



SUGAR METABOLISM OF PLANT AND RATOON CROPS IN SUGARCANE

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Received on 12th July, 2010, Revised and Accepted on 21st Feb., 2011

SUMMARY

Investigation on the sugar metabolism through various enzymatic assay viz. sucrose phosphate synthase (SPS), sucrose synthase (SS), acid and neutral invertase (AI & NI) activities, carbon allocation in the form of reducing, non reducing and total sugar content in relation to juice quality of plant and ratoon crop was made in 11 sugarcane varieties during maturity phase. Results of the study revealed that, except acid invertase (AI), all these enzyme activity was significantly higher in ratoon crop as compared to plant crop. Though the AI activity negatively correlated with sucrose % juice both in plant ($r = -0.562^*$) and ratoon crop ($r = -0.235$), the association found to be significant only in plant crop. Among the varieties studied, Co 94012, CoC 671, Co 86032 and Co 99004 showed higher brix %, sucrose % and purity % than rest. Differences between plant and ratoon and among the varieties in juice quality might be related to differences in activities of sucrose metabolic enzymes viz., SPS, SS, and AI & NI between plant and ratoon crops. Apart from these enzymes, higher carbon allocation particularly non reducing sugars towards economic sink might also contribute for high sugar in ratoon crop.

Key words: Acid and neutral invertases, juice quality, sugarcane, sugar metabolism, sucrose phosphate synthase, sucrose synthase

INTRODUCTION

Sucrose accumulation by sugarcane is regulated at the level of the sink, where cycling of carbon between hexoses and sucrose is believed to be an important determinant of sink strength (Lingle 1997 & 1999). The sucrose synthesized in its leaves is translocated to the stem where it is hydrolyzed by the cell wall invertases before the hexose moieties are taken up by the storage cells (Hatch and Glasziou 1963). Though several enzymes are involved in the regulation of sucrose synthesis, cytosolic fructose 1, 6-bisphosphate and sucrose phosphate synthase (SPS) are recognized to be the key enzymes for sucrose synthesis (Stitt *et al.* 1988). Attempts have already been made to correlate invertase activity with internodal growth and sucrose content of

sugarcane stalk (Venkataramana *et al.* 1991, Lingle 1999, Zhu *et al.* 2000, Vidyasekar 2007). Increase in cell wall acid invertase activity with internode age is concomitant with the increase in sucrose and sucrose to total sugar ratio, thus they contribute sucrose accumulation in sugarcane (Lingle 1999). Sugarcane neutral invertase had a high specific activity than soluble acids invertase (apoplasmic and vacuolar) in the sucrose accumulating region of sugarcane stem.

Ratooning of sugarcane is common throughout the world and ratoon occupies a sizable proportion of the total area under sugarcane cultivation. Ratoon crops, in general, mature earlier than plant crop and give high sugar recovery than plant crop when crushed early in the season (Mohan Rao *et al.* 1956). Apart from the factors

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like early shoot growth, lower hydration and lack of nitrogen responsible for higher sugar content of a ratoon crop (Mohan Rao and Narasimhan 1954) some of the biochemical factors like enzymes responsible for sucrose synthesis (SPS and SS) and accumulation (Acid and Neutral invertase) might be responsible for high sugar recovery in ratoon crop. Since, research in this area is very much scanty, the present study reports on sucrose synthesizing and accumulating enzyme activities in relation to juice quality of plant and ratoon crops of sugarcane varieties.

MATERIALS AND METHODS

A field experiment was conducted at Sugarcane Breeding Institute, Coimbatore during first week of February, 2008 with popular varieties (CoC 671, Co 8021, Co 85019, Co 86032, Co 94012, Co 95020, Co 97008, Co 97009, Co 99004, Co 99008 and Co 2000-10) replicated thrice in RBD. Each variety was planted in six rows in each replication, with a gross plot size of 32.4 m² (6.0 x 0.9 x 6). The 2- budded setts were spread over the furrow with a standard count of 80 buds per row of 6.0 m length, spaced 0.9 m apart (75,000 per hectare), respectively. On completion of harvest of the plant crop (2nd week of February, 2009) the same field was allowed for raising ratoon crop (4th week of February, 2009) and the cultural operations of ratoon crop were followed as per the recommendations. The data on the sucrose accumulation pattern through various enzymatic sucrose phosphate synthase (SPS) and sucrose synthase (SS) was assayed during 6th, 7th, 8th & 9th month, acid and neutral (AI & NI) invertases assayed at monthly intervals during maturity phase (8th, 9th & 10th month) of the plant and ratoon crops. Reducing, non-reducing and total sugars were assayed at 10th month. Juice quality parameters were estimated at 10th and 12th month of plant and first ratoon crops.

Agro metrological condition

Data on weather parameters on maximum and minimum temperatures, after noon relative humidity, total rainfall and rainy days were for two cropping seasons (2008-2009 & 2009-2010). In plan crop season (Feb 2008 to Jan. 2009), the mean maximum and minimum temperatures were 35.70 °C and 30.40 °C respectively,

and the average afternoon relative humidity ranged from 81.0 to 89.10 % (Fig.1). During this season, a total rainfall of 680.70 mm was received in 52 rainy days (data not shown). In ratoon crop season (Feb 2009 to Feb 2010), the mean maximum and minimum temperatures were 37.3 °C and 29.40 °C, respectively and the average after noon relative humidity ranged from 81.50 to 90.50% (Fig.1). Total rainfall received during the season was 688.10 mm in 49 rainy days (data not shown)

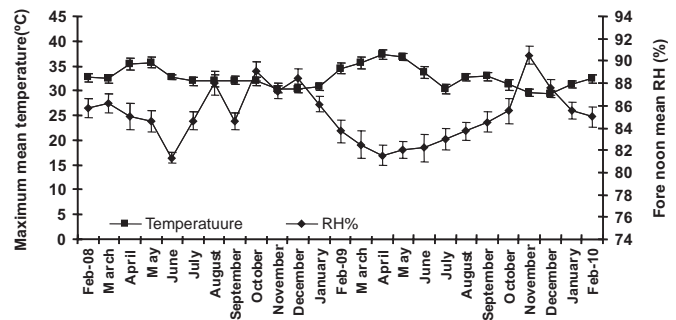


Fig. 1. Agro meteorological parameters of 2008- 2009 crop season (plant crop) and 2009 -2010 crop season (ratoon crop). Vertical bars represent the standard error ($P < 0.05\%$)

Extraction & quantification of sucrose synthesizing enzymes (SS and SPS)

Sucrose synthase and Sucrose phosphate synthase activity were estimated according to Huber *et al.* (1989). 100 mg of fresh leaf sample was ground with 5 ml of cold phosphate buffer (pH 7) containing 20 μ l mercaptoethanol by using pre chilled pestle and mortar. The filtrate was centrifuged at 10,000 rpm for 15 minutes and the resultant supernatant was used for estimating SPS and SS activity. The reaction mixer for SPS enzyme assay was Tris HCl (0.7 ml), fructose-6-phosphate (0.02 ml), MgCl₂ (0.2 ml), UDPG (0.2 ml), NAF (0.2 ml) & enzyme extract (0.5 ml). The reaction mixer for SS enzyme assay was Tris HCl (0.7 ml), Fructose (0.02 ml), and MgCl₂ (0.2 ml), UDPG (0.2 ml), Distilled water (0.2 ml) & enzyme extract (0.5 ml). Both the tubes were incubated at 30°C and reaction was stopped by adding arsenomolybdate reagent and the colour developed was read at 700 nm by using spectrophotometer. The amount of sucrose formed was quantified against a standard prepared with known concentration of glucose in identical experimental conditions and expressed as μ g g⁻¹ hr⁻¹.

Extraction and quantification of sucrose accumulating enzymes (SS and SPS)

Acid and neutral invertase was estimated according to Huber *et al.* (1989). The stem samples of each variety from each samples were cut into discs and pre chilled. The pre chilled cane discs (1g) were treated with 5 ml cold ethyl acetate for 20 min and ethyl acetate was decanted and then discs were washed with distilled water until the smell goes off. The invertase activity was estimated in a reaction mixture containing (10 ml). The reaction mixture for acid invertase is 5 ml of 0.05 M citrate buffer (pH 5), 2 ml of 0.2 mM sucrose, 0.1 ml of toluene, and 3 ml of distilled water. The reaction mixer for neutral invertase is 5 ml of 0.05 M Na₂PO₄ buffer (pH 7), 2 ml of 0.2 mM sucrose 0.1 ml of toluene, 3 ml of distilled water. 0.5 ml of aliquot were taken from each reaction mixture and 1 ml of copper reagent was added and kept in water bath for 10 minutes and then the tubes were incubated at 30°C and reducing sugar was estimated by adding 1 ml of arsenomolybdate reagent at an interval of 15 minutes and 45 minutes. The amount of reducing sugar formed was read at 700 nm by using spectrophotometer and quantified against standard curve prepared with known concentration of glucose in identical experimental condition.

Estimation of Sugars

Total sugars content in stem was estimated from the alcohol extract by using anthrone method adopted by Dubois *et al.* (1956) and it was expressed as mg g⁻¹ dry weight. The sucrose content (non reducing sugars) in the sample was estimated by resorcinol method described by Ashwell (1957) and it was expressed as mg g⁻¹ dry weight. Reducing sugars was calculated by deducting the non reducing sugars from total content and expressed as mg g⁻¹.

Estimation of juice quality parameters

Juice samples were drawn from the composite juice of five canes of each variety for analyzing various quality parameters. The brix value was recorded with refractrometer (Make: Bellingham Stanley LTD, Model: RFM 340) and the temperature corrected reading was expressed as brix percentage. The sucrose percent juice

was estimated by clarifying the juice with Horne's dry lead sub-acetate and the clarified juice was fed into a saccharimeter (Make: Rudolph, Model: Autopol 880). From the brix and pol readings, the sucrose per cent juice was calculated and expressed as sucrose percentage. Purity per cent of the juice was calculated by using the data on sucrose % and brix %. The data were statistically analyzed as per the method of Panse and Sukhatme (1961).

RESULTS AND DISCUSSION

Sucrose metabolism

In the present study, the enzyme responsible for sucrose synthesis viz., sucrose phosphate synthase (SPS) and sucrose synthase (SS) activities was assayed at 6th, 7th, 8th & 9th month age of both plant and ratoon crops. Significant varietal variation in SPS and SS activity was found both in plant and ratoon crops. Generally, SS activity followed similar varietal trend as that of SPS, however the activity was higher than that of SPS activity. Irrespective of the varieties and crops, SPS & SS activity increased gradually from 6th to 7th month and thereafter declined at 8th and 9th month of the crop (Fig. 2A & B). At all the stages and varieties, the ratoon crop recorded a higher enzyme (SPS and SS) activity as compared to plant crop, however the difference in SPS activity between plant & ratoon crop was significant (wider) at 7th month and later stages (8th and 9th) the difference was very narrow (Fig. 2A). At 7th month, the SPS enzyme activity varied from 9.00 (Co 95020) to 17.50 $\mu\text{mol g fw}^{-1} \text{h}^{-1}$ (Co 94012) in plant, while in ratoon it varied from 12.50 (Co 97008) to 20.20 $\mu\text{mol g fw}^{-1} \text{h}^{-1}$ (Co 94012). The variation in SS activity at 7th month was 12.00 (Co 97008) -19.50 $\mu\text{mol g fw}^{-1} \text{h}^{-1}$ (Co 94012) in plant crop, while in ratoon the variation was 14.00 (Co 97009) -21.10 $\mu\text{mol g fw}^{-1} \text{h}^{-1}$ (Co 94012). As these enzymes significantly positively correlated with sucrose % juice in present study, variation in the SPS and SS enzyme activities between plant and ratoon crop at grand growth phase (7th month) resulted in difference in sucrose per cent juice, hence SPS and SS enzyme activities could be used as biochemical marker for identifying source strength at early stages of the crop. These results confirm the findings of Stitt *et al.* (1988),

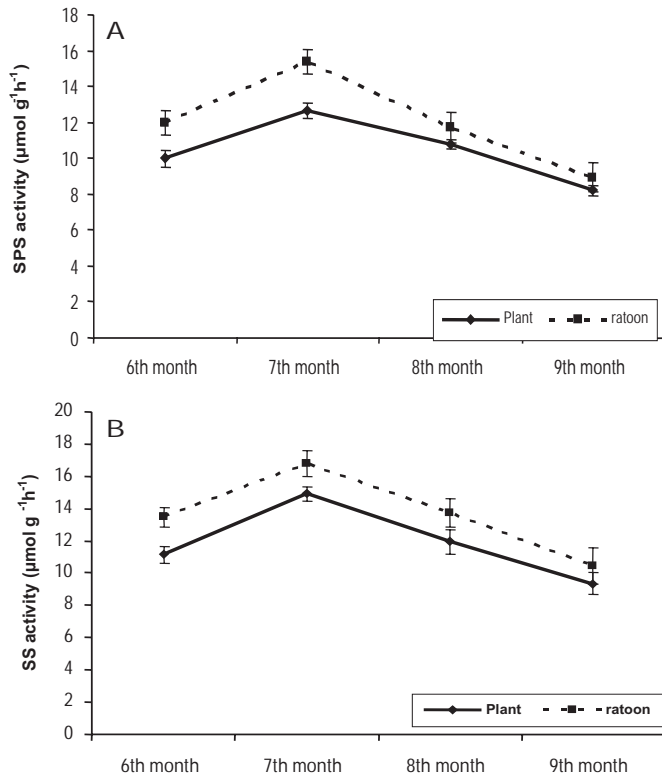


Fig. 2A & 2B. SPS and SS ($\mu\text{mol g}^{-1}\text{h}^{-1}$) activities of plant and ratoon crops during maturity phase of sugarcane. Each values represents mean of three replications. Vertical bars represent the standard error ($P < 0.05\%$)

Zhu *et al.* (1997), Lingle (1999 and 2001) and Vidyasekar (2007) in sugarcane.

In both the plant and ratoon crop, high sugar varieties CoC 671, Co 86032, Co 94012 and Co 99004 recorded higher SPS and SS activity at all the stages compared to the low sugar varieties Co 97008, Co 95020 and Co 97009. Similar varietal variation in SPS and SS was reported in sugarcane (Huber and Huber 1996, Zhu *et al.* 1997 and 2000, Lingle 2001) and further they stated that high sucrose phosphate synthase (SPS) and low acid invertase (AI) activity at early stages of growth may prove as a useful index for the production of sucrose in sugarcane cultivars.

Sucrose accumulation in plant and ratoon crops

In the present study, acid and neutral invertases were estimated to assess the relative ability of the varieties

to import and allocate sucrose in storage parenchyma of sink tissue. The acid invertase (AI) activity was assayed at 8th, 9th, 10th and 11th month of plant and ratoon crops and in all the stages, plant crop recorded a higher enzyme activity compared to ratoon crop. The AI activity showed declining trend from 8th to 11th month both in plant and ratoon crops (Fig. 3A). At 8th month the enzyme activity varied from 8.28 (Co 99004) to 38.43 $\mu\text{mol g}^{-1}\text{h}^{-1}$ (Co 2000-10) in plant crop, while in ratoon crop it varied from 24.87 (CoC 671) to 44.71 $\mu\text{mol g}^{-1}\text{h}^{-1}$ (Co 2000-10). Interestingly, the difference in the AI activity between plant and ratoon crops was wider particularly during 8th month of the crop. Since, acid invertase (AI) play a pivotal role in cane elongation, the difference in the enzyme activity between plant (higher AI activity) and ratoon (lower AI activity) crops resulted in difference in the cane growth among the crops and varieties. Zhu *et al.* (2000) observed a significant, curvilinear regression between elongation rate and acid

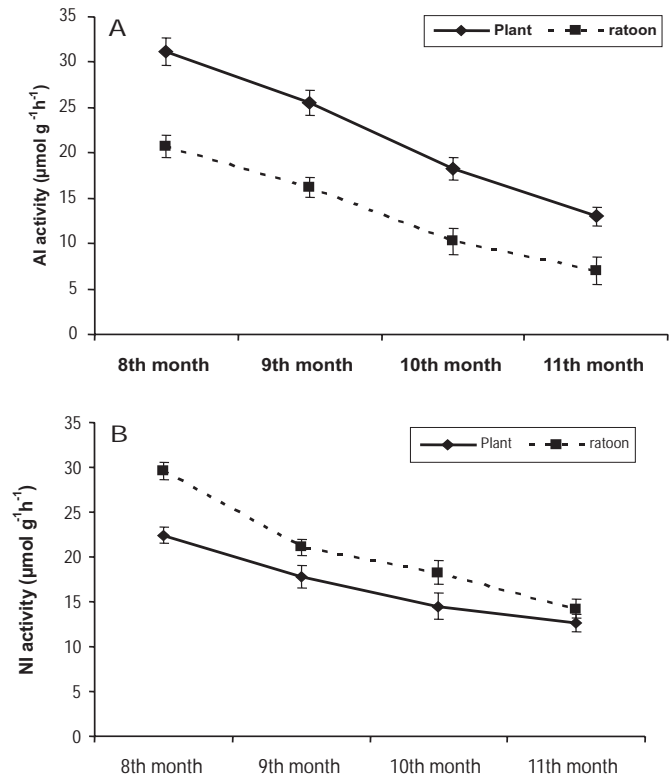


Fig. 3A & 3B. AI & NI ($\mu\text{mol g}^{-1}\text{h}^{-1}$) activities of plant and ratoon crops during maturity phase of sugarcane. Each value represents mean of 3 replications. Vertical bars represent the standard error ($P < 0.05\%$)

invertase activity ($R^2=0.4$), but not with SPS, SS & NI enzymes assayed. Hence, in present study the differences in the AI enzyme activity between the crops and varieties might be resulted in reduction in internodal length and stunted growth of ratoon crop (data not shown).

In both plant and ratoon crops high sugar varieties CoC 671, Co 94012, Co 85019, Co 86032 and Co 99004 recorded lower AI enzyme activity as compared to low sugar varieties (Co 97008, Co 2000-10). Though the AI activity negatively correlated with sucrose % juice both in plant ($r = -0.562^*$) and ratoon crop ($r = -0.235$), the association found to be significant only in plant crop. The result indicated that the difference in the association between the AI activity and crops might be reason for the variation in cane growth and sugar accumulation between plant and ratoon crops. Compartment of these enzymes and their physiological function has reported in sugarcane (Batta & Sing 1986, Zhu *et al.* 1997, Lingle 1997, Vidyasekar 2007). According to them, soluble acid invertase activity found to possess an inverse relationship with sucrose accumulation in sugarcane that is in the whole stalk and within individual sugarcane internodes. Further, they reported that concentration of AI was usually high in tissues that are fast growing, such as cell and tissue cultures, and immature stem internodes but decreases rapidly during internode growth and development.

Neutral invertase is present in considerable amount in mature tissues and catalyzes the hydrolysis of terminal non-reducing β - fructo-furanoside. It may also play a key role in the control of hexose concentrations in the cytosol of sugarcane stem cells, thus affecting control over the expression of sugar responsive genes (Vorster and Botha 1998). The neutral invertase (NI) activity assayed at 8th (22.38 $\mu\text{mol g}^{-1}\text{h}^{-1}$), 9th (17.79 $\mu\text{mol g}^{-1}\text{h}^{-1}$) and 10th (14.51 $\mu\text{mol g}^{-1}\text{h}^{-1}$) of plant crop and ratoon crops 8th (29.56 $\mu\text{mol g}^{-1}\text{h}^{-1}$), 9th (21.07 $\mu\text{mol g}^{-1}\text{h}^{-1}$) and 10th (18.26 $\mu\text{mol g}^{-1}\text{h}^{-1}$) of ratoon crop, indicated that the ratoon crop recorded higher enzyme activity as compared to plant crop; however the difference in the NI activity between the crops was high only at 8th month (Fig.3B). Both plant and ratoon crops of varieties varied significantly in NI activity, the high sugar varieties CoC 671, Co 86032, Co 94012, Co 85019 and Co 99004

recorded higher NI activity compared to the low sugar varieties Co 97008, Co 95020, Co 97009. Similar varietal variation in NI activity was reported in sugarcane (Moore 1995, Lingle 1997, Voster and Botha 1998, Gomathi and Thandapani 2004). NI activity was significantly and positively associated with sucrose % juice both in plant ($r=0.534^*$) and ratoon ($r= 0.508^*$). Thus, in the present study, higher sucrose % in ratoon crop was associated with higher SPS, SS and NI and lower AI activity.

Variation in carbon allocation in plant and ratoon crops

Carbohydrate fractions reducing (fructose), non-reducing (sucrose) and total sugar was estimated in stem tissues at 10th month to compare the differences in allocation of carbon compounds between the varieties and crop types. Results indicated that in both the crop highest reducing sugar content was observed in low sugar varieties Co 95020 and Co 2000-10 and the lowest content was found in high sugar varieties (Co 99004, CoC 671 and Co 94012) and *vice versa* for non reducing sugars (data not shown). Significant variation in reducing sugar content among the crops and varieties in the present study were supported the findings of Dendsay *et al.* (1992) and Sehtiya and Dendsay (2000), who observed negative association between sucrose % juice with reducing sugar content in sugarcane. In present study, higher purity % in ratoon crop might be due to accumulation of higher concentration of non-reducing

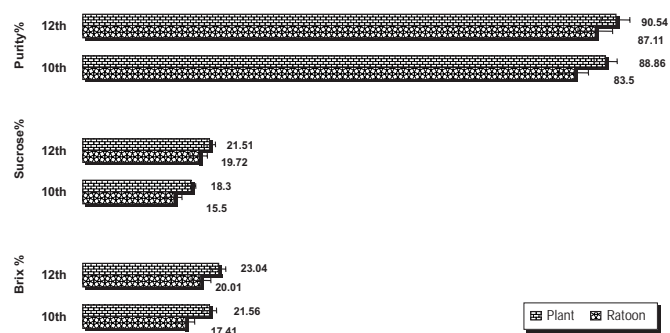


Fig. 4. Differences in carbon allocation (RS-reducing sugars, NRS- Non reducing sugars & TS- Total sugars) between plant and ratoon crop during maturity phase of sugarcane. Each points represents mean of 3 replications. Horizontal bars represent the standard error ($P < 0.05\%$)

(NS) and lower level of reducing sugars (RS) as compared to plant crop (Fig. 4). Hence, apart from the total sugar (TS) accumulation regulation of carbon allocation towards non reducing sugar (sucrose) particularly economic sink (stem) and lower level of reducing sugars (RS) favors in higher sucrose % in high sugar types particularly in ratoon crop.

Juice quality parameters in plant and ratoon crops

Sugarcane quality is normally expressed in terms of sucrose percent juice. Different juice quality parameters such as brix, pol percent, sucrose estimation, and cell size and invertase activity may help in evaluating the quality of sugarcane clones. In present study, juice quality parameters viz., brix, sucrose, and purity % was compared between plant and ratoon crops at 10th and 12th month and in both crops the juice quality parameters reached maximum peak only at 12th month. Thus, in ratoon crop early ripening was absent as found in sub tropical condition (Mohan Rao *et al.* 1956). Irrespective of the varieties and stages ratoon crop recorded higher juice quality parameters compared to plant crop (Table 1). However, the difference in juice quality between the plant and ratoon crop was wider at 10th month stage than at 12th month stage and also varied with varieties (Fig. 5). At 10th month the ratoon crop showed 14.37, 18.06 and 4.32 % increase in brix, sucrose and purity % juice over the plant crop. The increase was comparatively less at 12th (6.38, 9.06 & 1.89 % in brix, sucrose and purity % respectively). The varieties varied significantly in sucrose % juice at all the stages studied. Similar varietal

variation was reported by Bhatt and Shukla (1989), Lingle (1999) and Nalini (2008).

However, ratoon recorded higher juice quality parameters in all the varieties studied compared plant crop. Irrespective of the crops the varieties, Co 94012, CoC 671, Co 86032, and Co 99004 showed higher brix %, sucrose %, purity % and CCS % than rest of the varieties. Higher juice quality parameters in ratoon crop associated with the higher SPS, SS and AI enzyme activity and lower AI activity. Hence, in present study both the acid invertase (inversely) and neutral invertase (positively) activities highly significantly associated with sucrose % juice over the period and this enzyme activities might be taken as a biochemical indicator for high sugar types. Thus, differences among varieties and plant types in sugar accumulation rates may be related to differences in activities of sucrose metabolism enzymes as reported by Batta and Singh (1986) and Zhu *et al.* (2000). According to them the acid invertase enzyme activity is negatively correlated with cane elongation, while neutral invertase is related to sucrose content (Voster and Botha 1998, Lingle 1999, Vidyasekar 2007). Among the juice quality parameters studied, sucrose percent was most reliable indicator of juice quality compared to brix percent as reported by Sreekumar *et al.* (1994).

Varieties, Co 94012, CoC 671, Co 86032 and Co 99004 showed higher brix %, sucrose % and purity % than other varieties studied. Differences among the crops and cultivars in juice quality of plant and ratoon crops may be related to differences in activities of sucrose metabolic enzymes ie. higher the SPS, SS and AI enzyme activity and lower AI activity. Hence, in present study the acid invertase (inversely) and neutral invertase (positively) activities highly significantly associated with sucrose % juice over the period and these enzymatic differences might be taken as a biochemical indicator for higher sugariness in ratoon crop. These findings are in accordance with the work of Sehtiya and Dendsay (2000) and Vidyasekar (2007), who also observed a similar differences in invertase activity in high and low sugar yielding cultivars. The high sucrose phosphate synthase and low acid invertases activity have also been considered as a useful index for sucrose accumulation potential of sugarcane cultivars

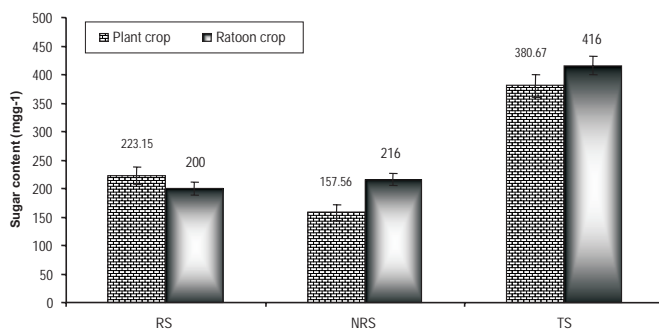


Fig. 5. Differences in juice quality parameters between plant and ratoon crop during maturity of sugarcane. Each points represents mean of 3 replications. Vertical bars represent the standard error ($P < 0.05\%$)

Table 1. Varietal variations in juice quality parameters in plant and ratoon crops of sugarcane at 10th and 12th month

Varieties	Brix%				Sucrose%				Purity%			
	10 th month		12 th month		10 th month		12 th month		10 th month		12 th month	
	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon
CoC 671	18.35	19.25	22.45	23.00	16.60	19.46	21.04	22.00	88.46	90.09	90.28	92.61
Co 8021	15.25	19.55	20.05	22.65	13.11	17.01	17.68	21.65	83.96	87.00	88.26	89.21
Co 85019	18.45	20.51	21.75	23.50	16.67	18.26	20.44	21.50	86.35	89.05	90.45	92.26
Co 86032	16.85	21.00	22.00	23.85	16.81	19.32	19.05	21.85	83.76	90.00	86.76	89.48
Co 94012	20.25	20.05	23.70	24.60	18.85	19.15	22.03	23.60	80.08	91.55	92.41	93.50
Co 95020	16.55	19.60	20.75	22.50	14.35	18.02	18.22	20.50	82.70	89.93	85.95	87.15
Co 97008	18.55	19.25	22.40	23.25	14.71	17.76	19.63	19.25	77.29	82.25	87.78	89.00
Co 97009	16.44	20.25	20.00	22.95	14.08	17.23	19.64	19.95	83.64	85.08	89.43	91.62
Co 99004	17.70	19.50	22.10	23.75	16.45	19.74	21.15	21.75	87.93	84.23	87.96	90.65
Co 99008	16.80	20.70	21.65	21.90	14.50	17.02	19.38	20.90	84.30	82.22	89.57	91.56
Co 2000-10	17.00	20.45	20.35	21.80	14.30	18.17	18.66	19.80	80.11	86.85	87.58	88.91
Mean	17.41	20.01	21.56	23.04	15.50	18.30	19.72	21.51	83.50	87.11	88.86	90.54
SED	0.368*	0.448*	0.604**	0.408*	0.330*	0.452*	0.538**	0.408*	1.32*	1.80*	2.10*	1.80*
CD(P=0.05)	0.750*	0.888*	1.90**	1.75*	0.780*	1.17*	1.69**	1.75*	2.60*	3.60*	4.20*	3.60*
% increase	14.37%	6.38%	18.06%	9.07%	4.32%	1.89%						

(Lingle 1997). The present study also confirms the importance of these enzymes during developmental stages as well as at maturity.

CONCLUSION

The present study confirms that differences in juice quality between plant and ratoon and among the varieties might be related to differences in activities of sucrose metabolic enzymes viz., SPS, SS, and AI and NI between plant and ratoon crops. Thus, it is concluded that activities of these enzymes at appropriate growth phase and their association with sucrose % decides the juice quality of ratoon crop. The study also confirms that, higher carbon allocation particularly non reducing sugars towards economic sink might be reason for high sugar content in ratoon crop with better juice purity.

ACKNOWLEDGEMENT

The authors thank The Director and The Head Crop Production, Sugarcane Breeding Institute (ICAR), Coimbatore to carry out the study and their constant encouragement for article writing.

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