

Original Research Article

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***In Vitro* Culture of Elite Sugarcane Cultivar (*Saccharum* spp) by Shoot Apical Meristem and Effect of Plant Hormones on Shoot Regeneration**

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ABSTRACT

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The present investigation was carried out at Tissue Culture Laboratory located in Sugarcane Research Station, Cuddalore on *in vitro* shoot regeneration of sugarcane apical meristem during the period of 2018-2019. The regeneration of sugarcane variety CoC25 with supplementation of cytokinin 0.1mg/l BAP, 0.015mg/lkin, 0.5mg/lGA₃ was used in sugarcane on defined MS media for shoot induction. The concentration of 0.25mg/lBAP, 0.5mg/lkin, 1.0mg/lGA₃, 0.5mg/l NAA involved in shoot regeneration in variety CoC25 showed the highest shoot length, number of tillers and chlorophyll content. The concentration of ½ MS, 4mg/l NAA and 40g/l sucrose as only auxin involved in root initiation showed the highest number of roots and root length. The sugarcane plantlets were acclimatized in greenhouse before planting in the field.

Introduction

Sugarcane (*Saccharum* spp.) is a member of the Poaceae family and is an imperative cash crop. Sugarcane produces around 70% to 80% of sugar worldwide and 100% in India (Thorat *et al.*, 2017). In India, sugarcane is cultivated under a wide range of agro-climatic conditions and secured a distinct position after cotton, and as an agro-industrial crop because of the

prominent source of efficiently vital product (sugar) as well as by-products (bagasse, molasses and press mud) playing a major role in the economic progress of small and large-scale industrial sectors.

In a tropical climate, the cultivation of sugarcane is more successful in terms of cane yield and sugar recovery throughout the year (Singh and Shami, 2017).

Sugarcane is recognized as the most competent crop, which converts solar energy into harvestable chemical energy in the form of sucrose and biomass (Joyce *et al.*, 2010). Sugarcane varieties are highly heterogeneous and usually multiplied by stem cutting method. The stems of sugarcane with two to three nodes are generally used for plantation in many tropical countries (Behara and Sahoo, 2009). Owing to the unavailability of standardized multiplication procedure and contamination by systematic microorganism is the serious challenge to multiply an elite genotype of sugarcane (Thorat *et al.*, 2016). However, plant tissue culture of sugarcane has received extensive research attention because of its economic consequence (Behara and Sahoo, 2009). During the past four decades, plant tissue culture is used for the large-scale micro propagation in commercial horticulture and agriculture worldwide (Das *et al.*, 1996).

Generally, plant tissue culture technique is used for providing the highest number of healthy plantlets within the short duration, which is useful for rapid spreading of newly released sugarcane varieties in short duration (Fildmann *et al.*, 1994). In sugarcane, explant like apical meristem (Devarumath *et al.*, 2007), were used for rapid multiplication. During the process, to maintain genetic fidelity of the micro-propagated plantlets with respect to the mother plants are major concerns. Micro propagation is the only realistic means of achieving rapid, large-scale production of disease-free seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane (Lorenzo *et al.*, 2001; Taylor and Dukie, 1993). Barba *et al.*, (1978) reported that within 9 months of apical meristem produce planting material from a single spindle which was sufficient to plant a hectare of land. Lee (1987) also reported shoot tip culture for mass propagation of Sugarcane. This study was carried out to develop protocol

for multiplication of elite sugarcane variety CoC25 grown in Tamil Nadu.

Materials and Methods

The variety CoC25 was selected based on their early maturity, high productivity and resistance to pests in different areas of Tamil Nadu. The explant plant materials were collected from the seed plot of Sugarcane Research Station, Cuddalore. All the experimental work was carried out at Sugarcane Research Station, Cuddalore during July 2018 to April 2019. The explant materials were taken from six months old sugarcane plants. Size of the apical meristem taken was 4 - 6 mm. The explants were then cultured on MS (Murashige and Skoog's medium 1962) supplemented with various combinations of BAP, GA₃ and Kin for initiation of cultures, BAP, NAA, GA₃ and tender coconut in liquid medium for the multiplication of cultures and with various levels of NAA for the rooting of cultures.

The media composition used for shoot initiation are T₁- MS+30g sucrose, T₂- MS+0.1mg/l BAP+0.015mg/l kin+0.5mg/l GA₃ +30g sucrose+3.5g/l activated charcoal, T₃- MS+0.2mg/l BAP+0.1mg/l kin+0.5mg/l GA₃+30g sucrose, T₄- MS+0.3mg/l BAP+0.5mg/l GA₃+30g sucrose, T₅- MS+0.5mg/l kin+0.5mg/l GA₃+30g sucrose+3.5g/l activated charcoal. The media composition used for shoot initiation and proliferation are T₁- MS+30g sucrose, T₂- MS+0.1mg/l BAP+0.1mg/l kin+0.5mg/l GA₃+30g sucrose, T₃- MS+0.1mg/l BAP+0.1mg/l kin+0.5mg/l GA₃+0.1mg/l NAA+30g/l sucrose, T₄-MS+0.2mg/l BAP+0.25mg/l kin+0.5mg/l GA₃+0.25mg/l NAA +30g /l sucrose, T₅- MS+0.25mg/l BAP+0.5mg/l kin+1.0mg/l GA₃+0.5mg/l NAA +30g/l sucrose. The media composition used for root initiation are T₁- ½ MS+0.5mg/l NAA+30g sucrose, T₂- ½ MS+1mg/l

NAA+40g/l sucrose, T₃- ½ MS+2mg/l
NAA+40g/l sucrose, T₄- ½ MS+3mg/l
NAA+40g/l sucrose, T₅- ½ MS+4mg/l
NAA+40g/l sucrose, T₆- ½ MS+4mg/l
NAA+40g/l sucrose. Data in case of shoot initiation were recorded 20 days after culturing, for shoot multiplication 30 days after culturing and for rooting 20 days after culturing. The documented data on various observations were analyzed statistically.

Results and Discussion

Shoot tips with apical meristem were found excellent starting material for the micro propagation of sugarcane. Anita *et al.*, (2000) also used shoot tip as explant source for mass micro propagation of sugarcane crop, which supported strongly our choice of explant selection. Rapid shoot growth occurred when shoot tips were used as explants for micro propagation inoculated on MS medium.

Interaction of shoot initiation with hormones BAP, Kin and GA₃

The protocol identified through the present study was an easy and efficient one for sugarcane mass micro propagation which can be easily repeated for the improvement of sugarcane crop. The different hormonal combinations attributed highly significant ($P < 0.01$) impact on growth percentages of shoot tips (Figure 1). Growth percentages for different hormones ranged from 18.33 to 85.50%, where MS+30g sucrose (T₁) showed the lowest value of 18.33% and MS+0.1mg/l BAP+0.015mg/l kin+0.5mg/l GA₃ +30g sucrose+3.5g/l activated charcoal (T₂) showed the highest value of 85.50% (Table 1). Our results are in line with those of Anita *et al.*, (2000), because they also reported shoot tip as explant source for *in vitro* mass micro propagation of sugarcane crop. The reason might be that shoot tip is much safer and fast growing portion of the plant.

The hormones combinations recorded highly significant ($P < 0.01$) interaction. The different hormones combinations showed highly significant ($P < 0.01$) impact on average shoots length of shoot tips. The average shoot length ranged from 2.5 cm for MS+30g sucrose (T₁) to 9.2 cm for MS+0.1mg/l BAP+0.015mg/l kin+0.5mg/l GA₃ +30g sucrose+3.5g/l activated charcoal (T₂) (Table 1).

Razi-ud-Din *et al.*, (2004) reported maximum shoot growth of 83.3% with average shoot length of 3.7 cm when using MS medium supplemented with 5.0 mg/l BAP and 1.0 mg/l GA₃, but the present study we observed that maximum growth of 85% for the variety CoC25 at 0.1mg/l BAP, 0.015mg/l kinetin and 0.5 mg/l GA₃. Similarly different hormones combinations showed highly significant ($P < 0.01$) impact on leaves number. The average leaves number ranged from 2 to 7 where MS+30g sucrose (T₁) showed the lowest value of 2 and MS+0.1mg/l BAP+0.015mg/l kin+0.5mg/l GA₃+30g sucrose+3.5g/l activated charcoal (T₂) showed the highest value of 7 (Table 1).

Shoot multiplication of variety CoC25

Multiplication of cultures, initiated on solid medium was carried out on liquid medium with four different combinations of BAP, Kin, NAA and GA₃ along with control.

Effects of different concentrations and combinations of BAP, Kin, NAA and GA₃ on multiplication of sugarcane variety

Chattha *et al.*, (2001) and Jadhav *et al.*, (2001) reported that different genotypes give shoots on different media. Wongkaew and Fletcher (2004) used MS medium containing 0.5 mg/l NAA, 0.5 mg/l BAP and 15% coconut water for the growth initiation of meristem tips. The different hormones combinations showed

highly significant ($P < 0.01$) impact on average shoot length (Figure 1). The shoot length ranged from 1.5 cm for MS+30g sucrose (T_1) to 13.25 cm for MS+0.25mg/l BAP+0.5mg/l kin+1.0mg/l GA_3 +0.5mg/l NAA +30g/l sucrose (T_5) (Table 2). Khan and Rashid (2003) reported shoot tip initiation on Kin and GA_3 with 5 leaves per plant which is very much similar to our results.

The different hormones combinations also showed highly significant ($P < 0.01$) impact on number of tillers. The number of tillers ranged from 0 for MS+30g sucrose (T_1) to 7.5 for MS+0.25mg/l BAP+0.5mg/l kin+1.0mg/l GA_3 +0.5mg/l NAA +30g/l sucrose (T_5) (Table 2). Cheema and Hussain (2004) observed 29 shoots per plant at 0.4 mg/l BAP in combination with 0.4 mg/l Kin.

The different hormones combinations showed significant ($P < 0.05$) impact on Chlorophyll content of 1.134mg/g for MS+30g sucrose (T_1) to 8.652mg/g for MS+0.25mg/l BAP+0.5mg/l kin+1.0mg/l GA_3 +0.5mg/l NAA +30g/l sucrose (T_5) (Table 2).

The leaves were dark green in color initially but changed to light green and in some cases yellowish after some time. It was observed that chlorophyll content is highest in the *in vitro* plants (8.652mg/g) as they were growing under optimal nutritional as well as temperature and illumination condition provided in the growth chamber. The experimental plants experience a climate change during acclimatization which affects the overall growth of the plant but it regains its normal growth after acclimatization when transferred to the field conditions.

Rooting of sugarcane plantlets

The shoots of 8 to 10 cm in size with multiple leaves were transferred to rooting medium. Growth hormone NAA with six different

concentrations were used (Figure 1). From the study it was found that root initiation started in this variety of sugarcane after 6 to 10 days.

Rooting response of this variety were observed on half strength liquid Murashige and Skoog medium supplemented with various concentrations of NAA with 40 g/l sucrose. There were differences in the response of different concentrations however NAA 0.5 - 5.0 mg/l responded well.

Effects of different concentrations of NAA on rooting of variety CoC25

The average root number for the variety CoC25 were recorded highly significant ($P < 0.01$) different. The average number of roots ranged from 6 to 35. The highest value (35) was observed at 5.0 mg/l NAA while the lowest value (6) was observed for 0.5 mg/l NAA (Table 3). The control for low concentration of NAA hormone failed to produce any root in CoC25. Root length for variety CoC25 was highly significant ($P < 0.01$) for different treatments. The mean root length ranged from 1.0 cm at 0.5 mg/l NAA and 3.05 cm at 5.0 mg/l NAA. Baksha *et al.*, (2002) used 5.0 mg/l NAA for best response of rooting in half strength MS medium. This suggests use of high concentration of NAA for rooting purpose as the best root initiation growth hormone with highest number of 41 roots per plant. Mamun *et al.*, (2004) obtained best results of rooting on MS medium supplemented with auxins (NAA +IBA) 0.5 mg/l for each one. From the present study it was found that NAA 5.0mg/l was best for optimum root growth for the variety CoC25 but at the low concentration of NAA produced only 6 roots per plant. Ali and Afghan (2001) observed only 6 - 7 roots after 3 weeks on MS medium containing 2 mg/l IBA and 6% sucrose but the present study reported 35 roots per plant which are much higher number as compared to their results.

Table.1 List of media composition used for shoot initiation in sugarcane varCoC25

S.No	Treatments	Growth percentage	Shoot length cm	No. of leaves
T ₁	MS+30g sucrose	18.33	2.5	2
T ₂	MS+0.1mg/l BAP+0.015mg/lkin+0.5mg/lGA ₃ +30g sucrose+3.5g/l activated charcoal	85.50	9.2	7
T ₃	MS+0.2mg/l BAP+0.1mg/lkin+0.5mg/lGA ₃ +30g sucrose	60.20	6.3	5
T ₄	MS+0.3mg/l BAP+0.5mg/lGA ₃ +30g sucrose	78.23	7.5	6.6
T ₅	MS+0.5mg/lkin+0.5mg/lGA ₃ +30g sucrose+3.5g/l activated charcoal	56.50	5.5	4
	SEm+	3.8	0.48	0.56
	CD	12.47	1.56	1.83

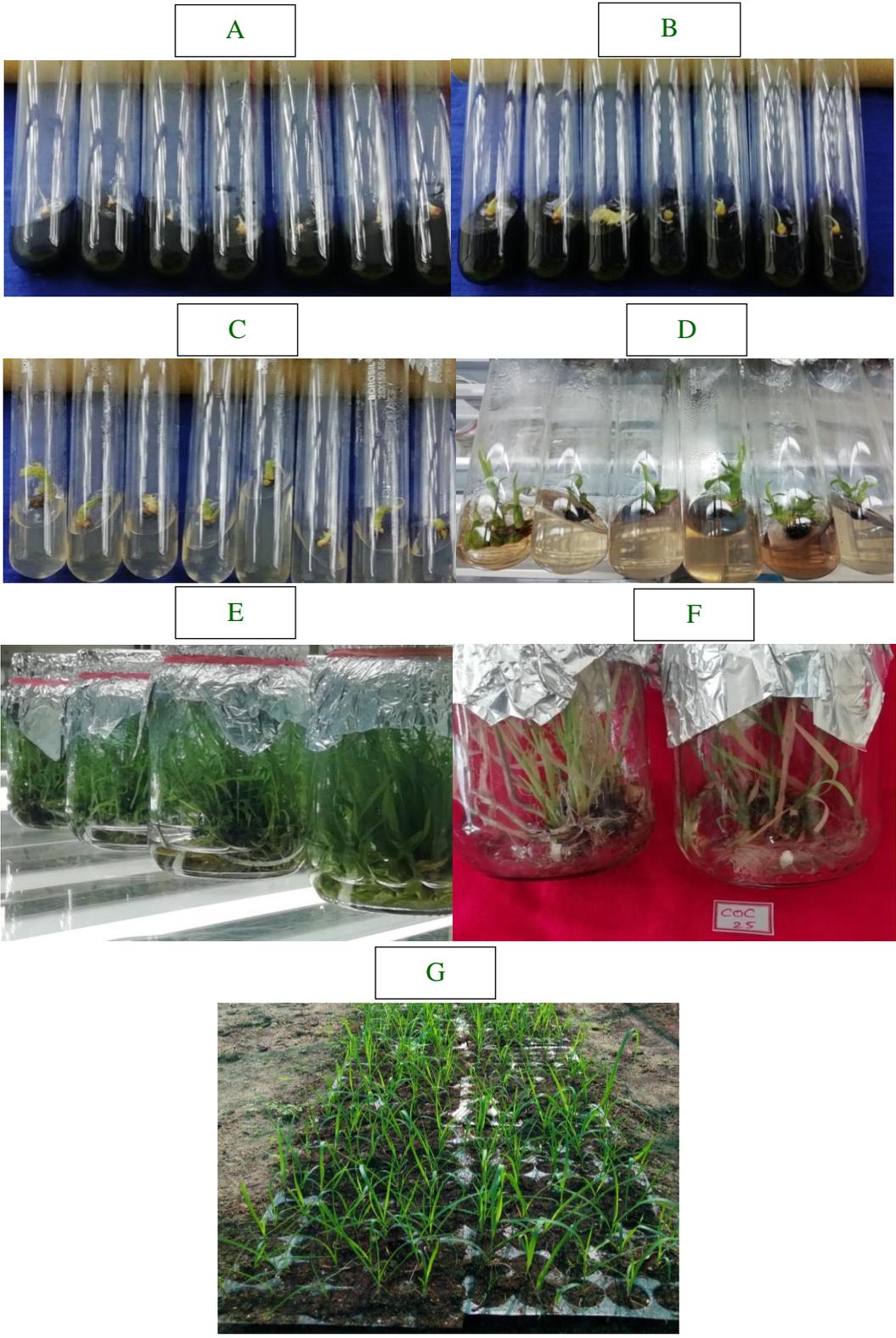
Table.2 List of media composition used for shoot initiation and proliferation in sugarcane varCoC25

S.No	Treatments	Shoot length cm	Number of tillers	Chlorophyll content mg/g
T ₁	MS+30g sucrose	1.5	0.0	1.134
T ₂	MS+0.1mg/lBAP+0.1mg/lkin+0.5mg/lGA ₃ +30g sucrose	8.5	5	3.421
T ₃	MS+0.1mg/lBAP+0.1mg/lkin+0.5mg/lGA ₃ +0.1mg/l NAA+30g/l sucrose	9.1	3.5	4.760
T ₄	MS+0.2mg/lBAP+0.25mg/lkin+0.5mg/lGA ₃ +0.25mg/l NAA+30g /lsucrose	11.52	5.5	6.785
T ₅	MS+0.25mg/lBAP+0.5mg/lkin+1.0mg/lGA ₃ +0.5mg/l NAA+30g/l sucrose	13.25	7.5	8.652
	SEm+	0.49	0.26	0.22
	CD	1.59	0.85	0.72

Table.3 List of media composition used for root initiation in sugarcane varCoC25

S.No	Treatments	No. of roots	Root length (cm)
T ₁	½ MS+0.5mg/lNAA+30g sucrose	6	1.00
T ₂	½ MS+1mg/l NAA+40g/l sucrose	15	1.40
T ₃	½ MS+2mg/l NAA+40g/l sucrose	19	2.15
T ₄	½ MS+3mg/l NAA+40g/l sucrose	23	1.75
T ₅	½ MS+4mg/l NAA+40g/l sucrose	29	2.95
T ₆	½ MS+4mg/l NAA+40g/l sucrose	35	3.05
	SEm+	1.01	0.38
	CD	3.20	1.20

Apical meristem inoculated test tube, C,D- Grownup Apical meristem, E- Shoot multiplication
F- Shoots in multiplication medium, G- Grownup plants



Acclimatization

The rooted plantlets were transferred to portray and covered with polythene bags for high humidity for 2 - 3 days. After that these were transferred to soil in growth room for few days and then these were transferred to green house to assess their potential for further hardening. The acclimatization potential was 70 - 80%. Micropropagation of sugarcane from shoot tip may become the successful method to cope with the present day demand. It will be an easy way for obtaining intensive number of plants in limited time under controlled conditions. Through the use of tissue culture technique it may be easy to obtain disease free plants. The protocol used in the present study can be used for rapid multiplication of sugarcane especially for the variety CoC 25.

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