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Investigation on the Interplay of Sucrose Phosphate Synthaseand SucroseSynthase in Relation to Sucrose Accumulation in High and Low Sugarcane Parents and Progeny

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ABSTRACT: *Sugarcane is the prime source of sugar and its increased accumulation in the cane stalk through better understanding of source- sink communication is the prime focus of the research today. Under the varying climate conditions as well as cultivars differing in maturity, the activities of sucrose synthase and sucrose phosphate synthase and invertases controls the synthesis and storage of sucrose in the cane stalk.The level of sucrose increased steadily during the development and the bottom portion of the developing cane recorded much higher increase in sucrose in high sugar cultivars. Hexoses in the high and low sucrose accumulating cultivars decreased with age and the concentration in mature internodes were lower than in immature internodes. The immature portions of the developing canes recorded vary high level of hexose pool at the early stage of the development and which subsequently declined during maturity. The SPS activity increased gradually during cane maturation in both top and bottom portion of high and low sucrose sugarcane varieties and showed positive correlation with sucrose and negativecorrelation with hexose sugars.The activity profile of sucrose synthase in both high and low sucrose varieties decline during maturation and the activity was significantly lower in the mature internodes than immature internodes. The high and low sugar progenies of the crosses when evaluated for the activity profile of the sucrose metabolizing enzymes also exhibited similar trend as observed in mature and immature internodes during development.*

Keywords: *Day after planting.Sucrose phosphate synthas.Sucrose synthase. Hexose Sugar.*

INTRODUCTION

Sugarcane (*Saccharum spp*. complex) is a commercially important cash crop grown in more than hundred countries and accounts for approximately 70% of the world sugar production. Sucrose is the prime product of sugarcane, hence continued research is needed to improve its level in the cane stalk. In India, a distinct difference in sucrose content (pol% cane) is observed in tropical and subtropical varieties which ultimately lead to variation in sucrose recovery percent which is mainly determined by the accumulation of sucrose in the stem. The sucrose content and the recovery of the Indian varieties show distinct variations compared to the varieties of Brazil, China and Australia. In order to understand these variations, concentrated efforts are needed to better understand the sucrose metabolism, transport and source - sink interactions that regulate sucrose accumulation which ultimately provide details about the processes governing overall stalk sucrose concentrations.At the global level, contrary to the demand of high sucrose containing genotypes, sugarcane improvement especially sucrose content during last 50 years have been largely through the increase of cane yield rather than sucrose content per unit mass (Jackson, 2005). These situation warrant vertical improvement in cane yield *vis-a-vis* sucrose content per unit area as the only option left to the Indian sugar industries.

Development of improved sugarcane varieties efficient in sucrose accumulation has become the key component of all the advanced sugarcane breeding programmers (Lingle *et al.,* 2009). Mature sugarcane has the capacity to store upto 25% sucrose on fresh weight basis under favorable conditions but in practical sense it has never been achieved. A positive relationship between free space in the internode

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Table 1
Sucrose content at different developmental stages in top and bottom portion of high and low sugar varieties

			Sucrose (mg g^{-1} fresh wt.)					
		Top				*Bottom*		
DAP/Variety	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*
240	160.00	145.00	86.00	130.33	174.00	150.00	110.00	144.67
270	175.00	150.00	94.00	139.67	180.00	163.00	122.00	155.00
300	183.00	156.00	102.00	147.00	197.00	172.00	138.00	169.00
330	190.00	159.00	122.00	157.00	215.00	176.00	150.00	180.33
360	200.00	161.00	130.00	163.67	223.00	180.00	165.00	189.33
390	205.00	163.00	136.00	168.00	230.00	183.00	167.00	193.33
Mean	185.50	155.67	111.67	150.94	203.17	170.67	142.00	171.94
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	1.680	4.828		Variety	1.269	3.659	
	DAP	2.376	6.828		DAP	1.794	5.174	
	Var.XDAP	4.115	11.826		Var.XDAP	3.107	8.962	

Sucrose (mg g^{-1} fresh wt.)

parenchyma tissue and pol% support the role of free space in sucrose accumulation and possibly this can be used as a physiological indicator for sucrose yield. In sugarcane, sucrose phosphate synthase and sucrose synthase play important role in sucrose metabolism. Sucrose phosphate synthase, the pivotal sucrose synthesizing enzyme needs to be addressed along with invertase enzyme as the difference in the activities of these enzymes reflect the sucrose accumulation in sugarcane.

The advances in research on sugarcane have indicated that attributes like delayed leaf senescence, increased sucrose loading rates in source tissuesand high photosynthetic activity (electron transport rate) are also found to be associated with the high total sugar phenotype of a sugarcane line having ability to accumulate higher level of sucrose in the culm. Biochemical markers especially associated with high sucrose phenotype that can be measured at the early

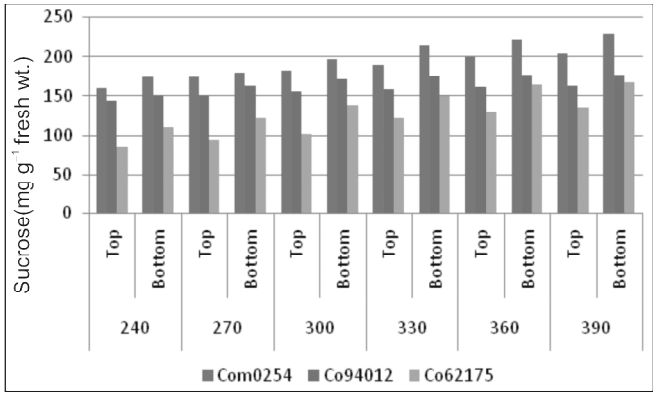

Figure 1: Sucrose content at different developmental stages in top and bottom portion of high and low sugar varieties

developmental stages would have better utility. Among the category of synthesis and cleavage of sucrose, enzyme sucrose phosphate synthase has been reported as a useful marker when measured in the uppermost internodes of young plants which have

Table 2
Hexose content at different developmental stages in top and bottom portion of high and low sugar varieties

			Hexose (mg g^{-1} fresh wt.)					
		TOP				*Bottom*		
DAP/Variety	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*
240	5.72	7.22	21.59	11.51	2.92	4.42	17.49	8.28
270	4.82	6.32	21.00	10.71	2.32	3.82	16.29	7.48
300	3.72	5.22	15.79	8.24	1.32	2.82	10.39	4.84
330	1.02	2.52	10.59	4.71	1.23	2.72	6.69	3.55
360	0.92	2.40	8.29	3.87	0.8	2.30	5.26	2.79
390	0.77	2.27	6.00	3.01	0.35	1.85	3.50	1.90
Mean	2.828	4.325	13.877	7.01	1.489	2.988	9.937	4.805
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.043	0.124		Variety	0.033	0.094	
	DAP	0.061	0.175		DAP	0.046	0.133	
	Var.XDAP	0.106	0.303		Var.XDAP	0.080	0.230	

Hexose (mg g^{-1} fresh wt.)

Table 3
Sucrose phosphate synthase (SPS) activity at different developmental stages in top and bottom portion of high and lowsugar varieties

	SPS activity (imole sucrose formed mg^{-1} protein min^{-1})							
	TOP				*Bottom*			
DAP/Variety	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*
240	0.591	0.320	0.227	0.379	3.476	3.061	2.653	3.063
270	0.678	0.367	0.260	0.435	4.360	3.867	3.328	3.852
300	0.762	0.413	0.293	0.489	4.675	4.118	3.568	4.120
330	0.844	0.457	0.324	0.542	5.306	4.674	4.050	4.677
360	0.926	0.501	0.356	0.594	5.545	4.885	4.233	4.888
390	0.969	0.525	0.372	0.622	5.683	5.007	4.338	5.009
Mean	0.795	0.431	0.305	0.510	4.841	4.269	3.695	4.268
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.005	0.015		Variety	0.048	0.139	
	DAP	0.007	0.021		DAP	0.068	0.197	
Var.XDAP	0.013	0.037		Var.XDAP	0.118	0.342		

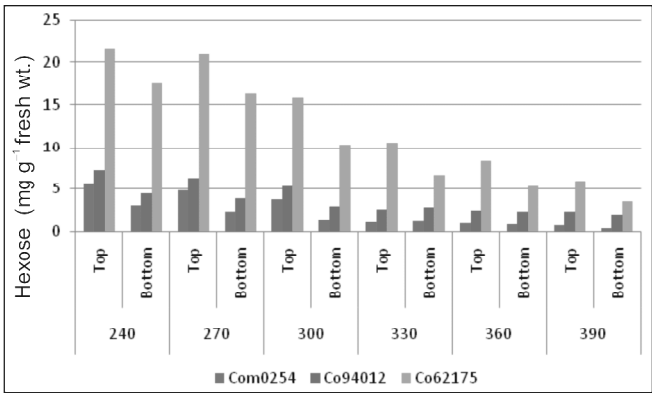

Figure 2: Hexose content at different developmental stages in top and bottom portion of high and low sugar varieties

grown past the elongation phase possessing six internodes (Grof *et al*. 2007). The high and low sucrose parents and the high and lowsucrose progenies of the crosses have been evaluated for majorsucrose metabolizing enzymes during development in relation tosucrose accumulation. An increase in sucrose content is likely be achieved by better understanding of the enzyme activity profile of sucrose metabolizing enzymes during cane development.Despite extensive studies on sucroseaccumulation in sugarcane, the biochemical processes controllingthe yield of sucrose remain poorly understood hence the present investigation is an attempt in thisdirection.

MATERIALS AND METHODS

Four sugarcane varieties differing in sucrose content *viz*. CoM 0254 and Co 94012 (high sucrose), CoM 0265 (mediumsugar) and Co 62175 (low sugar) were planted on the P.G. farm of M.P.K.V., Rahuri during 2011-12 and 2012-2013. Simultaneously,some crosses of sugarcane cultivars were also effected to improve

Table 4
Sucrose phosphate synthase (SPS) activity at different developmental stages in progenies of crosses having high sucrose content

	SPS activity (imole sucrose formed mg^{-1} protein min^{-1})							
	TOP				Bottom			
DAP/ Variety	CoM0265 xCoC671	CoM 0265 x CoM0254	Co740 x CoC671	Mean	CoM0265 xCoC671	CoM 0265 XCoM0254	Co740 x CoC671	Mean
240	0.520	0.736	0.910	0.722	1.547	1.656	5.261	2.821
270	0.597	0.485	1.044	0.828	1.775	1.899	6.004	3.226
300	0.674	0.950	1.174	0.932	1.996	2.136	6.665	3.599
330	0.743	1.052	1.299	1.032	2.210	2.366	7.380	3.985
360	0.812	1.154	1.426	1.132	2.424	2.595	8.242	4.420
390	0.854	1.208	1.493	1.184	2.538	2.717	8.629	4.628
Mean	0.700	0.991	1.224	0.972	2.081	2.228	7.030	3.780
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.013	0.038		Variety	0.021	0.061	
	DAP	0.019	0.054		DAP	0.030	0.086	
	Var.XDAP	0.032	0.093		Var.XDAP	0.051	0.149	

Table 5
Sucrose phosphate synthase (SPS) activity at different developmental stages in progenies of crosses having low sucrose content

	SPS activity (ìmole sucrose formed mg^{-1} protein min^{-1})							
	TOP				*Bottom*			
DAP /Variety	*CoM0265 xCoC671*	*CoM 02654 x CoM025*	*Co740 x CoC671*	*Mean*	*CoM0265 xCoC671*	*CoM 0265 x CoM025*	*Co740 x CoC671*	*Mean*
240	0.454	0.562	0.616	0.544	0.516	1.288	1.406	1.070
270	0.511	0.644	0.707	0.621	0.633	1.478	1.613	1.241
300	0.574	0.725	0.794	0.698	0.710	1.662	1.814	1.395
330	0.636	0.802	0.880	0.773	0.785	1.860	2.008	1.551
360	0.698	0.880	0.965	0.848	0.866	2.018	2.203	1.696
390	0.731	0.935	1.010	0.892	0.905	2.114	2.306	1.775
Mean	0.600	0.758	0.829	0.730	0.736	1.737	1.892	1.455
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.010	0.029		Variety	0.016	0.047	
	DAP	0.014	0.041		DAP	0.023	0.067	
	Var.XDAP	0.025	0.075		Var.XDAP	0.040	0.116	

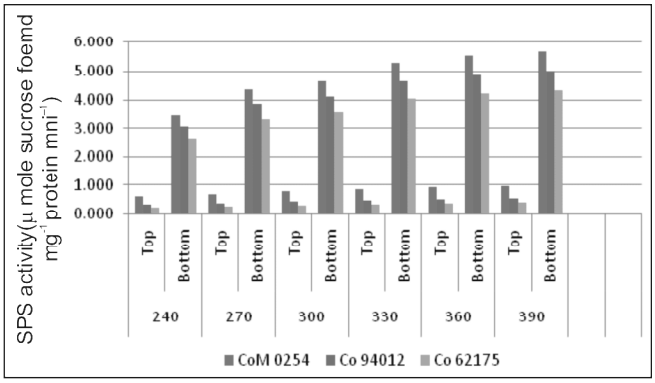

Figure 3: SPS activity at different developmental stages in top and bottom portion of high and low sugar varieties

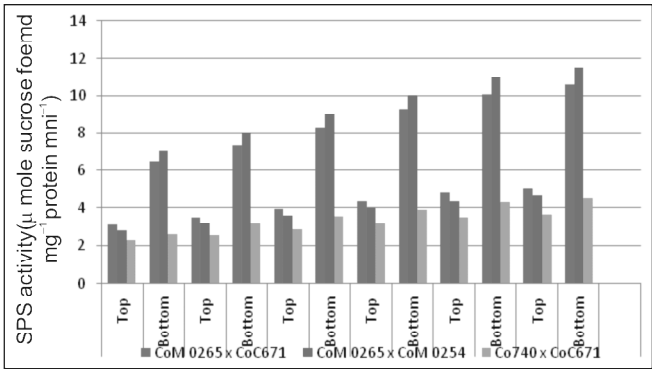

Figure 4: SPSactivity at different developmental stages in progenies of crosses having low sucrose content

the sucrose content of CoM 0265, a widely cultivated sugarcanevariety occupying an area of more than 85 % in the state and whichis salt tolerant by making crosses with the high sucrose sugarcanevarieties CoM 0254 and CoC 671. Another sugarcane variety Co 740 was also used for effecting the crosses. The progenies of suchcrosses were evaluated for sucrose content and the high and lowsucrose progenies were planted on the research farm of C.S.R.S., Padegaon by following the recommended agronomic practices.

Table 6
Sucrose synthase (SuSy) activity at different developmental stages in top and bottom portion of high and low sugar varieties

	SuSy activity (ìmole sucrose formed mg^{-1} protein min^{-1})							
	TOP				*Bottom*			
DAP/Variety	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*	*CoM 0254*	*Co 94012*	*Co 62175*	*Mean*
240	2.396	3.668	3.993	3.352	1.310	1.350	1.770	1.477
270	2.216	3.393	3.693	3.101	1.209	1.246	1.633	1.363
300	2.032	3.143	3.420	2.865	1.009	1.040	1.364	1.138
330	1.839	2.817	3.066	2.574	0.895	0.924	1.212	1.010
360	1.626	2.491	2.711	2.276	0.469	0.706	0.998	0.724
390	1.388	2.125	2.313	1.942	0.374	0.555	0.784	0.571
Mean	1.916	2.940	3.199	2.685	0.878	0.970	1.294	1.047
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.010	0.028		Variety	0.014	0.040	
	DAP	0.014	0.040		DAP	0.020	0.057	
	Var. XDAP	0.024	0.070		Var. XDAP	0.034	0.098	

Table7
Sucrose synthase (SuSy) activity at different developmental stages in progenies of crosses having high sucrose content

SuSy activity (ìmole sucrose formed mg^{-1} protein min^{-1})

		TOP				*Bottom*		
DAP /Variety	*CoM0265 xCoC671*	*CoM 0265 x CoM0254*	*Co740 x CoC671*	*Mean*	*CoM0265 xCoC671*	*CoM 0265 x CoM0254*	*Co740 x CoC671*	*Mean*
240	15.249	9.281	8.543	11.024	4.890	4.626	3.286	4.267
270	14.105	8.585	7.902	10.197	4.524	4.280	3.039	3.948
300	13.063	7.951	7.452	9.489	4.190	3.963	2.815	3.656
330	11.709	7.127	6.560	8.465	3.755	3.552	2.523	3.277
360	9.989	6.302	5.980	7.424	3.321	3.141	2.231	2.898
390	8.521	5.378	5.101	6.333	2.833	2.680	1.903	2.472
Mean	12.106	7.437	6.923	8.822	3.919	3.707	2.633	3.420
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.090	0.258		Variety	0.070	0.203	
	DAP	0.127	0.365		DAP	0.100	0.287	
	Var.XDAP	0.220	0.633		Var.XDAP	0.070	0.203	

Sampling Methods

Theplant samples were collected after 240, 270, 300, 330, 360 and 390 DAP at monthly intervals. The stem was further divided into twoequal portions *viz*. top and bottom representing the immature andmature storage tissues respectively and were immediately frozen inliquid nitrogen to stop metabolic activity and to avoid diurnalvariation in enzyme activity and sugar levels. Brix and sucrose in cane juice was determined by usinga "Brix Hydrometer" and Saccharimeter according to the A.O.A.C. 1995. Sugar recovery percentage was calculated according to theequation as described by Yadav and Sharma (1980).

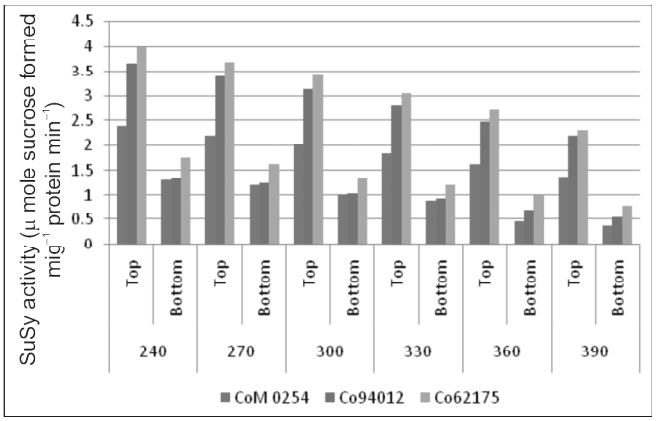

Figure 5: SuSy activity at different developmental stages in top and bottom portion of high and low sugar varieties

Extraction and Assay of Enzymes

The activity of both sucrose phosphate synthase and sucrose synthase were assayed as per the modified method of Hawker (1967). The reaction mixture for sucrose synthase contained 125 µl 0.015 M UDPG, 125 µl 0.05 M fructose, 700 µl 0.2 M Tris-HCl buffer (pH 8.2) containing 0.025 M $MgSO_4$ and 50 µl of enzyme preparation in total volume of 1.0 ml.

Table8
Sucrose synthase (SuSy) activity at different developmental stages in progenies of crosses having low sucrose content

SuSy activity (ìmole sucrose formed mg^{-1} protein min^{-1})

		TOP				*Bottom*		
DAP /Variety	*CoM0265 xCo671*	*CoM 0265 x CoM0254*	*Co740 x Co671*	*Mean*	*CoM0265 xCo671*	*CoM 0265 x CoM0254*	*Co740 x Co671*	*Mean*
240	8.203	6.258	3.823	6.095	4.504	3.114	1.842	3.153
270	7.587	5.789	3.536	5.637	4.166	2.881	1.704	2.917
300	7.027	5.361	3.275	5.221	3.859	2.668	1.578	2.702
330	6.318	4.805	2.935	4.686	3.458	2.391	1.414	2.421
360	5.570	4.250	2.596	4.139	3.050	2.115	1.251	2.139
390	4.752	3.625	2.214	3.530	2.609	1.804	1.067	1.827
Mean	6.576	5.015	3.063	4.885	3.608	2.496	1.476	2.526
		SE (±)	CD at 5%			SE (±)	CD at 5%	
	Variety	0.096	0.277		Variety	0.085	0.246	
	DAP	0.136	0.391		DAP	0.121	0.348	
	Var.XDAP	0.236	0.677		Var.XDAP	0.209	0.603	

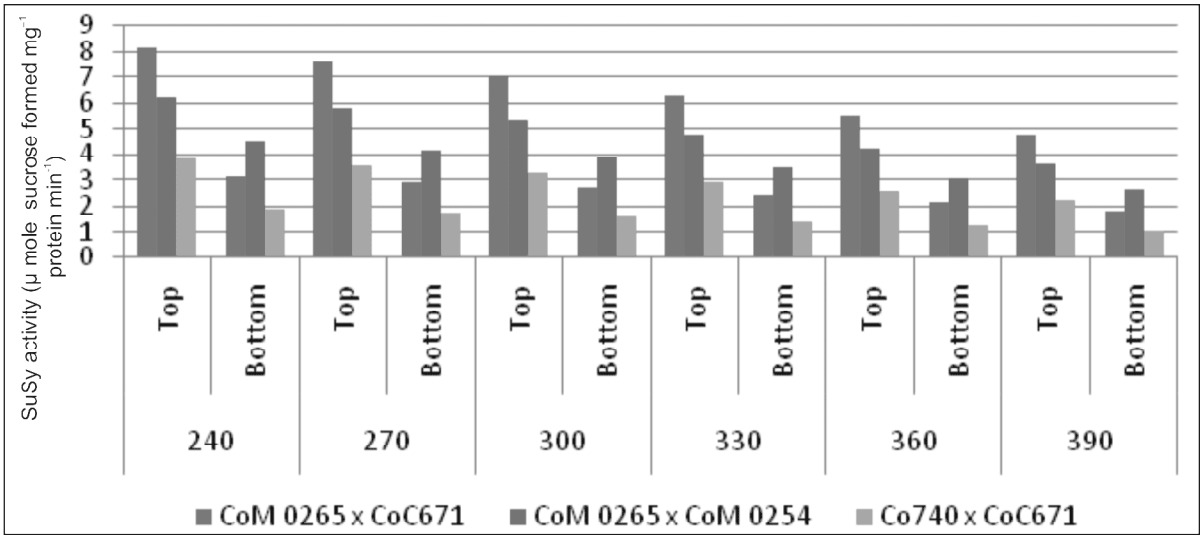

Figure 6: SuSyactivity at different developmentalstages in progenies of crosses having low sucrose content

Reaction mixture for sucrose phosphate synthase contained 125 µl 0.015 M UDPG, 125 µl 0.05M fructose–6-phosphate, 700 µl 0.2 M Tris-HCl buffer (pH 7.4) containing 0.025 M $MgSO_4$ and 0.4 M NaF (as phosphatase inhibitor) and 50 µl enzyme preparation in a total volume of 1.0 ml. reaction mixture. Both the SPS and SuSy activity was expressed as µmole sucrose formed mg^{-1} protein min^{-1}. Soluble protein was determined by the colorimetric method of Lowry *et al*., (1951) using bovine serum albumin as the standard protein.

RESULTS AND DISCUSSION

Sucrose and Hexose Contents

Top portion of high sugar variety CoM 0254 recorded significantly higher sucrose content (205.00 mg g^{-1} fr.wt.) than Co 94012 and Co 62175 (163.00 and 136.00 mg g^{-1} fr.wt.) respectively at 390 DAP. When bottom portion of the cane was analyzed for sucrose content similar trends of increased in sucrose content was noticed and bottom portion of high sugar variety CoM 0254 recorded significantly higher sucrose content (230.00 mg g^{-1} fr.wt.) than Co 94012 and Co 62175 (183.00 and 167.00 mg g^{-1}fr.wt.) respectively at 390 DAP. The data of sucrose content showed significant varietal difference as well as difference during the development stages (Table 1). Prathima *et al*. (2012) reported that, sucroseconcentration significantly increased from top internodes to middle and bottom internodes in both high and low sugar genotypes. Batta *et al*. (2011) reported that, sucrose contentincreased from stem elongation stage to maturity stage in both parents and progenies. Jain *et al*. (2013) reported that, improvement in sucrose content might be due to changein activity pattern of sucrose synthesizing enzymes which alsohelped increase CCS yield.

The hexose level decreased gradually both intop and bottom portion of the cane as the crop attend the maturity. Low sugar variety Co 62175 recorded significantly higher decreased in hexose (6.00 and 3.50 mg g^{-1} fr.wt.) respectively both in top and bottom portion of the cane than high sugar variety Co 94012 and CoM 0254 (Table 2). Pan *et al*. (2009) reported that, the hexosecontent in young internodes was found higher as compared to olderinternodes, where it decreased pronouncedly and become almostabsent. The hexose content of low sugar variety was higher ascompared to high sugar variety.Hexoses in high and low sucrose accumulating cultivarsdecreased with age and concentration in mature internodes werelower than in immature internodes (McCormick *et al*., 2006, 2009). Prathima *et al*. (2012) reported that, low sugargenotypes had higher concentration of total hexoses as comparedto high sugar genotypes. There was significantly decrease in total hexose concentration when the crop reached fully mature stage. Verma *et al*. (2011) reported that, in both high and lowsucrose accumulating cultivars, hexose sugar decreased withadvancement of developmental stages.

Sucrose Phosphate Synthase Activity

High sugar variety CoM 0254 recorded significantly higher SPS activity (0.969 and 5.683μ mole sucrose formed mg^{-1} protein min^{-1}) respectively than Co 94012 and Co 62175 both in top and bottom portion of the cane. The data of SPS activity showed significant varietaldifferences as well as differences during the developmental stages (Table 3). Lingle (1998) reported that, the sucrose phosphatesynthase activities increased during development. Pan *et al*. (2009) earlier reported the lower SPS activityin the young internodes compared to maturing and maturedinternodes and it showed increasing trends with increasing in theage of the internodes. Verma *et al*. (2010, 2011) and Lingle (1998) reported thathigh sugar cultivars showed increased enzyme activity of SPS compared to low sugar cultivars at all developmental stages. SPS activity was positively correlated with sucrose and negativelycorrelated with hexose sugars. Luo (2006) reported that the activity of SPS enzymesincreased gradually with stalk development and decreased withmaturity of sugarcane.

Progeny of high sugar cross Co 740X CoC 671 recorded significantly higher (1.493 and 8.629μ mole sucroseformed mg^{-1} protein min^{-1}) respectively both in top and bottom portion of cane than CoM 0265 X CoM 0254 and CoM 0265 X CoC 671 at 390 DAP. Similar trend was also observed when both top and bottom portion of progeny of low sugar crosses were analyzed for SPS activity (Table 4 and 5).

Sucrose Synthase Activity

SuSy activity decreased gradually both in top and bottom portion of cane during cane development from 240 to 390 DAP. Low sugar variety Co 62175 recorded significantly higher SuSy activity (3.993 and 1.770μ mole sucrose formed mg^{-1} protein min^{-1}) respectively both in top and bottom portion of cane than high sugar varieties Co 94012 and CoM 0254 at 240 DAP. As the crop attend the maturity, SuSy activity goes decreased gradually and low sugar variety Co 62175 recorded significantly higher decreased in SuSy activity (2.313 and 0.784 μmole sucrose formed mg^{-1} protein min^{-1}) than Co 94012 and CoM 254 respectively at 390 DAP both in top and bottom portion of the cane (Table 6).

Progeny of high sugar cross CoM 0265 × CoC 671 recorded higher SuSy activity (15.249 and 4.890 μmole sucroseformed mg^{-1}protein min^{-1}) at 240 DAP than CoM 0265 × CoM 0254 and Co 740 × CoC 671and showed decreased trend as the crop attend the maturity and significantly higher decreased in SuSy activity was observed in CoM 0265 × CoC 671 (8.521 and 2.833μ mole sucroseformed mg^{-1}protein min^{-1}) than CoM 0265 × CoM 0254 and Co 740 × CoC 671 both in top and bottom portion of the cane at 390 DAP. Similar trend was also observed in progeny of low sugar crosses (Table 7 and 8). Lingle(1996) reported that, higher SuSy activity was correlated with sucroseaccumulation. However, Zhu *et al*. (1997) did notfound any correlation between SuSy and sucrose accumulationwhile analyzing the segregating F-1 population. The higher SuSyactivity in the young internodes suggests that it may be anindicator of sink for sucrose import. The data of SuSy activity showed significant differencesin crosses involving different parents as well as differences duringthe developmental stages.

CONCLUSIONS

The high sugar variety CoM 0254 recorded significantly higher sucrose content with minimum hexose pool. The SPS activity was significantly higher even at the early stage of cane development in the high sugar varieties and also in progenies of the crosses having high sucrose. The activity profile of these enzymes was almost two fold higher in the early stage of the development in both high sugar varieties. The higher SPS activity both in top and bottom portion was correlated with higher sucrose and CCS percentage. The SuSy activity was significantly higher in the top i.e. maturing portion of the cane than in bottom portion of the cane and was higher in the low sugar varieties at all the developmental stages. The progeny of the cross Co740 × CoC 671 with high sucrose content recorded lowest SuSy activity.

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