

Research Article**Effects of various growth regulators on *in vitro* shoot tip culture of sugarcane (*Saccharum officinarum*) varieties Co-86032 and CoC-90063****A. Pauline Fatima Mary* and Dr. (Mrs).S.Usha¹**

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DOI: 10.5281/zenodo.7440547**ABSTRACT**

In the present study, the two varieties of CoC-90063 and Co-86032 of *Saccharum officinarum* were selected and *in vitro* studies were done. The explant chosen was shoot tip. The hormones taken for the present study BAP, BAP+Kn, BAP+IBA, BAP+NAA were employed in different concentrations ranging from 0.5 mg/l – 2.0 mg/l. Co-86032 exhibit faster growth than variety CoC-90063. In both the varieties shoot regeneration percentage, number of shoot per explants was minimum in BAP treatment. BAP + NAA combination exhibit very good response in both varieties CoC-90063 and Co-86032. Multiple shoot per explants varied from 3 to 12 shoots/explants.

Keywords: *In vitro*, Sugarcane, Shoot tip, Explants, Growth hormone, Micropropagation.

INTRODUCTION:

Sugarcane is a major source of edible sugars. Sugar is a highly placed commodity in consumer products. Day by day increasing use of sugar and its relevant products have created a challenging situation for sugarcane researcher and growers. Malik (1990) reported that yield potential of sugarcane varieties is deteriorating day by day due to segregation, susceptibility to diseases, insects, admixture, changes adaphic and climatic environment. Moreover, the lack of rapid multiplication procedures has long been a serious problem in sugarcane breeding programs as it takes 10- 15 years of work to complete a selection cycle. Sugarcane is a tall growing monocotyledonous crop plant which is cultivated in the tropical and subtropical regions of the World primarily for its ability to store high concentration of sucrose or sugar in the internodes of the stem (Cox *et al.*, 2000). Nand Lal and Singh (1994) revealed that the method for rapid multiplication of sugarcane has been developed

using shoot tip culture. Tissue culture techniques play an important role in the genetic improvement of vegetatively propagated crops like sugarcane (Liu and Chen (1984), Krishnamurthy 1986, Siddique *et al.*, 1994). Kenia Tiel *et al.*, (2006) reported the methodology for rapid sugarcane shoot regeneration using meristematic tissue. Recently, plant biotechnology and molecular biology have created unprecedented opportunities and promises in the field of agriculture. The spectacular findings in plant tissue culture have generated outstanding interest, enthusiasm and optimism over the world. Methods have been developed for the propagation of genotype, more and efficient regeneration through micropropagation. Micropropagation is an important tool used in commercial propagation through plant tissue culture. In sugarcane, apical meristem culture is used for rapid multiplication of new promising cultivars and for rejuvenating and prolonging the life span of outstanding

cultivars. In the present investigation plant regeneration protocol through shoot tip culture in sugarcane (*Saccharum officinarum*) using two varieties CoC-90063 and Co-86032.

MATERIALS AND METHODS:

The major plant source for this study was the Noble cane *Saccharum officinarum* L. (Poaceae). In this two varieties CoC-90063, Co-86032 were taken. Which were collected from Aringar Anna Sugar Mill, Kurungkulam, Thanjavur (Dt). The shoot tip explants of 2-4 mm were excised with the help of sterile forceps and sterile blade from 3-4 months old grown plants were used for the present study. The hormones taken for the present study was BAP, BAP+Kn, BAP+ IBA, BAP+NAA and the hormonal effect in different concentration ranging from 0.5 µg/l – 2.0 µg/l were undertaken.

The explants were washed thoroughly in running tap water for 30 minutes and placed in detergent solution for 10 minutes. Then the explants were washed in running tap water to remove the traces of detergent solution followed by washing in double distilled water. Then explants were transferred disinfected with 0.1 percent v/v aqueous mercuric chloride (HgCl₂) for a period of 3-5 minutes in front of laminar airflow. After rinsing in sterilized water several times then disinfected shoot tips were taken for inoculation.

The shoot tips were inoculated vertically oriented on the MS medium containing different combinations and concentrations of growth regulators. Culture tubes were transferred to the inoculation room. The culture room was maintained at temperature of 25⁰ ± 2⁰C. After inoculation the following parameters viz, number of days taken for shooting, number of shoot per explants and shoot regeneration percentage were calculated.

RESULTS AND DISCUSSION:

In the present study two varieties of *S. officinarum* were taken and *in vitro* studies were conducted. The explants chosen were shoot tip. Initial attempts to regenerate plants through *in vitro* technique were made in sugarcane by Nickell (1964), Heinz and Mee (1969). Protocol for *in vitro* plant regeneration of sugarcane through callus culture, axillary bud and shoot tip culture

has been developed by many authors Barba *et al.*,(1978), Sauvaine and Glazy (1978), Heinz *et al.*, (1977), Baksha *et al.*, (2002).

In the present study, Variety Co-86032 exhibited faster growth than variety CoC-90063. Shoot regeneration percentage varies from 50-100 percent. 100 percent shoot regeneration was achieved in BAP+ NAA combinations in the concentrations of 0.5mg/l BAP +0.5 mg/l NAA in the variety Co-86032. The number of days for shooting ranges from 9-10 days. Multiple shoot per explants varied from 3-12 shoots explants. The highest number of multiple shoots in the variety CoC-90063 was produced in the 0.5 µg/l BAP+0.5 mg/l NAA concentration. The highest number of multiple shoot in the variety Co-86032 was observed in the concentration of 0.5 mg/l BAP + 0.5 mg/l NAA.

Baksha *et al.*, (2002) reported that plant regeneration from shoot tip was the highest on MS medium supplemental with BAP 2.0 µg/l and IBA 0.5 mg/l. Neelamathi *et. al.*, (2005) reported that the induction of axillary shoots in sugarcane has been obtained by using MS medium supplemented with riboflavin 10 µg/l along with growth regulators BAP (0.2 µg/l), & Kinetin (1.0 µg/l). Radha Jain *et. al.*,(2005) reported that the shoot tip explants of nine sugarcane varieties were established on a solid MS medium containing 0.8% agar, 3% sucrose and 0.5 mg/l of IAA, IBA & Kinetin.

Kenia Tiel *et al.*, (2006) reported that the methodology for Rapid sugarcane shoot regeneration using meristematic tissue from *in vitro* plantlets. The highest regeneration efficiency, averaging 9.34 shoots/explants was obtained from cut meristematic tissue previously induced in the presence of 4.52-µm 2,4-D.

Kambaska and Santilata (2009), Standardized the protocol for induction of callus and regeneration of plantlets was established through *in vitro* culture using young meristems of Sugarcane (*Saccharum officinarum* L. cv- Nayana) as an explants. The multiple shoot regeneration at various frequencies was observed by using different concentration and combination of growth regulators. The highest percentage of callus induction was observed in MS medium supplemented with 2.5 mg/l, 2-4 D. The best

response in terms of multiple shoot induction was observed on MS medium with BAP 2.0 mg/l + NAA 0.5 mg/l. When *in vitro* shoot tips were inoculated on to the half-strength MS basal media supplemented with 3.0 mg/l NAA, rooting was more profuse. Rooted shoots were transplanted in the green house for hardening and their survival rate was 90% in the field condition.

Shweta Pathak *et al.*, (2009) an experiment was carried out to study the effect of growth regulators on *in vitro* multiplication of shoot cultures and their rooting in sugarcane varieties CoS 99259 and CoSe 01235. About 1.5 cm long shoot tip explants comprising apical meristems and 1-2 leaf primordia were carefully excised from the apical region, sterilized and inoculated on agar (8.0 g/l) gelled MS medium supplemented with various concentrations and combinations of BAP, kinetin and NAA. The results showed that MS medium supplemented with BAP, Kinetin and NAA (0.5

mg/l each) was most suitable for producing optimum number of shoots in both the varieties. For rooting, the multiplying cultures were separated in smaller groups and transferred on half strength MS liquid medium containing 50g/l sucrose and different concentrations of auxins (NAA and IBA). Maximum 94% shoot cultures developed vigorous root system on half strength MS liquid medium containing 50 g/l sucrose and 5.0 mg/l NAA. Gopitha *et al.*, (2010) reported that the Best regeneration of shoot was achieved when they were cultured on MS medium supplemented with BAP 1.0mg/L and IBA 0.5mg/L. Among the different media tested with 3mg/L NAA and 5% sucrose supplemented media proved best production of roots. Lal M. *et al.*, (2014) reported the tissue culture technique could be beneficially utilized for rapid multiplication of sugarcane, if the constraints especially high cost of production and somaclonal variation are taken care off.

Table 1- Effects of Cytokinins (BAP, Kn) and Auxins (NAA, IBA) on different concentrations and combinations in MS medium in shoot regeneration from shoot tip of variety Co-86032.

Name of the Hormone	Hormonal concentration mg l ⁻¹	Variety Co-86032			
		Days to shoot	No. of shoot per explants	Average length of shoot (cm)	Shoot regeneration (%)
BAP	0.5	12	3	2.4	50
	1.0	14	5	2.6	60
	2.0	15	4	1.8	50
BAP + Kn	0.5 + 0.5	12	7	4.4	70
	1.0 + 0.5	13	6	2.5	60
	2.0 + 0.5	11	7	2.4	50
BAP + IBA	0.5 + 0.5	10	10	5.1	80
	1.0 + 0.5	9	9	5.4	60
	2.0 + 0.5	11	9	4.7	60
BAP + NAA	0.5 + 0.5	9	12	4.2	100
	1.0 + 0.5	10	11	5.8	70
	2.0 + 0.5	11	10	4.6	70

Table 2- Effects of Cytokinins (BAP, Kn) and Auxins (NAA, IBA) on different concentrations and combinations in MS medium in shoot regeneration from shoot tip of variety CoC-90063.

Name of the Hormone	Hormonal concentration mg l ⁻¹	Variety CoC-90063			
		Days to shoot	No. of shoot per explants	Average length of shoot (cm)	Shoot regeneration (%)
BAP	0.5	14	3	1.7	60
	1.0	15	4	2.1	50
	2.0	13	4	3.0	50
BAP + Kn	0.5 + 0.5	13	5	3.5	60
	1.0 + 0.5	14	6	4.2	50
	2.0 + 0.5	13	5	4.7	70
BAP + IBA	0.5 + 0.5	10	7	5.0	60
	1.0 + 0.5	9	9	5.7	80
	2.0 + 0.5	11	7	5.3	70
BAP + NAA	0.5 + 0.5	11	10	4.8	90
	1.0 + 0.5	10	8	5.8	70
	2.0 + 0.5	11	8	4.5	80

BAP = Benzyl Amino Purine, Kn = Kinetin, IBA = Indole Butyric Acid, NAA=Naphthalene Acetic Acid.

Figure 1- Effects of Cytokinins (BAP, Kn) and Auxins (NAA, IBA) on different concentrations and combinations in MS medium in shoot regeneration from shoot tip of variety Co-86032.



1. Initiation of axillary shoot on MS medium with BAP + Kn (15 days)



2. Initiation of axillary shoot on MS medium with BAP + NAA (15 days)



3. Development of multiple shoot on MS medium with BAP + NAA (20)



4. Development of multiple shoot on MS medium with BAP + NAA (25)



5. Shoot Elongation on MS medium with BAP (30 days)



6. Shoot Elongation on MS medium with BAP + Kn (30 days)

Figure 2- Effects of Cytokinins (BAP, Kn) and Auxins (NAA, IBA) on different concentrations and combinations in MS medium in shoot regeneration from shoot tip of variety CoC-90063.



1. Initiation of axillary shoot on MS medium with BAP (15 days)



2. Initiation of axillary shoot on MS medium with BAP + IBA (20 days)



3. Development of multiple shoot on MS medium with BAP + IBA (25 days)



4. Development of multiple shoot on MS medium with BAP + NAA (25 days)



5. Shoot Elongation on MS medium with BAP (30 days)



6. Shoot Elongation on MS medium with BAP + Kn (30 days)

CONCLUSION:

Sugarcane (*Saccharum officinarum*) is a major agricultural crop in tropical and sub tropical region of the world. A protocol for tissue culture studies in the two cultivars of *Saccharum officinarum* using shoot tip as explants was developed. Multiple shoots were obtained in sugarcane culture on MS medium supplemented with BAP, BAP+Kn, BAP+IBA and BAP+NAA.

Co 86032 exhibit faster growth than the variety CoC-90063. In both varieties cent percentage of shoot regeneration was observed in the medium containing 0.5mg/l BAP+0.5mg/l NAA. BAP+NAA combination exhibit very good response in both varieties CoC 90063 and Co 86032. The highest number of multiple shoot in the variety Co 86032 was observed in the concentration of 0.5mg/l BAP+0.5mg/l NAA.

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Conflict of Interest: No potential conflict of interest declared.

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