
Sucrose Catabolism in Developing Roots of Three *Beta vulgaris* Genotypes with Different Yield and Sucrose Accumulating Capacities

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ABSTRACT

The functions of individual sucrolytic enzymes in sugarbeet (*Beta vulgaris*) roots are poorly understood, although a positive association between sucrose synthase activity and root size, and a negative association between soluble acid invertase activity and sucrose concentration have been documented. To test the veracity of these relationships and determine whether any sucrolytic activities were associated with root yield or sucrose accumulation, sucrolytic enzyme activities, fresh and dry mass, and the content of sucrose, glucose, fructose and cell wall materials were measured in roots of three *B. vulgaris* genotypes with differing yield and sucrose accumulating capacities at five stages in their development. Across all genotypes and developmental stages, sucrose synthase activity was positively associated with root mass, water content, and accumulation of cell wall materials. No meaningful association was observed between alkaline invertase, soluble acid invertase or insoluble acid invertase activities and any of the physical or chemical properties examined. These results suggest that sucrose synthase activity may be a marker for root yield, a factor in root sink strength, and a promoter of cell wall biosynthesis.

Key words: acid invertase, alkaline invertase, *Beta vulgaris* L., carbohydrate partitioning, fodder beet, sucrose synthase, sugarbeet

Abbreviations used: Alln, alkaline invertase; DTT, dithiothreitol;

IAIn, insoluble acid invertase; SAIN, soluble acid invertase; SuSy, sucrose synthase; UDP, uridine 5'-diphosphate; UDP-glc, uridine 5'-diphosphate-glucose.

Sugarbeet (*Beta vulgaris* L.) root growth and development are dependent on sucrose cleaving enzymes to provide substrates for respiration and the biosynthesis of cellular structures and metabolites (Kruger, 1997). Sugarbeet root size and carbohydrate content are also likely to be influenced by sucrolytic enzymes since these activities have been demonstrated in other plant species to affect sink strength (Sung et al., 1989; Zrenner et al., 1995) cell expansion (Pfeiffer and Kutschera, 1995), mitotic activity (Cheng and Chourey, 1999), osmotic conditions (Gibeaut et al., 1990), phloem unloading (Eschrich, 1980), and cell wall biosynthesis (Amor et al., 1995).

Three enzymes, sucrose synthase, acid invertase and alkaline invertase, catalyze sucrose cleavage in sugarbeet. Sucrose synthase (SuSy; UDP-D-Glc:D-Fru 2- α -glucosyltransferase, EC 2.4.1.13) catalyzes the reversible reaction of sucrose with uridine 5'-diphosphate (UDP) to yield fructose and UDP-glucose. Localized in the cytoplasm, SuSy is primarily a sucrose degrading enzyme, although it is also capable of sucrose synthesis (Xu et al., 1989). SuSy is the predominant sucrolytic activity in sugarbeet roots and is present at high activities at all but the earliest stages of development (Giaquinta, 1979; Klotz and Finger, 2002). Acid and alkaline invertases (β -D-fructofuranoside fructohydrolase, EC 3.2.1.26) catalyze the irreversible hydrolysis of sucrose to fructose and glucose, but differ in their pH optima and their cellular location. Acid invertases exhibit maximum activity at pH values of 4.5 to 5.5, and occur as soluble enzymes in the vacuole (SAIN, soluble acid invertase) and insoluble enzymes bound to the cell wall (IAIn, insoluble acid invertase). SAIN and IAIN activities occur at high levels in seedling roots, but rapidly decline to low levels as taproots develop (Pavlinova and Prasolova, 1973; Klotz and Finger, 2002). Alkaline invertase (Alln), with a pH optimum of 7.0 to 8.0, is a cytoplasmic enzyme. Alln activity is present throughout root development, with a level of activity that is generally intermediate to acid invertase and sucrose synthase activities (Masuda et al., 1987; Klotz and Finger, 2002).

The individual functions of sucrolytic enzymes in the growth and sucrose economy of sugarbeet roots have yet to be determined. A positive association between SuSy activity and root size (Pavlinova and Prasolova, 1973; Klotz and Finger, 2002), and a negative association between soluble acid invertase activity and sucrose accumulation (Giaquinta, 1979; Berghall et al., 1997), however, have been noted.

These observations have led to speculation that SuSy activity may be a determinant of root yield and SAI activity may influence sucrose content. Although knowledge of the factors that influence root yield and sucrose content would be useful for devising strategies for enhanced sucrose yield, the relationships between sucrolytic activities and the principal components of sucrose yield, root yield and sucrose content, have not, to our knowledge, been further examined.

In the present study, three diverse *B. vulgaris* genotypes were used to examine the relationships between individual sucrolytic activities and the accumulation of mass, sucrose, glucose, fructose and cell wall materials. The objectives of this research were (1) to test the veracity of the observed relationships between sucrose synthase activity and root size, and soluble acid invertase and sucrose accumulation, and (2) to determine whether any sucrolytic activity was associated with root yield or sucrose content. The three genotypes were chosen to maximize differences in yield and sucrose accumulating capacities. The genotypes were (1) a commercial fodder beet variety, selected for its large root size but relatively low sucrose content; (2) an inbred sugarbeet breeding line, L19, noted for its high root sucrose content but small size (Theurer and Doney, 1989); and (3) a commercial sugarbeet hybrid, selected to be intermediate in root size and sucrose content to the fodder beet and L19 breeding line.

MATERIALS AND METHODS

Plant Material

Plants of three *B. vulgaris* genotypes were greenhouse grown in Sunshine Mix #1 (Sun Gro Horticultural Products, Seba Beach, Alberta, Canada) in 15 liter pots with supplemental light under a 16 h light/8 h dark regimen. All plants were watered once every two days. A slow release fertilizer (36 g of Osmocote 14-14-14, Scotts-Sierra Horticultural Products, Marysville, OH, USA) was added to pots at time of sowing and nine weeks after sowing. Genotypes were the fodder beet hybrid 'Monovigour' (Danisco, Holeby, Denmark), the commercial sugarbeet hybrid VDH66156 (Van der Have, Rilland, Netherlands) and the inbred sugarbeet line L19 (PI590690). Roots were harvested 4, 6, 8, 12 and 16 weeks after seeds were planted. Ten roots of each genotype were harvested and weighed at each sampling time. Whole roots or representative longitudinal sections from the center of the root were rapidly frozen in liquid nitrogen at time of sampling, lyophilized and ground to a fine powder. Care was taken to insure that root sections were representative of whole roots and included crown and tail tissue as well as epidermal and internal parenchyma and vascular tissue. Water content was calcu-

lated from the difference in mass before and after lyophilization.

Enzyme Extraction

Lyophilized tissue was homogenized in ten volumes (w/v) of extraction buffer (100 mM Hepes-NaOH, pH 7.2, 10 mM Na₂SO₃, 5 mM DTT, 1 mM MgCl₂) and the homogenate was passed through a 20 µm filter. The filtrate was centrifuged at 17,000g. The insoluble materials from filtration and centrifugation were combined, washed three times with extraction buffer and used for insoluble acid invertase activity assays. An aliquot of the supernatant from centrifugation was dialyzed against dialysis buffer (10 mM Hepes-NaOH, pH 7.2, 1 mM DTT, 1 mM MgCl₂) and subsequently used for sucrose synthase activity assays. An equal volume of cold acetone was added to the remaining supernatant to concentrate the extract for soluble invertase assays. Precipitated proteins were pelleted by centrifugation at 10,000g for 15 min, washed with 50% cold acetone and resuspended in dialysis buffer. Acetone precipitation did not affect invertase activity, as similar activities for soluble acid invertase and alkaline invertase were observed for extracts concentrated by acetone precipitation or ultrafiltration (data not shown). All manipulations were performed at 4°C.

Enzyme Activity Assays

Sucrose synthase, alkaline invertase, and soluble and insoluble acid invertase activities were determined using spectrophotometric end point assays as previously described (Klotz and Finger, 2002). All assays were run in duplicate. Control reactions were run on all samples by assaying in the absence of UDP or sucrose for sucrose synthase and invertase assays, respectively. Total protein was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Carbohydrate Analysis

Sugar concentrations were determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) using lactose as an internal standard (Klotz and Finger, 2002). Lactose (2.5 µmol) was added to finely ground, lyophilized tissue (50 mg) and the mixture extracted twice with refluxing 80% ethanol (4 ml) for 20 min. Soluble carbohydrates were separated from cell debris by centrifugation. The cell debris was dried in a 80°C incubator and used as a measure of nonextractable dry matter content. Soluble extracts were combined and evaporated of ethanol. A 200 µl aliquot was passed over a 300 mg C18 Maxi-Clean SPE Cartridge (Alltech Associates, Deerfield, IL, USA) and eluted with 1 ml of water. The eluate was diluted five-fold, filtered through a 0.22 µm filter, and 20 µl injected on a 250 x 4 mm

Dionex CarboPak PA-10 column (Sunnyvale, CA, USA) equipped with a 50 x 4 mm CarboPak PA-10 guard column. Carbohydrates were eluted isocratically with 60 mM NaOH at 1.0 ml min⁻¹ and detected with an ESA Coulochem II electrochemical detector (Chemsford, MA, USA) equipped with a gold working electrode and operating in pulsed amperometric mode using the manufacturer's recommended settings for carbohydrate analysis.

Data Analysis

Linear regression analysis was conducted between root soluble sucrolytic activities and root physical and compositional traits across genotypes and sampling dates, or within a genotype across sampling dates using SigmaStat for Windows 2.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Root physical and compositional differences of *B. vulgaris* genotypes

The physical and compositional properties of roots of three *B. vulgaris* genotypes differed significantly as a function of root age and genotype (Figure 1). Differences between genotypes were apparent early in development and continued through sixteen weeks of growth. Root mass was greatest in the fodder beet variety, intermediate in the sugarbeet hybrid and lowest in L19 (Figure 1a). The rate of mass accumulation was greatest in the fodder beet variety and lowest in L19 roots, and by sixteen weeks after planting, roots of the fodder beet variety and the sugarbeet hybrid were 3.3-fold and 2.4-fold greater in mass than L19 roots, respectively. The accumulation of dry matter as a function of time for the three genotypes was similar to the accumulation of fresh mass (data not shown). Averaged over all sampling dates, the accumulation of dry matter in the roots of fodder beet and the sugarbeet hybrid was three-fold and two-fold greater than in L19 roots, and by sixteen weeks after planting, the root dry masses of the fodder beet variety, the sugarbeet hybrid, and L19 were 217, 173, and 94 g, respectively.

Root water concentration, expressed as a percent of fresh mass, was significantly different in the three genotypes (Figure 1b). Over all stages of development, the fodder beet roots averaged 5.4% more water than L19 roots, and sugarbeet hybrid roots averaged 3.4% more water than L19 roots. Generally, water concentration declined with root age for all three genotypes.

Sucrose concentration, expressed as the mass of sucrose per gram dry mass, was greatest in L19 roots, and averaged 40 and 17% more than

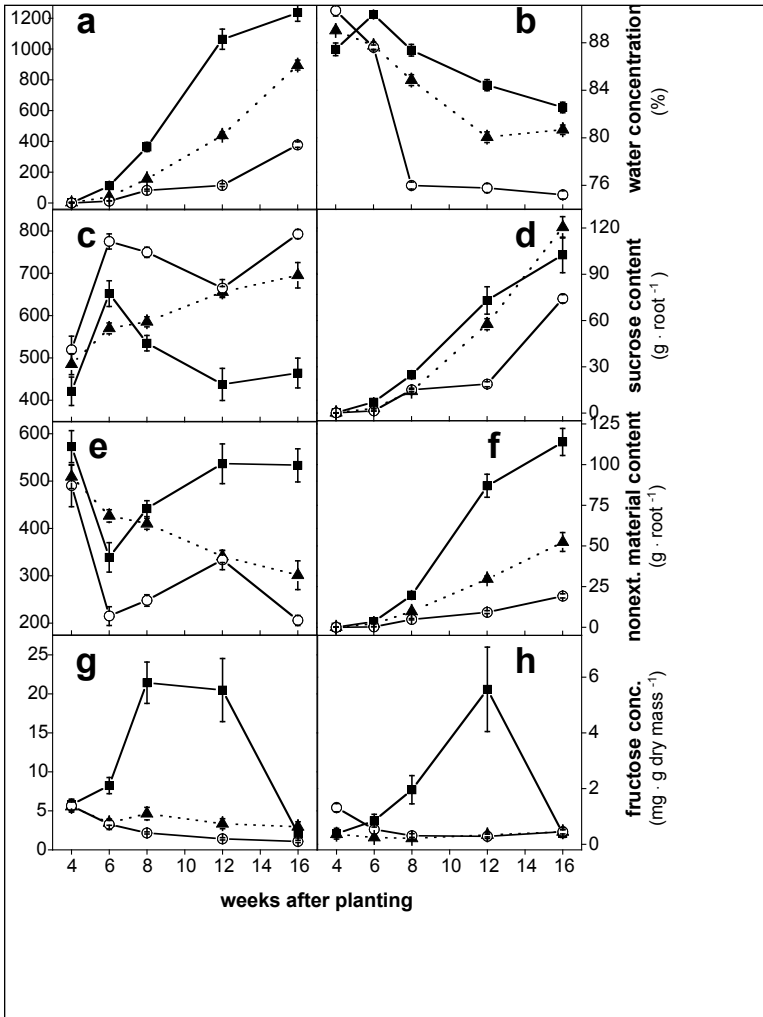


Fig. 1. Root physical and compositional properties for the fodder beet variety Monivigour (■), the commercial sugarbeet variety VDH66156 (▲), and the sugarbeet breeding line L19 (○) as a function of weeks after planting. (a) fresh mass, (b) water concentration, (c) sucrose concentration, (d) sucrose content, (e) nonextractable material concentration, (f) nonextractable material content, (g) glucose concentration, and (h) fructose concentration. Nonextractable material was the dry matter remaining after exhaustive extraction with refluxing 80% ethanol. Error bars are ± 1 standard error of the mean ($n = 10$), where these exceed the size of the symbol.

fodder beet and sugarbeet hybrid roots, respectively (Figure 1c). Sucrose concentration increased between four and six weeks after planting in all genotypes. After six weeks, sucrose concentration generally declined in fodder beet roots, was relatively unchanged in L19 roots, and increased steadily in sugarbeet hybrid roots, indicating that carbon partitioning to sucrose storage during this period decreased in fodder beet roots, was unchanged in L19 roots and increased in sugarbeet hybrid roots. Total sucrose content, expressed as grams of sucrose per root, was greatest in sugarbeet hybrid roots, intermediate in fodder beet roots and lowest in L19 roots (Figure 1d). Sucrose content increased with root development in all genotypes, although the rate of sucrose accumulation, evidenced by the slope of the sucrose content vs. time curve, was different for the three genotypes.

The concentration (Figure 1e) and accumulation (Figure 1f) of nonextractable dry material provided an estimate of cell wall formation. Nonextractable dry material was the cellular material remaining after exhaustive extraction with refluxing 80% ethanol. Cell walls, cell membranes and insoluble proteins were the major components of this material with cell wall polysaccharides responsible for most of this fraction's mass (Bohn et al., 1998). The concentration and accumulation of nonextractable material were greatest in fodder beet roots, lowest in L19 roots, and intermediate in sugarbeet hybrid roots. The concentration of nonextractable material generally increased in fodder beet roots and decreased in sugarbeet hybrid roots between six and sixteen weeks after planting, indicating an increase in partitioning of imported carbon into cell wall synthesis in the fodder beet and a decrease in carbon partitioning into cell wall biosynthesis in the sugarbeet hybrid. The concentration of nonextractable material was variable in the sugarbeet breeding line L19 during this period of development.

Generally, the concentrations of glucose (Figure 1g) and fructose (Figure 1h) were unchanged in roots of the sugarbeet hybrid and L19 as they matured beyond six weeks after planting. Glucose and fructose concentrations in fodder beet roots, however, fluctuated more than ten-fold during development. When averaged over all sampling dates, root glucose concentrations were 4.3 and 1.5-fold greater in the fodder beet and the sugarbeet hybrid, respectively, than in L19, and root fructose concentrations were 5.7 and 1.8-fold greater in fodder beet and L19, respectively, than in the sugarbeet hybrid.

Sucrolytic enzyme activities of *B. vulgaris* genotypes

Sucrose synthase was the predominant sucrolytic activity in all genotypes, at all stages of development examined (Figures 2a). When averaged over all sampling dates, SuSy accounted for 89, 86 and 84% of the soluble sucrolytic activity in roots of fodder beet, the sugarbeet

hybrid and L19, respectively. Generally, SuSy activity, expressed as the rate of sucrose cleavage per gram dry mass, was greater in fodder beet roots, lower in L19 roots and intermediate in the sugarbeet hybrid roots. The three genotypes differed in their developmental expression of SuSy activity; as roots matured, SuSy activity generally declined in fodder beet roots, was relatively unchanged in L19 roots, and was variable in the sugarbeet hybrid roots.

Alkaline invertase was present at substantially lower activities than sucrose synthase in all genotypes (Figures 2b). When averaged over sampling dates, AIn accounted for 3.5, 6.0 and 7.2% of the soluble sucrolytic activity of roots of fodder beet, the sugarbeet hybrid and L19, respectively. In all genotypes, AIn activity was greatest four weeks after planting, and declined during the following four weeks of growth. AIn activities in fodder beet and sugarbeet hybrid roots were similar and generally two-fold greater than in L19 roots.

Soluble acid invertase activity was a significant sucrolytic activity in roots four weeks after planting, and accounted for 20, 35 and 25% of the total soluble sucrolytic activity at this stage of development in roots of fodder beet, the sugarbeet hybrid and L19, respectively (Figure 2c). After four weeks, SAI activity declined rapidly in all genotypes, and was barely detectable in L19 roots by six weeks after planting and in fodder beet and sugarbeet hybrid roots by twelve weeks after planting. SAI activity was generally greatest in fodder beet roots, lowest in L19 roots and intermediate in sugarbeet hybrid roots, although exceptions to this general trend were observed in fodder beet roots at twelve weeks after planting and sugarbeet hybrid roots sixteen weeks after planting.

Insoluble acid invertase activity was measured using the insoluble material remaining after extraction of soluble proteins, and its activity was expressed as a function of the dry mass of the insoluble material (Figure 2d). In all genotypes, IAI activity was greatest four weeks after planting and declined significantly by six weeks after planting. IAI activity was similar in roots of fodder beet and the sugarbeet hybrid and approximately three-fold greater, on average, in L19 roots.

Associations between physical and chemical properties and sucrolytic activities

Linear regression analysis was used to determine associations between root physical and compositional properties and individual sucrolytic activities. Each sucrolytic activity was individually compared with root mass, water content, sucrose content, glucose content, fructose content, and nonextractable material content. Analyses were

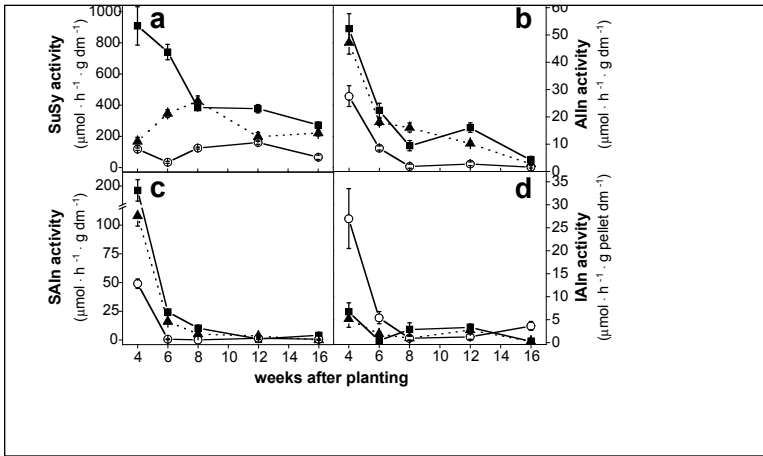


Fig. 2. Root sucrolytic activities for the fodder beet variety Monovigour (■), the commercial sugarbeet variety VDH66156 (▲), and the sugarbeet breeding line L19 (○) as a function of weeks after planting. (a) sucrose synthase activity, (b) alkaline invertase activity, (c) soluble acid invertase activity, and (d) insoluble acid invertase activity. Activities of soluble enzymes (a-c) are expressed as $\mu\text{mole sucrose cleaved} \cdot \text{h}^{-1} \cdot \text{g dry mass}^{-1}$. Activity of insoluble acid invertase (d) was measured on the insoluble material remaining after extraction of soluble proteins and is expressed as $\mu\text{mole sucrose cleaved} \cdot \text{h}^{-1} \cdot \text{g dry mass of pellet}^{-1}$. Error bars are ± 1 standard error of the mean ($n = 10$), where these exceed the size of the symbol.

conducted across genotypes and sampling dates and within a genotype across sampling dates.

Root mass was positively associated with sucrose synthase activity ($R^2 = 0.95$) when SuSy activities for all three genotypes at the five sampling dates (4, 6, 8, 12 and 16 weeks after planting) were compared to the corresponding mass for each genotype at each sampling date (Figure 3a). Water, sucrose and nonextractable materials, which collectively account for more than 98% of the root mass, differed in their association with sucrose synthase activity. SuSy activity was closely associated with water content ($R^2 = 0.96$, Figure 3b) and nonextractable materials content ($R^2 = 0.95$, Figure 3d), but was less closely associated with sucrose content ($R^2 = 0.64$, Figure 3c). No other significant association between any other physical or compositional property and SuSy activity were found.

Across genotypes and developmental stages, a statistically significant positive association occurred between alkaline invertase and

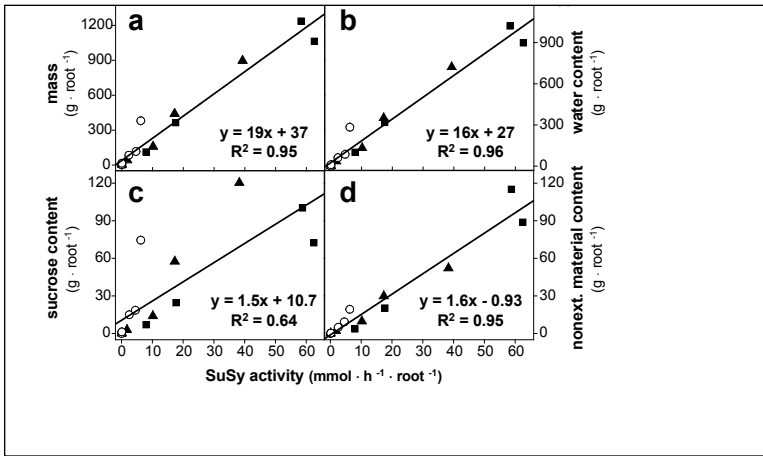


Fig. 3. Association between root sucrose synthase activity and root (a) mass, (b) water content, (c) sucrose content, and (d) nonextractable dry material content. Data points are the individual means ($n = 10$) for roots of the three genotypes (fodder beet variety Monovigour [■], commercial sugarbeet hybrid VDH66156 [▲], and sugarbeet breeding line L19 [○]) at five stages in their development (4, 6, 8, 12, and 16 weeks after planting). Nonextractable dry material was the dry matter remaining after exhaustive extraction with refluxing 80% ethanol.

glucose ($R^2 = 0.87$) or fructose content ($R^2 = 0.86$). Regression analysis, however, was dominated by a single data point and no significant association between glucose and fructose content and Alln activity was found when this data point was removed from the analysis. Therefore, no meaningful association between glucose or fructose content and Alln activity was likely. No other significant association between any other physical or compositional property and Alln activity were found.

No close association between any physical or compositional trait and soluble acid invertase activity was observed in this study. Linear regression analysis conducted individually for each genotype across sampling dates revealed no further associations than were observed when the analysis was conducted across all genotypes and sampling dates.

DISCUSSION

The differences in physical and compositional traits of the three

Beta vulgaris genotypes allowed comparisons of sucrolytic activities in roots with different capacities to accumulate mass, accumulate sucrose, and partition carbon between storage and utilization. Differences in mass, sucrose concentration and carbohydrate partitioning among the three genotypes were expected because breeding efforts of the past two centuries have selected fodder beets for their size and sugarbeet roots for their sucrose content, and the breeding line, L19, is documented for its unusually small size and high sucrose concentration (Ford-Lloyd and Williams, 1975; Theurer and Doney, 1989). Generally, developing fodder beet roots were greater in mass, contained more water, had a lower sucrose concentration and partitioned a greater fraction of their dry matter into nonextractable material than developing sugarbeet hybrid roots, and developing sugarbeet hybrid roots were greater in mass, contained more water, had a lower sucrose concentration, and partitioned a greater fraction of their dry matter into nonextractable material than developing L19 roots.

Linear regression analysis relating root physical and compositional traits with sucrolytic enzymes activities of the three *B. vulgaris* genotypes throughout development indicated a close positive association between root mass and sucrose synthase activity. A positive association between SuSy activity and growth or mass accumulation has been demonstrated in other sink organs including potato tubers, tomato fruit, pea seeds, carrot taproots and radish hypocotyls (Sung et al., 1989; Wang et al., 1993; Zrenner et al., 1995; Déjardin et al., 1997; Tang and Sturm, 1999; Usuda et al., 1999). In these plants, SuSy is believed to be both a marker and a determinant of sink strength. The closeness of the association between mass and sucrose synthase activity indicates that SuSy activity may be a marker for root mass in sugarbeet, and as mass accumulation is an indicator of sink strength (Sun et al., 1992), suggests a possible role for SuSy as a determinant of sink strength.

Sucrose synthase activity was positively associated with the content of water, sucrose, and nonextractable material in *B. vulgaris* roots. Collectively, these three fractions account for more than 98% of the total root mass in all three genotypes. Sucrose synthase activity was closely associated with the content of water ($R^2 = 0.96$) and nonextractable material ($R^2 = 0.95$), but was less closely associated with sucrose content ($R^2 = 0.64$) across all genotypes and sampling dates. The close positive association between SuSy activity and nonextractable materials suggests a role for SuSy activity in cell wall biosynthesis. SuSy is most likely the major source of UDP-glucose, the principal substrate for cellulose biosynthesis and for conversion to the various nucleotide diphosphosugars that are substrates for hemicellulose and pectin

biosynthesis. Although UDP-glucose pyrophosphorylase (EC 2.7.7.9) may also generate UDP-glucose, this enzyme is present at low levels in sugarbeet roots (Pavlinova, 1972) and is more likely to degrade UDP-glucose than to synthesize it (Delmer and Amor, 1995). A role for SuSy in cell wall biosynthesis has been proposed in other plant species based on an association of SuSy activity with cellulose (Amor et al., 1995; Nolte et al., 1995), callose (Chourey and Miller, 1995) and hemicellulose (Buckeridge et al., 1999) biosynthesis.

The association between SuSy activity and water content observed for the three *B. vulgaris* genotypes is possibly a consequence of SuSy activity on cell wall synthesis and/or sink strength. Promotion of cell wall biosynthesis by SuSy, as suggested by the close association between SuSy activity and the content of cell wall material, would likely result in an increase in cell wall surface area, with a concomitant relief of cellular turgor pressure and an increase in water uptake (Cosgrove, 1981). Alternatively, enhancement of sink strength by SuSy, as suggested by the close association between SuSy activity and the accumulation of root mass, would be associated with an increase in carbohydrate import into the root, causing an increase in cell osmotic potential and an increase in water uptake (Kehr et al., 1999).

The association between SuSy activity and sucrose content was also significant, suggesting that SuSy may be a factor in sucrose accumulation. The coefficient of determination for this association, however, was relatively low, indicating that other factors are likely to be important in the control of sucrose accumulation. Clearly, SuSy's influence on sucrose accumulation was not as dominant as its influence on root mass, water content or content of cell wall materials. Whether the association of SuSy activity with sucrose content is a consequence of the overall effect of SuSy on sink strength remains to be determined.

Alkaline invertase, soluble acid invertase, and insoluble acid invertase activities were not meaningfully associated with any root physical or chemical characteristic in this study. In contrast to reports of an inverse relationship between soluble acid invertase activity and sugarbeet root sucrose content (Giaquinta, 1979; Berghall et al., 1997), no association between soluble acid invertase and sucrose content or concentration was found in this study. The lack of an association between any sacrolytic activity and physical or compositional property, however, does not preclude the existence of a relationship because tissue and cellular localization of enzymes and metabolites were not taken into account.

The associative data obtained in this study provide clues of enzymatic function, but are insufficient to conclusively demonstrate function in *B. vulgaris* roots. Future research will seek to confirm and describe

the roles of SuSy in root yield, sink strength, cell wall biosynthesis, and sucrose accumulation.

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