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Research Article

STANDARDISATION OF CALLUS CULTURE TECHNIQUES FOR EFFICIENT SUGARCANE MICROPROPAGATION

***Yadav S. and Ahmad A.**
Department of Botany, G.F. College, Shahjahanpur, U.P., INDIA
**Author for Correspondence*

ABSTRACT
Standardization of protocol for induction of callus and shoot regeneration was established through in vitro culture of sugarcane. The best callus induction was observed at 3.0 mg/l of 2,4-D. The best response in terms of multiple shoot formation was observed that on MS medium supplemented with BAP, Kinetin and NAA (0.5mg/l each). Best rooting was observed in half-strength MS medium supplemented with 3mg/l NAA among different auxin.

Key Words: *Callus Culture, in vitro, Saccharum Officinarum L.*

INTRODUCTION
Sugarcane belongs to the genus Saccharum officinarum L., of the tribe Andropogoneae in the grass family (Poaceae). This tribe includes tropical and subtropical grasses including the cereals genera Sorghum and Corn. Sugarcane is one of the most efficient photosynthesizer, C- 4 plant in plant kingdom and commercially propagated through stems cuttings. Sugarcane breeding programmes have focused on the production of varieties with high yield, higher sucrose content, pest and disease resistance, tolerance to abiotic stress and improved rooting ability (Brumbley *et al.,* 2008). The growing demand of newly released varieties could not be fulfilled by only conventional propagation methods as the multiplication rate through sett is 1:8. This restricts fast spread of superior varieties. Therefore, application of plant tissue culture techniques provides an alternative method for the crop improvement (Sengar *et al.,* 2011). Targeted research on mass multiplication and sugarcane biotechnology began only in 1960s. The pioneer works on induction of callus and production of roots on callus was achieved at Hawaiian Sugar Planters (Nickel, 1964; Heinz and Mee, 1969). Plant tissue culture offers the best methodology through micropropagation of sugarcane for quality and phytosanitary planting material at a faster rate in a shorter period of time. Tissue culture can increase the propagation potential by 20-35 times (Geijskes *et al.,* 2003; Snyman *et al.,* 2006). In addition, plants can be disease indexed (Snyman *et al.,* 2007) and healthy material multiplied in half the time compared to the conventional vegetative route. Numerous studies on sugarcane plant regeneration have been reported. Essentially, successful culture and regeneration of plants from protoplasts, cells, callus and various tissue and organs have been achieved in this crop. The objective of this study was to standardize protocol for induction of callus and shoot regeneration in sugarcane.

MATERIALS AND METHODS
Shoot top of explant was collected from field grown 8-10 months old of sugarcane variety CoSe 01235. Leaf cylinders provided by immature leaf rolls were used for callus induction. Explant was washed thoroughly under running tap water followed by 70 % alcohol for 1 hr. The explant material was taken into laminar flow cabinet and surface sterilized with 0.1% $HgCl_2$ for 7 minutes and rinsed 3 times with sterile double distilled water. The explants were then aseptically cultured on modified MS medium supplemented with different concentration of IBA, NAA and 2, 4-D for callus induction. Media were prepared with 3% sucrose, 0.8 % agar pH was adjusted to 5.6 and autoclaved at 121º C for 20 min. All cultures were incubated at 25±2º C and kept under 14 h photoperiod of fluorescent tube light. Callus was then transferred on different concentrations and combinations of cytokinin (BAP, Kinetin) and auxin

(IBA, NAA) for shoot regeneration. The in vitro grown micro-shoots were inoculated in the half strength MS media supplemented with different concentrations of auxin (IBA, NAA and IAA).

RESULTS AND DISCUSSION

The leaf sheath explants of sugarcane on MS media supplemented with varying concentration of IBA, NAA and 2, 4-D. Table 1 showed callus initiation at the cut margins of the explants after 12-15 days of inoculation. Maximum 70% leaf sheath explants showed callus initiation on MS medium supplemented with 3.0mg/l of 2,4-D. At lower concentration of 2, 4-D, low frequency of callus initiation was observed. Callus culture of sugarcane have also been successfully established using young leaves and young inflorescence as explants on MS medium containing 2,4-D and coconut milk (Nadar *et al.*, 1978; Liu and Chen, 1984; Bhansali and Sing, 1984). In sugarcane culture, 2, 4-D has proved to be indispensable for callus induction, proliferation and even embryogenesis (Brisible *et al.*, 1994, Chengalrayen and Gallomeaghar, 2001; Kenia *et al.*, 2006).

The data presented in Table (2) demonstrated that BAP or Kinetin alone could not induce sufficient shoot regeneration. A significant increase in shoot regeneration frequency (43%) was observed when both BAP and Kinetin were used simultaneously. Addition of NAA (0.5 mg/l) further enhanced the frequency of shoot regeneration (65%). Number of shoots per culture and length of shoots was also influenced by the growth regulators. The highest shoots per culture (8.6) and shoot height (6.1 cm) were observed in presence of BAP, Kinetin, NAA (0.5mg/l each). The present results are in agreement with previous reports given by other investigators who used cytokinin in combination with auxins in tissue culture of sugarcane (Chen *et al.*, 1988; Seema *et al.*, 2001). However, a high level of cytokinin and a low level of auxin were essential for differentiation of adventitious shoot in sugarcane leaf sheath callus.

Different types of auxins (IBA, NAA and IAA) were used at different concentrations and combinations to regenerate adventitious roots.

Table 1: The effect of different concentration of IBA, NAA and 2, 4-D on callus induction from leaf sheath explants of sugarcane

Plant growth regulator	Concentration mg/l	No. of explants inoculated	No. of explants showed callus	% of explants with callus induction
IBA	0.5	10	0	0 %
	1.0	10	1	10%
	2.0	10	2	20%
	3.0	10	1	10%
	5.0	10	2	20 %
NAA	0.5	10	00	0%
	1.0	10	1	0%
	2.0	10	2	10%
	3.0	10	1	20%
	5.0	10		10%
2,4-D	0.5	10	1	10%
	1.0	10	4	40%
	2.0	10	5	50%
	3.0	10	7	70%
	5.0	10	5	50%

(Table-3) Among them NAA and IBA were found to be comparatively responded better than IAA for profuse rooting. NAA+ IBA combination showed positive enhanced result. Best rooting was observed in half-strength MS medium supplemented with 3 mg/l NAA and the highest number of roots per micro-

shoots was 13.8 with average root length 4.6 cm. Gosal *et al.,* (1998) reported rapid multiplication in liquid MS medium on BAP (0.5 mg/l) and Kin (0.5 mg/l) and rooting on NAA (5 mg/l) and sucrose 70%. Sorory and Hosien (2000) reported that the use of 6% sucrose concentration enhanced shoot regeneration in sugarcane. Lal and Singh (1994) reported that the most efficient auxin for root initiation was NAA. Roots grow from the nodal primordial when the plantlets are well developed (Khan *et al.,* 1998). Rooting was highly influenced by the different types and concentrations of auxin used (Appropriate amounts of auxin in the rooting medium are crucial for root induction).

Table 2: Effect of the cytokinin (IBA, kin) and auxin (IBA, NAA) at different concentrations and combinations on shoot regeneration from callus tissue

Plant Growth Regulator	Concentration (mg/l)	% of microshoots rooted	Average No. of roots per micro shoots	Average length of roots (cm)
IBA	0.5	32	6.4	1.3
	1	66	8.2	2.3
	3	87	11.6	3.5
	5	71	7.9	3.8
	7	42	6.2	2.3
NAA	0.5	40	9.7	1.2
	1	64	10.8	1.3
	3	94	13.8	4.6
	5	79	8.7	3.5
	7	52	6.8	3.2
IAA	0.5	-	-	-
	1	18	6.3	1.2
	3	56	7.9	1.5
	5	31	3.6	2.4
	7	-	-	-
NAA+IBA	0.5+0.5	-	-	-
	0.5+1.0	42	6.4	2.1
	1.0+0.5	51	7.1	2.4
	1.0+1.0	64	8.9	3.8

Table 3: Effect of different auxins on *in vitro* rooting of shoots developed from leaf sheath callus

Plant Growth Regulator	Concentration mg/l	% of explants produced shoots	No. of shoots per explant	Average length of the shoots(cm)
BAP	0.5	15	4.3	3.6
	1.0	59	5.9	3.2
	2.0	47	4.7	3.0
Kinetin	0.5	14	2.6	2.9
	1.0	45	4.6	3.0
	2.0	31	2.5	2.7
BAP+ Kinetin	0.5+0.5	43	6.7	4.3
	1.0+0.5	34	5.8	5.1
	1.0+1.0	49	7.2	5.4
BAP+ Kinetin + NAA	0.5+0.5+0.5	65	8.6	6.1
	1.0+1.0+0.5	55	5.6	5.3
	2.0+2.0+0.5	46	3.9	4.9

Many workers also reported that 5 mg/l NAA was good for rooting (Larkin, 1982; Shukla *et al.*, 1994; Alam *et al.*, 1995) and more than 5 mg/l NAA inhibits rooting. In conclusion, 3.0 mg/l of 2, 4-D was best for callus formation. MS medium supplemented with BAP, Kinetin and NAA (0.5mg/l each). Was best for multiple shoot formation was observed that on MS medium supplemented with BAP, Kinetin and NAA (0.5mg/l each). Best rooting was observed in half-strength MS medium supplemented with 3mg/l NAA.

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