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Rapid Multiplication of Promising Sugarcane Varieties Through *in Vitro* Technique

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ABSTRACT

Propagation of sugarcane *(Saccharum* species hybrid*)* through conventional method is limited to low multiplication rates, it's time demand and potential transmission of pathogens through seed cane from generation to generation. To overcome such limitations of conventional propagation, *in vitro* propagation is the best alternative approach to produce healthy and vigour seed cane. The success of sugarcane production programme depends on the rate of seed multiplication of new varieties and it not only helps in rapid multiplication but also is quick adoption of new promising varieties over a large area. A Tissue Culture Lab was established in Haidergarh unit (Barabanki) of Balrampur Chini Mills Ltd., during 2021. Production of Tissue Culture plantlets was achieved as 1 lac, 2 lac. and 3 lac during 2021-22, 2022-23 and 2023-24, respectively. Well grown shoots approximately 4.0 to 5.5 cm. length and having approximate 11 roots / plantlets were transferred to green houses for hardening. The survival % of tissue culture plantlets in green house and field condition was approximately 90% and 94%, respectively. Crops raised though tissue culture were observed far better than normal crops. Samples of mother stock of Co 0118, Co 15023 and CoLk 14201 and their saplings were tested in ICAR – Indian Institution of Sugarcane Research, DBT-Accredited test laboratory, Lucknow and reported that plantlets produced from Haidergarh tissue culture lab are genetically true to type and free form sugarcane bacilli form virus, sugarcane mosaic virus, sugarcane yellow leaf virus and sugarcane streak mosaic virus.

Keywords: Sugarcane, apical meristem, *in vitro study*, healthy seed cane, genetic purity, sugarcane virus elimination.

INTRODUCTION

Sugarcane (*Saccharum* species hybrid) is one of the most important perennial field crop widely cultivated in tropical and subtropical regions globally which belongs to the Poaceae family. Sugarcane is a major commercial crop in India. Sugarcane is also recognized as an important energy crop because of its ability to produce ethanol directly from cellulose and molasses. It is also most efficient crops in the world which converts star energy into chemical energy.

Sugarcane is an industrial crop and one of the most important crops for earning foreign exchange in India. For commercial cultivation, sugarcane is propagated vegetatively and requires a considerable quantity of seeds. Good planting material (seed) contributes to higher cane yields as well as sugar recovery. Therefore, the availability of healthy seed cane is the main prerequisite for improving the productivity of the sugarcane crop. Availability of disease free and true to type planting material is essential for achieving a high yield of sugarcane cultivation. The success of sugarcane production programme depends on the rate of seed multiplication of new varieties as it not only helps in rapid spread but also in quick adoption of new promising varieties over a large area.

As sugarcane is propagated vegetatively, it favors the accumulation of pathogens. Therefore, along with seed canes, disease-causing pathogens are also being introduced into new races. The slow accumulation of different pathogens changes minor disease into major

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diseases (Sharma and Tamta, 2015). In sugarcane, the production of sufficient quantity of field material of new variety takes several years (8-10 years) through conventional methods of seed multiplication by the time of varieties started several investigators have suggested different protocols during the past three decades for *In vitro* micro-propagation of sugarcane (Hendre et al. 1975, 1983; Pathak 2009; Sauvaire and Galzy, 1978; Lee, 1987; Lal and Singh, 1994; Shukla, 1994). There are also chances of perpetuation of sett-borne diseases. *In vitro* micro-propagation techniques are now emerging as a powerful tool to overcome such problems. The most popular and economically beneficial variety Co 0238 occupied approx. 80-90% of the area covered in the eastern part of Uttar Pradesh during the last 8-10 years. Now this variety is highly affected by red-rot disease. The replacement of seed as well as other new varieties are now a big problem. Farmers always want to plant early maturing and high yielding varieties in order to maximize their profit, but it's also critical to educate them about the value of varietal balance and the potential consequences of relying too heavily on monoculture.

Keeping this view in mind a modern Tissue Culture Lab with all required facilities was established during 2021 in Balrampur Chini Mills Ltd., Unit-Haidergarh, Dist. (Barabanki). The objective of this lab was the production of healthy and disease-free sugarcane tissue culture plants (micro propagated plants) on a large scale in the laboratory and to provide the growers quality seed material of Balrampur group units by using three-tier system of sugarcane seed production.

MATERIALS AND METHOD

This study was conducted during 2021-22, 2022-23 and 2023-24 in Tissue Culture Lab at Haidergarh Chini Mills Ltd., a unit of Balrampur Group, which is located in Barabanki district near Lucknow, Uttar Pradesh. Mother plants of three varieties namely Co 0118, Co 15023 and CoLk 14201 that were used as source of explants were raised from stem cutting (setts) obtained from Indian Institute of Sugarcane Research, Lucknow, U.P. and Sugarcane Research Station Karnal, Haryana and were planted properly at the Haidergarh factory farm by using all the recommended agronomical practices.

After age of 8 months, crop shoot tops containing apical meristem were collected from the factory farm early in the morning. The leaf sheaths were removed carefully and cut into about 5 to 6 cm. length. The segments were washed under running tap water for 20 minutes followed by 10 min. rinsing with1% detergent solution. After washing with water several times, the segments were finally surface sterilized with 0.1% aqueous mercuric chloride ($HgCl_2$) solution for 10 minutes followed by 3 times washing with distilled water and the surface sterilized explants were excised and size to 1.25 to 1.5 cm. long for cutting.

Full strength MS basal medium (Murashige and Skoog, 1962) were used as a culture medium. MS based medium consisted of 30 gm/l sucrose for initiation of apical meristem and initiation. Whereas 60 gm/l sucrose for rooting. The pH of the medium was adjusted to 5.8 and agar was used @ 7.0 g/l and auto claved at 121^0C 15 psi for 20 minutes. The medium (20 ml/bottle) was dispensed into glass culture bottles for culturing and stored under aseptic condition until use for shoot initiation. At 8-10 days interval the developed shoots were transferred to fresh medium. Established cultures were transferred on multiplication medium with addition to NAA & GA3 (0.5 mg/l each) multiplied shoots were then transferred after 10-12 days in fresh multiplication medium until production of sufficient number of shoots to initiate. Well grown 3-5 cm long shoots were aseptically transferred to MS basal medium containing 0.500 mg/100 ml solution of NAA. Fully developed rooted plantlets were separated individually and planted in poly bags having soil: sand: compost in 1:1:1 ratio and placed in greenhouse for hardening. After 40-45 hardened plantlets were transferred from green house to open field condition (Singh, 2020; Shukla, 1994).

The plantlets were transplanted for their multiplication at the factory farms *i.e.* Haidergarh (Barabanki), Rauzagaon (Ayodhya), Balrampur (Balrampur), Kumbhi (Lakhimpur-Khiri), Akbarpur (Ambedkar Nagar), Maizapur (Gonda), Mankapur (Gonda) and Babhnan (Gonda) units at the distance of 90 x 45 cm. All the recommended agronomical and cultural practices were followed to grow the crop. The response of varieties in field condition were observed during 2022-23 and 2023-24 at four locations *i.e.* Haidergarh (Barabanki),

Rauzagaon (Ayodhya), Balrampur (Balrampur) and Kumbhi (Lakhimpur-Khiri). Twenty clumps of each variety were selected randomly during the experimentation years in both the planting season (Spring & Autumn) and characters mean were calculated and presented in (Table 3). To test the tissue culture plantlets and mother stock for "True to Type and Virus Free" the samples of all three varieties *viz.* Co 0118, Co 15023 and CoLk 14201 were collected randomly under the guidance of IISR, Lucknow. The mature seed cane was harvested and distributed amongst the growers on bud basis to raise the foundation seed nursery at their own farm under the supervision of our technical team.

RESULTS AND DISCUSSION

The result is based on three consequent years mean data *i.e.* 2021-22, 2022-23 and 2023-24. Suitable nutrient media, its chemical composition and physical form are essential for the successes of *In vitro* culture of plant. An efficient and low-cost protocol for tissue culture of sugarcane on large-scale using apical meristem explants has been developed and were followed. Result during 2021-22 approximately 100000, during 2022-23, 200000 and during 2023-24, 300000 plantlets were produced. The total production capacity of the Lab is approximate 300000 plantlets per year. Nutrient media based on Murashige and Skoog (MS) was used as a basal medium (Salokhe, 2021). Shoot initiation from the apical meristem explants was observed within two weeks after inoculation of explants on MS medium. The results showed that shoot apical meristem culture initiation or establishment responses in the sugarcane varieties were dependent on varieties. Similar results were also reported earlier by Pathak et al. (2009), Hailu et al. (2018), Singh et al. (2020) and Gupta et al. (2021). Result showed that 9 to 15 days are required for shoot initiation, and it takes about 60 to 84 days for transfer in multi medium from initiation medium. 105-171 days are required from shoot initiation to bunching, depending upon the varietal behavior. Co 15023 took more time compared to Co 0118 and CoLk 14201. Root emergence was observed in 8 to 14 days depending upon varietal behavior, 8 days required for CoLk 14201 while 14 days for Co 0118 (Table 1). Shoot initiation percentage was higher in CoLk 14201 (82%) followed by Co 0118 (72.20%) and Co 15023 (59%) (Table 2). Similarly, 70, 68 and 51 percent culture were established finally in CoLk 14201, Co 0118 and Co 15023, respectively. Similar

Table 1 : Days required for different stages and rooting behavior in varieties

Varieties	Days required to shoot initiation	Days required to transfer in multimedia	Days required to bunching	Days required to root emergence	No. of roots per shoot	Length of root (cm.)
Co 0118	11	72	131	14	11	1.04
Co 15023	15	84	171	11	9	1.12
CoLk 14201	09	60	105	8	13	0.98
Mean	12	72	136	11	11	1.04

Table 2 : Response of varieties in tissue culture lab (based on three years mean data)

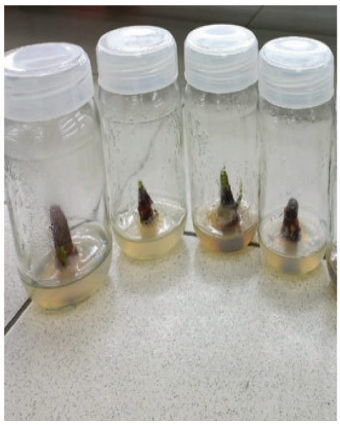
S. No.	Particulars	Varieties			Mean
		Co 0118	Co 15023	CoLk 14201	
1	Shoot initiation (%)	72	59	82	71
2	Culture established (%)	68	51	70	63
3	No. of shoots/culture bottle	10	4	12	9
4	Average shoot height in culture bottle (cm)	5.5	5.4	4.0	5.0
5	Rooting in plantlets (%)	92	86	95	91
6	Survival of plantlets in Green House (%)	94	86	94	91
7	Survival of plantlets in field (%)	95	92	96	94

results were also reported earlier by (Getnet, et. al. 2017); (Jahangir et al. 2014); reported that hormonal supplementation was not the only factor for regeneration, but potential of a specific variety is equally affecting. For shoot establishment the antibiotics are important to prevent bacterial contamination of the mother or the source plant has systemic disease. Besides genotypes, genetically dependent on phenolics oxidation determined *In vitro* survivability and regeneration of explants at the stage of micro-propagation shoot establishment. The number of shoots/bottle were found lowest in Co 15023 (4 nos. plantlets) and higher in CoLk 14201 (12 nos. plantlets) average height of the shoots ranged from 4.0 cm to 5.5 cm. Well grown shoots approximately 4.0 to 5.5 cm length were transferred to rooting medium and root initiation was found is 86% to 95% with a mean value of 91% plantlets. The rooted plantlets were separated individually and washed with running tap water. Soil mixture was filled in poly bags and the rooted plantlets were transferred in the bags. In green-house the survival of the plantlets was observed from 86 to 94% with an average of 91%.

The well-developed plantlets having 4-5 leaves (40-45 days old) were transferred to our different units and transplanted at our factory farm under the supervision of our technical staff. The survival percentage of the plantlets was also higher, which varied from 92 to 96%.

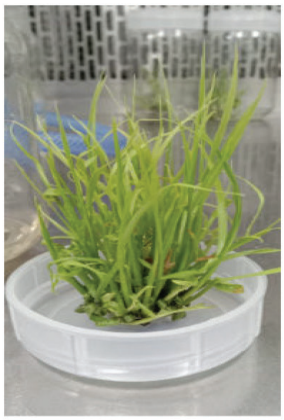
Remarkable improvement was recorded in number of shoots and millable canes in tissue culture crop over the normal crop, it ranged from 13 to 50% with a mean value of 30% in number of shoots and 18 to 31% with an average of 25% in number of millable canes. Number of shoots and millable cane per clump were higher in CoLk 14201 followed by Co 0118 and Co 15023. Improvement in Tissue Culture crop of Co 0118 over the normal crop was recorded 27% and 31 % in number of shoots and number of millable cane per clump, respectively. Similarly, number of shoots increased upto 50% in Co 15023. Number of buds per cane and per clump were counted in both the crops and it ranged from 21 to 24 and 69 to 134 in tissue culture plants and 19 to 22 and 53 to 59 in general crop respectively. The performance of CoLk 14201 was better than both the varieties in respect of number of buds. Improvement in Tissue Culture crop as compared to normal crop was recorded significantly agreed in earlier reports of (Jaleja et al. 2008) and Behera and Sahoo 2009. Maximum improvement was found in cane length in Co 0118 (8.08%) followed by Co 15023 (5.71%) and CoLk 14201 (4.76%). The thickness of tissue culture cane was slightly lower as compared to normal cane, similarly single cane weight was also numerically lesser than normal cane on the other hand improvement in total clump weight was found 15.19 to 18.42% over the normal cane. The current result obtained was in agreement with the earlier report of Salokhe, 2021, Ramanad, 2006 and (Sood, et al. 2006); (Figure 1). It may be due to improvement in cane height and number of millable cane/clumps in tissue culture crop as compared to normal crop (Table 3).

A. Sugarcane top of CoLk 14201

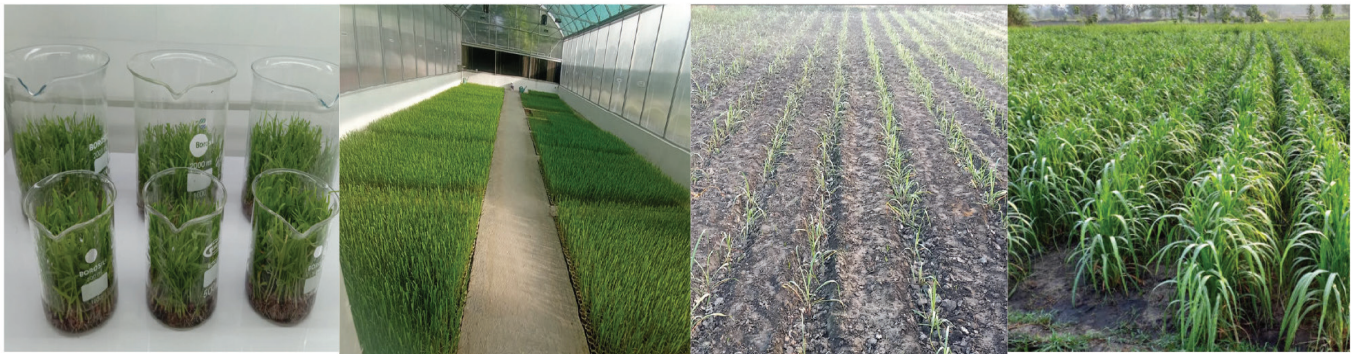


B. Shoot Formation

C. Tiller Formation



D. Profuse tillering



E. Rooted and separeled | **F. Hardening in green house** | **G. Transplanting in field** | **H. Standing crop**

Figure 1 : Different stages of plantlets developed from laboratory to green house and field

Table 3 : Response of tissue culture and normal crop in field condition based on two years and four locations mean data

S. No.	Particulars	Crop		Varieties		Mean
			Co 0118	Co 15023	CoLk 14201	
1	No. of Shoots / clump	Tissue Culture	14	9	17	13
		Normal	11	6	15	11
		Improvement (%)	27	50	13	30
2	No. of Millable Canes /clump	Tissue Culture	4.6	3.3	5.6	4.5
		Normal	3.5	2.8	4.5	3.6
		Improvement (%)	31	18	24	25
3	No. of Buds / stalk	Tissue Culture	21	21	24	22
		Normal	19	19	22	20
		Improvement (%)	11	11	9	10
4	No. of Buds / clump	Tissue Culture	97	69	134	100
		Normal	67	53	99	73
		Improvement (%)	45	30	35	37
5	Cane Length (cm.)	Tissue Culture	2.14	2.22	1.98	2.11
		Normal	1.98	2.1	1.89	1.99
		Improvement (%)	8.08	5.71	4.76	6.03
6	Cane Thickness (cm.)	Tissue Culture	2.1	2.19	2.09	2.13
		Normal	2.16	2.22	2.16	2.18
		Improvement (%)	-2.78	-1.35	-3.24	-2.29
7	Cane Weight (kg.)	Tissue Culture	0.89	0.99	0.89	0.92
		Normal	0.91	1.02	0.92	0.95
		Improvement (%)	-2.20	-2.94	-3.26	-3.16
8	Clump Weight (kg.)	Tissue Culture	3.64	3	4.5	3.71
		Normal	3.16	2.51	3.8	3.16
		Improvement (%)	15.19	19.52	18.42	17.41

Table 4 : Details of foundation seed nursery at growers field in different units of balrampur chini mills limited.

S.N.	Units	Co 0118		Co 15023		CoLk 14201		Total	
		No. of growers	Area (ha.)	No. of growers	Area (ha.)	No. of growers	Area (ha.)	No. of growers	Area (ha.)
1	Haidergarh	31	3.26	51	4.26	211	24.83	293	32.35
2	Rauzagaon	81	11.87	109	16.99	328	36.25	518	65.11
3	Balrampur	286	21.48	247	20.84	782	94.58	1315	136.90
4	Babhnan	56	3.98	340	24.40	240	16.19	636	44.57
5	Akbarpur	133	5.40	130	5.90	1628	146.50	1891	157.70
6	Mankapur	98	6.94	58	4.35	109	6.69	265	17.98
7	Tulsipur	21	1.76	-	-	60	6.84	81	8.60
8	Maizapur	25	3.19	30	4.14	226	38.37	281	45.70
9	Kumbhi	138	8.53	94	6.18	494	30.98	726	45.69
10	Gularia	02	0.26	31	4.10	238	34.50	271	38.86
	Total-	871	66.67	1090	91.16	4316	435.73	6277	593.46

Breeder seed nursery was raised at the factory farms under close supervision in a scientific manner. All the agronomical recommended practices were fully followed to raise the crop. At the 8-10 months age of mature crop, the seed was distributed amongst the progressive growers on bud basis to raise foundation seed nursery. In this regard, during 2022-23 and 2023-24, the breeder seed amongst 871, 1090 and 4316 growers of varieties Co 0118, Co 15023 and CoLk 14201, respectively was distributed. Thus, sugarcane foundation seed nursery though tissue culture seed was raised at fields of 6277 growersconveying an area of 593.46 ha area. This was a remarkable achievement of the BCML group in (Central & East UP) ofNorth India.

Samples of mother stock of Co 0118, Co 15023 and CoLk 14201 and their saplings were tested in ICAR – Indian Institution of Sugarcane Research, DBT-Accredited test laboratory, Lucknow and reported that plantlets produced from. Haidergarh Tissue Culture Lab are genetically true to type and were free form different sugarcaneviruses;sugarcane bacilliform virus, sugarcane mosaic virus, sugarcane yellow leaf virus and sugarcane streak mosaicvirus (data not shown).

CONCLUSION

The tissue culture technique for rapid multiplication of sugarcane seed is the most satisfactory and valuable method for healthy and pathogen free seed production. An effective protocol for *In vitro* rapid multiplication of sugarcane and hardening of plantlets in green house as well as in field condition was developed and standardized. The breeder seed nursery was developed at the different factory farms of the Balrampur Chini Mills Ltd., and the foundation seed nursery was developed at farmers fields under the close supervision of technical staff. Remarkable improvement was recorded in Tissue Culture crop over the normal crop in respect of cane population and number of buds in all three sugarcane varieties. Improvement was recorded up to 50% in shoot population and 31% in number of millable canes. Total clump weight was also higher in Tissue Culture crop, while canes were slightly thinner than normal cane. Plantlets were found to be genetically true-too-type and disease-free. Sugarcane foundation seed nursery was developed through Tissue Culture crop and raised at the fields of 6277 growers conveying 593.46 ha. of area. This is a remarkable achievement of the BCML group units. Thus, it is concluded that the Tissue Culture lab of Balrampur Chini Mills Ltd., is successfully working and achieving the main objective to produce true to type and healthy seed cane in sufficient number.

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