Original Research Article

Assessment of regenerative response of mid-late sugarcane varieties (*Sacchurum officinarum* L.) under in vitro condition

Abstract

With the aim to assess the regenerative response of six mid late sugarcane clones under *in vitro* conditions with four different types of growth media and the research was conducted in Sugarcane Hi-tech Laboratory, SRI, DRPCAU, Pusa, Bihar. Shoot apex explant culture of these cultivars on media M_1 (MS basal) and M_2 (ms basal + 0.5 mgl^1 IAA + 0.5 mgl^1 + 0.5mgl^1 KIN) suggested no definite role of medium on establishment of cultures. Shoot apex culture of the six selected clones on media M_3 (MS basal +0.1 mgl1 IAA + 2.0 mgl1 BAP + 1.0 mgl1 KIN) and M_4 (MS basal +0.1 mgl1 IAA + 2.0 mgl1 BAP + 1.0 mgl1 KIN) resulted in shoot proliferation and elongation. A genotypic difference for all tissue culture responses were found in the six selected cultivars. CoP16437 has the best response among these six cultivars, while CoP9301 has the lowest response.

Keywords: Sugarcane, tissue culture, elongation, proliferation, mid-late

INTRODUCTION

Sugarcane (*Sacchurum officinarum* L.) is the superlative and seductive source of raw material for the sweetening agent. Sugarcane farming is primarily driven by global sugar demand. It is a major cash crop in India due to its profitability and economic importance. It is one of the most important foreign-exchange earning crops, accounting for approximately 1.1% of India's GDP. The sugar industry in India is second only to the textile industry in terms of importance and it employs a large number of people. Sugarcane growers are generally focused on increasing cane yield, while the sugar industry is concerned with sugar recovery. Among these early maturing clones which were selected for the experiment, one is well adopted variety which increases sugar recovery in early crushing season. Early maturing and mid late varieties of sugarcane fulfill the conditions to run the sugarcane industries conveniently for a longer period in the ratio of 30:70 respectively.

Global production of sugarcane is 1.84 billion tonnes. Brazil alone produced 41% of the world's sugarcane, while India produced 17% of it. India occupies an area of 4.608 million hectare with the yield of 67.43 tonnes/hectare and production 341.20 million tonnes, whereas in Bihar, it covers an area 2.43 lakh hectare with yield 67.94 tonnes/hectare and production 11.15 lakh tonnes. The status of sugarcane cultivation in Bihar is decreasing due to syndrome factors. The downfall may be due to the long duration nature of the crop and shifting of the land from sugarcane to other short duration crops. The long duration also exposed it to a wide variety of pathogens causing disease at various stages of its growth. One of the main constraint is seed multiplication of newly developed varieties of sugarcane. If a breeder wants to develop a desirable clone, it typically takes 6-7 years to produce adequate quality seed material. This long period is creating a great bottleneck in breeding programmes. For this purpose, micro-propagation provides a practical and rapid method for clonal material mass propagation. Sugarcane is the suitable crop for the plant biotechnology and genetic engineering tool due to its complex genomic structure, poly-aneuploidy, rare flowering and poor fertility. But, the performance of a genotype under *in-vitro* selection programme depends on its aptitude to *in-vitro* culture in respect of callus culture induction, embryogenic callus production (Badawy *et al.,* 2008) and direct shoot regeneration from shoot apex without intervening callus.

Materials and Methods

The present research was conducted at Sugarcane Research Institute at Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India. The material included in the experiment consisted of six early maturing clones of sugarcane *viz.* CoP9301, CoP11437, CoP11438, CoP16437, CoP18437 and BO153 employed as the source of explants. Healthy and disease-free sugarcane tops obtained from plots of sugarcane form standing crop.

Explants containing shoot apex were taken from each genotype, sterilized by a standard procedure (Siddiqui *et al.,* 1994) and cultured on modified MS medium (Murashige & Skoog, 1962) supplemented with different concentrations of growth regulators. The supplement to be incorporated such as sucrose 30g/l into the basal medium were added before final adjustment of the volume prepared by double distilled water and further plant growth regulators were added and the pH of the medium was adjust at 5.8 ± 0.5 using either 0.1 NaOH or 1N HCl. Data on cultivar establishment, shoot proliferation and shoot elongation were recorded and shootlets were

then sub-cultured on shoot elongation and multiplication media (M_1) MS basal, (M_2) MS basal + IAA(0.5^{-1}) + BAP ($0.5mgl^{-1}$) + KIN($0.5mgl^{-5}$), (M_3) MS basal + IAA($0.1mgl^{-1}$) + BAP ($2.0\ mgl^{-1}$) + KIN($1.0mgl^{-1}$) and (M_4) MS basal + IAA($0.1mgl^{-1}$) + BAP ($2.0mgl^{-1}$) + KIN ($2.0\ mgl^{-1}$). The explants were kept in the dark for 15, 30, 45 and 60 days of intervals were subjected to check the establishment on (M_1 & M_2 media), proliferated shoots and elongated shoots (M_3 & M_4 media). The inoculated culture tubes and bottles were transferred to the tissue culture chamber having controlled environment conditions such as temperature $25^o \pm 2^oC$ and relative humidity (RH) 50% to 80%. The continuous light of about 2 kilo lux was maintained through tube lights. The tissue culture responses were assessed with respect to their frequency as percentage of cultures showing that response and their magnitude either in numbers or in groups namely, low, moderate, good and excellent. The % rate of survival of explants calculated out of total tubes cultured. The average number of proliferated shoots of each cultivar was calculated by random selection of five culture bottles out of total established bottles, whereas one culture bottle was selected out of the total established cultures to measure average shoot elongation.

Results and Discussion

Tissue culture techniques suits as a strong tool for examine and resolve basic problems in plant improvements. Varieties of crop plants differ in their response under *in vitro* condition. Thus, the present investigation has been undertaken for improved cultivars *i.e.* CoP16437, BO153, CoP11437, CoP9301, CoP11438 and CoP18437 of sugarcane grown at SRI, Pusa with the objective to assess the comparative response of these sugarcane varieties under *in vitro* condition with respect to cultivar establishment, shoot proliferation and elongation. Initially, shoot apex explants of the six cultivars were cultured on media M_1 (MS basal) and M_2 (MS basal + $0.5\ mgl^{-1}$ IAA + $0.5mgl^{-1}$ BAP + $0.5mgl^{-1}$ KIN) to assess the explants establishment in culture. The average rate of survival of the explants was 73.3%. There was no definite or clear cut role of MS medium as well as MS basal media with additives on the establishment of cultured shoot apices of the selected cultivars (Table 1).

Table 1: Regeneration potential of establishment in selected cultivars of sugarcane .

Sl. No.	Name of cultivar	M_1 (MS basal)		M_2 (MS basal + 0.5 mgl^{-1} IAA + 0.5 mgl^{-1} BAP + 0.5 mgl^{-1} KIN)	
		No. of explants inoculated	% rate of survival	No. of explants inoculated	% rate of survival
1	CoP16437	60	90.00	60	85.0
2	BO153	60	76.66	60	70.00
3	CoP11437	60	73.33	60	76.66
4	CoP9301	60	56.6	60	48.38
5	CoP18437	60	86.44	60	80.00
6	CoP11438	60	63.33	60	66.66

The average number of differentiated shoots per shoot apex explant culture of the six selected cultivars of sugarcane was observed to be the highest in the cultivar *i.e.* CoP16437, followed by CoP18437, CoP11437, CoP11438 and BO153, while the lowest in the cultivar, CoP9301 at 15, 30, 45 and 60 days interval on media M_3 with IAA (0.1mgl^{-1}) + BAP (2.0mgl^{-1}) + KIN (0.1mgl^{-1}) and M_4 with IAA (0.1mgl^{-1}) + BAP(2.0mgl^{-1}) + KIN (2.0$mgl^{-1)}$ on which shoot proliferation was observed highest in the cultivar CoP16437 (7.0) followed by CoP11437 (6.2), CoP18437 (5.8), CoP11438 (4.4) and BO153 (2.4), while the lowest in the cultivar CoP9301 (0.4) at 15 days interval. After 30 days, it was observed highest in the cultivar, CoP16437 (20.6) followed by CoP18437 (19.4), CoP11437 (17.4), CoP11438 (7.4) and BO153 (6.2), whereas the lowest in the cultivar CoP9301 (4.4). At 45 days interval the cultivar, CoP18437 (40.6) showed the highest average number of shoot proliferation, followed by CoP16437 (38.0), CoP11437 (34.4), CoP11438 (26.8) and BO153 (17.6), while lowest was observed in the cultivar CoP9301 (8.6). After 60 days, it was observed highest in the cultivar CoP16437 (72) followed by CoP18437 (61.4), CoP11438 (34.6) and BO153 (33.6) while the lowest was found in the cultivar COP9301 (26.4) (Table 2).

Table 2: Regeneration potential of shoot proliferation in selected cultivars of sugarcane on M_3 medium.

Sl. No.	Name of	Average no. of shoot proliferation on medium M_3 (MS basal + IAA (0.1 mgl^{-1}) + BAP (0.2 mgl^{-1}) + KIN (1.0mgl^{-1})

	Cultivar	15 days	30 days	45 days	60 days
1.	CoP16437	6.2	16.6	40.6	70.8
2.	BO153	3.6	07.0	16.8	34.2
3.	CoP11437	4.0	14.0	35.8	64.4
4.	CoP9301	0.0	04.0	09.4	25.0
5.	CoP18437	4.2	15.6	39.2	66.4
6.	CoP11438	3.2	08.4	26.4	39.4

Thus, the average number of shoot proliferation during the four intervals was observed highest in the cultivar, CoP16437 followed by CoP18437, CoP11437, CoP11438 and BO153 and the lowest in the cultivar CoP9301 on medium M_4 with 0.1mgl^{-1} IAA + 2.0mgl^{-1} BAP + 2.0 mgl^{-1} KIN (Table 3).

Table 3: Regeneration potential of shoot proliferation in selected cultivars of sugarcane on M_4 medium

SL. NO.	Name of cultivar	Average no. of shoot proliferation on medium M_4 (MS basal + IAA (0.1mgl^{-1}) +BAP(2.0 mgl^{-1}) +KIN (2.0 mgl^{-1})			
		15 days	30 days	45days	60days
1.	CoP16437	7.0	20.6	38.0	72.0
2.	BO153	2.4	06.2	17.6	33.6
3.	CoP11437	6.2	17.4	34.5	61.4
4.	CoP9301	0.4	04.4	08.6	26.4
5.	CoP18437	5.8	19.4	40.6	69.2
6.	CoP11438	4.4	07.4	26.8	34.6

Table 4: Regeneration potential of shoot elongation in selected cultivar of sugarcane on M_3 medium.

Sl. No.	Name Of Cultivar	Average shoot elongation (cm) on M_3 medium (MS basal + IAA (0.1 mgl$^{-1)}$ + BAP(2.0 mgl^{-1}) + KIN (1mgl^{-1})

		15 days	30 days	45 days	60 days
1.	CoP16437	1.5	4.4	8.4	10.5
2.	BO153	1.0	4.6	8.8	11.0
3.	CoP11437	1.2	3.3	7.6	09.8
4.	CoP9301	0.0	2.1	4.2	06.8
5.	CoP18437	1.3	4.1	8.1	10.2
6.	CoP11438	0.8	3.5	7.3	08.9

On medium M_3 with 0.1 mgl^{-1} IAA + 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} KIN, at 15 days interval, the average shoot elongation was observed to be the highest in the cultivar, CoP116437 (1.5cm) followed by CoP18437 (1.3cm), CoP11437 (1.2cm), BO153 (1.0cm) and CoP11438 (0.8cm), while no response was observed in the cultivar CoP9301. After 30 days, the cultivar BO153 (4.6 cm) showed the highest shoot elongation followed by CoP16437 (4.4 cm), CoP118437 (4.1cm), CoP11438 (3.5 cm) and CoP11437 (3.3 cm), whereas the lowest was observed in the cultivar, CoP9301 (2.1 cm). Furthermore, at 45 days interval, shoot elongation was observed to be the highest in the cultivar, BO153 (8.8 cm), followed by CoP16437 (8.4 cm), CoP18437 (8.1 cm), CoP11437 (7.6 cm) and CoP11438 (7.3 cm), while the lowest in the cultivar CoP9301 (4.2 cm). After 60 days of inoculation, the shoot elongation was observed highest in the cultivar BO153 (11 cm) followed by CoP16437 (10.5 cm), CoP18437 (10.2cm), CoP11437 (9.8 cm) and CoP11438 (8.9 cm), whereas, the lowest was observed in cultivar CoP9301 (6.8 cm) (Table 4). Thus, the average shoot elongation during the four intervals was observed to be the highest in the cultivar, BO153, followed by CoP16437, CoP18437, CoP11437 and CoP11438, whereas the lowest in the cultivar CoP9301.

Table 5: Regeneration potential of shoot elongation in the selected cultivars of sugarcane on M_4 medium.

Sl. No.	Name of cultivar	Average shoot elongation (cm) on M_4 medium (MS basal + IAA (0.1 $mgl^{-1)}$ + BAP(2.0 mgl^{-1}) + KIN (2.0mgl^{-1})			
		15 days	30 days	45 days	60 days
1.	CoP16437	1.80	5.8	8.5	10.1
2.	BO153	1.50	5.5	9.0	10.8
3.	CoP11437	1.60	3.8	8.1	09.2

4.	CoP9301	0.45	2.8	5.2	07.2
5.	CoP18437	1.50	5.2	9.1	11.3
6.	CoP11438	1.00	4.2	7.8	10.2

On medium M_4 with 0.1mgl^{-1} IAA + 2.0mgl^{-1} BAP + 2.0 mgl^{-1} KIN the average shoot elongation was observed after 15 days highest in the cultivar CoP16437 (1.8 cm) followed by CoP11437 (1.6 cm), BO153 (1.5 cm), CoP18437 (1.5 cm) and CoP11438 (1.0 cm), while the lowest was observed in the cultivar, CoP9301 (0.45 cm). Further, after 30 days of inoculation, shoot elongation was observed to be the highest in the cultivar, CoP164367 (5.8 cm), followed by BO153 (5.5 cm), CoP18437 (5.2cm), CoP11438 (4.2 cm), CoP11437 (3.8cm), whereas the lowest was observed in the cultivar, CoP9301 (2.8 cm). After 45 days, the highest shoot elongation was observed in cultivar, CoP18437 (9.1 cm), followed by BO153 (9.0 cm), CoP16437 (8.5 cm), CoP11437 (8.1 cm), CoP11438 (7.8 cm) while the lowest was observed in cultivar CoP9301 (5.2 cm). After 60 days interval the highest shoot elongation was observed in cultivar CoP18437 (11.3 cm) followed by BO153 (10.8 cm), CoP16437 (10.2cm), CoP16437 (10.1 cm) and CoP11437 (9.2 cm) whereas, the lowest was observed in the cultivar CoP9301 (7.2 cm) (Table 5). Thus, the average shoot elongation during the four intervals was observed to be the highest in the cultivar CoP18437 followed by BO153, CoP16437, CoP11438 and CoP11437 while the lowest in the cultivar CoP9301.

Discussion

In the present study, for the assessment of establishment, two media M_1 (MS basal) and M_2 (MS basal + 0.5mgl^{-1} IAA +0.5mg^{-1} KIN) were used in all the six cultivars of sugarcane. There is no definite or clear cut role of MS basal medium as well as MS basal with media additives on the establishment of cultured shoot apex of the selected cultivars of sugarcane. Contrary to the findings of the current study, Shukla *et al.* (1994) discussed both auxin and cytokinin as important for achieving good establishment in sugarcane shoot apex. Similar results were obtained by Biradar *et al*., (2009) they revealed that the per cent established varied between different levels of BAP in sugarcane. Contrary to the findings of the current study, the frequency of cultivar establishment was highest with BAP @ 2.0 mg/l (72%), on which shoots were active and healthy. The most established cultivar under *in vitro* culture on both selected media was CoP16437 followed by CoP18437, BO153, CoP11437 and CoP11438 while the least established cultivar was CoP9301. Thus, the sugarcane cultivars showed the differential frequency of survival indicating effect of the varieties on establishment.

Many factors influence the engagement of competent morphogenesis cells including complex interactions between genotypes, explant and the culture medium. Morphogenesis is triggered usually after competent cells are sub-cultured into a less complex medium allowing the expression of new developmental potential (Thorpe, 1983). It is believed that only a single factor, which needs to be applied to the cells not only in right amount but also on right sequence and under right culture conditions for morphogenesis (Stewards *et al*., 1964). *In vitro* plant propagation processes consist mainly of three steps: initiation and multiplication of shoot buds, elongation of shoot buds into shoots and *in vitro* and *ex vitro* rooting of shoots in order to form plantlets. The method has many advantages such as propagation is quick, rapid and plants obtained are true to nature, cultures can be started from very small segments of the mother plants, propagation is possible in most species throughout the year, greater control over chemical, physical and environmental factors and possibilities of rejuvenation from mature tissues (Ahuja, 1986).

The shoot proliferation from cultured shoot apex was observed in all the selected six cultivars of sugarcane namely CoP16437, BO153, CoP11437, CoP9301, CoP18437 and CoP11438. The pooled average number of shoot proliferation on both the medium M_3 with $0.1mgl^{-1}$ IAA + $2.0mgl^{-1}$ BAP+ $1.0mgl^{-1}$ KIN and M_4 with 0.1 mgl^{-1} IAA + 2.0 mgl^{-1} BAP + 2.0 mgl^{-1} KIN for all the selected six cultivars was also observed. The highest shoot proliferation was observed in the cultivar CoP16437 followed CoP18437, CoP11437, CoP11438 and BO153, while the lowest in the cultivar CoP9301 at 15, 30, 45 and 60 days interval. The pooled average shoot elongation on both medium M_3 with $0.1mgl^{-1}$ IAA + $2.0mgl^{-1}$ BAP + $1.0mgl^{-1}$ KIN and M_4 $0.1mgl^{-1}$IAA + $2.0mgl^{-1}$ BAP + $2.0mgl^{-1}$ KIN for the selected six cultivars was observed to be the highest in the cultivar, CoP16437 followed by BO153, CoP18437, CoP11437, CoP11438 and the lowest in the cultivar CoP9301 at 15, 30, 45 and 60 days interval.

Genotype has been also considered as an important factor determining the type and magnitude of responses in tissue culture responses through dominant and additive effect of nuclear genes and cytoplasm factors (Peng and Hodges, 1989). Thus, a genotype difference has been found in tissue culture responses of many plants (Kumar and Mazumdar, 1988; Kumar, 1999). In tissue culture, genotype of sugarcane also played an important role in determining type and magnitude of responses.

Conclusion

As a concluding remark, the best and highly recommended cultivar for shoot proliferation was CoP16437 followed by CoP18437, CoP11437, CoP11438 and BO153 whereas the poorest was CoP9301.

And for shoot elongation was CoP16437 followed by BO153, CoP18437, CoP11437 and CoP11438 whereas, the poorest was CoP9301. Thus, theses sugarcane cultivars showed differential response under *in vitro* conditions suggesting development of specific tissue culture protocol for individual genotype and for future breeding programme.

References

Ahuja MR (1986) *Curr. Sci.*, 55: 217-224.

Anablagan S, Kalmani A and Sakila M (2000) In vitro propagation of Sugarcane: Nature of callus, direct regeneration , regeneration through callus and morphological variations. Res. On crops., 1:138-140.

Badawy OM, Nasar MI ansdAlhendawi RA (2008) Response of sugarcane (Saccharum species hybrid) genotypes to embryogenic callus induction and invitro salt stress. Sugar Tech., 10: 243-247.

Baksha R., Alam R., Karim M. Z., Paul S. K. and Hossain M. A. 2002.*In vitro* shoot tip culture of sugarcane (*Saccharum officinarum* L.) variety lsd28.Biot., 1(3): 67-72.

Belay T., Mulugeta D. and Derbew B. 2014. Effects of 6-Benzyl aminopurine and Kinetin on *In Vitro* Shoot Multiplication of Sugarcane (*Saccharum officinarum* L.)Varieties. Adv. Crop. Sci. Tech., 2 (3): 1-5.

Biradar S., Biradar B. P., Patil V. C. and Kambar N. S. 2009. *In vitro* plant regeneration using shoot tip culture in commercial cultivars of sugarcane.Karnat. J. Agri. Sci., 22: 21-24.

Brown, D. C., & Thorpe, T. A. (1986). Plant regeneration by organogenesis. *Cell culture and somatic cell genetics of plants*, *3*, 49-65.

Dhumale, D.B.; Ingole, G.C. and Durge D.V. (1994).In vitro regeneration of sugarcane by tissue culture.*Ann.of Plant Physiol.,* 8 (2): 192-194.

Gautheret RJ (1938) Plant tissue culture: A History.*The Botanical Magagine*, 96:393-410. Heinz, D.J., Krishnamurthi, M., Nickell, L.G. and Maretzki, A. (1977).Cell tissue and organ culture in sugarcane improvement. In: *Applied and Fundamental Aspects of Plant Cell andOrgan Culture* (eds. Reinert, J. and Bajaj, Y.P.S.) Springer, Berlin. pp. 3-17.

Heinz, D.J., Krishnamurthi, M., Nickell, L.G. and Maretzki, A. (1977).Cell tissue and organ culture in sugarcane improvement. In: *Applied and Fundamental Aspects of Plant Cell andOrgan Culture* (eds. Reinert, J. and Bajaj, Y.P.S.) Springer, Berlin. pp. 3-17.

Kavita, Saxena, S., Anand, A. and Lal, M. (2015). Use of antibiotics to control bacterial contamination during *in vitro*micropropagation of sugarcane.*Agrica* 4: 41-44.

Kefeli, V. and Kuttacek, M. (1977). Phenolic substances and their possible role in plant growth regulation In: *Plant Growth Regulation* (ed. Pilet, P.E.) Springer Verlag, Berlin. pp. 181-188.

Krishnamurthi, M. (1981).Sugarcane tissue culture, an example for crop improvement Proc. *Int. workshop on improvement of tropical crop.through tissue culture,* March 7-14, 1981, Ohakla, Bangladesh. pp. 12-22.

Kumar, H. Upadhyaya, Kumar, M., Shahi, V.K. and Nasar, S.K.T. (1998).Effect of antioxidant in controlling browning of culture during micropropagation of litchi. In: Proc. of *National Seminar on Plant Biotechnology for Sustainable Hill Agriculture,* DARL. DRDO, Pithoragarh.

Kumar, M.B., Barker, R.E. and Reed, B.M. (1999). Morphological and molecular analysis of genetic stability in micropropagated *Frageria x Ananassa cv Pocahontas. In Vitro Cell. Dev. Biol. – Plants*, 35: 254-258.

Siddiqui, S. H., Khan, I. A., Abdullah, K., & Nizamani, G. S. (1994). Rapid multiplication of sugarcane through micropropagation. *Pakistan Journal of Agricultural Research*, *15*(1), 134-136.

Shukla, R., Khan, A.Q. and Garg, S.K. (1994).*In vitro* clonal propagation of sugarcane: optimization of media and hardening of plants. *Sugarcane*, 4: 21-23.

Steward, F.C., Mapes, M.O. and Mears, K. (1958).Growth and organized development of cultured cells II.Organization in cultures grown freely suspended cells.*Amer. J. Bot.*, 45: 705-708.