

## RESEARCH ARTICLE

## SUCROSE SYNTHESIS AND ACCUMULATION IN CONTRASTING COMMERCIAL HYBRIDS OF SUGARCANE

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## Abstract

Sugarcane has been bred with a focus of obtaining high sugar varieties. Identifying associated traits assumes importance for genetic manipulation. Enzymes of sucrose metabolism were researched and reviewed over decades. In the present study sucrose content in individual internodes, and related enzymes *viz.*, invertases (acid and neutral) and sucrose phosphate synthetase (SPS) were assessed. Sucrose content ranged from 119 mg/g to 432 mg/g, while acid invertase (AI) ranged from 1.35  $\mu\text{mol g}^{-1}\text{h}^{-1}$  to 52.3  $\mu\text{mol g}^{-1}\text{h}^{-1}$ . Enzymes SPS did not show any specific trend among different internodes; however the SPS/AI ratio showed significant correlation with sucrose content. Significant differences were observed between high and low sugar varieties with regard to the parameters studied.

**Key words:** Sucrose p. synthetase, sucrose synthetase, Acid invertase, sucrose, total sugars

## Introduction

Sucrose is the major product from sugarcane worldwide. Sucrose yield can be enhanced either by increasing biomass or sucrose concentration per se. The later approach is considered to be more profitable as the cost of harvesting, transport and milling are not increased (Jackson et al. 2000). The scope of increasing sucrose concentration depends on enhanced synthesis and or reduced inversion processes. In this regard the most related enzymes of sucrose cycle *viz.*, sucrose phosphate synthetase and invertases play important role.

Hatch and Glasziou (1963) reported that acid invertase is present in immature sugarcane tissues which are replaced by neutral invertase activity in mature storage tissue. It is present in tissues undergoing rapid growth where sucrose was rapidly hydrolysed (Schaffer, 1986; Beruter and Studer, 1997) and the resultant hexoses were effectively utilized for growth and development. Alkaline or neutral invertase activities during

development has been reported in sugarcane (Hatch and Glasziou, 1963).

The function of neutral invertase is to catalyse sucrose hydrolysis in cells of storage organ that lack appreciable acid invertase activity. Neutral invertase is present in considerable amount in mature tissues and catalyses the hydrolysis of terminal non-reducing  $\beta$ -fructofuranoside. It may also play a key role in the control of hexose concentration in the cytosol of sugarcane stem cells, thus affecting control over the expression of sugar responsive genes (Vorster and Botha, 1998).

Several attempts have been made to correlate invertase activity with internode growth and sucrose content of sugarcane stalk (Hatch and Glasziou, 1963; Madan, 1981; Venkataramana et al. 1991). Sugar accumulation rate was not consistently correlated with activity of any enzyme assayed. However, the combined influence of SPS and AI as key players in determining sucrose content was strongly recommended, during maturation of sugarcane internodes (Lingle, 1999; Zhu et al.

2000). The ripening of sugarcane is associated with the increase in sucrose concentration in mature stem tissue (Moore,1995). Sucrose accumulation is not merely a function of time as the rate of accumulation significantly increases between young and mature internodes (Botha et al. 1996). It is in this respect the present work was attempted to elucidate the role of invertases and SPS on sucrose concentration in sugarcane internodes and any variability between high and low sugar types.

### Materials and Methods

A field experiment was conducted at Sugarcane Breeding Institute, Coimbatore (76.59° E longitude and 11.02° N latitude), 426.72 m (TMSL), India, during 1999-2004, utilizing 12 sugarcane (*Saccharum officinarum*, L.) hybrid varieties representing high and low sugar content at harvest. Typical high sugar varieties CoC 671 and Co 1282 both belonging to early maturing group were utilized for the study.

### Brix (%)

Total solid sugar present in the juice sample was estimated using brix refractometer. About 200 $\mu$ l of the extracted juice of each internode tissue was placed on a refractometer and the total solid sugar measured by the refractometer was recorded.

### Sucrose content

The sucrose content in the sample was estimated as per Ashwell (1957). The juice extracted from each internode tissue was collected separately and desired dilutions were made. One ml of the extract was used for sucrose determination. Sucrose content was estimated using resorcinol reagent. The amount of sucrose present in the sample was determined using the standard graph.

### Acid and neutral invertases

Acid invertase was estimated as per Hatch and Glasziou (1963). Top 1 to bottom 20 internodes

were collected and 1g of representative intermodal tissue was used for invertases assay. A modified method of Hatch and Glasziou was followed (Venkatramana et al. 1991). The amount of reducing sugars formed was determined against a standard curve prepared with known concentrations of glucose as in identical experimental condition.

The neutral invertases was extracted and assayed principally in similar method, but the citrate buffer (pH ) was replaced with phosphate (pH 7.0).

### Sucrose phosphate synthetase

SPS activity was estimated by the method described by Hubbard et al. (1989). Internodal tissue was cut into small pieces and ground with 5ml of cold phosphate buffer (pH 7.0) containing 20mM  $\beta$ -mercaptoethanol and using prechilled pestle and mortar. The filtrate was centrifuged at 10,000 rpm for 15 minutes. The resultant supernatant was used for estimating SPS.

## Results and Discussion

### Sucrose accumulation

Brix value, a measure of soluble solids in the juice ranged from 11.8 to 18.3 in low sugar variety (Co 1282) and variation among the internodes was not substantial (Fig.1). There was no specific trend in HR brix% from top 1 internode to bottom 20<sup>th</sup> internode. However, in the variety CoC 671 the brix% showed a definite increasing trend from top 1 to bottom 20<sup>th</sup> internode. From internode no.11 to 21 the brix % was >24 indicating higher sucrose levels in bottom internodes. Only top four internodes showed <15% brix in this variety. Sucrose accumulation commences from the bottom most internodes and progresses upwards and thus maturity becomes an internode to internode process regulated by the balanced activities of invertase enzymes. Sucrose content ranged from 136 mg/g to 432 mg/g in variety

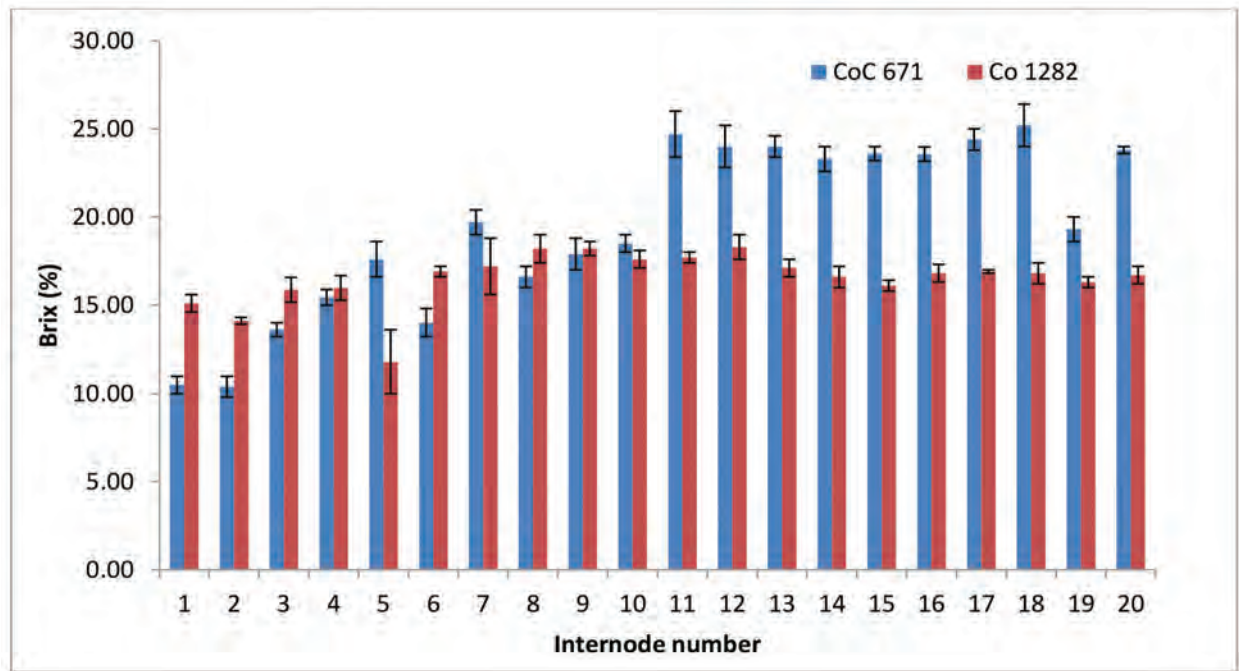


Fig. 1. Juice brix % in high (CoC 671) and low (Co 1282) sugar varieties from one to twenty internodes

CoC 671 and no. of internodes accumulating >400 mg/g, sucrose was ten. In the variety Co 1282 the sucrose content ranged from 119 to 409 mg/g with just one or two internodes accumulating about 400 add  $\mu\text{g/g}$  of sucrose (Fig. 2). The top five internodes recorded lower sucrose content.

Total sugars varied from 148 to 299 mg/g

(glucose equivalent) in internodes 1 to 20 in the variety CoC 671 (Fig. 3) and in Co 1282 the sugar content ranged from 133 to 289 mg/g among the internodes (Fig. 3). There is no specific trend in total sugar levels among the internodes in both the varieties studied.

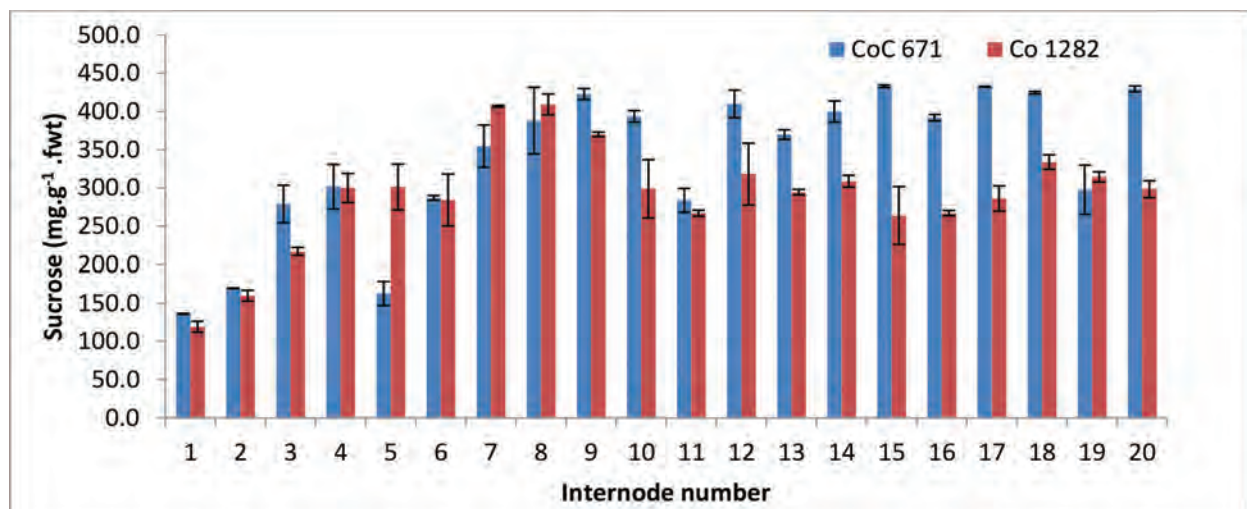


Fig. 2. Sucrose content in stalk tissue of high (CoC 671) and low (Co 1282) sugar varieties from one to twenty internodes

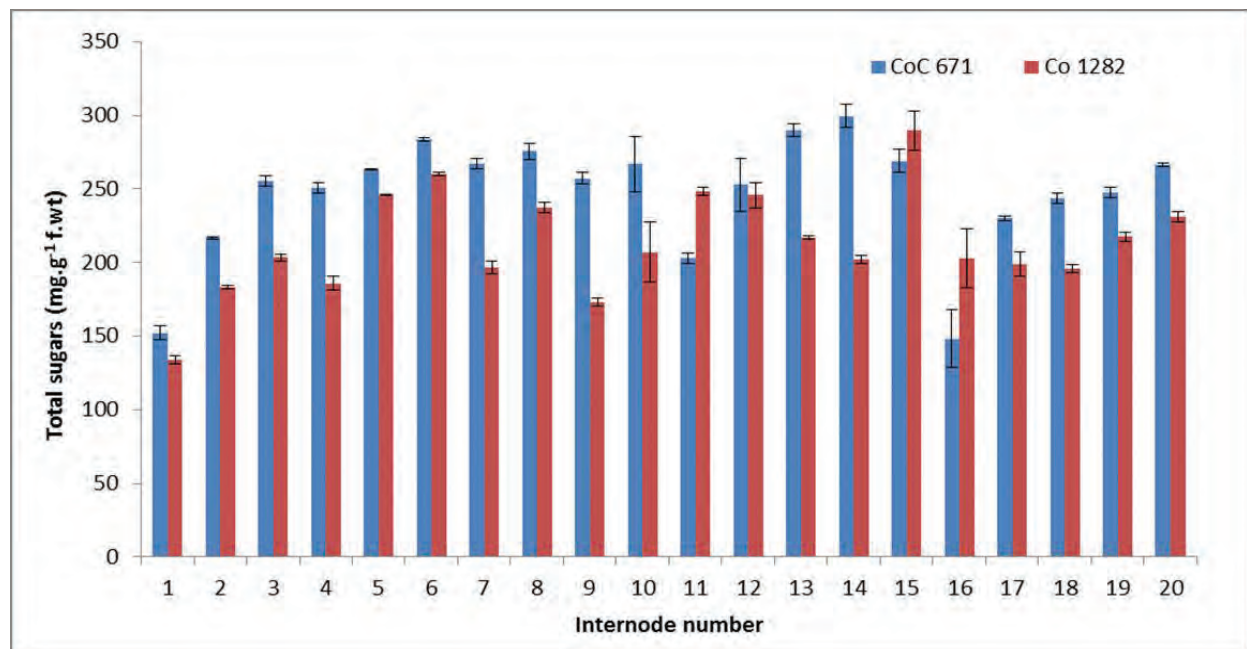


Fig. 3. Total sugar content in stalk tissue of high (CoC 671) and low (Co 1282) sugar varieties from one to twenty internodes.

**Table 1. Results of ANOVA (F-value) showing the relationship of SPS/AI ratio, acid invertase with sucrose in sugarcane genotypes**

Model	Df	SPS/AI ratio vs Sucrose	Acid invertase vs Sucrose
Regression	1		
Residual	38	11.61**	72.30**
Total	39		

\*\*Significant at 1%

The hexose level was more or less similar in both the varieties suggesting the energy availability for metabolic functioning. The sucrose and hexose levels were in accordance with earlier reports (Zhu et al. 2000).

### Enzymes of sucrose cycle

Among the enzymes studies in individual internodes acid invertase and SPS/AI ratio had significant relationship with sucrose content

(Table1). A significant and negative correlation existed between sucrose content and AI activity among the individual (20) internodes studied in both the varieties (Fig. 7a). Immature top internodes (from internode no.1 to 6) recorded very high activity of  $>20 \mu \text{mol sucrose hydrolysed g}^{-1} \text{h}^{-1}$ . However, the activity abruptly reduced to 1 and almost nil activity in bottom internodes in the variety CoC 671 (Fig. 4a). In the low sugar variety, Co 1282 the trend remained the same (Fig.4b), however, the activity was higher in the bottom internodes as compared to the similar internodes of high sugar variety CoC 671.

The neutral invertase activity also showed similar trend with top immature internodes recording higher activity while in bottom internodes the activity was less (Fig. 5a). Variety CoC 671 recorded higher activity ( $35.22 \mu \text{mol g}^{-1} \text{h}^{-1}$ ) in top 2 internodes and almost nil activity in bottom internodes ( $1.35 \mu \text{mol g}^{-1} \text{h}^{-1}$ ). Although the trend remained same in the variety Co 1282 (Fig.5b), the neutral invertase was higher in bottom internodes as compared to the variety CoC 671.



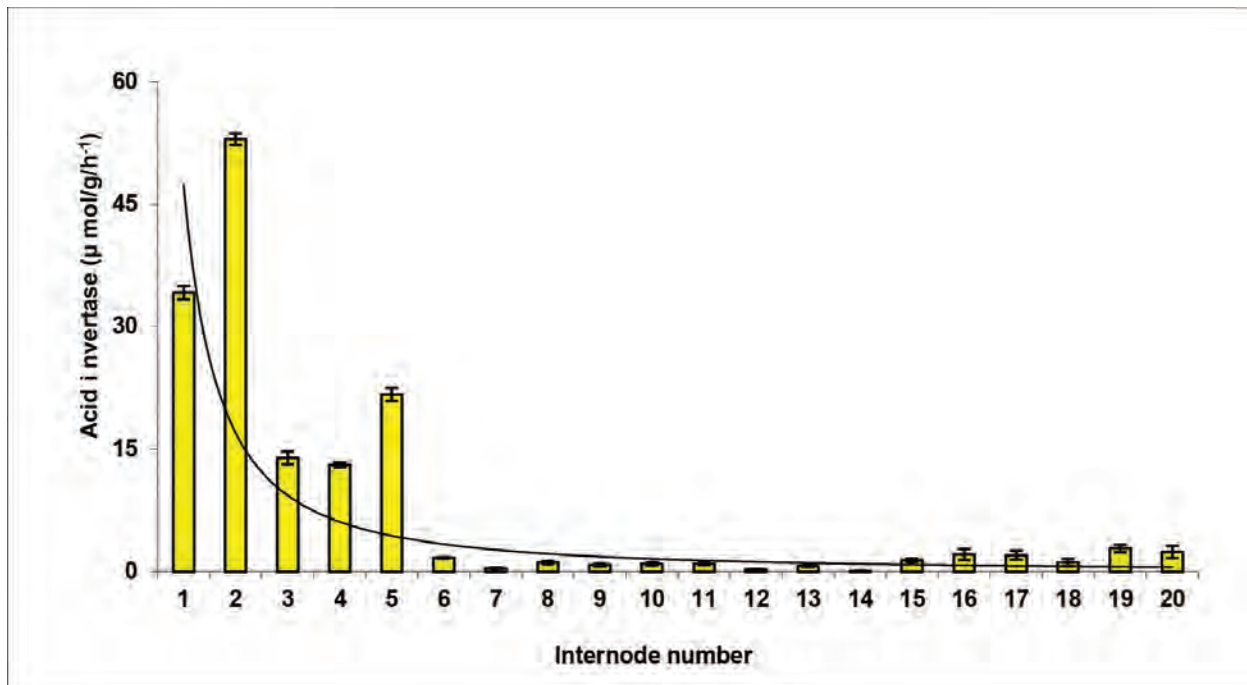


Fig. 4a. Acid invertase activity in stalk tissue of high (CoC 671) sugar variety from one to twenty internodes

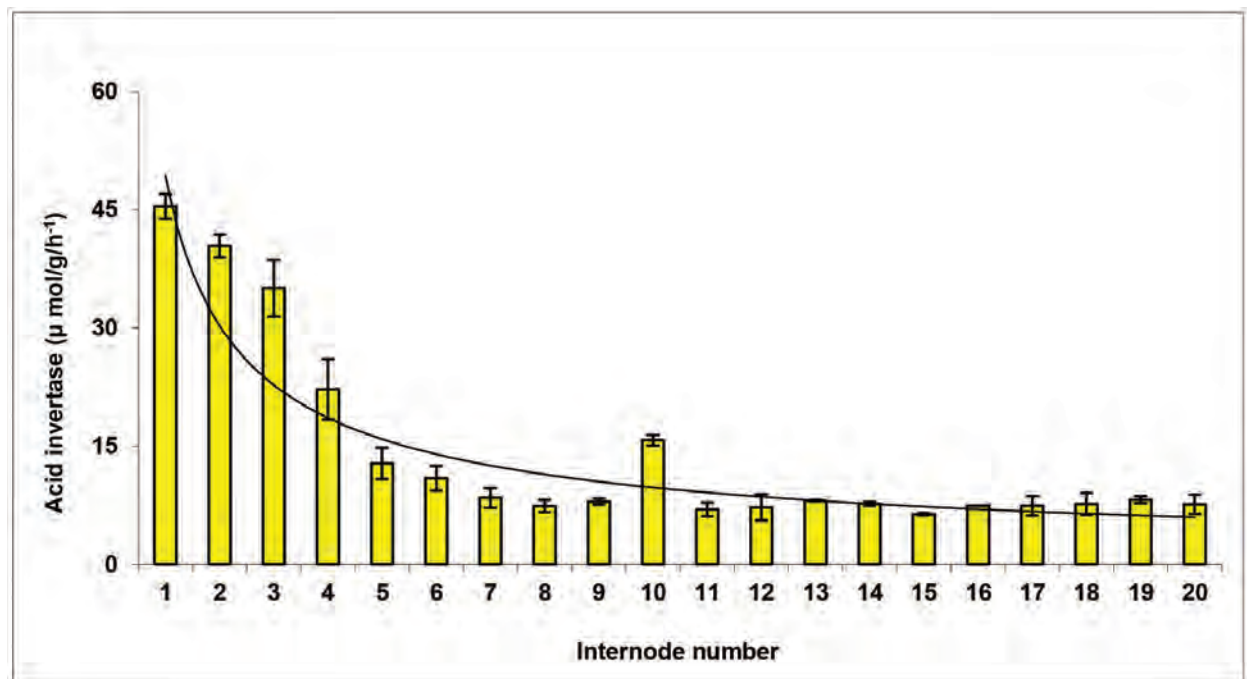


Fig. 4b. Acid invertase activity in stalk tissue of low (Co 1282) sugar variety from one to twenty internodes

A similar trend of acid and neutral invertases activity in young and old (mature) internodes was documented in sugarcane (Venkataramana and Naidu, 1993).

Sucrose phosphate synthase activity was higher in bottom internodes in the variety CoC 671 while, the top immature internodes recorded less activity (Fig. 6). The variety Co 1282 failed to show any

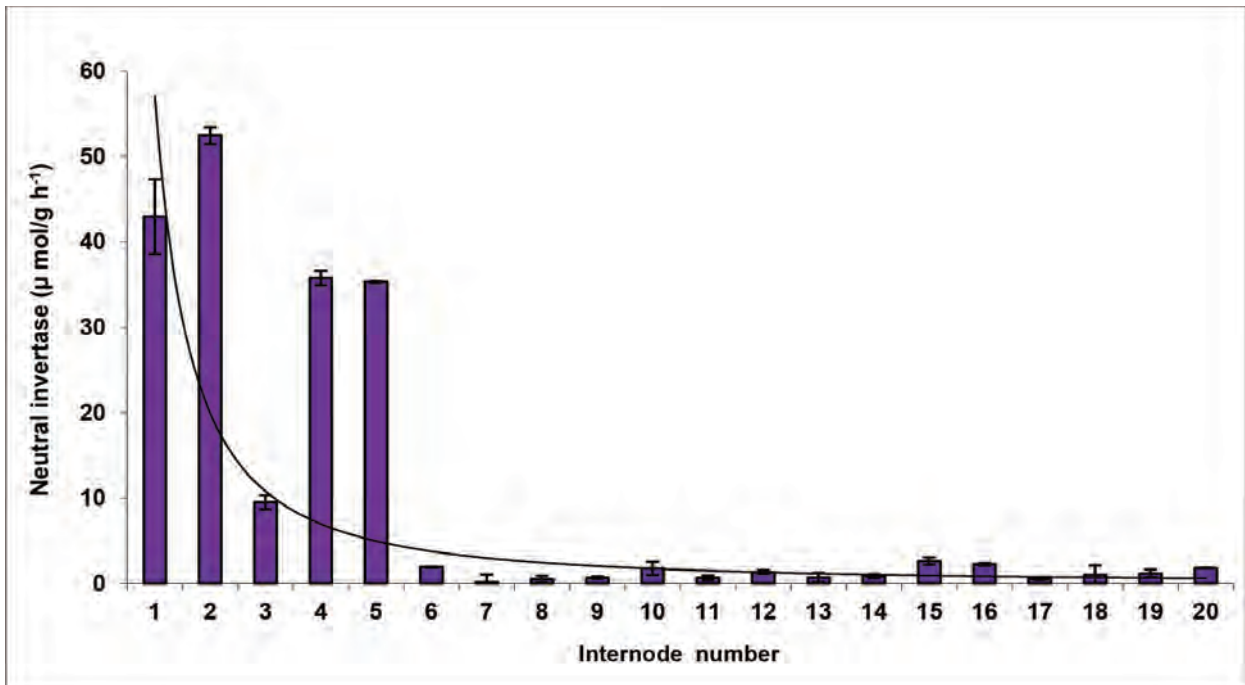


Fig. 5a. Neutral invertase activity in stalk tissue of high (CoC 671) sugar variety from one to twenty internodes

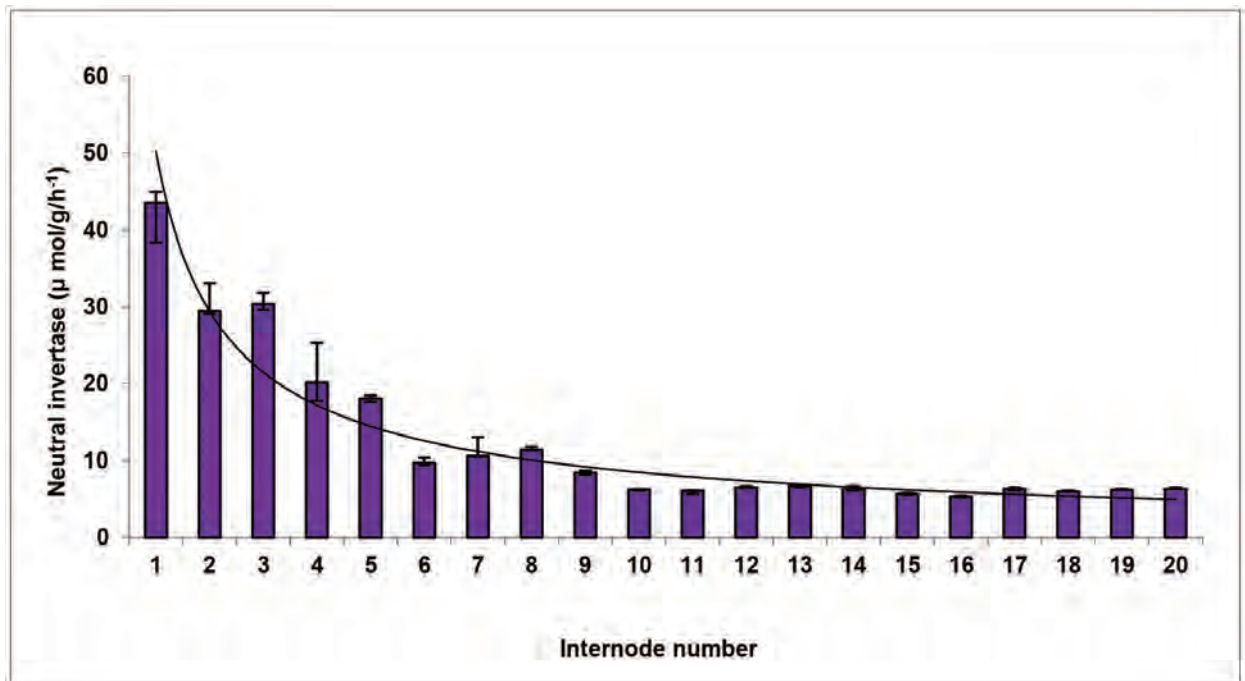


Fig. 5b. Neutral invertase activity in stalk tissue of low (Co 1282) sugar variety from one to twenty internodes

specific trend and top internodes recorded activity of  $7.0 \mu\text{mol g}^{-1} \text{h}^{-1}$  while, in bottom five internodes the activity was  $6.9 \mu\text{mol g}^{-1} \text{h}^{-1}$ . The comparable SPS and SS specific activity in sugarcane

internodal tissue during maturation was reported by Botha and Black in 2000. In older internodal tissue SPS activity was atleast three times higher than the soluble sucrose content (Botha and

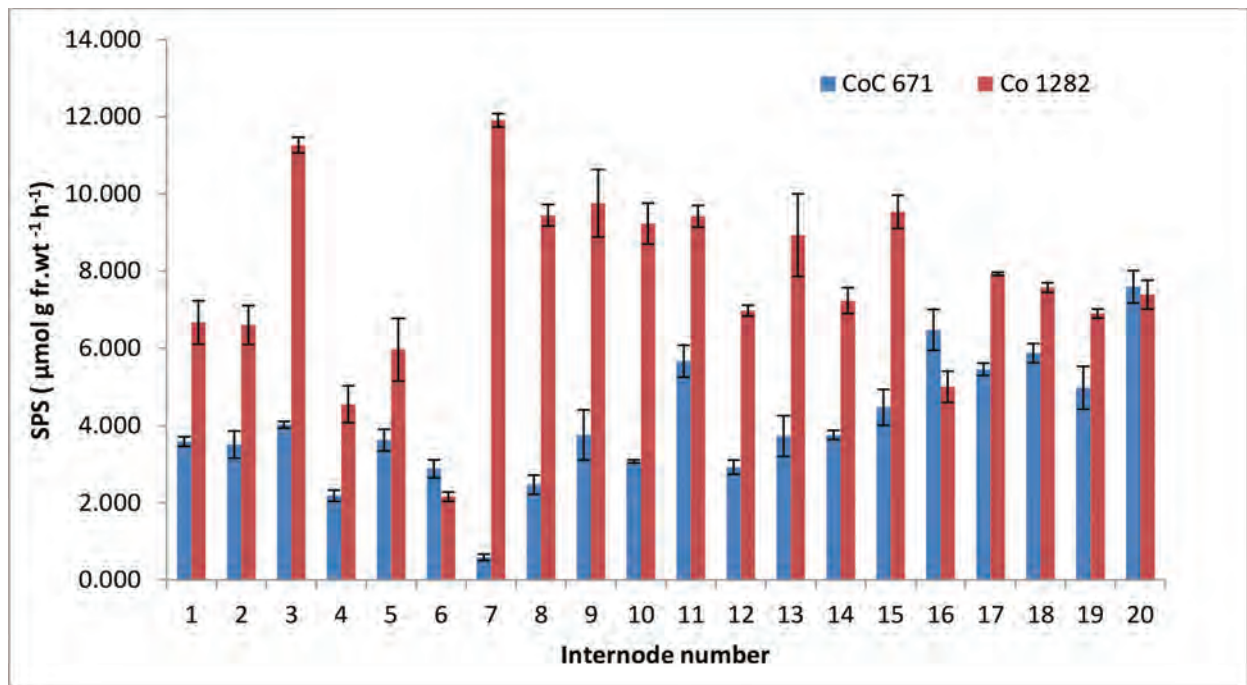


Fig. 6. SPS activity in stalk tissue of high (CoC 671) and low (Co 1282) sugar varieties from one to twenty internodes

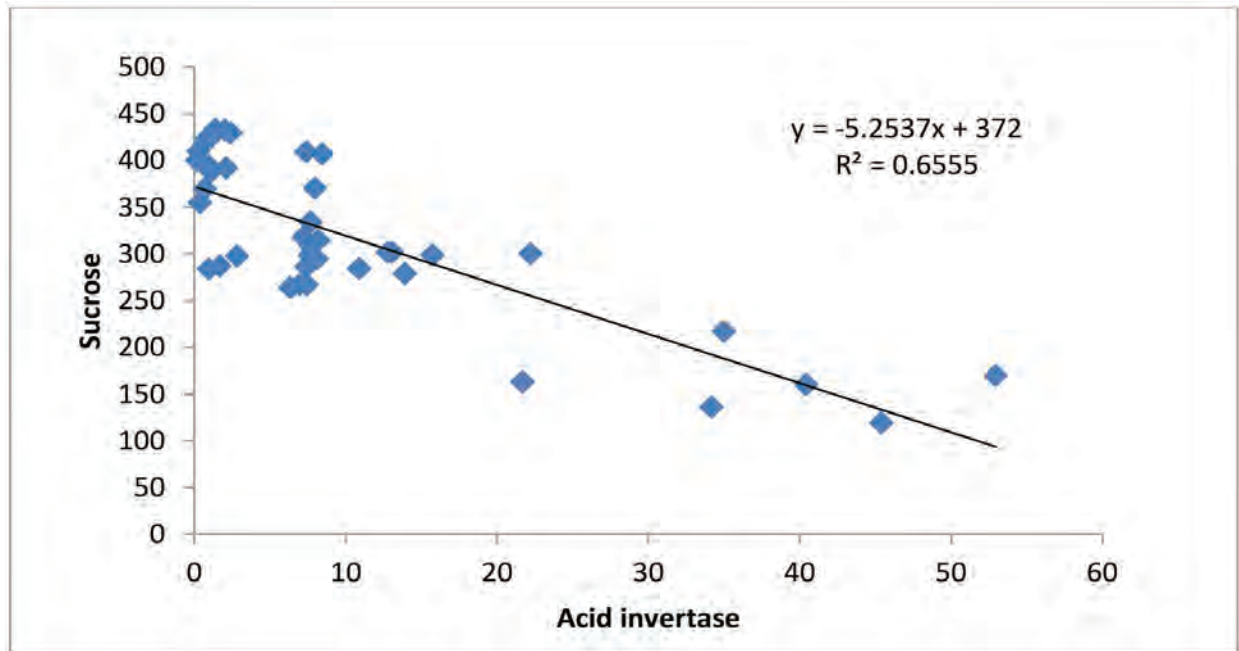
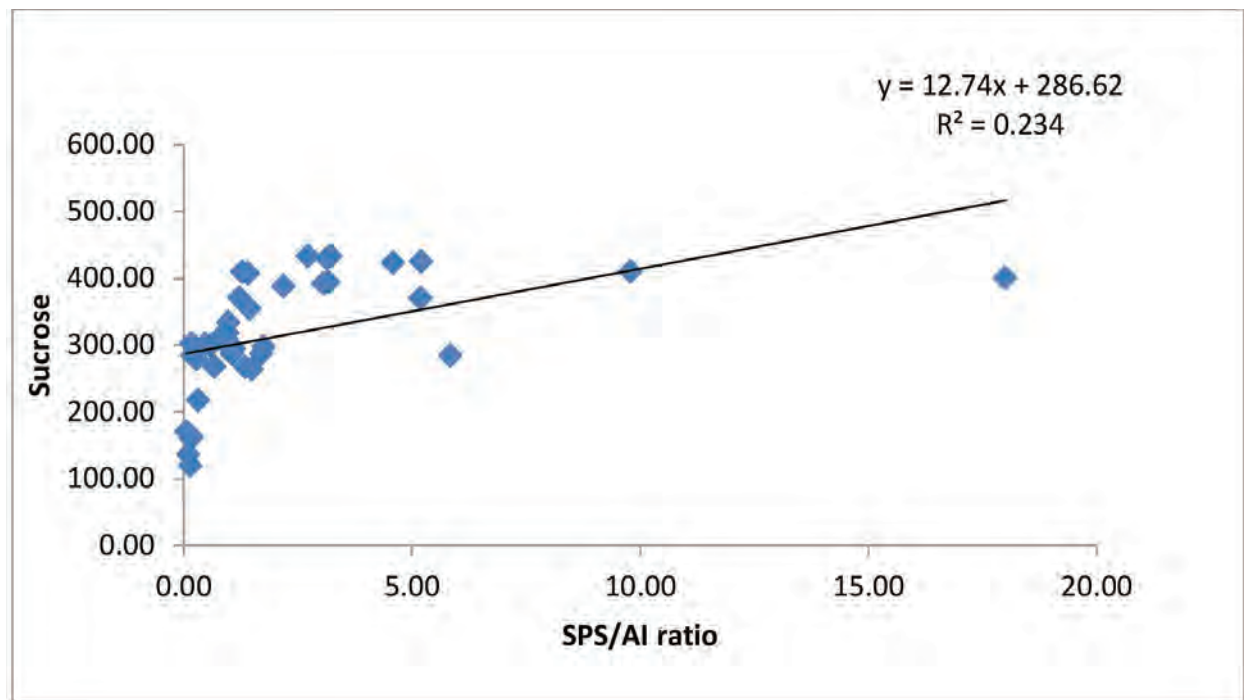


Fig. 7a. Association of acid invertase with stalk sucrose content in sugarcane genotypes

Black, 2000). SPS exerts more control at higher rates of photosynthesis and, has a greater control over maximum flux through the sucrose synthesis pathway (Huber & Huber 1996; Stitt 1989 and

Stitt & Quick 1989). SPS activity is well correlated with leaf sucrose content across the range of varieties (Christopher et al, 1998 and Grof et al., 2001).



**Fig. 7b.** SPS/AI ratio vs sucrose of acid invertase with stalk sucrose content in sugarcane genotypes

The ratio of SPS/AI correlated significantly and positively with sucrose content (Fig.7.b). The ratio of SPS to AI in internode tissue clearly indicates the advantageous position of sucrose accumulation and storage in higher sugar as compared to low sugar variety. The ratio suggests the higher net sucrose concentration in intermodal tissue (Zhu et al. 2000). Soluble acid invertase (SAI) activity is regarded to have an inverse relationship with sucrose accumulation in sugarcane, that is, sucrose (SUC) accumulation in the whole stalk and within individual sugarcane internodes was correlated with the down-regulation of soluble acid invertase (Zhu et al. 1997). SAI concentration is usually high in tissues that are fast growing, such as cell and tissue cultures, root apices, and immature stem internodes but decreases rapidly during internode growth and development (Zhu et al. 2000). It was found that low sugar varieties retain relatively high levels of SAI in the stalk as compared to high sugar types. Specifically, major differences in SUC accumulation among the

population were ascribed to differences between activities of SAI and SPS, provided SAI is below the critical threshold concentration (Zhu et al. 1997).

### Conclusion

The sucrose content distinctly varied among genotypes. Higher sucrose levels in cane as a single factor contribute for improved sugar recovery. Therefore, identifying high sucrose types is a priority component in breeding processes. In this regard the traits *viz.*, low invertase activity and a high sucrose phosphate synthetase/AI ratio would immensely contribute for identifying high sucrose types. Confirmation of the usefulness of these traits in a mapping population ultimately aid identifying high sucrose types in early stages of varietal evolution.

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