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Sucrose Accumulation in the Sugarcane Stem Is Regulated by the Difference between the Activities of Soluble Acid Invertase and Sucrose Phosphate Synthase[1]

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To assess the relative importance of morphological and biochemical factors in the regulation of sucrose (Suc) accumulation in the sugarcane (*Saccharum* spp. hybrids) stem, we investigated morphological and biochemical correlates of Suc accumulation among parents and progeny of a family segregating for differences. In contrast to the parents, no relationship was observed between morphology and the level of Suc accumulation among the progeny. The level and timing of Suc accumulation in the whole stalk and within individual internodes was correlated with the down-regulation of soluble acid invertase (SAI) activity. High SAI activity prevented most, but not all, Suc accumulation. There was a critical threshold of SAI activity above which high concentrations of Suc did not accumulate. This low level of SAI activity was always exceeded in the internodes of the lower-Suc-storing genotypes. However, low activity of SAI was not sufficient by itself to account for the Suc accumulation in the higher-Suc-storing genotypes. Major differences in Suc accumulation among the population were attributed to the difference between activities of SAI and Suc phosphate synthase, provided SAI is below the critical threshold concentration. This result is not unexpected, since the pathway of Suc transport for storage involves Suc hydrolysis and resynthesis.

Modern sugarcane cultivars are multispecies hybrids, primarily of *Saccharum officinarum* L., *Saccharum spontaneum* L., and *Saccharum robustum* Brandes et Jeswiet ex Grassl. Accessions of *S. officinarum* store up to 21% Suc in the juice of the stem (Sreenivasan and Nair, 1991), whereas accessions of *S. spontaneum* store less than 6% (Bull and Glasziou, 1963) and accessions of *S. robustum* store less than 10% (Rao et al., 1985). The large differences among species and hybrids of *Saccharum* in ability to accumulate Suc have been correlated with various morphological (Bull and Glasziou, 1963) and enzymatic (Hatch and Glasziou, 1963) factors. Within high-Suc-storing varieties, there are also large differences in Suc content as a function of plant age and the environment in which the plant is growing. The stem Suc content of high-Suc-storing genotypes is lower during periods of rapid growth and higher when growth is slowed because of either environmental constraints (cool temperatures or limited water and nutrients) or developmental maturation. Welbaum and Meinzer (1990) reported 108 mM Suc plus 109 mM hexose in immature internodes (no. 2 from top), increasing to 612 mM Suc plus 2 mM hexose in mature internodes (no. 20 from top). The gradient of sugar concentrations down the stem is more pronounced during periods of rapid growth and is reduced during periods of restricted growth (Fernandes and Benda, 1985).

Stems (stalks) of high-Suc-accumulating clones are generally high in moisture and low in fiber (Bull and Glasziou, 1963). Such stalks generally have a thick girth and high fresh weight. Stalks of low-Suc-storing clones are generally thin and fibrous and have a low fresh weight Therefore, part of the difference in Suc storage potential among genotypes might be based on limits set by morphological characters.

Invertase enzymes have been suggested as key regulators for accumulation of Suc in sugarcane stem storage parenchyma (Hatch and Glasziou, 1963; Sacher et al., 1963; Gayler and Glasziou, 1972a). NI occurs in the cytoplasm or metabolic compartment of cells (Hatch and Glasziou, 1963). In high-Suc-storing varieties, NI activities are low in meristematic cells but increase concomitantly with the increase in sugar accumulation in storage tissue (Hatch and Glasziou, 1963). SAI activities are usually high in tissues that are rapidly growing, such as cell and tissue cultures, root apices, and immature stem internodes. During internode growth and development, SAI activity can decrease by more than two orders of magnitude (Hatch and Glasziou, 1963). SAI activity occurs primarily in the vacuoles of storage parenchyma cells, but it is also reported to occur in the apoplastic cell wall space either as a soluble enzyme or bound to the cell wall fraction (Sacher et al., 1963; Gayler and Glasziou, 1972a; Hawker et al., 1991). Varieties within the *Saccharum* group that retain high levels of SAI activity in mature stem storage tissue do not store high levels of

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Abbreviations: NI, neutral invertase (β-fructofuranosidase, β-fructofuranoside fructohydrolase [EC 3.2.1.26]); SAI, soluble acid invertase (β-fructofuranosidase, β-fructofuranoside fructohydrolase [EC 3.2.1.26]); SPS, Suc-P synthase (UDP-Glc:D-Fru-6-P 2-α-D-glucosyltransferase [EC 2.4.1.14]); SS, Suc synthase (UDP-Glc:D-Fru 2-α-D-glucosyltransferase [EC 2.4.1.13]).

Suc (Hatch and Glasziou, 1963). The converse is only sometimes true. Apparently, SAI must be low before Suc can accumulate, but accumulation itself can be the result of other factors.

Although early research implicated the invertases as the principle enzymes regulating sugarcane growth and Suc accumulation (Hatch and Glasziou, 1963; Slack, 1965; Gayler and Glasziou, 1972a), later research discounted the importance of invertase (Veith and Komor, 1993). Other enzymes such as SS (Goldner et al., 1991), SPS (Hatch, 1964), or the balance of activities of several enzymes (Wendler et al., 1990; Veith and Komor, 1993; Komor, 1994) have also been considered key regulatory factors for Suc accumulation in sugarcane.

None of the sugarcane Suc accumulation studies can be considered conclusive, since data were obtained from different plant organs, among plants of different ages exposed to divergent environments, and among different plant genotypes (for review of the topic, see Moore, 1995). Therefore, we initiated research to test whether determinants of Suc storage could be identified in closely related genotypes (progeny), differing in capacity to store Suc under the same environmental conditions. We wished to identify the key enzymes involved in Suc accumulation in sugarcane so that we might later discover how these enzymes are regulated to affect Suc storage.

MATERIALS AND METHODS

Plant material consisted of 9-month-old stems of the parents and eight progeny clones of a cross between the high-Suc female parent Louisiana Purple (*Saccharum officinarum*, $2n = 80$) and the low-Suc male parent Molokai 5829 (Mol 5829, *Saccharum robustum*, $2n = 80$). The progeny of the cross were recognized as hybrids, and not selfs, since all progeny exhibited hairy leaf sheaths, which are characteristic of the male parent. The parents and 110 progeny clones were grown in replicated 1 × 1.5-m plots on the leeward plain of Oahu, HI. Plants were grown from vegetative cuttings using standard practices for water and fertilizer applications. Eight clones, identified from a previous study (Sills et al., 1995) as either high-Suc or low-Suc types, were selected for this study. Nonflowering, mature stalks of plants about 9 months old were harvested during November and December, 1994. The stalks were excised at the soil surface and leaves were removed, and the internodes were numbered sequentially down the stalk according to their stage of development. The internode immediately subtending the youngest, fully expanded leaf was designated internode no. 1, with lower internodes given sequentially increasing numbers (Clements and Ghotb, 1968). The stems were cut into individual internodes and weighed. The internode rind, consisting of a thick epidermis and sclerified vascular bundles, was removed and the remaining internode was cut into quarters lengthwise. Each quarter was chopped into small pieces and frozen in liquid nitrogen before being stored in a −80°C freezer until used for sugar and enzyme assays. The harvesting procedure was performed quickly in the field so that the time elapsed between excising the internode and freezing the storage tissue was kept to a minimum, usually less than 1 min.

Sugar Analyses

Sugar assays were performed on subsamples from the same internode that was used for enzyme activity assays. Frozen, chopped pieces from each internode were divided into two portions. One portion was thawed and homogenized in 70% ethanol, and the tissue and extract solution were boiled for 2 h. Aliquots from the supernatant were taken for measurements of reducing sugars and Suc. Reducing sugars were determined by the Somogyi-Nelson method (Nelson, 1944), and Suc was determined by the anthrone method (van Handel, 1968). The concentrations of sugars were calculated on each internode and expressed as micromoles per gram fresh weight. The whole-stalk concentrations were weight-averaged from individual internode nos. 2 through 11. The data presented are the mean values from at least three independent extractions. The reducing sugar concentrations did not significantly differ among clones on a whole-stalk basis and the data are not presented.

Enzyme Extraction

Frozen tissue of an internode was weighed and ground to a fine powder in liquid nitrogen in a chilled mortar. Quartz sand (Sigma) was added to facilitate cell disruption. Extraction buffer was added to the ground sample, and the slurry was ground to thoroughly mix the powdered tissue and buffer (Goldner et al., 1991). The ratio of fresh weight and extraction buffer was varied based on the internode age: internode nos. 1 to 4, 0.2 to 0.3 g fresh weight mL^{-1}; internode no. 5 and older, 0.4 g fresh weight mL^{-1}. The extraction buffer contained 50 mM Hepes (pH 7.5), 12 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 10 mM DTT, 2 mM benzamidine, 2 mM *N*-aminocapronate, and 10 mM diethyldithiocarbamate. When the extract started to melt, it was ground again to achieve full homogeneity. The homogenate was passed through two layers of Miracloth (Veratec, Walpole, MA) and the filtrate was centrifuged at 15,000*g* for 10 min at 4°C. The supernatant was desalted immediately on a Sephadex G-25 (Pharmacia PD-10) column. Dilute extracts were concentrated with Centricon-30 or Centriprep-30 (Amicon, Beverly, MA) at 4°C. Assays were conducted immediately after the extract was desalted or concentrated.

Enzyme activity recovery was determined for each of the sugarcane enzymes. An enzyme preparation of known activity was added to a sample of frozen sugarcane tissue, ground, and prepared as described above. The "spiked-sample" activity was compared with the sample prepared in parallel without the added enzyme preparation. The activity difference between the two extractions showed the lack of endogenous inhibitors of enzyme activity and was used to calculate the recovery of the sugarcane enzymes. Recovery of the soluble enzymes (SS, SPS, and invertases) was about 80%. Recovery for the cell wall-bound invertase varied between about 53 and 99%. Because we were not

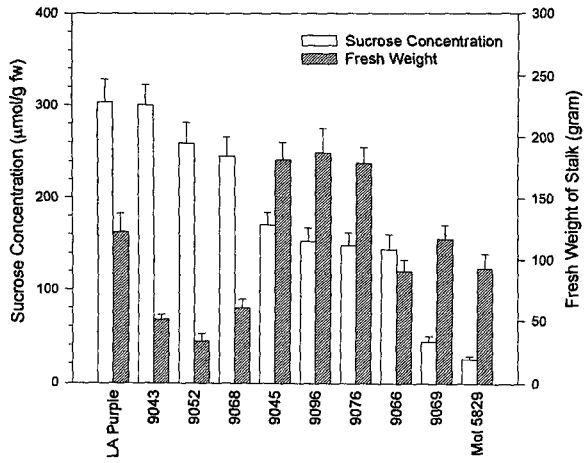

Figure 1. Mean concentration of Suc in stalks and stalk fresh weight of 9-month-old plants of the high-Suc *S. officinarum* parental clone Louisiana Purple (LA Purple), the low-Suc *S. robustum* parental clone Mol 5829, and a set of progeny from a cross between the two parents. The open bars represent the mean Suc concentrations with SE bars; the shaded bars represent the mean fresh weights (fw) with SE bars.

able to control the wide variance in recovery of cell wall-bound invertase, we have not presented those data.

Enzyme Assays

SAI activity at 37°C was assayed by adding 50 μL of desalted extract to 50 μL of 1 M sodium acetate (pH 4.5). The enzyme reaction was started by the addition of 100 μL of a 120 mM Suc solution. The reaction was stopped at 30 or 60 min by adding 30 μL of 2.5 M Tris base and boiling the mixture for 3 min. The NI activity assay was similar to that for SAI except that the reaction was conducted at pH 7.5 and no Tris base was added. For both SAI and NI assays, the concentration of Glc liberated was determined with the Glc test kit from Sigma.

SPS and SS activity assays were conducted at 37°C in the direction of synthesis at pH 7.5 (Hubbard et al., 1989). Fifty microliters of desalted enzyme extract was added to a 50-μL assay solution. The SS activity assay solution contained 50 mM Hepes (pH 7.5), 15 mM $MgCl_2$, 25 mM Fru, and 25 mM UDP-Glc. The SPS assay solution contained 100 mM Hepes (pH 7.5), 20 mM Glc-6-P, 4 mM Fru-6-P, 3 mM UDP-Glc, 5 mM $MgCl_2$, and 1 mM EDTA. For the control, UDP-Glc was not added in the assay solutions. Reactions were incubated at 37°C for 0, 30, and 60 min and then stopped by boiling for 3 min. Suc produced by these reactions was assayed using the anthrone assay (van Handel, 1968): 70 μL of reaction solution was added to 70 μL of 30% KOH, boiled for 10 min, and cooled to room temperature; 1 mL of freshly prepared anthrone reagent, containing 76 mL of H_2SO_4, 30 mL of H_2O, and 150 mg of anthrone, was added and the reaction was incubated at 37°C for 20 min. A_{650} was measured immediately in a v_{max} microplate reader (Molecular Devices, Menlo Park, CA).

Protein concentration was determined by the method of Bradford (1976) with BSA as the standard protein. All enzyme activities were calculated on each internode and expressed as micromoles of product formed per gram of total protein per minute. The genotype mean enzyme activities were calculated from weight-adjusted specific activity data from individual internode nos. 2 through 11. The data presented are the mean values from at least three independent extractions.

RESULTS

Suc Accumulation

The internode mean Suc concentration differed between the parents by one order of magnitude, ranging from 25.9 μmol g^{-1} fresh weight for Mol 5829 to 304 μmol g^{-1} fresh weight for Louisiana Purple (Fig. 1). The internode mean Suc concentrations of the eight progeny were intermediate between those of the parents. The difference between the parents in mean fresh weights of stalks calculated from internode nos. 2 through 11 was relatively small, and it was greatly exceeded by the difference in mean fresh weights of stalks of the progeny, which ranged from 29.5 to 165.8 g. High-Suc clones were found among clones with both the low and high stalk weight (Fig. 1). Therefore, stalk weight was not a good indicator of the Suc concentration in the stem.

Young internodes of all clones were low in Suc, whereas the older internodes accumulated Suc to different levels (Fig. 2). The high-Suc parent Louisiana Purple, but none of its progeny, showed Suc accumulation in internodes as young as no. 3. The low-Suc parent Mol 5829 showed little Suc accumulation in internodes as old as no. 11. The high-Suc and low-Suc progeny differed not only in the final Suc concentration reached in the older internodes but also in the pattern of Suc accumulation along the stalk. High-Suc

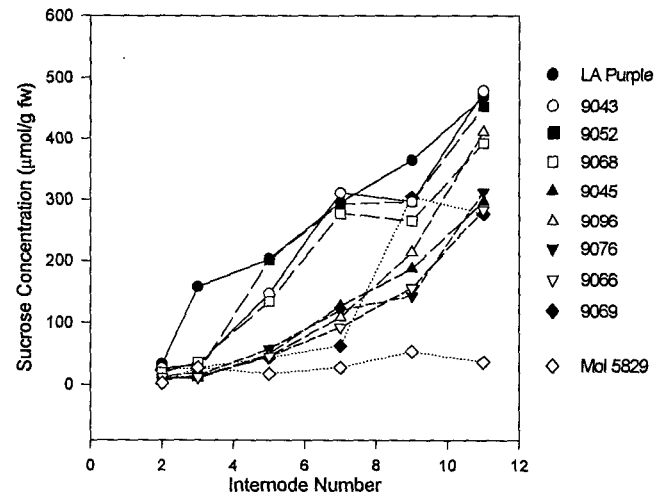

Figure 2. The mean Suc concentration in individual internodes of 9-month-old plants of the high-Suc *S. officinarum* parental clone Louisiana Purple (LA Purple), the low-Suc *S. robustum* parental clone Mol 5829, and a set of progeny from a cross between the two parents. Internode numbers increase down the stalk from the immature no. 1 internode to the fully elongated and mature internode no. 11 at the base of the stalk. fw, Fresh weight.

clones started accumulation of Suc three to four internodes earlier than did the low-Suc clones.

Enzyme Activity

SAI activity varied by internode age and genotypes, with highest activities in the young internodes of the low-Suc parent, Mol 5829, lowest in the high-Suc parent, Louisiana Purple, and intermediate in the progeny (Fig. 3a). The high SAI activity of young internodes of the Suc-accumulating clones declined rapidly, reaching basal levels by internode no. 3 or 5, as the internodes matured and accumulated Suc. NI (Fig. 3b), SS (Fig. 3c), and SPS (Fig. 3d) activities were relatively low and not significantly related to internode age, except SPS, which showed a tendency for increase during internode maturation in some clones.

When the enzyme activity and Suc level of each internode of all genotypes were compared simultaneously, no significant overall relationship was found between the activities of NI, SS, and SPS and Suc accumulation (Fig. 4, b–d). However, a significant nonlinear negative relationship between SAI activity and Suc accumulation did exist in individual internodes (Fig. 4a). When SAI activity was high, the Suc content was always low, but when SAI was low, the Suc content could be either low or high and fit a hyperbolic function ($r^2 = 0.70$, $P < 0.002$).

The net production of Suc, and hence the final Suc concentration, should be correlated with the difference between the rate of Suc synthesis and Suc hydrolysis, i.e. SPS minus SAI. Since the K_m of SAI for Suc is in the low millimolar range, the rate of hydrolysis by SAI can be considered to be independent of Suc throughout all internodes and represented by the in vitro rate. Although there was no significant correlation between SPS activity and Suc concentration in individual internodes, the differences in activities of SPS and SAI and Suc concentration (Fig. 4e) were strongly correlated ($r^2 = 0.71$, $P < 0.002$) with a hyperbolic function.

Because of the time lag between a shift of enzyme activities and its effect on Suc accumulation, and because whole-stalk analysis is common agronomic practice, the activities of SAI (Fig. 5a), NI (Fig. 5b), SS (Fig. 5c), and SPS (Fig. 5d) measured on individual internodes were weight-averaged to give a mean value for the whole stalk of each genotype. This value was compared with the mean Suc concentration of the whole stalk of each genotype. Among

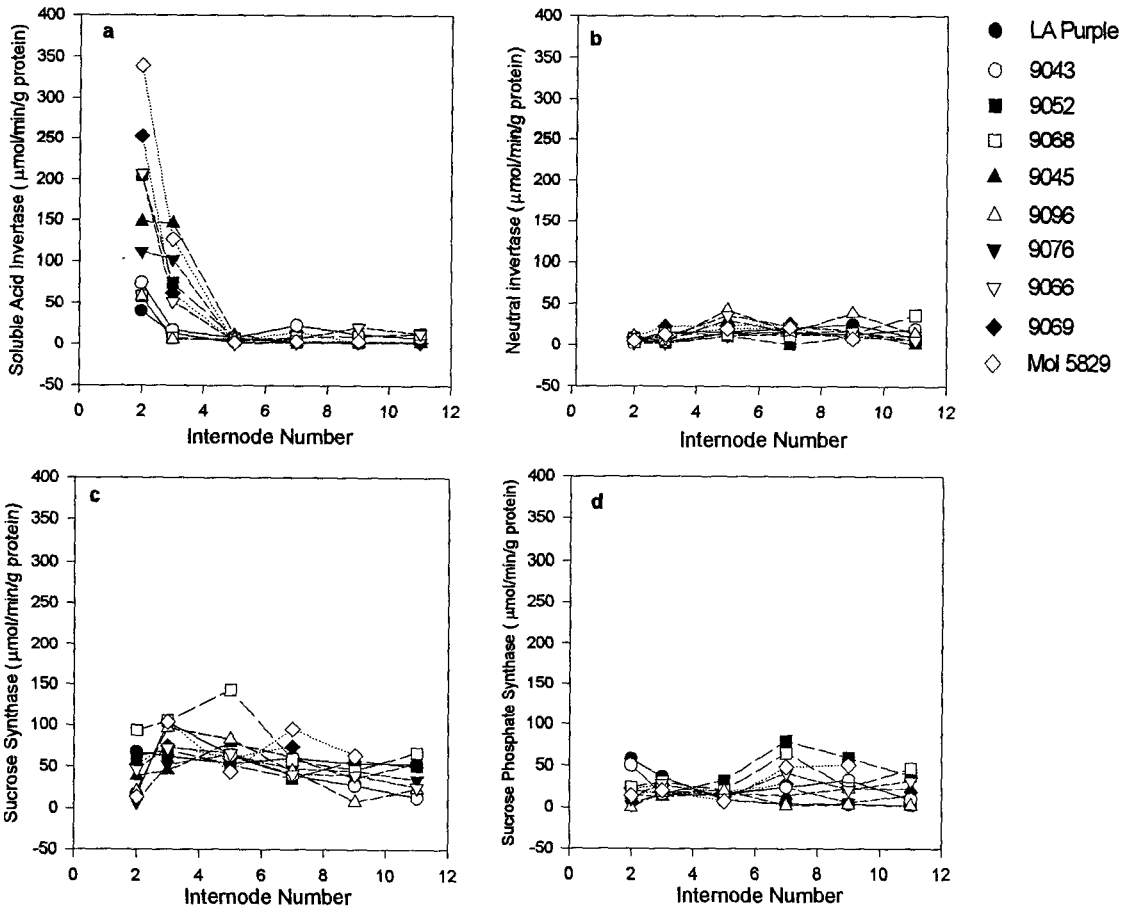

Figure 3. Specific activities of SAI (a), NI (b), SS (c), and SPS (d) in individual internodes of 9-month-old plants of the parental high-Suc *S. officinarum* clone Louisiana Purple (LA Purple), the parental low-Suc *S. robustum* clone Mol 5829, and a set of progeny from a cross between the two parents. Internode numbers increase down the stalk from the immature no. 1 internode to the fully elongated and mature internode no. 11 at the base of the stalk.

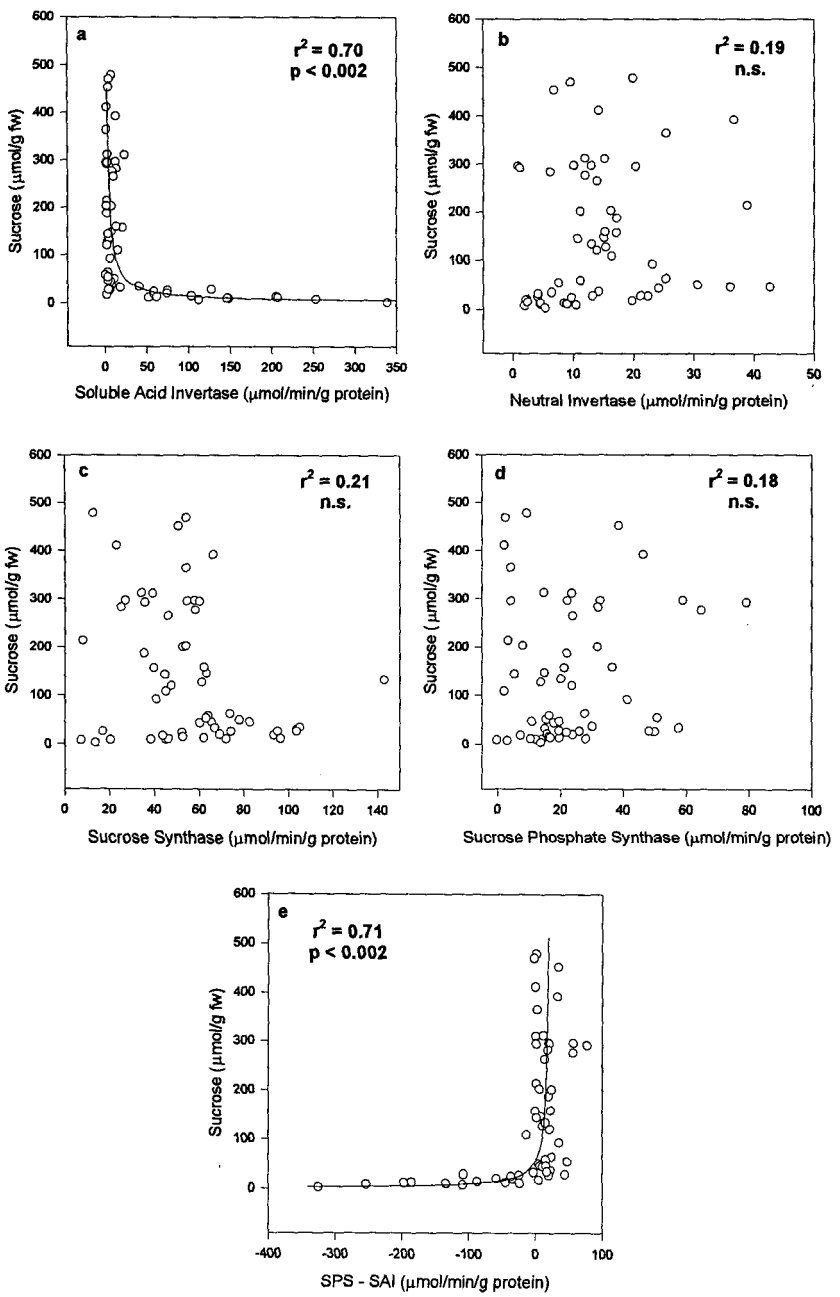

Figure 4. Relationship between Suc concentration and enzyme specific activities of SAI (a), NI (b), SS (c), SPS (d), and SPS minus acid invertase (e) in individual internodes of stalks of 9-month-old plants of the parental high-Suc *S. officinarum* clone Louisiana Purple, the parental low-Suc *S. robustum* clone Mol 5829, and a set of progeny from a cross between the two parents. n.s., Not significant; fw, fresh weight.

genotypes, there was no significant relationship between Suc concentration and activities of NI, SS, or SPS. However, the mean Suc concentration was negatively correlated (r^2 = 0.52, P < 0.03) with the stalk mean SAI activity (Fig. 5a). The correlation between enzyme activity and Suc concentration was greater and positive (r^2 = 0.86, P < 0.001) when activity was expressed as the difference between activities of SPS and SAI (Fig. 5e).

DISCUSSION

Our present analyses of internodes of different ages of two parents and a set of their progeny grown in a common environment suggest a critical role for SAI in limiting Suc accumulation in sugarcane. Developmentally, all clones had their highest activities of SAI associated with low levels of Suc in the immature stem internodes and, conversely, the lowest levels of SAI activity associated with high levels of Suc in the mature internodes. Major differences among genotypes in SAI activity and Suc storage included level of SAI activity in the immature internodes, the level of Suc accumulated in the mature internodes, and developmental timing of the decline of SAI activity with the onset of Suc accumulation.

On a whole-stalk basis, the relationship between SAI and Suc was a highly significant (r^2 = 0.52; P < 0.03), negative, linear one, suggesting a simple, perhaps major, gene difference among the genotypes differing in level of Suc

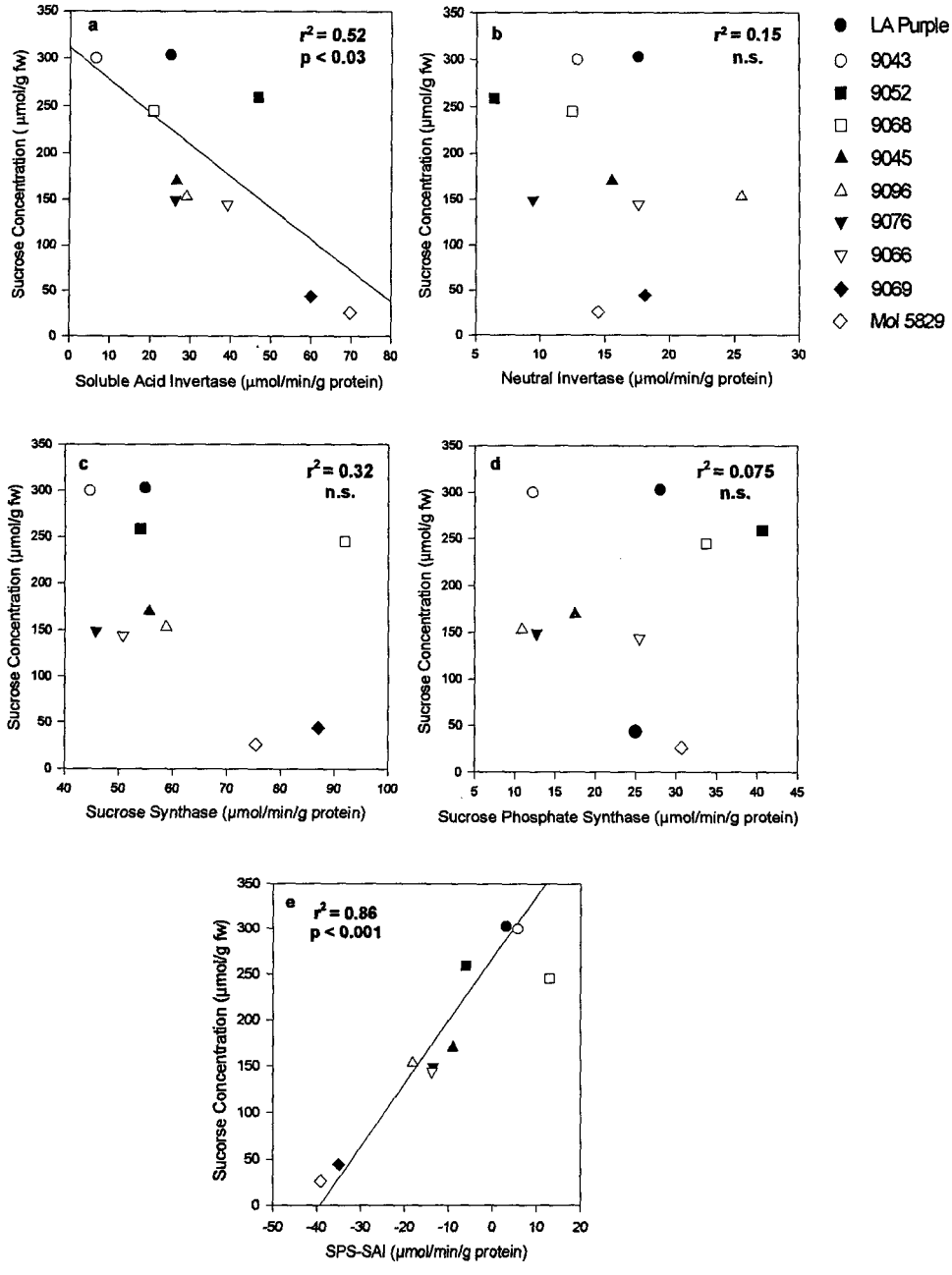

Figure 5. Relationship between mean internode Suc concentrations and mean enzyme specific activities of SAI (a), NI (b), SS (c), SPS (d), and SPS minus acid invertase (e) in whole stalks of 9-month-old plants of the parental high-Suc *S. officinarum* clone Louisiana Purple (LA Purple), the parental low-Suc *S. robustum* clone Mol 5829, and a set of progeny from a cross between the two parents. n.s., Not significant; fw, fresh weight.

stored. The Suc synthesis enzymes SS and SPS were not by themselves correlated with Suc accumulation. However, when activity of SPS was combined with that of SAI, there was an even stronger positive correlation (r^2 = 0.86; P < 0.001). Much of the strength of the correlation could be the result of the very high SAI activity and low Suc content in the low-Suc parent Mol 5829. However, if this clone was removed from the calculation, the correlation coefficient decreased only slightly to r^2 = 0.78 at the same level of significance (P < 0.001). It thus appears that, on the whole-stalk basis, the differences among genotypes in the accumulation of Suc is the result of the balance of Suc synthesized by SPS and the Suc cleaved by SAI.

The inhibitory role of SAI in Suc accumulation is supported by analyses combining individual internodes of all genotypes. In these analyses, none of the enzymes alone, except SAI, was significantly correlated with Suc concentration. On the basis of the strong correlation coefficient, SAI might be considered to be the major or key limiting enzyme. The relationship between SAI and Suc was not a

simple linear regression and correlation, as it should be for a fast, rate-limiting enzyme. Instead, the relationship was a simple, nonlinear regression and correlation indicative of a co-limiting enzyme or a long time delay between the activity of an enzyme and its effect on Suc storage. Assuming that Suc production is accomplished only by SPS, with SAI being the "destructive" participant, the measured in vitro rates of SPS would require 30 d to increase Suc concentration by 100 mM, a common difference between two internodes (Fig. 2), in the complete absence of SAI! Fast-growing sugarcane produces an internode approximately every 10 d, which means that the effect of SPS or SAI change will not show up until three internodes later. Most data, except the few values at the inflection between the level and the ascending arms of the curve, fit this hyperbolic relationship (Fig. 4a). The cluster of 11 points at the inflection between the arms that did not fit the regression line were mostly the intermediate-aged no. 5 internodes, an older internode of the two lowest Suc varieties, and a younger internode of the highest Suc progeny. These internodes represent the developmental stage at which the accumulation of Suc lags behind the decline in activity of SAI below a limiting threshold due to the time needed between a shift in enzyme rates and a measurable shift in Suc level. The time factor is incorporated in the whole-stalk analysis in which the enzyme activities, dominated by the young internodes, are related to the Suc level dominated by the old internodes. In this plot, consequently, a linear regression is seen. It is possible that the relationship between SAI activity and Suc accumulation is the result of two totally independent processes and should be represented by two linear regressions. However, the following discussion supports the idea that Suc storage is the result of co-limiting factors that include SAI and SPS activities and the response time of the system.

The negative hyperbolic relationship between SAI activity and Suc accumulation gives new insights about the Suc accumulation process. The data show that, when SAI activity was high, Suc accumulated but only to a relatively low level; however, when SAI activity was low, Suc accumulated to variable and sometimes high concentrations. To determine the SAI threshold activity below which high-Suc accumulation sometimes occurred, we included all data of the ascending arm in a linear regression to calculate SAI activity at 0 Suc. The calculated SAI activity was 7 μmol min^{-1} g^{-1} protein. Thus, a very low level of SAI activity was sufficient to prevent Suc accumulation. This raises a question of whether some low level of SAI activity, below the threshold, is necessary for maximum Suc accumulation or whether accumulation might be maximized if SAI activity decreased to 0. To estimate the amount of Suc that would be present if SAI activity declined to 0 in our experiments, we regressed the data of high SAI activities plotted on the horizontal arm of the hyperbola. This linear regression indicated that without any SAI activity the sugarcane plant would have attained a Suc concentration of only 24 μmol g^{-1} fresh weight (which is approximately the Suc concentration reached by synthesis via SS).

Low levels of SAI activity could be necessary and sufficient for hydrolyzing Suc in the apoplastic space either for maintenance of a concentration gradient for the unloading from phloem or for retrieval of sugars lost from the storage parenchyma cells. The fact that Suc concentrations were variable at low levels of SAI activity indicates that SAI activity alone is not sufficient for high accumulation of Suc; therefore, additional reactions are required. The additional reactions considered were enzyme activities of NI, SS, and SPS.

When Suc concentration was correlated with any combination of activities involving either NI or SS, there was no significant relationship. A co-limiting role in Suc accumulation for other enzymes was seen only when Suc concentration was plotted as a function of the differences between SPS and SAI activities. The upward bending of the hyperbolic curve, which is the net accumulation of Suc, started only when SPS synthesis of Suc exceeded the SAI hydrolysis of Suc. This value is positive and close to 0. The fact that there was very little or no Suc accumulation when SPS activity was less than SAI activity indicates that the measured enzyme activities in vitro reflect the in vivo activities. Therefore, we believe that the usually high SAI activity measured in vitro was real. There was no indication of an SAI inhibitor in the cells that might have been lost during the enzyme preparation. A relatively small shift to SPS activity in excess of SAI activity is sufficient to allow net Suc accumulation over an extended time, resulting in the vertical line shown in Figure 4e.

The low level of SAI activity that is present in the sugar-storing internodes may partly represent soluble extracellular invertase. Total SAI activity is partitioned between a larger fraction, which occurs in the vacuole, and a smaller fraction, which occurs in the apoplastic space (Sacher et al., 1963). The apoplastic SAI confined to the cell wall or cell surface is postulated to control the flow of Suc from the conducting tissue to the young, growing cells; the vacuolar SAI is concerned with the return of Suc from storage in the vacuole (Hatch and Glasziou, 1963; Sacher et al., 1963; Glasziou and Gayler, 1972). To the degree that this is a correct model of the distribution and function of SAIs, then the higher levels of SAI activity we measured and which prevent the high accumulation of Suc must largely represent the vacuolar fraction of SAI. In the present experiments, the low levels of SAI activity that accompany high Suc accumulation could be primarily the SAI activity of the apoplast.

Gayler and Glasziou (1972a, 1972b) found that apoplastic SAI activity was less than 10% of vacuolar SAI activity in young internodes; during stalk development SAI activity decreased in both compartments but the decrease was faster in the vacuole, resulting in the apoplastic SAI activity being 10 times greater than vacuolar SAI in the mature internodes. In our experiments, the threshold level of SAI activity at which high Suc accumulation occurred is about 10% of the high levels of SAI that restrict Suc accumulation.

The strong negative relationship between vacuolar acid invertase activity and Suc storage and the probably promotive effect of apoplastic acid invertase promise a good potential for manipulation of the expression of the invertase gene(s) to increase Suc accumulation in storage organs such as the stem internodes of sugarcane. These potentials

have been demonstrated in potato (Frommer and Sonnewald, 1995) and are under investigation for sugarcane (Peters et al., 1996; Zhu et al., 1996). For potato the coding region of the mature invertase protein of yeast was fused to the proteinase inhibitor II signal peptide and transformed into plants so that the invertase protein would be localized to the apoplastic space of the tubers (Sonnewald et al., 1991). Transformed potato plants showed improved tuber growth and increased accumulation of reducing sugars. There was up to a 30% increase in the total fresh weight of tubers per plant, thus demonstrating that the apoplastic space is involved in Suc metabolism in growing potato tubers (Frommer and Sonnewald, 1995). For sugarcane the SAI genes are being isolated, cloned, and characterized (Albert et al., 1996; Peters et al., 1996). Future research will attempt to modify the level of expression of SAI both in the apoplastic space and in the vacuole of sugarcane storage parenchyma cells to discover whether localized SAI activity regulates Suc accumulation in sugarcane.

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LITERATURE CITED

Albert H, Zhu YJ, Carr J, Moore PH (1996) Structure and expression of soluble acid invertase genes in the stem of high- and low-sucrose accumulating *Saccharum* species and hybrids. *In* JR Wilson, ed, Sugar 2000. Commonwealth Scientific and Industrial Research Organization, Division of Tropical Crops and Pastures, Brisbane, Australia, pp 102–104

Bradford MM (1976) A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Bull TA, Glasziou KT (1963) The evolutionary significance of sugar accumulation in *Saccharum*. Aust J Biol Sci **16:** 737–742

Clements HF, Ghotb A (1968) The numbering of leaves and internodes for sugarcane nutrition studies. Proc Int Soc Sugar Cane Technol **13:** 569–584

Fernandes AN, Benda GTA (1985) Distribution patterns of brix and fibre in the primary stalk of sugar cane. Sugar Cane **5:** 8–13

Frommer WB, Sonnewald U (1995) Molecular analysis of carbon partitioning in solanaceous species. J Exp Bot **46:** 587–607

Gayler KR, Glasziou KT (1972a) Physiological functions of acid and neutral invertases in growth and sugar storage in sugar cane. Physiol Plant **27:** 25–31

Gayler KR, Glasziou KT (1972b) Sugar accumulation in sugarcane: carrier-mediated active transport of glucose. Plant Physiol **49:** 563–568

Glasziou KT, Gayler KR (1972) Storage of sugars in stalks of sugar cane. Bot Rev **38:** 471–490

Goldner WM, Thom M, Maretzki A (1991) Sucrose metabolism in sugarcane cell suspension cultures. Plant Sci **73:** 143–147

Hatch MD (1964) Sugar accumulation by sugar-cane storage tissue: the role of sucrose phosphate. Biochem J **93:** 521–526

Hatch MD, Glasziou KT (1963) Sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants growth in controlled environments. Plant Physiol **38:** 344–348

Hawker JS, Jenner CR, Niemietz CM (1991) Sugar metabolism and compartmentation. Aust J Plant Physiol **18:** 227–237

Hubbard NL, Huber SC, Pharr DM (1989) Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol **91:** 1527–1534

Komor E (1994) Regulation by futile cycles: the transport of carbon and nitrogen in plants. *In* ED Schulze, ed, Flux Control in Biological Systems. Academic Press, San Diego, CA, pp 153–201

Moore PH (1995) Temporal and spatial regulation of sucrose accumulation in the sugarcane stem. Aust J Plant Physiol **22:** 661–679

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem **153:** 375–380

Peters KF, Grof CPL, Botella J, Albert H (1996) Isolation and genetic manipulation of invertase genes in sugarcane. *In* JR Wilson, ed, Sugar 2000. Commonwealth Scientific and Industrial Research Organization, Division of Tropical Crops and Pastures, Brisbane, Australia, pp 127–129

Rao TCR, Sreenivasan TV, Palanichami K (1985) Catalogue on Sugarcane Genetic Resources. III. *Saccharum barberi.* Jeswiet, *Saccharum sinense.* Roxb. Amend Jeswiet, *Saccharum robustum.* Brandes et Jeswiet ex Grassl, *Saccharum edule.* Hassk. Sugarcane Breeding Institute, Coimbatore, India

Sacher JA, Hatch, MD, Glasziou KT (1963) Sugar accumulation cycle in sugar cane. III. Physical and metabolic aspects of cycle in immature storage tissues. Plant Physiol **39:** 348–354

Sills GR, Bridges W, Al-Janabi SM, Sobral BWS (1995) Genetic analysis of agronomic traits in a cross between sugarcane (*Saccharum officinarum* L.) and its presumed progenitor (*S. robustum* Brandes and Jesw. ex Grassl). Mol Breed **1:** 353–363

Slack CR (1965) The physiology of sugar-cane. VIII. Diurnal fluctuations in the activity of soluble invertase in elongating internodes. Aust J Biol Sci **18:** 781–788

Sonnewald U, Brauer M, von Schaewen A, Stitt M, Willmitzer L (1991) Transgenic tobacco plants expressing yeast-derived invertase in either the cystol, vacuole or apoplast: a powerful tool for studying sucrose metabolism and sink/source interactions. Plant J **1:** 95–106

Sreenivasan TV, Nair NV (1991) Catalogue on Sugarcane Genetic Resources. III. *Saccharum officinarum* L. Sugarcane Breeding Institute, Coimbatore, India

van Handel E (1968) Direct microdetermination of sucrose. Anal Biochem **22:** 280–283

Veith R, Komor E (1993) Regulation of growth, sucrose storage and ion content in sugarcane cells, measured with suspension cells in continuous culture grown under nitrogen, phosphorus or carbon limitation. J Plant Physiol **142:** 414–424

Welbaum GE, Meinzer FC (1990) Compartmentation of solutes and water in developing sugarcane stalk tissue. Plant Physiol **93:** 1147–1153

Wendler R, Veith R, Dancer J, Stitt M, Komor E (1990) Sucrose storage in cell suspension cultures of *Saccharum* sp. (sugarcane) is regulated by a cycle of synthesis and degradation. Planta **183:** 31–39

Zhu J, Komor E, Moore PH (1996) Relationship of sucrose metabolism enzymes with sucrose storage in sugarcane. *In* JR Wilson, ed, Sugar 2000. Commonwealth Scientific and Industrial Research Organization, Division of Tropical Crops and Pastures, Brisbane, Australia, pp 89–91