



ISSN NO. 2320-5407

Journal Homepage: - [www.journalijar.com](http://www.journalijar.com)

## INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/1805  
DOI URL: <http://dx.doi.org/10.21474/IJAR01/1805>



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Journal DOI: 10.21474/IJAR01

### RESEARCH ARTICLE

#### REGENERATION OF SOMACLONES FROM SUGARCANE CULTIVAR CO-740 & COMPARISON OF THEIR AGRONOMIC CHARACTERS WITH LOCAL VARIETIES.

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#### Manuscript Info

##### Manuscript History

Received: 12 August 2016  
Final Accepted: 22 September 2016  
Published: October 2016

##### Key words:-

Sugarcane cultivars, Somaclone, Tissue culture, Callus, Sucrose, Growth parameters.

#### Abstract

The present study was carried out to select better performing somaclones generated from the tissue culture of local popular existing sugarcane cultivar Co-740. Three somaclones were selected & compared their growth & yield parameters with local cultivars (Co-740, Co-419 & CoC-671). These parameters studied at regular one month interval from 30<sup>th</sup> day–360<sup>th</sup> day. After 30 days of plantation, maximum germination was found in parent variety Co-740 followed by GSBT-9, and minimum in other selected canes during 2015-16. But all have shown poor germination rate in this climate. The somaclone GSBT-9 has shown maximum shoot length (more number of internodes), tillering, single cane weight & highest number of millable canes in a unit area compared to its parent variety Co740 & other varieties. Thus, improved performance of GSBT-9 is contributing to higher biomass yield. However, though GSBT-9 has higher crop yield (biomass) but it was failed to recover sugar as compared with GSBT-7 & 8 in which sugar recovery was highest among all studied canes. Hence, all selected somaclone have shown better performance in the growth, yield & sugar recovery than their parent variety Co-740 & other existing varieties. Further, GSBT-9 can survive longer period with sugar recovery in this semi-arid climate & late harvesting area.

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#### Introduction:-

Sugarcane has been major crop of India. Sugarcane (*Saccharum*) comprises five species, three are cultivated (*S. officinarum*, *S. barberi* & *S. sinense*) and two are wild (*S. spontaneum* & *S. robustum*). Broadly there are two distinct agro-climatic regions of sugarcane cultivation in India, viz., tropical & sub-tropical lying from 8°N to 33°N latitude except cold hilly areas. Tropical region covers around 45% of total cane area in the country, which includes the states of Maharashtra, AP, Tamilnadu, Karnataka, Gujarat, MP, Goa & Kerala. It is responsible for about 70% of World sugar production (Lakshmanan, et. al., 2002). Production of sugar in India during last five years is rotating around 24.3 to 26.3 million ton. In India, 35 million farmers grow sugarcane for livelihood & equal number of agricultural laborers earns their living by working in sugarcane farms. The main product of sugarcane is sugar; in

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addition many by-products of sugarcane industry like bagasse, molasses, press mud & green top being used for various purposes.

Sugarcane productivity during the last decade has not shown significant improvement inspite of increased input cost and extensive research. This decline in yield is mainly due to the stress conditions like alkalinity, salinity, drought, diseases, etc., or prevalent cropping systems mainly depleting ground water, or continuous adoption of cropping system having no time for summer ploughing resulted in incidence of white grub in sugarcane, and/or due to ever increasing World population on one hand & continued loss of prime agricultural land by diversion of land to housing & industries on the other hand, therefore, the use of more marginal land for high agricultural yield has become inevitable. To overcome these problems, there is a need to improve yield of plant varieties & to re-orient research on evolving stress tolerant crop cultivars using modern research techniques.

The limitations of conventional breeding have been overcome by plant tissue culture which takes advantage of the cellular totipotency proposed by Haberlandt (1902). Tissue culture technique in sugar cane can be used for rapid multiplication of newly developed high yielding, high sugar, disease resistant varieties and rejuvenation of outstanding varieties under cultivation. Tissue culture is now widely used in sugarcane improvement and breeding programme. Heinz & Mee (1969) who first demonstrated plantlets of sugarcane could be developed from callus culture, and callus can be initiated from almost any part of sugarcane tissue like shoot & root (Chen et al., 1988; Rehman, et al., 2002; Khan et al., 2004). Sauvaire & Galzy (1978) and Hendre et. al.,(1983) have developed methods for rapid multiplication of sugarcane by tissue culture. Sugarcane tissue culture has been successfully applied for breeding and propagation by many workers (Kalaw et.al., 1977; Koga & Kudo, 1977; Chagvardief et. al., 1981). Variations may pre-exist in the explants tissue but more usually it develops during in vitro growth of cells. (Heinz & Mee, 1969; Misra, 2009), and Liu & Chen (1976) reported sugarcane plantlets regenerated from callus has wide variation in chromosome number and several other important characters. Junito et. al., (1983) have studied agronomic performance of sugarcane clones derived from callus tissue. There have also successful attempts of isolating sugarcane cell lines tolerant to NaCl (Liu & Yeh, 1984; Fitch & Moore, 1984). Therefore, in this research work, we tried to select efficient somaclones generated during sugarcane tissue culture. Three pre-existing varieties of sugarcane such as Co-740, CoC-671 & Co-419 were used & three somaclones were generated (GSBT-7, 8 & 9) from parent cultivar Co-740. These clones were screened for their growth performance & quantity yield along with other local cultivars in agro-climatic conditions of Bidar.

## **Materials and Methods:-**

### **Planting in plots & parameters observed:-**

The trial experimental plot was prepared at BSSK Ltd (Sugar factory), Hallikhed, in Bidar district. Local popular late maturing cultivar Co-740 and early maturing cultivars CoC-671 & 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 & compared their growth parameters with pre-existing cultivars. The growth parameters studied at regular intervals were percentage of germination, No. of tillers, leaves, internodes, & length of shoot, leaf, weight of single cane, stalk diameter, millable cane/unti area, height of cane, and juice quality. These readings were taken at one month interval from 30<sup>th</sup> day–360<sup>th</sup> day.

### **Preparation of media for tissue culture:-**

The tissue culture was done in aseptic condition on M.S. medium as illustrated by Murashige & Skoog (1962); Brown, (1984); Bright & Jones (1984). The composition of the modified M.S. medium (Heinz & Mee, 1969) used for callus & shoot induction (table 1).

**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     | <b>Macronutrients (10X/1000ml)</b><br>NH <sub>4</sub> NO <sub>3</sub><br>KNO <sub>3</sub><br>MgSO <sub>4</sub> .7H <sub>2</sub> O<br>KH <sub>2</sub> PO <sub>4</sub><br>CaCl <sub>2</sub> .2H <sub>2</sub> O  | 1650.0<br>1900.0<br>370.0<br>170.0<br>440.0                 | 1650.0<br>1900.0<br>370.0<br>170.0<br>440.0                | 1650.0<br>1900.0<br>370.0<br>170.0<br>440.0                |
| 2     | <b>Micronutrients (100X/1000ml)</b><br>MnSO <sub>4</sub> .4H <sub>2</sub> O<br>H <sub>3</sub> BO <sub>3</sub><br>ZnSO <sub>4</sub><br>CuSO <sub>4</sub> .5H <sub>2</sub> O<br>CoCl <sub>2</sub> .6H <sub>2</sub> O<br>NaMoO <sub>4</sub> .2H <sub>2</sub> O<br>KI | 2230.0<br>620.0<br>860.0<br>2.5<br>2.5<br>25.0<br>83.0      | 2230.0<br>620.0<br>860.0<br>2.5<br>2.5<br>25.0<br>83.0     | 2230.0<br>620.0<br>860.0<br>2.5<br>2.5<br>25.0<br>83.0     |
| 3     | <b>FeEDTA (100X/100ml)</b><br>Na <sub>2</sub> EDTA<br>FeSO <sub>4</sub> .7H <sub>2</sub> O  | 3.73<br>2.78  | 3.73<br>2.78   | 3.73<br>2.78   |
| 4     | <b>Vitamins (100X/100ml) &amp; others</b><br>Glycine<br>Thiamine HCl<br>Nicotinic acid<br>Pyridoxine HCl<br>Myo-inocitol  | 100.0<br>50.0<br>50.0<br>50.0<br>100.0                      | 100.0<br>50.0<br>50.0<br>50.0<br>100.0                     | 100.0<br>50.0<br>50.0<br>50.0<br>100.0                     |
| 5     | <b>Polyvinyl pyrrolidone (PVP)</b>  | 50.0  | 50.0   | 50.0   |
| 6     | <b>Sucrose</b>  | 20gm  | 20gm   | 7%   |
| 7     | <b>Growth regulators</b><br>2,4-D<br>Kinetin<br>NAA<br>Coconut milk (CM)  | 3.0<br>--<br>--<br>--<br>100ml                              | --<br>2.0<br>--<br>--<br>100ml                             | --<br>--<br>7<br>100ml                                     |
| 8     | <b>Agar</b>   | 8gm   | 8gm  | --   |
| 9     | <b>pH</b>   | 5.8   | 5.8  | 5.8  |

**Sterilization of materials:-**

All glassware treated with 2% teepol (Glaxo detergent cleaner) & washed in tap water followed by rinsing in distilled water & autoclaved. To maintain aseptic conditions of lab, & 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°C with stirring, filtered & stored frozen. Stock solutions were prepared in distilled water. The hormone stock solutions of 2-4 D & NAA were prepared by dissolving 50mg of pure chemical in 2-5ml of ethanol heated slightly & 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 & gradually diluting to 10ml with distilled water. All the stock solutions were stored in the refrigerator.

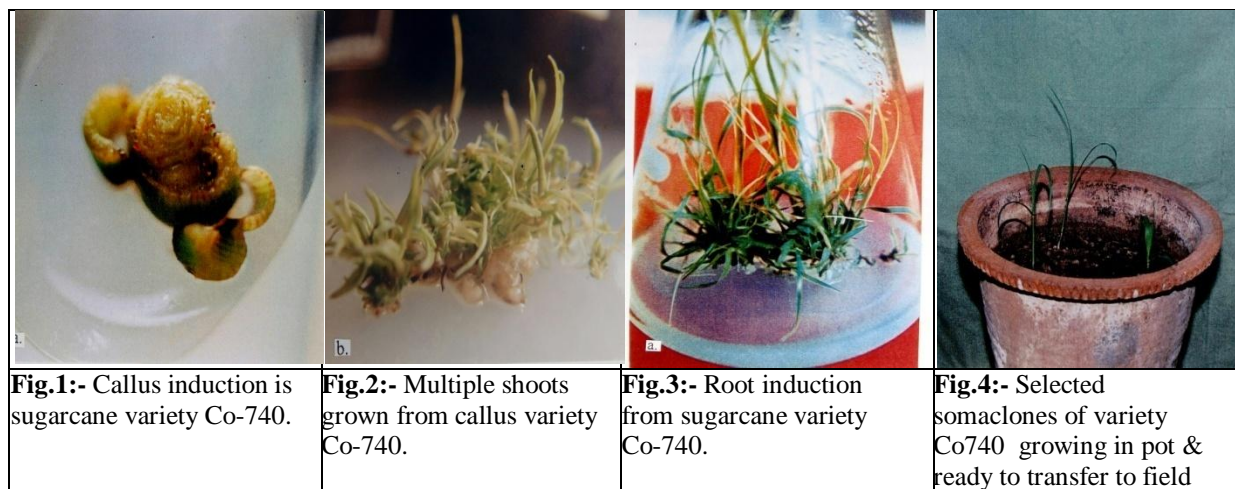
**Preparation of explants:-**

Apical young leaves & sub-apical meristem portion were cut into pieces of 2-3cm & 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<sub>2</sub> & 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  $26\pm 2^{\circ}\text{C}$  in dark condition. After 5-7 days, callus cells form from the broken regions of the explants (fig.1), later considerable mass of callus was found after six weeks. 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 & roots:-

Modified M.S. basal media were used for shoot & 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 ( $40\mu\text{Einstein/m}^2/\text{sec}$ ) and optimum temperature at  $27\pm 4^{\circ}\text{C}$ . Growth of plantlets were 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 & 0.22mg/l IBA were used in both liquid medium using filter paper & semi-solid medium. The better root growth was induced in gyratory shaker (fig.3).



#### Transfer of regenerated somaclone seedlings to pots & plots:-

First the seedlings were grown in test tubes on hormone free medium. Then, these were subjected to less quantity of sucrose till they become green & photosynthetic. At the same time agar concentration in the tubes was increased to enable the seedlings to become strong & adapt with field conditions, and were allowed to grow to height of 10-12cm. They were carefully removed from tubes & 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. It was sterilized in closed box for about 2hrs at  $150^{\circ}\text{C}$  in hot air oven, treated with 10M  $\text{MnCl}_2$ , kept aside for overnight & 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 & Arnon, 1950) for initial 10 days and then switched to tap water using atomizer & 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 pots containing sand & 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 & then clones were propagated further in the bigger plots. Finally, the clones selected in the present work were GSBT-7, GSBT-8 & GSBT-9.

#### Studies of sugarcane growth parameters:-

Quality seeds of local cultivars Co-740, Co-419 & CoC-671 obtained from Agriculture Research Center, Janawada & seeds of somaclones derived from Co-740 were treated with fungicide and planted in small pots (10mx10m). After 30 days of planting, the rate of germination was observed. The seedlings of all these canes were maintained providing all agronomic conditions. The shoot length was measured from surface of soil to the tip of canes during their growth period between 120 -360 days with 30 days of interval. Number of tillers & number of internodes were counted after 120, 240, & 360days of planting, Cane diameter (cm), single cane weight & number of millable cane in 10mx10m were taken after 360 days of growth.

**Analysis of quality parameters:-**

The parameters that are commonly measured for assessing the quality & maturity of sugarcane are Brix, Pol or sucrose percentage and purity.

**Juice Brix:-**

It refers to the total solids content present in the juice expressed in percentage. Brix indicates sugars as well as non-sugars. Brix can be measured in the field itself in the standing cane crop using a Hand Refractometer (HR Brix). Composite juice samples from several canes were collected in the field using pierce. Then a drop of the composite juice sample was placed in the Hand Refractometer and measured the Brix reading. The circular field got darkened relative to the Brix level, which could be easily read. The HR Brix meter has graduations from 0 to 32%. The HR Brix readings were separately taken for each variety. A narrow range indicated ripeness of the cane, while a wide difference indicated cane is not yet ripe. On the other hand, if the bottom portion of cane has lower Brix value than the top, it means that the cane is over ripened & reversion of sugar has taking place.

**Pol percent:-**

The juice sucrose percent is actual cane sugar present in the juice. It is determined by using a polarimeter; hence sucrose percent is also referred to as pol percent. Pol percent and sucrose percent are synonyms. Now days an instrument called Sucrolyser is also used for determination of sucrose percent in juice.

**Purity coefficient:-**

It refers to the percentage of sucrose present in the total solids content in the juice. A higher purity indicates the presence higher sucrose content out of total solids present in juice. The purity percentage along with sucrose percent aids in determining maturity time.

Purity percentage = (Sucrose % / HR Brix) 100

A cane crop is considered fit for harvesting if it has attained a minimum of 16% sucrose & 85% percentage commercial cane sugar (CCS). It refers to the total recoverable sugar percent in the cane. This could be calculated by the following (Sujeet, et.al. 2013) formula:

CCS (tons/ha) = [Cane yield (tons/ha) X Sugar recovery %]/100

Sugar recovery (%) = [S-0.4 (B-S)] x 0.73

where, S=Sucrose percent in juice, B=Corrected Brix percent

**Results:-****Growth performance of sugarcane varieties at experimental field:-****Germination percentage:-**

The germination observed after 30 days of plantation (figs.5) shown maximum germination in parent variety Co-740 followed by GSBT-9 and minimum in the GSBT-8 during 2015-16 (fig.6). This shows poor potential of sugarcane varieties planted in Bidar district, & nearly 50% planting material seems to be wasted due to various agronomic problems.

**Shoot length of sugarcane varieties:-**

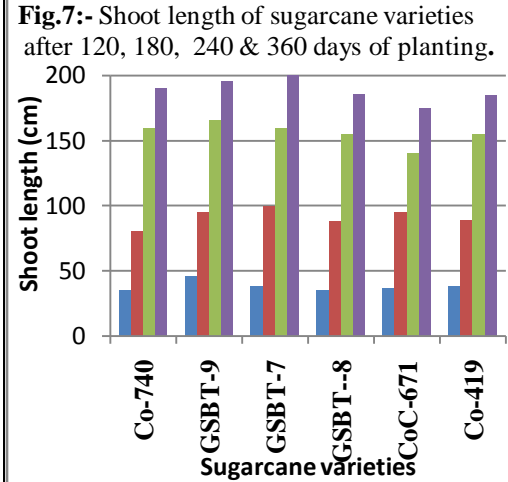
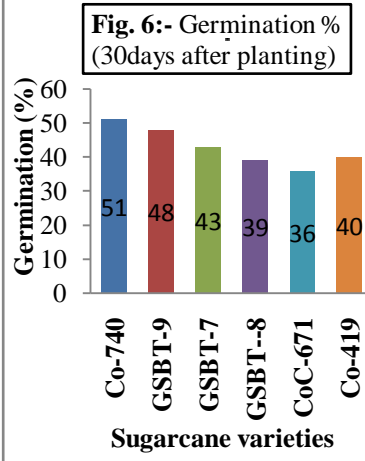
The selected somaclone variety GSBT-9 has shown maximum shoot length for growth period up to 240 days (fig.7 & 8a). GSBT-8 has shown poorest performance among cultivars (fig.8b). But GSBT-7 has shown faster & highest growth during after every stage of observations (fig.8c). GSBT-9 has an edge over other late maturing cultivars & its parent variety Co-740 (Fig.9). However, the cultivars CoC-671 & Co-419 had low growth rate at beginning could reach the maximum height of 200cm at 360 days of growth (figs.10 & 11).

**Number of tillers:-**

It is one of the important parameter in a crop like sugarcane as yield depends on quality & quantity of millable shoots. At the age of 120 days GSBT-9 has maximum tillering as compared to other varieties & it is about 50% higher than others but 18% more than its parent variety Co-740. This trend was similar at 240 days of growth (table 2 & fig.13).



**Fig.5:-** Experimental plot at Bidar, early growth of varieties under trial.



**Fig.8a:-** Well grown nine month old Sugarcane variety GSBT-9.



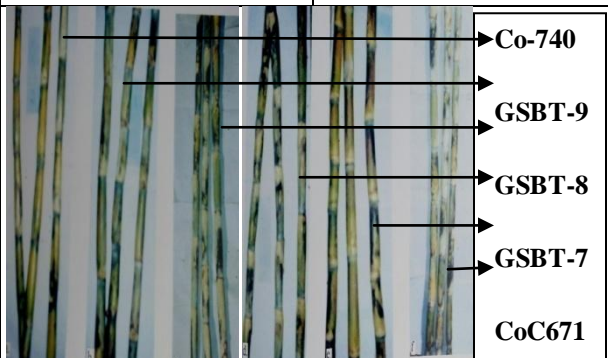
**Fig.8b:-** Well grown nine month old Sugarcane variety GSBT-8.



**Fig.8c:-** Well grown nine month old Sugarcane variety GSBT-7.



**Fig.9:-** Well grown nine month old Sugarcane variety Co-740.



|  |   |   |
|--|---|---|
| <b>Fig.10:-</b> Well grown nine month old Sugarcane variety CoC-671. | <b>Fig.11:-</b> Well grown nine month old Sugarcane variety Co-419. | <b>Fig. 12:-</b> Millable canes of all varieties after harvest. |
|--|---|---|

#### **Number of millable cane and single cane weight:-**

Sugarcane varieties were allowed to grow up to 360 days after planting and single cane weight (fig.14) & number of millable canes/unit area of 10'x10'(fig. 12) were recorded (table 2). GSBT-9 clone shown 13% more number of millable canes in a unit area than its parent variety Co740 & the number were low by more than 30% in other varieties as compared to GSBT-9 & CoC-671. The result of single cane wt. shown 38% higher productivity in the GSBT-9 clone than its parent variety Co740 which has minimum wt. among all varieties tested in the work. Both single cane wt. & no. of millable cane/unit area are important parameters in deciding the cane yield/hectare. Thus improved performance of GSBT-9 can be correlated with these growth parameters contributing to higher yield.

#### **Number of internodes and cane diameter:-**

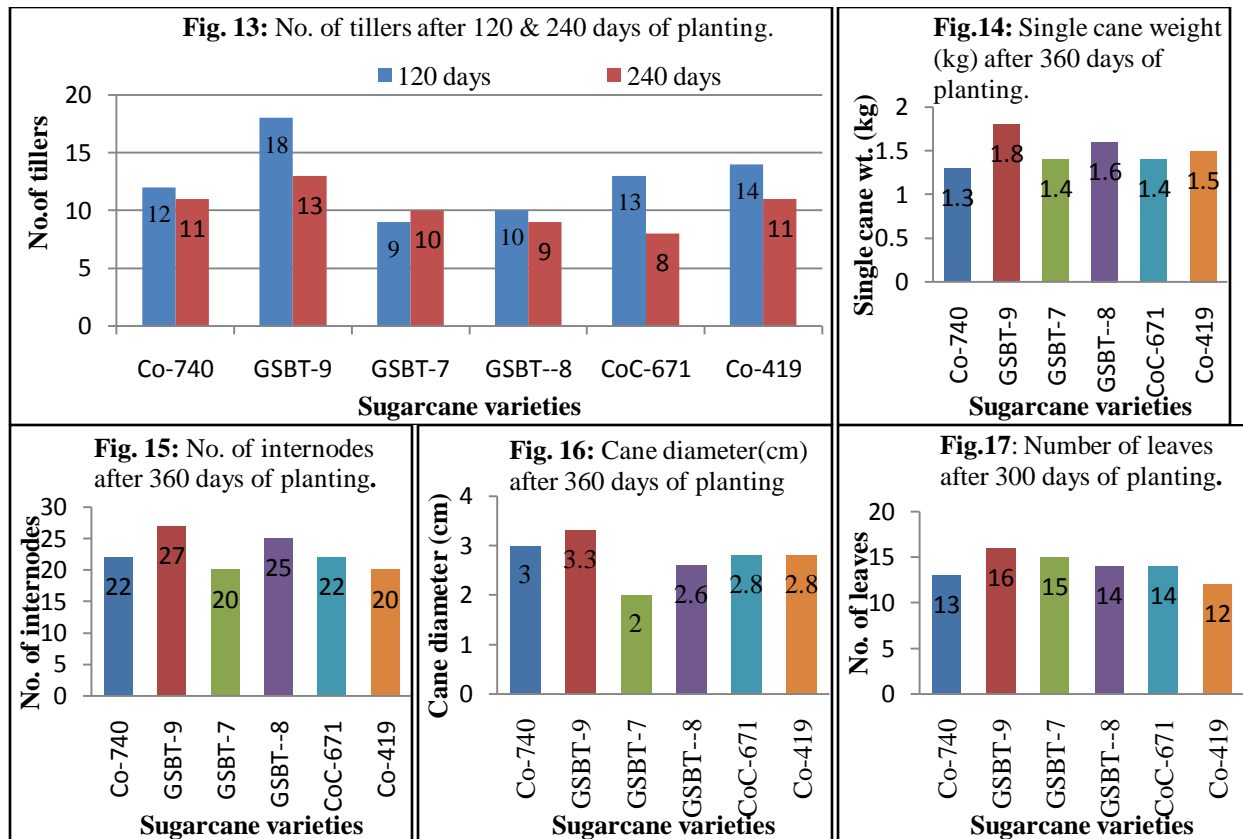
The cane characters not only depend on height of the millable cane but also on diameter & number of internodes. After 360 days of plantation, the minimum number of internodes was found in CoC-671 & Co-419, whereas maximum number seen in GSBT-9 clones (fig.15). It was 23% higher than its parent variety Co740 (table 2). The superiority of GSBT-9 clone is again depicted with respect to cane girth over the other local cultivars (fig.16). It was shown nearly 10% improved cane diameter than its parent variety (Table 2). Desirable characters with respect to cane height, no. of tillers, no. of internodes and diameter of GSBT-9 showed its better performance in this climatic conditions than its parent & other selected varieties.

#### **Yield and sugar recovery:-**

Cane yield was calculated on the basis of number of millable canes/unit area & single cane weight. The GSBT-9 clone contributes to final yield of about 77 metric tons/hectare which was more 55% yield over its parental variety Co-740. Slight higher yield (about 8%) was with GSBT-7 clone. Based on these data, it was calculated that, 56% more sugar/hectare can be obtained by GSBT-9 than its parent variety. As these values based on calculations, their actual yield can be confirmed by only growing in large scale trials in the field. Cane juice from standing cane of the varieties under trial was analyzed from sugar factory. The results of 10<sup>th</sup> month cane juice shown in table 3. Cultivar Co-419 being early maturing variety showed highest recovery (9.39) followed by CoC419 (9.27) among existing cultivars. Whereas, among somaclones, though GSBT-9 has higher crop yield but it was failed to recover sugar as compared with GSBT-7 & 8 in which sugar recovery was highest among all studied canes. The cultivar Co-740 & its three somaclones shoed about 21% lower recovery than cultivar CoC-671. However, the juice analysis after 11 months showed that, the cultivars Co-740 & its somaclones have better keeping quality than cane variety CoC-671.

#### **Number of leaves:-**

The rate of photosynthesis of any crop depends on number of leaves & their size. These two parameters were recorded after 240, 270, 300 & 360 days after planting (fig.17). GSBT-9 & 8 showed maximum no. of leaves after 240 days of growth & Co-671 has minimum. The length of leaf was also maximum in GSBT-9 & minimum was in its parent variety (table 2). This superiority of GSBT-9 clone continued in the standing canes after 270-300 days of growth. Thus more number of leaves & their higher length seems to contribute towards improved productivity in the clone of GSBT-9.



**Table 2:-** Growth parameters of sugarcane varieties after number of days of planting.

| Varieties | Number of tillers |          | Number of internodes | Number of leaves | Cane diameter (cm) | Single cane weight (kg) | Number of millable cane (10'x10'area) |
|-----------|-------------------|----------|----------------------|------------------|--------------------|-------------------------|---------------------------------------|
|           | 120 days          | 240 days | 360 days             | 300 days         | 360 days           | 360 days                | 360 days                              |
| Co-740    | 12                | 11       | 22                   | 13               | 3.0                | 1.3                     | 160                                   |
| GSBT-9    | 18                | 13       | 27                   | 16               | 3.3                | 1.8                     | 180                                   |
| GSBT-7    | 09                | 10       | 20                   | 15               | 2.0                | 1.4                     | 140                                   |
| GSBT-8    | 10                | 09       | 25                   | 14               | 2.6                | 1.6                     | 140                                   |
| CoC-671   | 13                | 08       | 22                   | 14               | 2.8                | 1.4                     | 140                                   |
| Co-419    | 14                | 11       | 20                   | 12               | 2.8                | 1.5                     | 156                                   |

**Table 3:-** 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     |

**Discussions:-**

During present investigation of plant tissue culture technology and field experiments were performed to study the biotechnological aspects of cane productivity in Bidar district. This is first of its kind study in this part of our state, which is rapidly emerging as a sugar bowl of north Karnataka. The technology of developing somaclones through callus culture was standardized during this work. Tissue culture in sugarcane is widely used as crop improvement



process in many parts of the World (Heinz & Mee, 1969; 1971; Naik, 2001). Liu (1981) has reported similar methods for selection of high yielding with high sucrose content line from Taiwan cane cultivars. He has seen significant yield and sucrose recovery differences in selected somaclones of the local varieties. Anbalogan et al.,(2000) reported some phenotypic variability as a result of physiological changes during in vitro conditions (Bairu et al., 2011). In this work also the various growth parameters clearly depicted the improved performance of somaclone GSBT-9 for its yield & GSBT-7 & 8 for their sugar recovery over its parent clone Co-740 and the recent entries CoC-671 & Co-419. This improvement was due to the profuse tillage of this somaclone and improved single cane weight. The yield of sugarcane is a quantitative character dependent upon various traits (Ahmed, et al., 2010). Correlation studies in sugarcane are helpful in selecting for improved clones (Kadian et al., 2006). Correlation studies reveal that the cane yield / plant was positively correlated with number of stalks per plant, cane diameter, cane height & wt. and numbers of internodes. There was lot of variations in growth parameters of selected clones. All 3-somaclones, GSBT-7, 8 & 9 have shown high polyploidy nature. These have shown lot of variations in leaf, stem & flowering characters. Similar results were reported by Saboohi (2014) Even though GSBT-9 shown higher cane productivity, it was failed to synthesize higher sucrose content and thus improved sugar recovery was observed (table 3). In general, all selected somaclone have shown better performance in the yield & sugar recovery than their parent variety Co-740 & other existing varieties. Further, as it was seen that, GSBT-9 has shown improved sucrose recovery in the late growth period i.e. beyond 9.6 month. This trend of better keeping quality was seen up to 12 months of crop standing in the field. These findings 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 & accumulation in sugarcane largely depends on the activity of three enzymes namely Invertase (I), Sucrose synthase (SS) & sucrose phosphate synthase (SPS) as reported by Hatch & Glaszton, (1963); Akzava,(1976); Federico et. al., (2002), Pan et. al.,(2009), and (Biradar et. al.,2016). The growth of sugarcane slows down with increased sucrose contents & productivity (Almeda et. al.,2003). High sugar cultivars (GSBT-9) may have more SPS activity compared to low sugar cultivars, and invertase & sucrose synthase activity may higher in low sugar cultivars like CoC-671 (Biradar et. al., 2016). These variations among somaclones 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 present investigation assumes importance as it starts from the whole plant level studies of cell & tissue culture by biotechnological approach to improve the cane productivity in one of the backward parts of sugarcane growing areas of our state.

### **Conclusion:-**

All the cultivars showed poor germination percentage under experimental condition during 2015-16. It is necessary to look in to the reasons for 50% loss in the quality of planting material during emergence. The somaclone GSBT-9 selection depicted improved growth in terms of leaf length, height of cane, tiller numbers & cane diameter. GSBT-8 has shown poorest performance & GSBT-7 has shown faster & highest growth. GSBT-9, also showed 30-40% high yield as calculated from the number of shoots per unit area and single cane weight, hence it can be considered as suitable line for further cultivation instead of its parent cultivar Co-740 & other varieties. However, though the GSBT-9 has higher crop yield (biomass) but it was failed to recover sugar as compared with GSBT-7 & 8 in which sugar recovery was highest among all studied canes. Hence, all selected somaclone have shown better performance in the yield & sugar recovery than their parent variety Co-740 & other existing varieties. But GSBT-9 can survive longer period with sugar cane recovery in this semi-drought & late harvesting area.

### **Acknowledgement:-**

We are thankful to our Principal, Dr B.S.Biradar for giving us necessary facilities to carryout the reaserch work. We also owe sense of gratitude to Dr.G.R.Naik, Gulbarga University, Kalaburagi for giving us valuable suggestions during the course of this work.

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