Sugar Accumulation Cycle in Sugar Cane. II. Relationship of Invertase Activity to Sugar Content & Growth Rate in Storage Tissue of Plants Grown in Controlled Environments [1]

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Introduction

Immature internodes of sugar cane contain an acid invertase with optimum activity between pH 5.0 and 5.5 (1, 2). The level of this enzyme shows marked seasonal variation, being high when growth is rapid and low otherwise. Acid invertase is absent from mature internodes of sugar cane varieties with a high capacity for sugar storage, which instead contain a neutral invertase with optimum activity at pH 7.0. In this paper, we report measurements of acid and neutral invertase activities, rates of elongation of internodes, and sugar contents of plants which were subjected to different temperature and water regimes in controlled environment greenhouses.

Methods & Materials

Growth Conditions. For environmental studies a hybrid cane (variety Pindar) was used. Plants were grown from single bud sets planted on the same day in a vermiculite and gravel mixture in 2 gallon perforated containers. Prior to the commencement of the experiment the plants were watered with a full nutrient solution twice each day.

When the plants were 15 weeks old the experiment was commenced by random allocation of eight plants to each combination of a five temperature, three watering regime factorial design. The temperatures used were 34°, 30°, 26°, 22°, and 18°. For each temperature, different groups of plants were supplied with sufficient nutrient solution to saturate the vermiculite medium either twice daily, four times weekly or twice weekly. Two replicates, each consisting of two plants, were used for each treatment.

Selection of Tissue. Nodes were numbered by regarding the node subtending the leaf showing the last visible dewlap as number 1. Internodes were numbered accordingly. Immature storage tissue was selected by taking the 4 to 5 cm of tissue below the apical meristem. Partially mature tissue was from internodes which had not fully expanded. The second and third internodes above the base of the stalk were sampled as fully mature tissue. In figures in which the internode number is quoted the stage of maturity according to the above selection system is also indicated. The rind was removed from the internodes and the tissue cut into small pieces. Portions of the randomized tissue were used for the various determinations.

Measurement of Growth. Three days prior to harvesting, the stem of each plant was marked by pushing a holder containing 12 needles spaced at 1 cm intervals through the leaf sheath. Upon harvesting and removal of the leaf sheaths the growth rate was measured by the increase in the distance between the needle marks.

Determination of Sugar Content. Three volumes (w/v) of 95% ethanol was added to a weighed portion of the tissue contained in bottles which were capped and heated to 80° for 10 minutes. After standing at room temperature for 3 days the bottles were shaken for 8 hours. Reducing sugars were determined by the method of Hoffman (3) adapted to use with a Technicon Auto-Analyzer. Total sugars were measured by the same method following hydrolysis with dilute HCl. Sucrose, glucose, and fructose are the only sugars which contribute significantly to the total sugar content of sugar cane.

Determination of Moisture Content and Dry Weight. Weighed samples of the tissue were dried at 70° for 5 days then reweighed.

Invertase Assays. Juice was expressed from the tissue and filtered through fine muslin. Preliminary experiments showed that the method of extraction did not alter the invertase content per unit volume of juice. The juice was then dialyzed for 24 hours against a large volume of 0.005 M citrate buffer (pH 7.0) at 2° to remove endogenous sugars. The dialysed juice was assayed at pH 5.4 and pH 7.1 for acid and neutral invertase activity respectively in reaction mixtures containing juice, 0.3 ml, 0.2 M sucrose, either 0.05 M phosphate buffer (pH 7.1) or 0.05 M citrate buffer (pH 5.4), and 0.05 ml of toluene in a total volume of 1 ml. Reactions were incubated for 4 hours at 30°. The reactions were stopped by the addition of 2 ml of ethanol and were then heated at 70° for 5 minutes. Reducing sugar formation was determined as previously de-

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scribed. Controls containing no sucrose were also run. Invertase activities of the mature tissue of different cane species was determined by the same method after the activity was concentrated from the juice by precipitation with $(NH_4)_2SO_4$ (2).

Results

The rate of elongation of internodes, the activity of the neutral and acid invertases and the total sugar content of the storage tissue at the commencement of the experiment are shown in figure 1. Expressed as a function of the physiological age of the tissue, internode elongation and acid invertase activity were directly related as also were total sugar content and neutral invertase activity.

Acid Invertase, Internode Elongation, and Sugar Content of Immature Storage Tissue. After 10 days in different environments the rate of internode elongation and acid invertase content changed proportionally by factors of up to 10 (fig 2). With adequate water at 18° the acid invertase activity remained approximately at the zero time level but increased with increasing temperature up to 34° (fig 3). On the low water regime the activity of the enzyme remained almost unchanged at all temperatures. As can be inferred from the results shown in figure 2 similar relationships are observed if internode elongation is plotted against temperature.

When adequate water was supplied to the plants the total and reducing sugar content of the tissue after 10 days was directly related to acid invertase activity (fig 4). As the temperature and hence the acid invertase activity decreased the ratio of sucrose to reducing sugars increased from 0.4 to more than 2. On the low water regime sugar content was not related to invertase activity, and at each temperature was approximately twice the sugar content of tissue from plants supplied with adequate water.

Qualitatively similar results were obtained after 30 days in the different environmental conditions.

Neutral Invertase Activity and Sugar Content of Mature Storage Tissue. The changes in the total sugar content and neutral invertase activity of mature storage tissue from plants grown for 30 days under different environments are shown in figures 5 and 6. When these data are plotted as change in sugar content against change in neutral invertase, a linear relationship is obtained. The internodes examined in

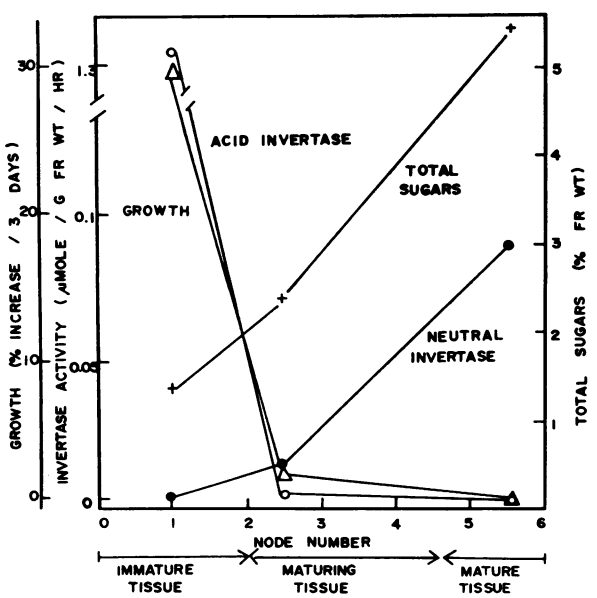


FIG. 1. Acid and neutral invertase activities, sugar content, and rate of elongation of internodes of different maturity at zero time. Zero time refers to the time the 15 weeks-old cane stalks were placed in different environmental conditions. Each value is the average of two determinations. The sampling procedure is described in the Methods and Materials section.

Table I

Relationship between Neutral Invertase & Sugar Content in Maturing Storage Tissue

Stalks were grown for 30 days at 18° under the conditions shown. Tissue was from internodes which had just attained full expansion.

Water regime	Neutral invertase*		Total sugar (% fr wt)	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
2×/day	0.47	0.34	5.8	5.3
4×/week	0.75	0.57	7.0	6.4
2×/week	2.15	2.08	8.9	10.0

* μmoles sucrose hydrolysed/g fr wt-hr.

Table II

Activity of Acid & Neutral Invertases in Mature Storage Tissue of Different Saccharum Species

No. of varieties examined*	Invertase (mμmoles sucrose hydrolysed/g fr wt-hr).** Neutral	Acid	Sucrose % fr wt	Reducing sugar % fr wt	Fiber*** % fr wt
	Saccharum officinarum hybrids				
6	0-151	0	11.0-15.8	0.2-0.6	10.8-14.0
	Saccharum hybrid water shoots				
2	15-75	6-134	3.7-3.9	2.0	5.2-6.2
	Saccharum officinarum****				
7	2-112	0-1	15.2-18.9	0.1-1.2	9.5-13.0
	Saccharum sinense				
1	53	0	13.5	0.1	15.7
	Erianthus varieties				
2	1.4-90	2.8-6.0	0.8-1.7	1.0-1.2	26.9-29.9
	Saccharum spontaneum				
6	0-130	6.1-120	3.8-6.5	0.3-0.9	28.2-42.0

* Where a number of varieties were examined the extremes of the individual estimates are given.

** The procedure for the preparation and assay of enzymes is described in the Methods and Materials section.

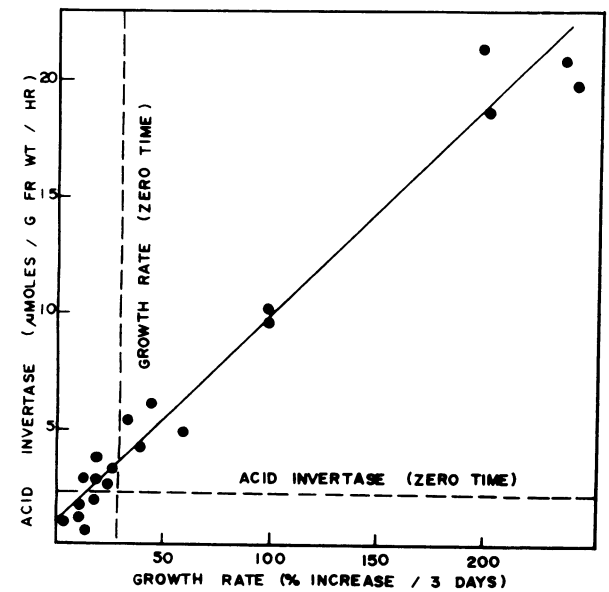
*** Fiber is calculated as non-sugar dry weight. It is mainly cell wall material. Tissue which included rind was used for the determination of the sucrose, reducing sugar, and fibre content.

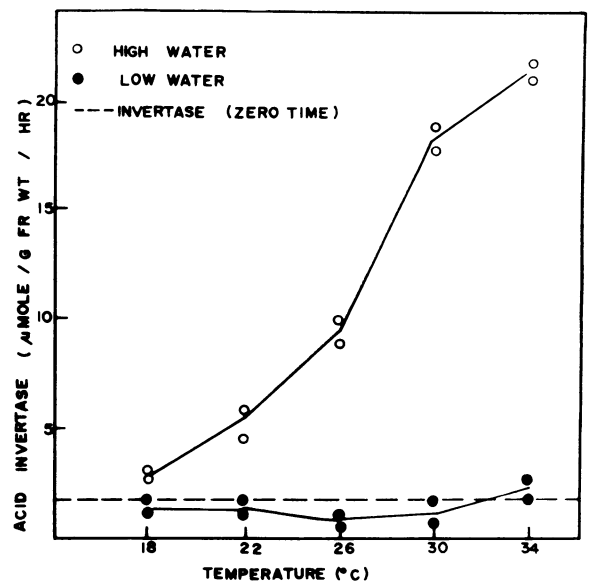
**** Acid invertase was detected in only one variety.

this study were identical with the internodes used at zero time, being the second and third internodes from the base of the stalk. In separate experiments it was shown that during maturation the appearance of the neutral invertase preceded the increase in sugar content of the tissue.

Similar results were obtained with less mature but fully expanded tissue taken from the sixth and seventh internode from the base of the stalk. However, in contrast to fully mature tissue, the neutral invertase activity and sugar content were dependent upon water levels (table I).

Invertase Activity in the Storage Tissue of Different Cane Varieties. Table II shows the distribution of acid and neutral invertase activities in different species of the Saccharum complex which have widely varying capacities for sucrose storage. The mature tissue of varieties of *Saccharum officinarum*, and hybrids thereof contained a neutral invertase but no acid invertase. These varieties are characterized by a high capacity for sugar storage. However, acid invertase was present in the mature storage tissue of the water shoots (tillers which develop under low light intensity and are characterized by having abnormally thick stalks & a low sugar & high water content). Erianthus and *Saccharum spontaneum* varieties are wild canes with a relatively low capacity for sugar storage. Acid invertase was present in the mature tissue of all these varieties. Neutral invertase was detected in all but two varieties examined and was distributed in a manner which was apparently unrelated to species. With the exception of water





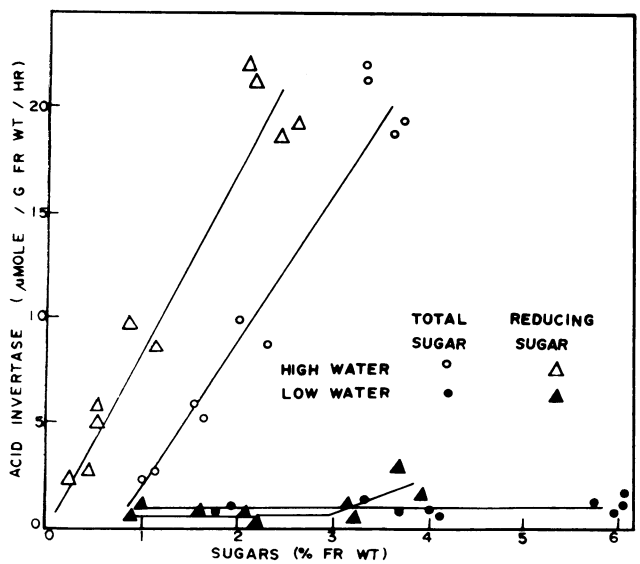
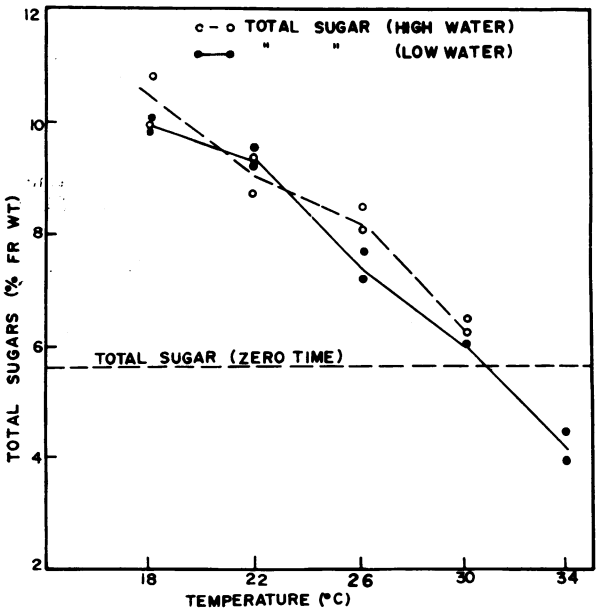


FIG. 2 (*upper left*). Relationship between rate of elongation of immature internodes and acid invertase activity. Plants were grown for 10 days under different conditions of temperature and water regime.

FIG. 3 (*upper right*). Effect of temperature and water on the acid invertase activity of immature storage tissue. Plants were grown under the conditions specified for 10 days. High water and low water refer respectively to plants watered twice each day and plants watered twice each week. Duplicate determinations are shown.

FIG. 4 (*lower left*). Relationship between acid invertase activity and sugar content of immature storage tissue after 10 days in different environmental conditions. Duplicate determinations are shown for plants grown at 18, 22, 26, 30, and 34°. High and low water treatments were as in figure 3.



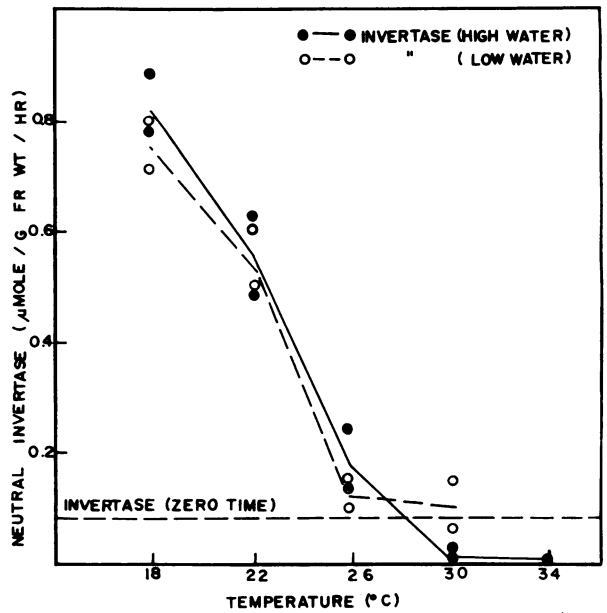


FIG. 5. Total sugar content of mature tissue after 30 days in different environments. Duplicate determinations are shown. High and low water treatments were as in figure 3.

FIG. 6. Neutral invertase activity of mature tissue after 30 days in different environments. Duplicate determinations are shown. High and low water treatments were as in figure 3.

shoots the non-sugar dry matter content of the tissue was much higher in varieties which contained acid invertase.

Discussion

We have suggested that invertases have a key role in regulating the movement of sucrose from conducting tissue and its subsequent utilization for growth or storage (4). In our present studies, the rate of elongation of internodes remained correlated with acid invertase activity irrespective of whether the independent variable was age of tissue (fig 1), temperature or water regime (fig 2 & 3).

The sugar content of mature storage tissue was closely related to the neutral invertase activity when the independent variable was either tissue maturity (fig 1) or temperature (fig 5 & 6), being high when sugar was moving into storage and low otherwise. We consider that this enzyme regulates movement of sucrose from vascular to storage tissue in mature internodes (4). Assuming a regulatory function for neutral invertase, changes in invertase activity and sugar content over a period would not necessarily show a close relationship. Influx of sugar into storage would depend upon the average invertase activity over the period while eflux would vary depending upon the concentration of stored sugar.

The major organic constituents of storage tissue are sugars and cell wall material. Under environmental conditions which gave rapid growth of immature internodes, there was no net sugar storage in the mature internodes of the same stalk. The reverse was also true, indicating that growth and storage are reciprocally related, presumably because of competition for the available photosynthate.

A theory has been advanced (5) that translocation from leaves to other parts of the plant is faster at lower temperatures i.e. has a negative temperature coefficient. The rapid increase of sugars in sugar beet and in stems of sugar cane following a series of cold nights was considered to support this thesis. A similar observation was made in the present investigation. However we suggest that the failure of plants to store sugars at high temperature is due largely to the utilization of the available photosynthate for growth; thus, in the case of cane, the negative temperature coefficient is an artifact of measurement.

Summary

After transferring 3-month-old sugar cane plants to combinations of a five temperature and three watering regimes, the changes in invertase activities of storage tissue were measured and related to changes in growth rates and sugar contents of the tissue. With the hybrid variety used for environmental studies immature storage tissue contained an acid invertase which disappeared as the tissue matured. A neutral invertase was present in mature tissue but was not detected in immature tissue. Upon changes of environment the acid invertase level of immature tissue changed by a factor of up to tenfold in 10 days. The acid invertase content and

rate of elongation of immature internodes were linearly related, both increasing with increasing temperature provided the plant had adequate water. Changes in the total sugar content of mature tissue were related to the neutral invertase activity of the tissue. The neutral invertase activity and sugar content of mature internodes increased as the temperature was decreased. The results are discussed in relation to the concept that invertases of sugar cane storage tissue contribute to the partitioning of available carbohydrate from photosynthesis between the processes of sugar storage and growth.

Acknowledgment

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Sugar Accumulation Cycle in Sugar Cane. III. Physical & Metabolic Aspects of Cycle in Immature Storage Tissues[1]

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Introduction

Sugar cane storage tissues accumulate sugar against a concentration gradient using energy provided by respiration (2). Kinetic and tracer studies show that tissue discs placed in sugar solutions have three distinct compartments (6, 7). These compartments are redefined with specific reference to sugar accumulation. The outer space which includes the cell walls is the zone of the tissue in rapid diffusion equilibrium with sugars and ions in the bathing solution. The metabolic compartment is a zone in which hexoses are phosphorylated and interconverted. Movement of anions including phosphorylated sugars between the bathing solution and this zone is restricted. It is also assumed to be the locus of synthesis of a sucrose derivative termed sucrose-X, cleavage of which provides the necessary energy for accumulation of sucrose against a gradient into the storage compartment. The storage compartment is the zone in which sucrose, glucose, and fructose are accumulated and which is bounded by a permeability barrier resistant to diffusion of sugars.

For tissue slice studies on sugar storage we use the following terms. Uptake describes the total amount of sugar removed from the medium and retained by the tissue after washing, irrespective of its form. That part of the total sugars taken up which appears as sugar in the storage compartment is defined as accumulation.

Sucrose is virtually the sole sugar transferred from the metabolic to the storage compartment. Reducing sugars in the storage compartment are derived from hydrolysis of accumulated sucrose (7). All sugars diffuse slowly from the storage compartment, reducing sugars diffusing more rapidly than sucrose (6). Immature storage tissue contains an invertase which is optimally active between pH 5.0 and 5.5 (8, 11). The enzyme is absent from more mature tissue which contains higher total sugar but

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