet roots and allows control and flexibility in the regulation of sucrose catabolism to balance the root's metabolic needs with its function as a sucrose storage organ. Understanding these enzymes and their role in sugarbeet sucrose catabolism is essential for understanding growth and sucrose accumulation in the sugarbeet crop and is needed to understand their impact on crop and extractable sucrose yield.

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LITERATURE CITED

Amor, Y., C. Haigler, S. Johnson, M. Wainscott and D. Delmer. 1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc. Natl. Acad. Sci. USA 92: 9353-9357.

Anguenot, R., S. Yelle and B. Nguyen-Quoc. 1999. Purification of tomato sucrose synthase phosphorylated isoforms by Fe(III)-immobilized metal affinity chromatography. Arch. Biochem. Biophys. 365: 163-169.

Berghall, S., S. Briggs, S.E. Elsegood, L. Eronen, J.O. Kuusisto, E.J. Philip, T.C. Theobald and P. Walliander. 1997. The role of sugar beet invertase and related enzymes during growth, storage and processing. Zuckerind. 122: 520-530.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Cheng, W-H. and P.S. Chourey. 1999. Genetic evidence that invertase-mediated release of hexoses is critical for appropriate carbon partitioning and normal seed development in maize. Theor. Appl. Genet. 98: 485-495.

Chin, C.K. and G.D. Weston. 1973. Distribution in excised *Lycopersicon esculentum* roots of the principal enzymes involved in sucrose metabolism. Phytochem. 12: 1229-1235.

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

Chourey, P.S., E.W. Taliercia, S.J. Carlson and Y-L. Ruan. 1998. Genetic evidence that two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis. Mol. Gen. Genet. 259: 88-96.

D'Aoust, M-A., S. Yelle, and B. Nguyen-Quoc. 1999. Antisense inhibition of tomato fruit sucrose synthase decreases fruit setting and the sucrose unloading capacity of young fruit. Plant Cell 11: 2407-2418.

Dejardin, A., L.N. Sokolov and L.A. Kleczkowski. 1999. Sugar/osmoticum levels modulate differential abscisic acid-independent expression of two stress-responsive sucrose synthase genes in *Arabidopsis*. Biochem. J. 344: 503-509.

Eschrich, W. 1980. Free space invertase, its possible role in phloem unloading. Ber. Dtsch. Bot. Ges. 93: 363-378.

Fay, L. and A. Ghorbel. 1983. Studies on β-fructosidase from radish seedlings. III. Comparative structure of soluble and wall bound forms. Plant Sci. Let. 29: 33-48.

Fu, H. and W.D. Park. 1995. Sink and vascular-associated sucrose synthase functions are encoded by different gene classes in potato. Plant Cell 7: 1369-1385.

Gabriel, O. and S-F. Wang. 1969. Determination of enzymatic activity in polyacrylamide gels. I. Enzymes catalyzing the conversion of nonreducing substrates to reducing products. Anal. Biochem. 27: 545-554.

Giaquinta, R. 1977. Sucrose hydrolysis in relation to phloem translocation in *Beta vulgaris*. Plant Physiol. 60: 339-343.

Giaquinta, R.T. 1979. Sucrose translocation and storage in the sugar beet. Plant Physiol. 63: 828-832.

Gibeaut, D., N. Karuppiah, S. Chang, T. Brock, V. Vadlamudi, D. Kim, N.S. Ghosheh, D.L. Rayle, N.C. Carpita and P.B. Kaufman. 1990. Cell wall and enzyme changes during graviresponse of the leaf-sheaf pulvinus of oat (*Avena sativa*). Plant Physiol. 94: 411-416.

Goldstein, A., and J. O. Lampen. 1975. β-D-Fructofuranoside fructohydrolase from yeast. Meth. Enzym. 42: 504-511.

Gupta, M., P.S. Chourey, B. Burr and P.E. Still. 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.

Hatch, M.D. and K.T. Glasziou. 1963. Sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments. Plant Physiol. 38: 344-347.

Hedley, P.E., G.C. Machray, H.V. Davies, L. Burch and R. Waugh. 1994. Potato (*Solanum tuberosum*) invertase-encoding cDNAs and their differential expression. Gene 145: 211-214.

Kruger, N.J. 1997. Carbohydrate synthesis and degradation. In: Dennis D.T., Turpin D.H., Lefebvre D.D., Layzell D.B., eds. Plant Metabolism. Essex, England: Longman Press, 83-104.

Kutschera, U. 1991. Osmotic relations during elongation growth in hypocotyls of *Helianthus annuus* L. Planta 184: 61-66.

Lee, H-S. and A. Sturm. 1996. Purification and characterization of neutral and alkaline invertase from carrot. Plant Physiol. 112: 1513-1522.

Leigh, R.A., T. ap Rees, W.A. Fuller and J. Banfield. 1979. The location of acid invertase activity and sucrose in the vacuoles of storage roots of beetroot (*Beta vulgaris*). Biochem. J. 178: 539-547.

Lowell, C., P. Tomlinson and K. Koch. 1989. Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. Plant Physiol. 90: 1394-1402.

Masuda, H., T. Takahashi and S. Sugawara. 1987. The occurrence and properties of alkaline invertase in mature roots of sugar beets. Agric. Biol. Chem. 51: 2309-2314.

Masuda, H., T. Takahashi and S. Sugawara. 1988. Acid and alkaline invertase in suspension cultures of sugar beet cells. Plant Physiol. 86: 312-317.

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

Morris, D.A. and E.D. Arthur. 1984. Invertase and auxin-induced elongation in internodal segments of *Phaseolus vulgaris*. Phytochem. 23: 2163-2167.

Morris, D. and E. Arthur. 1985. Invertase activity, carbohydrate metabolism and cell expansion in the stem of *Phaseolus vulgaris* L. J. Exp. Bot. 36: 623-632.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.

Pavlinova, O.A. and M.F. Prasolova. 1973. Physiological role of sucrose synthetase in sugar beet roots. Sov. Plant Physiol. 19: 780-785.

Pfeiffer, I. and U. Kutschera. 1995. Sucrose metabolism and cell elongation in developing sunflower hypocotyls. J. Exp. Bot. 46: 631-638.

Pressey, R. 1967. Invertase inhibitor from potatoes: purification, characterization, and reactivity with plant invertases. Plant Physiol. 42: 1780-1786.

Ricardo, C.P.P. and T. ap Rees. 1970. Invertase activity during the development of carrot roots. Phytochem. 9: 239-247.

Ricardo, C. and D. Sovia. 1974. Development of tuberous roots and sugar accumulation as related to invertase activity and mineral nutrition. Planta 118: 43-55.

Ross, H.A., D. McRae and H.V. Davies. 1996. Sucrolytic enzyme activities in cotyledons of the faba bean. Plant Physiol. 111: 329-338.

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

Sturm, A. and G-Q. Tang. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth, and carbon partitioning. Trends Plant Sci. 4: 401-407.

Sung, S., D. Xu, C. Galloway and C. Black. 1988. A reassessment of glycolysis and gluconeogenesis in higher plants. Physiol. Plant. 72: 650-654.

Sung, S., D. Xu and C. Black. 1989. Identification of actively filling sucrose sinks. Plant Physiol. 89: 1117-1121.

Taliercio, E.W., J-Y. Kim, A. Mahe, S. Shanker, J. Choi, W-H. Cheng, J-L. Prioul and P.S. Chourey. 1999. Isolation, characterization and expression analyses of two cell wall invertase genes in maize. J. Plant Physiol. 155: 197-204.

Tang, G-Q., M. Lscher and A. Sturm. 1999. Antisense repression of vacuolar and cell wall invertase in transgenic carrot alters early plant development and sucrose partitioning. Plant Cell 11: 177-189.

Tymowska-Lalanne, Z. and M. Kreis. 1998*a*. The plant invertases: physiology, biochemistry and molecular biology. Adv. Bot. Res. 28: 71-117.

Tymowska-Lalanne, Z. and M. Kreis. 1998*b*. Expression of the *Arabidopsis thaliana* invertase gene family. Planta 207: 259-265.

Winter, H. and S.C. Huber. 2000. Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. Crit. Rev. Plant Sci. 19: 31-67.

Xu, D-P., S-J. Sung, T. Loboda, P.P. Kormanik and C.C. Black. 1989. Characterization of sucrolysis via the uridine diphosphate and pyrophosphate-dependent sucrose synthase pathway. Plant Physiol. 90: 635-640.

Xu, J., W. T. Avigne, D.R. McCarty and K.E. Koch. 1996. A similar dichotomy of sugar modulation and developmental expression affects both paths of sucrose metabolism: evidence from a maize invertase gene family. Plant Cell 8: 1209-1220.

Zrenner, R., M. Salanoubat, L. Willmitzer and U. Sonnewald. 1995. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). Plant J. 7: 97-107.