Sucrose Catabolism during Sugarbeet Root Development: Changes in Sucrolytic Isoenzyme Activities and Carbohydrate Accumulation during Growth

Karen L. Klotz[1] and Fernando L. Finger[2]
[1]USDA/ARS Northern Crop Science Laboratory, Fargo, ND 58105-5677
[2]Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brazil

Introduction

Sucrose catabolism is essential for growth, development and sucrose partitioning in sugarbeet roots. Sucrose catabolism fuels growth and development by providing metabolic energy and substrates for the synthesis of cellular structures. Sucrose degradation also governs root growth by affecting cell expansion, phloem unloading, carbon partitioning and sink strength. In these ways, sucrose catabolism significantly impacts sugarbeet root growth, sucrose utilization and sucrose accumulation and, therefore, affects the yield and value of the sugarbeet root crop.

Sucrose catabolism occurs primarily by the action of three enzyme activities. Acid invertase, alkaline invertase and sucrose synthase catalyze the conversion of sucrose to its constituent monosaccharides. Acid invertase catalyzes the irreversible hydrolysis of sucrose to glucose and fructose. Acid invertase exhibits optimum activity at pH values of 4.5 to 5.5 and is found solubilized in the cell vacuole or insolubilized in the cell wall. Alkaline invertase catalyzes the same irreversible hydrolysis reaction as acid invertase, but is most active at pH 7.0 to 8.0, and is located in the cell cytoplasm. Sucrose synthase is a cytoplasmic enzyme that catalyzes the reversible cleavage of sucrose in the presence of uridine 5'-diphosphate (UDP) to form UDP-glucose and fructose.

The function of these enzyme activities in sugarbeet roots is largely unknown. Attempts to determine the role of these enzymes have been 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. To aid in understanding how these enzymes participate in sugarbeet root sucrose catabolism, the number of isoenzymes for each of the major sucrolytic activities was determined and their contribution to sugarbeet root sucrolytic activity during development was examined. The relationship between these activities and root growth and carbohydrate accumulation was also determined.

Materials and Methods

Protein extraction and enzyme activity assays

Greenhouse grown sugarbeet roots of commercial hybrid VDH66156 were harvested 2, 4, 6, 8, 12 and 16 weeks after sowing. Ten roots were collected at each harvest date, rapidly frozen in liquid N_2 and lyophilized. Soluble proteins were extracted from root samples by homogenization in extraction buffer (100 mM HEPES, pH 7.2, 10 mM Na_2SO_3, 5 mM DTT and 1mM $MgCl_2$) and centrifugation to remove cell wall debris. The crude extract was dialyzed overnight against 10 mM HEPES, pH 7.2, 1 mM DTT and 1 mM $MgCl_2$ to remove sugars. The protein extract was assayed for acid and alkaline invertase activity by the method of Goldstein and Lampen (1975) at pH 4.7 and 7.2 for acid and alkaline invertase, respectively. Sucrose synthase activity was measured by the

method of Somogyi (1952). Insoluble acid invertase activity was measured with proteins extracted from the cell wall pellet. The cell wall pellet was washed three times with extraction buffer, extracted with 100 mM HEPES, pH 7.2, 10 mM Na_2SO_3, 5 mM DTT, 2 M NaCl and 15 mM EGTA, centrifuged to remove particulate matter and dialyzed overnight. Insoluble acid invertase activity was assayed as described above for soluble acid invertase. Total protein was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Activity stained isoelectric focusing gel electrophoresis

Isoenzymes for each enzyme family were determined by activity staining of isoelectric focused polyacrylamide gels with ampholines in the pH range of 3.5 to 9.5. Focused gels were incubated for 30 minutes in substrate and stained with 0.1% (w/v) 2,3,5-triphenyltetrazolium chloride (Gabriel & Wang, 1969). Substrates used were 100 mM sucrose for invertase activity and 100 mM sucrose and 10 mM uridine 5'-diphosphate for sucrose synthase activity. Acid invertase, alkaline invertase and sucrose synthase activities were assayed at pH 4.7, 7.8 and 6.5, respectively. Buffers used were 100 mM NaOAc, pH 4.7, 100 mM HEPES-NaOH, pH 7.8 and 100 mM MES-HCl, pH 6.5. Control gels were incubated in the appropriate buffer without substrate and stained as above.

Carbohydrate assays

Sucrose content was determined by high performance anion exchange chromatography with pulsed amperometric detection using lactose as an internal standard. A lactose standard was added to lyophilized tissue and extracted twice with refluxing 80% EtOH. Cell debris was removed by centrifugation, and EtOH was removed by evaporation. The extract was passed over a C_{18} SPE cartridge, filtered and injected onto a 25 cm Dionex CarboPak PA-10 column. Carbohydrates were eluted isocratically with 60 mM NaOH at 1.0 ml/min, and detected electrochemically with a gold working electrode operating in pulsed amperometric mode.

Results and Discussion

Growth, sucrose accumulation and the activities of the major sucrolytic enzymes were measured in greenhouse grown sugarbeet roots during sixteen weeks of development. Sugarbeet roots increased rapidly in weight during the early stages of growth (Fig. 1A). Rate of growth was greatest in seedling roots and declined as sugarbeet roots aged. Accumulation of total root mass increased with root development (Fig. 1B). Accumulation of mass was minimal until roots were at least six weeks old, and mostly occurred late in development. Sucrose accumulation was evident throughout most of root development. Sucrose concentration increased rapidly between two and four weeks of age, indicating the ability of roots to store sucrose even when young (Fig. 2A). Most of the sucrose stored in sugarbeet roots, however, accumulated late in development, and total sucrose content of roots was minimal until sugarbeet roots were greater than six weeks of age (Fig. 2B).

Acid invertase was the predominant sucrose degrading activity in the roots of sugarbeet seedlings. It was a minor sucrolytic activity during all other stages of development. Soluble and insoluble acid invertase activities were found at high levels in two week old roots (Fig. 3A-B). These activities dropped precipitously after two weeks of growth, and were barely detectable by six weeks of age. Soluble acid invertase activity was due to the activities of two isoenzymes as determined by

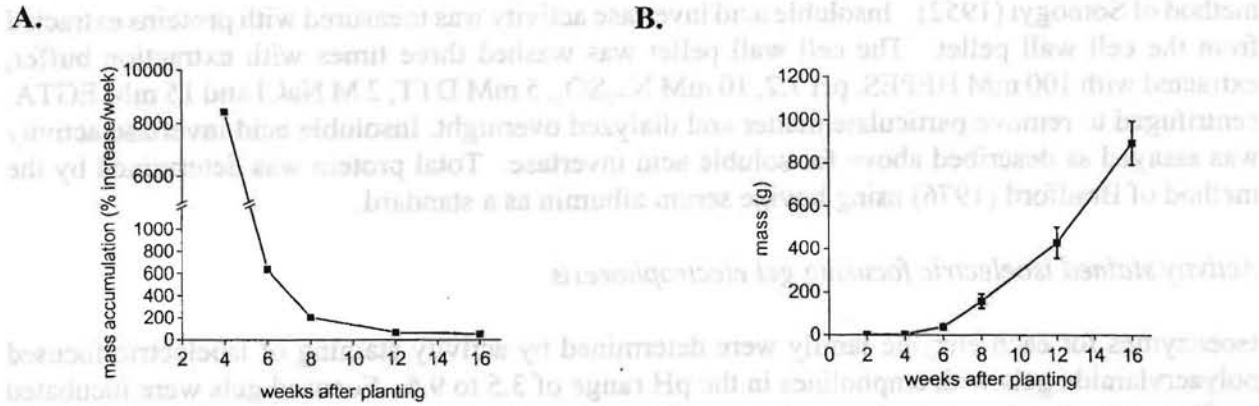

Figure 1: Growth of sugarbeet roots during sixteen weeks of development. **A.** Change in rate of growth. **B.** Change in total root mass. Error bars = one standard deviation.

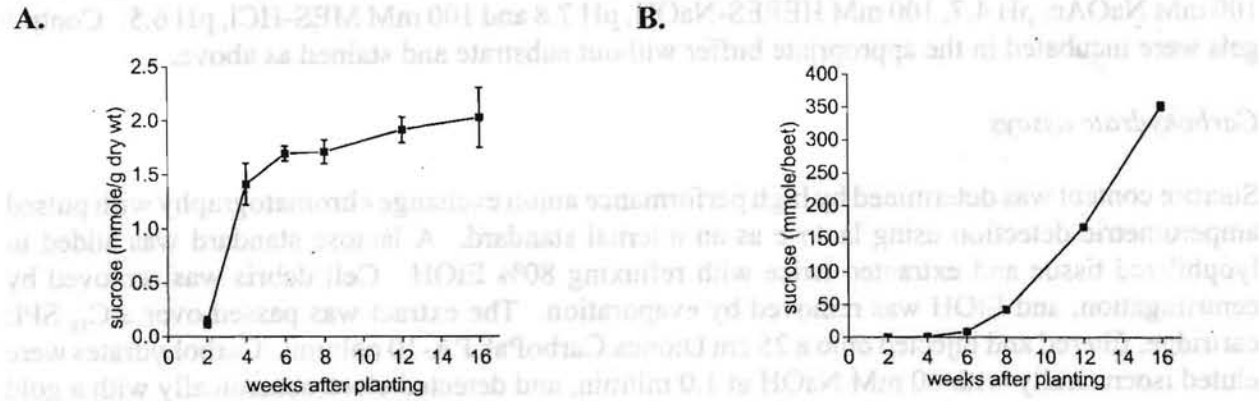

Figure 2: Sucrose accumulation in sugarbeet roots during sixteen weeks of development. **A.** Change in sucrose concentration. **B.** Change in total sucrose content. Error bars = one standard deviation.

isoelectric focusing polyacrylamide gels (data not shown). A major soluble acid invertase isoenzyme was found throughout development. A second minor isoenzyme was evident only in two week old roots. Acid invertase activity closely paralleled sugarbeet root growth rate (Fig. 1A) and was inversely proportional to root sucrose concentration (Fig. 2A). Acid invertase activity is likely to be important for the rapid growth observed in young sugarbeet roots. High acid invertase activity has been observed in many plant species in tissues or organs undergoing rapid growth (Morris & Arthur, 1985; Pfeiffer & Kutschera, 1995; Ricardo & Sovia, 1974). Acid invertase is believed to provide the hexose substrates required to maintain rapid growth. High acid invertase activity and a rapid growth rate, however, occurred at the expense of sucrose storage. An inverse relationship between acid invertase and sucrose content has been observed in other plant species (Hatch & Glasziou, 1963; Ricardo & ap Rees, 1970; Ricardo & Sovia, 1974).

Alkaline invertase activity was present at low levels throughout most of sugarbeet root development (Fig. 3C). Alkaline invertase activity was not found in the root of young seedlings, but was evident

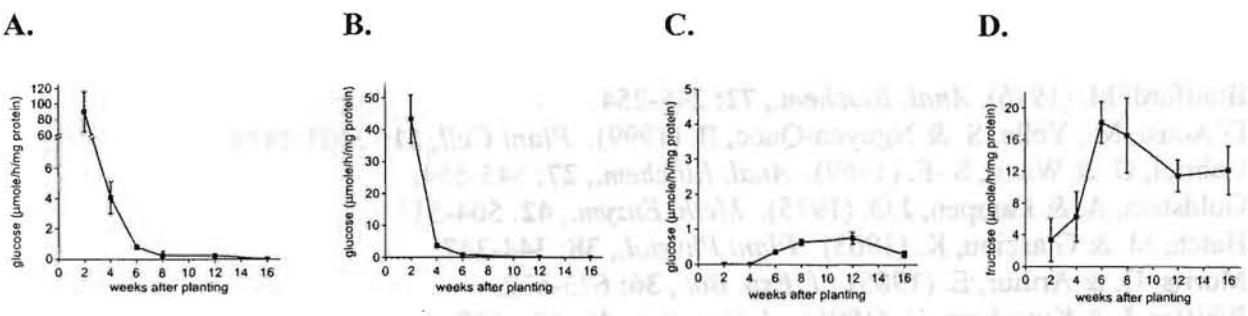

Figure 3: Sucrolytic enzyme activities in sugarbeet roots during sixteen weeks of development. **A.** soluble acid invertase activity, **B.** insoluble acid invertase activity, **C.** alkaline invertase activity and **D.** sucrose synthase activity. Error bars = one standard deviation.

by six weeks of age. Alkaline invertase activity was due to the activities of two isoenzymes (data not shown). Although total alkaline invertase activity was relatively unchanged as sugarbeet roots matured beyond six weeks of age, the contribution of the individual isoenzymes to this activity changed. Both isoenzymes were present in sugarbeet roots between six and sixteen weeks of age. One isoenzyme exhibited maximum activity at eight weeks and declined with subsequent development. The second isoenzyme increased in activity as roots aged beyond six weeks. No relationship was observed between either alkaline invertase isoenzyme and root growth or sucrose accumulation. Presently, no function is known for alkaline invertase in sugarbeet or other plant species.

Sucrose synthase was the predominant sucrose degrading activity at all but the earliest stages of development and was responsible for nearly all sucrolytic activity in mature roots (Fig. 3D). Sucrose synthase activity increased during the first six weeks of growth and remained at high levels with subsequent development. Two sucrose synthase isoenzymes contributed to sucrose synthase activity (data not shown). One isoenzyme was present throughout root development. A second isoenzyme was found only in sixteen week old roots. Sucrose synthase was the major sucrolytic activity when nearly all accumulation in root weight (Fig. 1B) and sucrose content (Fig. 2B) occurred. Although its function in sugarbeet roots has not been determined, sucrose synthase has been implicated in the regulation of carbon partitioning and sink strength in other plant species (D'Aoust *et al.*, 1999; Zrenner *et al.*, 1995). Sucrose synthase may have a similar function in sugarbeet roots.

Acknowledgments

The authors thank Craig L. Nerby for technical assistance, the Beet Sugar Development Foundation for financial support, and CAPES/MEC (Brazil) for Dr. Finger's scholarship. Mention of trademark or proprietary product names does not constitute a guarantee or warranty of the product by the USDA, and the use of the name implies no approval of the product to the exclusion of others that may also be suitable.

Literature Cited

Bradford, M. (1976). *Anal. Biochem.*, **72:** 248-254.
D'Aoust, M., Yelle, S. & Nguyen-Quoc, B. (1999). *Plant Cell*, **11:** 2407-2418.
Gabriel, O. & Wang, S.-F. (1969). *Anal. Biochem.*, **27**: 545-554.
Goldstein, A. & Lampen, J.O. (1975). *Meth. Enzym.*, **42**: 504-511.
Hatch, M. & Glasziou, K. (1963). *Plant Physiol.*, **38:** 344-347.
Morris, D. & Arthur, E. (1985). *J. Exp. Bot.*, **36:** 623-632.
Pfeiffer, I. & Kutschera, U. (1995). *J. Exp. Bot.*, **46:** 631-638.
Ricardo, C. & ap Rees, T. (1970). *Phytochem.*, **9:** 239-247.
Ricardo, C. & Sovia, D. (1974). *Planta*, **118:** 43-55.
Somogyi, M. (1952). *J. Biol. Chem.*, **153**: 375-380.
Zrenner, R., Salanoubat, M., Willmitzer, L., & Sonnewald, U. (1995). *Plant J.*, **7**: 97-107.