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RESEARCH ARTICLE

PRODUCTION OF SOMACLONES FROM SUGARCANE CULTIVAR CO-740 and THEIR BIOCHEMICAL GROWTH PARAMETERS

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ABSTRACT

Present work was carried out to generate new somoclones by local existing sugarcane cultivar Co740. Three somoclones of this variety were generated such as GSBT-7, 8 and 9, and their growth parameters with respect to productivity were studied along with other local existing sugarcane cultivars (early maturing variety CoC-671 and late maturing varieties Co-740 and Co-419). These clones were screened along with other local cultivars in agro-climatic conditions of Bidar. The developed somaclones were analyzed for the study of quanto-quality yield and biochemical characterizations like enzymes involved in sucrose metabolism, protein profile, etc. All 3-somaclones, GSBT-7, 8 and 9 have shown high polyploidy nature. Increase in sugar content found especially in lower internodes at final stages of growth with decreased sucrose synthase activity. The Invertase activity was higher in 4th and 5th internodes during early growth period but its activity has shifted gradually in 1st and 2nd internodes during later stage of growth. The average activity of SS enzyme declined during maturity i.e. from 1st to 5th internode indicating its activity is more in growing region. Activity of SPS enzyme was gradually increased from immature stage (5th month) to mature stage (10th month) of growth of all sugarcane varieties proportionately with accumulation of sucrose in mature internodes. The polymorphism using total protein was not conspicuous to provide any confirmed conclusion at this stage of investigation. GSBT-9 compared to other late maturing varieties has shown improved sucrose recovery in the late growth period. This trend of better keeping quality was seen up to 10th months of crop standing in the field that encouraged us to recommend cultivation of GSBT-9 instead of Co-740 in the areas of late crushing of sugarcane.

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INTRODUCTION

Sugarcane is one of the economic crop grown this area. Sugar industries which are mainly located in the rural areas have improved the economic conditions of the farmers as well as agricultural laborers engaged in sugarcane farming. Sugar (sucrose) and cane byproducts (molasses and Bagasse) of sugar industry play a major role in national economy. But sugarcane productivity in the recent years has not shown any significant improvement inspite of extensive research and increase in input cost. The decline in yield is mainly due to biotic and abiotic stresses. Due to the ever increasing population, there is a need to improve the yield of sugarcane.

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The environmental stresses and the limitations of conventional breeding have been overcome by plant tissue culture which takes advantages of the cellular totipotency (Haberlandt, 1902) and genome variations (Larkin and Scowcroft, 1981). One of the most essential concerns in the sugarcane industry is to determine the price of the sugarcane that are delivered and sold to the miller. The price of sugarcane is estimated by its sucrose content. Therefore, increasing sugar content is the primary objective of sugarcane improvement programmes. One of the best programme for improvement of sugarcane (sucrose) productivity against environmental stresses is tissue culture. Plant regeneration from tissue cultures of sugarcane improvement was successfully done by many workers (Heinz, *et al*., 1977; Chagvardiff, *et al*., 1981). Many plant varients were developed by shoot meristem culture. There have been reports of others (Junito and Manuel, 1983) that somaclones derived from tissue culture have shown better agronomic

performance when compared to pre-existing varieties. Regeneration from callus, leaf explants or other parts of plant leads to the generation of considerable variation known as somaclone variations. These variations may include aneuploidy, sterility, morphological traits of economic importance or biochemical changes due to mutation that leads to higher yield or resistant to extremities (like high or low temperature, salts, scarecity of water, diseases). This high degree of genetic instability results to permanent heterogenicity. Liu (1981) has selected of high yielding and more sucrose content lines from Taiwan cane cultivars, and seen significant yield and sucrose recovery differences in selected lines. He also worked on the use of comical and physical mutagens on sugarcane callus for induced variations, but no change in the genome with respect to desired characters was found. Therefore, generation of new somaclones of sugarcane by tissue culture is the best and final option than any other methods. John and Meretzki (1983) have isolated from sugarcane cultures of varients resistant to antimetabolites, such desirable results can be expected from somoclones derived from tissue culture. Since sucrose plays a central role in plant growth and development and its turnover depends metabolizing enzymes, the enzymatic profiles of sucrose phosphate synthase, sucrose synthase and invertase were analysed.

Therefore, this research work was carried out select efficient somaclones developed during sugarcane tissue culture. In this work, three pre-existing varieties of sugarcane such as Co-740, CoC-671 and Co-419 were used and three somaclones were generated (GSBT-7, 8 and 9) from parent cultivar Co-740. These clones were screened along with other local cultivars in agro-climatic conditions of Bidar. The developed somaclones were analyzed for the study of quanto-quality yield and biochemical characterizations like enzymes involved in sucrose metabolism, protein profile, etc.

MAERIALS AND METHODS

Planting in plots and parameters observed

Local popular early maturing variety CoC-671 and late maturing varieties Co-740 and Co-419 were obtained from Agriculture Research Centre, Janwada. Seed-sets were treated by fungicides, and then planted in small plot (10mx10m). The cultivar Co-740 was used as parent plant for generating new somaclones and compared their biochemical changes during the growth of these varieties. These readings were taken at one month interval from 30^{th}day–360^{th}day. The internodes of 5^{th} month standing cane numbered 1^{st}, 2^{nd},3^{rd},4^{th} and 5^{th} (top to bottom) and only their biochemical parameters were measured till end of the work. Every time, they were brought to the lab. for biochemical and enzyme analysis. The sugar analysis from juice was done in the lab. of sugar factory.

Table 1. Components of M.S. Medium in different combinations used in tissue culture

S.No.	Ingredients	Concentration of MS medium for Callus initiation(MS-C) mg/l	Concentration of MS medium for Shoot initiation(MS-S) mg/l	Concentration of MS medium for Callus induction(MS-R) mg/l
1	Macronutrients (10X/1000ml)			
	NH_4NO_3	1650.0	1650.0	1650.0
	KNO_3	1900.0	1900.0	1900.0
	$MgSO_4.7H_2O$	370.0	370.0	370.0
	KH_2PO_4	170.0	170.0	170.0
	$CaCl.2H_2O$	440.0	440.0	440.0
2	Micronutrients (100X/1000ml)			
	$MnSO_4.4H_2O$	2230.0	2230.0	2230.0
	H_3BO_3	620.0	620.0	620.0
	$ZnSO_4$	860.0	860.0	860.0
	$CuSO_4.5H_2O$	2.5	2.5	2.5
	$CoCl_2.6H_2O$	2.5	2.5	2.5
	$NaMoO_4.2H_2O$	25.0	25.0	25.0
	KI	83.0	83.0	83.0
3	FeEDTA (100X/100ml)			
	Na2EDTA	3.73	3.73	3.73
	FeSO4.7H2O	2.78	2.78	2.78
4	Vitamins (100X/100ml) & others			
	Glycine	100.0	100.0	100.0
	Thiamine HCl	50.0	50.0	50.0
	Nicotinic acid	50.0	50.0	50.0
	Pyridoxine HCl	50.0	50.0	50.0
	Myo-inocitol	100.0	100.0	100.0
5	Polyvinyl pyrroliodine (PVP)	50.0	50.0	50.0
6	Sucrose	20gm	20gm	7%
7	Growth regulators			
	2,4-D	3.0	--	--
	Kinetin	--	2.0	--
	NAA	--	--	7
	Coconut milk (CM)	100ml	100ml	100ml
8	Agar	8gm	8gm	--
9	pH	5.8	5.8	5.8

Preparation of media for tissue culture

The tissue culture was done in aseptic condition on M.S. medium as illustrated by Murashige and Skoog (1962); Brown, (1984); Bright and Jones (1984). The composition of

the modified M.S. medium (Heinz and Mee 1969) used for callus and shoot induction (Table 1).

Sterilization of materials

All glassware treated with 2% teepol (Glaxo detergent cleaner) and washed in tap water followed by rinsing in distilled water and autoclaved. To maintain aseptic conditions of lab, and other instruments required in the tissue culture were sterilized with 70% alcohol followed by exposure to UV-rays for half an hour.

Preparation of stock solution

The coconut milk was prepared every week by collecting liquid from several nuts, heated to 80^{o}C with stirring, filtered and stored frozen. Stock solutions were prepared in distilled water. The hormone stock solutions of 2-4 D and NAA were prepared by dissolving 50mg of pure chemical in 2-5ml of ethanol heated slightly and gradually diluted to 100ml with water. Similarly 1.0mM kinetin was prepared dissolving 21.5mg of kinetin in a small volume of 0.15N HCl by heating gently and gradually diluting to 10ml with distilled water. All the stock solutions were stored in the refrigerator.

Preparation of explants

Apical young leaves and sub-apical meristem portion were cut into pieces of 2-3cm and washed with sterilized distilled water by adding few drops of teepol as wetting agent. Then the material was surface sterilized in 70% alcohol for 3minutes, then rinsed with water to remove the trace of alcohol. Then, sterilized with 0.1% $HgCl_2$ and rinsed in distilled water. Now the material was cut into 3-4mm size. These were inoculated aseptically on M.S. medium immediately; otherwise, they secrete polyphenols which inhibit the growth of callus. The inoculated tissues were incubated at 26±2^{o}C in dark condition. After 5-7 days, callus cells form from the broken regions of the explants, later considerable mass of callus was found after six weeks (Fig.1). The callus mass was sub-cultured onto same medium for more callus proliferation. The subculture of callus was done every 4-5 weeks intervals.

Callus induction for growth of shoots and roots

Modified M.S. basal media were used for shoot and root induction. For shoot induction, 2-3/gm of callus mass of sugarcane cultivar Co-740 was placed on MS-S medium. Cultures were kept for incubation under florescent light (40µEinstins/m^2/sec) and optimum temperature at 27±4^{o}C. Growth of plantlets was induced from callus upon replacement of 2, 4 D with cytokinin (6-benzylaminopurine 0.5mg/l) for shoot initiation (Figs.2). For root induction, 0.05mg/l NAA and 0.22mg/l IBA were used in both liquid medium using filter paper and semi-solid medium. The better root growth was induced in gyratory shaker.

Transfer of regenerated somaclone seedlings to pots and plots

First the seedlings were grown in test tubes on hormone free medium (Fig. 3). Then, these were subjected to less quantity of sucrose till they become green and photosynthetic. At the same time agar concentration in the tubes was increased to enable the seedlings to become strong and adapt with field conditions, and were allowed to grow to height of 10-12cm. They were carefully removed from tubes and washed with distilled water to remove agar traces attached to roots as even traces of agar invites fungi. Further, the roots of seedlings were treated with IBA (0.05 mg/l), and then transferred to small pots having sterilized sand.

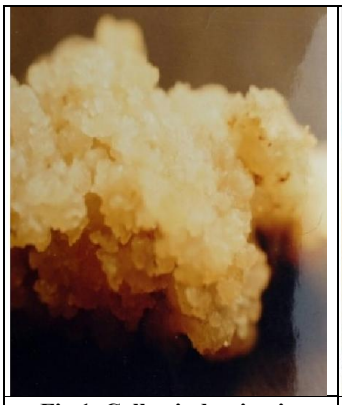


Fig.1. Callus induction is sugarcane variety Co-740

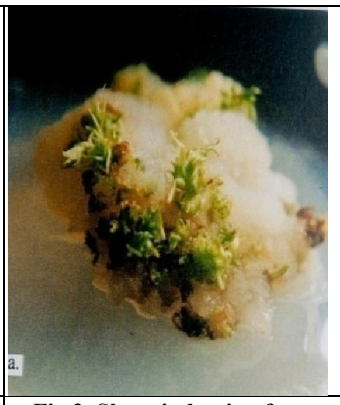


Fig.2. Shoot induction from callus on modified M.S. medium

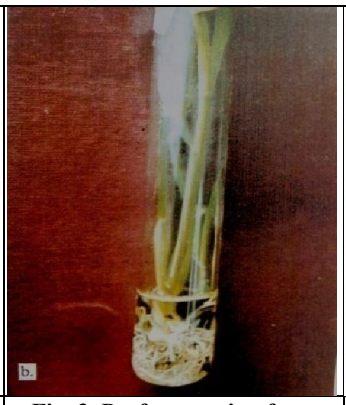


Fig. 3. Profuse rooting from variety Co740 & somaclones ready to transfer to pot

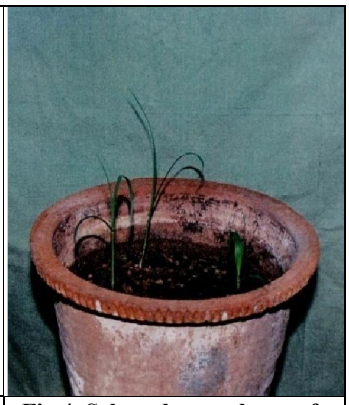


Fig.4. Selected somaclones of variety Co740 growing in pot & ready to transfer to field

It was sterilized in closed box for about 2hrs at 150^{o}C in hot air oven, treated with 10M $MnCl_2$, kept aside for overnight and then filled in pots. The seedlings were treated with 2mg/l Bavistin (fungicide) to avoid fungal infection. The pots with seedlings sprayed frequently with Hoagland solution (Hoagland and Arnon, 1950) for initial 10 days and then switched to tap water using atomizer and were covered with polythene bags for maintaining humidity.

They were then gradually exposed to the open environment by removing polythene bags for specific time. When plants got acclimatized to natural conditions, and then were transferred to bigger pot containing sand and soil (1:1) mixture (Fig.4). The plants were added with additional nutrients through Hoagland solution. After 6-7 days, they were transferred to field plots. Canes of 7-8 months old with sufficient internodes cut down and then clones were propagated further in the bigger plots. Finally, the clones selected in the present wok were GSBT-7, GSBT-8 and GSBT-9.

Determination of biochemical parameters

Extraction of sugar: Plant internodes after 300, 330 and 360 days of planting were harvested and used for sugar analysis. These were slices and dried. 10gm of cane sample has taken into 400ml Mason jar containing 300ml of boiling 98.5% ethanol. Then Soxlet extraction apparatus were applied for extraction of sugar sample. The extract was reduced on water bath below at 78°C. This concentrated the extract by removing alcohol.

Quantitative analysis of Sugar: 1ml of liquid sugar samples were diluted using 50ml of distilled water to bring the concentration of sugar within limit. Each test tube containing sugar was added with 2ml of assay reagent and mixed within 2minutes, and then all test tubes were kept in water bath at 37°C for 30minues. At the end the reactions in the test tubes were stopped by addition of 2ml of 12N H_2SO_4 in each tube. The absorbance of each tube against blank was measured at 540nm.

Table 2. Determination of sugar from calibration curve

Tubes	Water (ml)	Sample (ml)	Sucrose (mg/l) standard (ml)	Sucrose absorbance at 540nm
Blank reagent	50	--	--	0
1	49	--	1	0.006
2	48	--	2	0.012
3	47	--	3	0.021
4	46	--	4	0.030
5	45	--	5	0.039
6	44	--	6	0.047
7	43	--	7	0.054
8	42	--	8	0.064
9	41	--	9	0.071
10	40	--	10	0.081
Cane sugar sample	48	2	--	0.068

Extraction of enzymes: The fresh sugarcane varieties (GSBT-7, 8, 9, Co740 and CoC-671) with 1-5 internodes (Benda, 1969) brought from experimental plot, cleaned with tap water and used for enzyme extraction. They were kept at 4°C and 1gm of frozen tissue was grounded in fine powder using liquid nitrogen. The HEPES buffer (pH 7.2) was used to extract enzymes and filtered through muslin cloth, and the filtrate was centrifuged at 15000rpm for 10minutes at 4°C to get enzyme extract. It was desalted on Sephadex G 25 and used for enzyme assay.

Invertase (I) assay: The Invertase activity of all selected varieties was determined by homogenizing the first five internodes of the standing canes from 5^{th}-10^{th} month old. Soluble Invertase activity was assayed (Zhu et al., 1997) taking 50µl of enzyme extract and mixed with 50µl of 1M sodium acetate (pH 4.5). The enzyme reaction was started by the addition of 100µl of 120mM sucrose solution. The reaction was stopped at 30 and 60 minutes by adding 30µl of 2.5M Tris-base and boiling the mixture for 3 minutes. The concentration of glucose was determined by glucose kit obtained from Dr. Reddy's lab. Hyd.

Sucrose synthase (SS) assay: Its activity measured at 37°C, pH 7.5 (Hubbard, *et al*., 1989) by taking 50µl of enzyme extract and it was mixed with 50µl of 1M sodium acetate (pH 4.5). The enzyme reaction was started by the addition of 100µl of 120mM sucrose solution. For control UDP Glucose was not added in the assay solution. The reaction was incubated at 37°C for 0, 30 and 60 minutes, and then stopped by boiling 3minutes. Glucose produced in the reaction was determined by anthrone assay (Van Handel, 1968). 70µl of reaction solution was added to 70µl 30% KOH, boiled for 10minures and cooled to room temperature. 1ml of anthrone reagent (150mg of anthrone dissolved in 76ml of H_2SO_4 and 30ml of H_2O) was added and incubated at 37°C for 20minutes. Absorbance at 650nm was measured immediately.

Sucrose phosphate synthase (SPS) assay: Its activity was conducted similar to glucose synthase activity except for assay buffer (pH 7.5). Protein concentration for both enzymes was determined by the method of Barford (1976) with BSA as std. protein. All activities were calculated for each internode and expressed as µm of product/gm protein/minute.

Quantitative analysis of proteins: The leaves of the standing cane (3^{rd} node from top) of each sugarcane varieties were crushed in liquid nitrogen at -196oC. 1gm of crushed leaf was homogenized with HEPES buffer (pH7.2). The homogenate was filtered through muslin cloth, subjected to sonication and centrifuged at 10,000rpm for 10minutes at 4oC. The supernatant was stored at -20oC till future use. The total proteins were determined according to Barford method (1976).

Qualitative analysis of proteins by SDS-PAGE: The molecular basis of variation can be studied by under taking marker assisted profiling of proteins and nucleic acids (Naik, 2001). During present work, the partially purified protein was subjected to investigate protein banding pattern using SDS-PAGE electrophoresis. The protein bands were stained by using both Commise Brilliant Blue-G as well as silver staining methods.

Preparation of SDS-Polyacrylamide gels

Acrylamide and N, N-methylene bisacrylamide → A stock solution containing 29% (w/v) acrylamide and 1% (w/v) N.N-methylene bisacrylamide was prepared in distilled water. It was stored in dark bottle at room temperature and prepared fresh after every two months.

Sodium dodecyl sulphate (SDS) → 10% (w/v) stock solution was prepared in distilled water and stored at room temperature.

Resolving gel buffer (pH 8.8) 1.5M Tris Cl → 18.17g of Tris dissolved in 75ml distilled water, the pH 8.8 was adjusted with 6N HCl and final volume made to 100ml with distilled water.

Stacking gel buffer (pH 6.8) 1.5M Tris Cl → 18.17g of Tris dissolved in 75ml distilled water, the pH was adjusted with 6N HCl and final volume made to 100ml with distilled water.

N, N, N, N-tetramethyl diamine (TEMED) → It is sold by several manufacturers and it was used for acceleration of

polymerization of acrylamide by catalyzing the formation of free radicals from ammonium persulphate.

Ammonium persulphate solution → A small amount of 10% (w/v) stock solution was prepared in distilled water and stored at 4°C. It was prepared fresh weekly.

Tris-Glycine electrophoresis buffer → A 5x stock buffer was made by dissolving 15.1g Tris base and 94g of glycine in 900ml distilled. Then 50ml of stock solution of 10% (w/v) was added and final volume made 1000ml with distilled water.

1x SDS gel loading buffer (Lammli buffer) → 50mm of Tris Cl (pH 6.8), 100mM 2-mercaptoethanol, 2%SDS, 0.1% bromophenol blue and 10% glycerol were mixed toprepare 1x SDS- loading buffer and stored at room temperature. 2-mercaptoethanol was added just before the buffer was used from1M stock.

Table 3. Solutions prepared for resolving gels for Tris-glycine SDS-PAGE

Solution components	Volume (ml)	ml/gel mold volume
10%	5.0	10.0
Water	1.9	4.0
30% acrylamide	1.7	3.3
1.5 M Tris (pH 8.8)	1.3	2.5
10% SDS	0.05	0.1
10% APS	0.05	0.1
TEMED	0.002	0.004

Table 4. Solutions prepared for staking gels for Tris-glycine SDS-PAGE

Solution components	Volume (ml)	ml/gel mold volume
	2.0ml	3.0ml
Water	1.4	2.1
30% acrylamide	0.33	0.5
1.5 M Tris (pH 8.8)	0.25	0.38
10% SDS	0.02	0.03
10% APS	0.02	0.03
TEMED	0.002	0.003

Pouring of SDS-polyacrylamide gels

Gel plates were assembled according to Genei manufacturer's instructions. All components of resolving gel (Table 3) mixed and poured into the gap between glass plates with the help of micropipette. Sufficient space was left at the top for stacking gel (up to teeth length of comb + 1cm). The gel was placed at room temperature for 30 minutes. After polymerization, the overlaid material was poured off and washed the top of gel with distilled water to remove any unpolymerized acrylamide. Now, all components of staking gel (Table 4) were mixed and poured on the surface of resolving gel. Immediately, Teflon comb was inserted into it and kept the gel at room temperature for stability.

Loading of protein samples for electrophoresis: The samples were prepared by heating them at 100°C for 5minutes in 1x SDS-gel loading buffer. The denatured protein samples were centrifuged at 10,000rpm for 10 minutes. The comb from gel was removed after 30 minutes and the wells washed with distilled water to remove any unpolymerized staking gel. Tris-glycine electrophoresis buffer was added in top and bottom reservoirs of the apparatus. Bromophenol blue was mixed with 30µl i.e. 150µg of crude protein sample and 120µg of purified protein sample, each was loaded in separate wells. The electrophoresis apparatus was connected to an electric power supply and applied the voltage 8v/cm of gel. After dye front moved into resolving gel, then voltage was increased to 15v/cm and allowed to run the samples until they reach the bottom of resolving gel (about 4 hrs). The apparatus was disconnected from the power supply and glass plates were removed from it, placed on a paper towel, and then the plates were separated from the gel. The gel can now fix and stained with silver nitrate.

Staining of protein bands on gel with silver nitrate

Fixing solution →50ml of methanol was mixed with 10ml of acetic acid and made the final volume of 100ml with distilled water (i.e.5:1:4 ratio). 50% ethanol: 50ml of absolute alcohol was mixed with equal amount of distilled water.

Pre-treatment solution (0.02%) → 20mg of sodium thiosulphate dissolved into 100ml of distilled water.

Silver nitrate solution → 200mg of $AgNO_3$ dissolved in 0.075% of formaldehyde (75µl of formaldehyde into 95.25ml of distilled water).

Developer solution → 6gm of sodium carbonate dissolved in to 0.05% formaldehyde solution and then 4mg of sodium thiosulphate dissolved in it.

The gel was fixed for 2hours in fixing solution and then washed thrice with 50% ethanol. Later, the gel was treated with pre-treatment solution for 1minute and then washed thrice with distilled water. Finally the gel was soaked in $AgNO_3$ solution for 20minutes and then placed it into a developer solution till the protein bands appeared on gel (Fig.5).

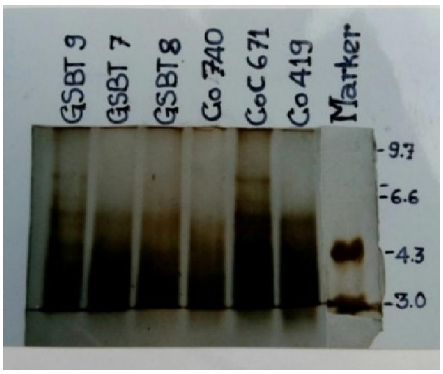


Fig. 5. SDS-PAGE protein profile

RESULTS

Even though GSBT-9 shown higher cane productivity, it was failed to synthesize higher sucrose content and thus improved sugar recovery was observed (Table 5). As it was seen that, CoC-671 shown early maturity and high sucrose content till 9th month of the growth whereas, GSBT-9 has shown improved sucrose recovery in the late growth period i.e. beyond 9.6 month. Sugar in sugarcane leaves and its accumulation is

regulated by sucrose synthesizing and hydrolyzing enzymes. Three important enzymes associated with these functions such as Invertase, Sucrose synthase and Sucrose phosphate synthase (Hongmei *et al*., 2000). Sugarcanes grown in Bidar climate have shown different maturity pattern. Naturally this parameter depends on the level of these three enzymes during growth stages in the life cycle of the crops.

Invertase activity: The enzyme activity showed ranging from 8.3 to 12.8µ (table 6). The activity was maximum during 5th, 6th, and 10th months of growth. The average activity during 5-10 months varied in each internode (fig.6). This trend however is not seen in Co-671 which shown highest activity after 9th and 10th months. The Invertase activity was higher in 4th and 5th internodes during early growth period (5th and 6th months), however, its activity has shifted gradually in 1st and 2nd internodes during later stage of growth (8th , 9th and 10th months) and the total activity of this enzyme seized during 7th month.

Sucrose synthase activity: It is related with sucrose accumulation as well as its breakdown (Table 7). The cultivar CoC-671 depicted maximum activity during the entire growth period. The average activity of this enzyme declined during maturity i.e. from 1st to 5th internode (Fig. 7). Its activity was higher than Co-740 and 9.4% higher than Co-419 at 5th month of growth. This trend remains constant during the growing season with maximum activity was seen even after 10th month of growth.

Sucrose phosphate synthase activity: SPS was found to be main enzyme for the synthesis of sucrose. The results of enzyme extracted from 1st-5th node depicted the best performance of the somaclone GSBT-9 as compared to early maturing variety CoC-671 and late maturing varieties Co-740 and Co-419 (Table 8).

Table 5. Percent sugar observed in all varieties after 300, 330 and 360 days after planting

Cane Variety	300 days				330 days				360 days			
	Brix	Pol.	Purity	Recovery	Brix	Pol.	Purity	Recovery	Brix	Pol.	Purity	Recovery
Co-740	17.79	13.87	77.96	7.63	19.00	15.65	82.37	19.0	19.89	16.23	81.59	9.26
GSBT-9	17.39	13.63	78.49	7.55	18.30	14.60	79.78	8.19	19.90	16.03	83.54	9.31
GSBT-7	16.89	13.19	78.09	7.27	18.30	14.85	81.15	8.44	18.79	16.00	85.15	9.42
GSBT-8	19.39	15.76	82.31	9.17	18.10	14.57	80.50	8.23	19.09	16.12	84.44	9.43
CoC-671	18.89	14.05	74.38	7.34	18.00	14.62	81.12	8.39	18.59	15.78	84.88	9.27
Co-419	18.89	15.26	80.78	8.64	17.69	14.92	84.34	8.73	20.09	16.41	81.68	9.39

Table 6. Invertase activity of internodes of sugarcane varieties at different growth periods

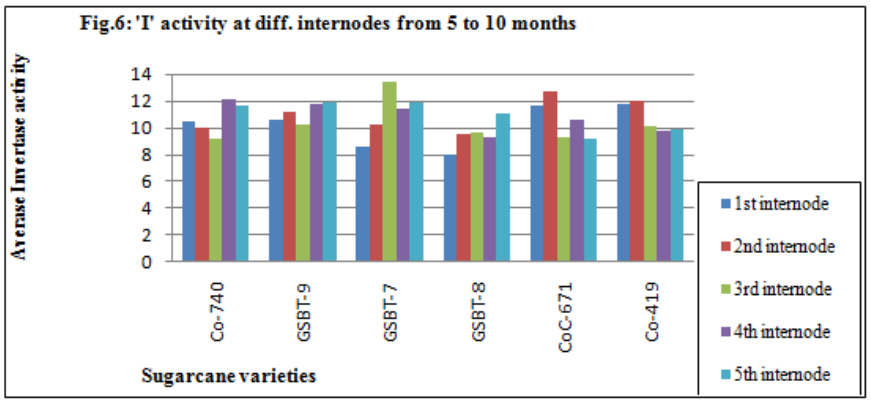
Cane Variety	5th month					6th month					7th month				
	Internodes					Internodes					Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	2.3	5.1	9.8	18.0	24.0	2.8	3.9	8.0	22.0	18.0	8.2	9.7	6.8	9.4	9.0
GSBT-9	2.9	6.0	16.0	18.0	19.0	2.8	7.0	16.0	19.0	19.0	4.9	8.0	13.0	10.0	7.4
GSBT-7	2.6	5.0	9.0	18.3	24.0	3.0	5.0	14.7	20.2	19.6	8.0	6.7	8.8	9.0	9.0
GSBT-8	2.0	4.8	7.3	17.0	21.0	2.0	6.8	11.3	16.3	17.0	6.0	6.4	13.4	9.7	9.0
CoC-671	3.3	4.2	8.7	20.0	23.0	3.4	4.8	13.2	18.4	12.0	12.0	14.3	7.8	5.4	5.0
Co-419	7.2	6.3	8.0	14.0	16.0	7.0	12.2	13.7	17.0	14.0	11.3	12.0	9.0	8.7	8.2
Cane Variety	8th month					9th month					10th month				
	Internodes					Internodes					Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	12.0	8.7	9.0	9.3	8.7	18.4	16.7	11.0	6.8	5.4	19.4	15.7	10.4	7.3	5.0
GSBT-9	12.4	10.3	9.8	7.3	8.0	17.0	19.4	10.0	7.0	6.4	21.0	20.7	9.6	8.7	4.5
GSBT-7	11.9	11.3	12.0	9.4	9.0	13.7	11.4	14.0	6.8	7.3	19.0	17.7	9.9	5.7	6.9
GSBT-8	12.3	12.4	11.4	8.2	6.3	11.7	12.3	7.9	7.5	7.0	19.0	14.3	6.8	7.0	5.9
CoC-671	14.0	18.3	9.3	4.8	3.7	21.2	19.4	8.7	4.2	3.0	16.4	15.7	7.8	10.7	8.2
Co-419	11.0	12.8	9.8	6.7	7.0	19.4	16.8	9.4	5.5	6.0	14.7	12.0	7.6	7.0	8.0

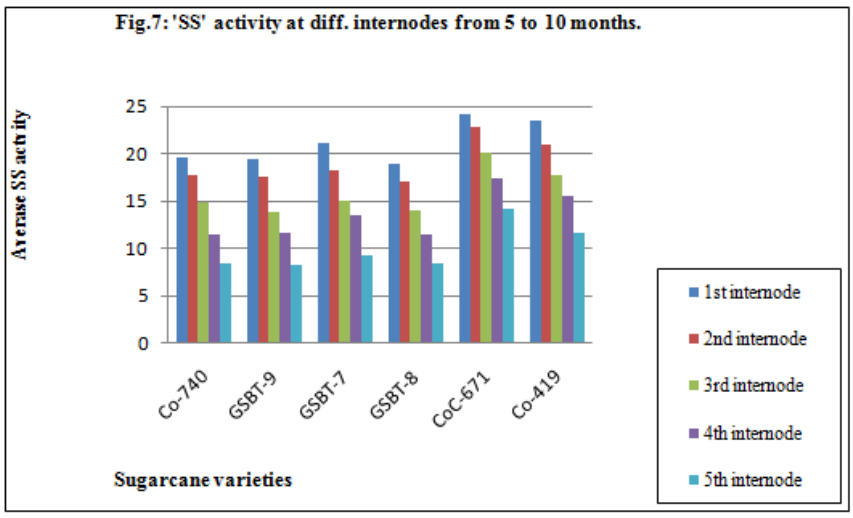
Table 7. SS activity of internodes of sugarcane varieties at different growth periods

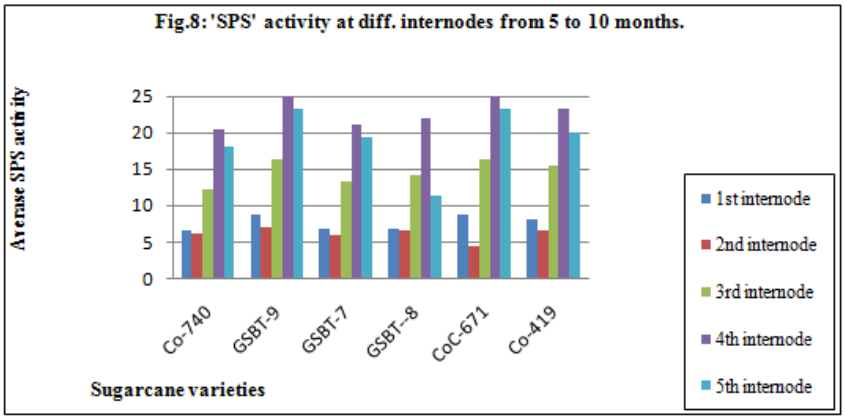
Cane Variety	5th month					6th month					7th month				
	Internodes					Internodes					Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	18.4	20.3	17.6	19.7	16.4	16.8	13.3	10.6	7.3	5.2	20.6	18.8	15.9	11.8	8.6
GSBT-9	19.5	23.6	18.4	22.8	17.3	18.2	15.9	11.6	6.4	4.3	19.3	16.8	12.4	10.6	8.7
GSBT-7	19.8	23.2	20.6	22.9	18.7	19.2	15.6	11.2	9.8	4.4	19.6	16.5	14.1	11.0	7.8
GSBT-8	20.4	24.2	21.3	22.5	19.6	17.6	14.3	10.7	8.2	6.1	16.4	13.8	10.7	7.6	3.4
CoC-671	24.6	27.3	25.4	28.1	26.0	20.6	17.4	14.3	10.8	7.1	24.3	21.2	19.6	16.8	13.9
Co-419	22.8	25.3	21.9	24.2	19.2	20.0	16.8	13.3	9.6	6.4	20.6	17.9	15.1	12.8	10.2
Cane Variety	8th month					9th month					10th month				
	Internodes					Internodes					Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	19.9	16.3	11.8	7.2	3.9	20.6	17.8	14.9	9.8	6.4	21.4	19.6	18.2	12.9	9.8
GSBT-9	20.2	17.4	14.6	10.8	6.3	18.9	15.6	12.4	7.5	4.3	19.9	16.3	13.9	11.2	8.8
GSBT-7	22.4	17.5	13.9	10.2	6.8	21.4	15.8	11.5	9.8	4.5	24.0	21.2	18.9	17.4	13.3
GSBT-8	19.8	15.6	11.9	7.4	3.2	19.1	16.3	13.4	10.2	8.0	20.3	18.2	15.9	12.6	10.2
CoC-671	28.2	23.6	18.5	13.9	8.8	26.7	21.9	18.3	14.8	10.9	20.9	25.3	23.9	20.0	17.9
Co-419	26.2	21.5	16.8	12.3	7.3	24.8	20.1	17.8	15.4	11.1	26.4	23.5	21.8	18.4	15.4

Table 8. SPS activity of internodes of sugarcane varieties at different growth periods

Cane Variety	5th month Internodes					6th month Internodes					7th month Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	3.8	5.9	10.1	13.6	16.4	3.4	6.1	9.8	13.4	16.1	2.4	5.9	8.6	12.3	16.5
GSBT-9	4.3	7.8	12.1	16.3	19.1	3.9	7.4	11.5	15.6	18.8	3.7	7.8	12.1	15.5	19.4
GSBT-7	2.5	5.6	8.3	12.1	15.4	2.0	6.3	10.1	14.2	17.6	3.1	6.8	10.3	13.9	16.8
GSBT-8	3.6	6.1	9.9	13.2	17.4	2.9	5.3	8.4	12.6	15.9	2.8	7.1	11.4	15.3	18.2
CoC-671	2.9	4.8	7.2	11.4	14.3	3.7	7.3	10.1	14.4	17.5	1.9	6.0	10.4	14.3	17.2
Co-419	3.9	6.4	9.2	13.4	16.8	2.9	5.7	10.0	14.3	17.8	3.8	6.7	11.2	16.4	18.9
Cane Variety	8th month Internodes					9th month Internodes					10th month Internodes				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Co-740	9.3	6.5	14.9	29.4	20.3	10.4	7.2	15.8	28.4	19.8	9.8	5.1	14.6	23.2	19.4
GSBT-9	13.2	5.6	20.3	34.6	29.2	12.8	6.3	20.7	32.8	24.6	14.5	7.2	21.4	36.4	28.3
GSBT-7	11.4	6.3	18.4	29.9	21.0	9.4	5.1	14.6	26.3	19.4	12.3	6.1	18.8	30.4	25.6
GSBT-8	10.3	7.2	17.3	28.6	22.0	10.4	5.5	17.4	29.9	20.3	11.3	7.8	20.3	32.4	26.0
CoC-671	8.6	5.8	11.0	20.8	17.2	11.6	6.2	19.4	30.1	24.2	10.8	6.8	17.7	28.3	21.0
Co-419	12.6	6.6	19.8	30.4	24.3	12.0	7.5	19.7	30.4	23.2	13.4	6.8	23.3	35.4	19.8







The GSBT-9 showed 50% higher and Co-419 shown 37% higher SPS activity than Co-740 after 10th month of harvesting of crop. SPS activity in CoC-671, GSBT-7 and 8 was higher in late growth stage (10th month) and activity was widely varied from Co-740. The CoC-671, GSBT-7 and GSBT-8 had higher SPS activity during late growth stage, i.e., activity was increased 17.3% in CoC-671, 35.6% in GSBT-7 and 29.3% in GSBT-8. Activity was gradually increased from immature stage (5th month) to mature stage (10th month) of growth of all sugarcane varieties proportionately with accumulation of sucrose (table 6). The average SPS activity was gradually increased from 1st- 5th internodes (especially in 4th internode) of all varieties. Further, the average SPS activity was higher in GSBT-9 clone followed by CoC-671 compared to others (Fig. 8).

DISCUSSIONS

There was lot of variations in growth parameters of canes. All 3-somaclones, GSBT-7, 8 and 9 have shown high polyploidy nature. Improved sucrose recovery in the late growth period GSBT-9 clone than other existing late maturing cultivars indicates better keeping quality of GSBT-9 up to 10th months of crop standing in the field. This finding encouraged us to recommend cultivation of GSBT-9 instead of Co-740 in the areas of late crushing of sugarcane. The farmers may be advised to cultivate GSBT-9 in the locations of low water regime and with chances of late cane harvesting. The genotypic variations of cane maturity and sugar accumulation were thought to be of interest to undertake sucrose accumulating enzyme in these varieties. Sucrose synthesis, translocation and accumulation in sugarcane largely depends on the activity of three enzymes namely Invertase (I), Sucrose synthase (SS) and sucrose phosphate synthase (SPS) as reported by Hatch and Glaszion, (1963); Akzava,(1976); Federico et al. (2002) and Pan *et al*., (2009). The growth of sugarcane slows down with increased sucrose contents and productivity (Almeda *et al*., 2003).

The major sugar in canes is sucrose which is disaccharide hydrolyzed by Invertase enzyme into glucose and fructose molecules. Therefore, Invertases play an essential role in portioning photosynthates between storage and growth. The enzymes along with other growth influencing factors can regulate many aspects of growth and development of plants (Roitsch and Gonalez, 2004 and Fotopoulus, 2005). The active sucrose seems to be same in sugarcane regardless of tissue maturity; however, there were differences between tissues of different internodes with respect to sucrose accumulation due to their concentration of Invertases and their need to grow (Alexander, 1973). The variation in Invertase activity not only depends on the maturity of cultivars but also on the different somaclones. Thus data showed interesting relation between the different cane varieties, seasons of analysis and age of nodes. The shifting of Invertase activity from 4th and 5th internodes during early growth period to 1st and 2nd internodes during later growth period may be due to higher sugar hydrolysis energy is required in upper side for growth than in basal side where growth was lesser. Similarly others reported that, the Invertase activity was high in tissues of root apex, leaves, stem apex and stem internodes during floral development (Zeleneva and Khavki, 1980; Greenland and Lewis, 1981). In mature tissues, where sucrose is stored, the levels of Invertase are usually low as also reported by Gordon and Flood (1980).

SPS and SS/I are key enzymes in synthesis and breakdown of sucrose respectively (Federico et al., 2002). SPS activity was higher in mature internodes compared with immature internodes in all cultivars. However, high sugar cultivars showed SPS activity compared to low sugar cultivars at all developmental stages. I and SS activity was higher in immature internodes in all cultivars. These results were evidenced by the reports of Verma et al. (2011). There are very few reports of such studies in Indian cultivars differing in maturity and sugar accumulation level/recovery. In this work substantial evidence was obtained with respect to activity of SPS and high sugar content in the variety GSBT-9. Sucrose synthase was high activity in CoC-671 during entire growth period as compared to other entries Co-740, GSBT 8, 7, Co-419 and CoC671. Enzyme Invertase was low active in the late maturing variety than the early maturing variety CoC-671 during the entire growth period. The variations in the different somaclones of variety Co-740 was confirmed at the enzymatic level. These variations depend upon the age of crop and node number of standing cane. It is necessary to undertake detailed investigation at molecular level for these three important enzymes in selected varieties of somaclones. The molecular basis of variation can be studied by under taking marker assisted profiling of proteins and nucleic acids (Naik, 2001). During present work, the partially purified protein was subjected to investigate protein banding pattern using SDS-PAGE electrophoresis. The total protein content was very low in cane leaves, and higher content of phenolics made the study little difficult.

The protein band was stained by using both Commise Brilliant Blue-G as well as silver staining method. The polymorphism using total protein was not conspicuous to provide any confirmed conclusion at this stage of investigation. However, the investigation has helped in standardizing the methodology for protein profiling in sugarcane cultivars for future research. The pattern could shown a tendency of variation in the different varieties including somaclones and its parent (Co-740) approx 8Kd protein band was missing in the varieties GSBT-7, Co-740 and Co-419 which was quite visible in GSBT-8, 9 and CoC-671.The present investigation assumes importance as it starts from the whole plant level studies of cell and tissue culture through enzyme analysis and molecular level approach of protein profiling. It also assumes important as the investigation look into the biotechnological approach to improve the cane productivity in one of the backward parts of sugarcane growing areas of our state.

Conclusions

All 3-somaclones, GSBT-7, 8 and 9 have shown high polyploidy nature. Increase in sugar content found especially in lower internodes at final stages of growth with decreased sucrose synthase activity. The Invertase activity was higher in 4th and 5th internodes during early growth period but its activity has shifted gradually in 1st and 2nd internodes during later stage of growth. The average activity of SS enzyme declined during maturity i.e. from 1st to 5th internode indicating its activity is more in growing region. Activity of SPS enzyme was gradually increased from immature stage (5th month) to

mature stage (10th month) of growth of all sugarcane varieties proportionately with accumulation of sucrose in mature internodes. The polymorphism using total protein was not conspicuous to provide any confirmed conclusion at this stage of investigation. GSBT-9 has shown improved sucrose recovery in the late growth period. This trend of better keeping quality was seen up to 10th months of crop standing in the field that encouraged us to recommend cultivation of GSBT-9 instead of Co-740 in the areas of late crushing of sugarcane.

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