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Effect of different concentrations of 2, 4-dichlorophenoxy acetic acid on callus induction of sugarcane (*Saccharum officinarum* l.) Var. Co 86032 and com 0265

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Abstract
Standardization of protocol for callus induction and response of callus to different concentrations of 2, 4-D of two sugarcane genotypes (Co 86032, CoM 0265) was established through callus culture using leaf whorl and meristem as an explant. The results showed that meristem as well as leaf whorl explants were suitable for callus induction in sugarcane. MS medium supplemented with 3 mg/l and 4 mg/l of 2, 4-D was found most suitable for callus induction and multiplication in both the varieties. This treatment also took minimum number of days for callus induction and maximum callus induction percentage in both the genotypes. Profuse and good quality callus induction was observed in MS medium + 3 mg/l and 4 mg/l 2, 4-D in both the explants genotypes.

Keywords: Sugar cane, Meristem, Leaf whorl, Tissue Culture

1. Introduction
Sugarcane is one of the most important industrial crop in both tropical and subtropical region of the world and a major export product of many developing countries. It is a principal raw material for sugar industry as world's 75% sugar comes from sugarcane [Naik *et al.* (2001), Anon. (2013)] [15, 1]. In addition to sugar production, it is raw material for paper, alcohol, plywood, industrial enzyme and animal feed. Lack of rapid multiplication has been a serious problem in Sugarcane breeding [Ali and Afghan (2001)] [2]. Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology. During the last thirty years, micro-propagation and other *in vitro.* techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop plants [George and Sherrington (1984), Das *et al.* (1996] [6,5]. Tissue culture can increase the propagation potential by 20-35 times [Geijskes *et al.* (2003), Snyman *et al.* (2006)] [7, 18]. In addition, plants can be disease indexed [Snyman *et al.* (2007)] [19]. 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 of sugarcane variety Co 86032 and CoM 0265 using leaf whorl and meristem explants.

Materials and Methods
The experiment was conducted at sugarcane tissue culture laboratory, Main Sugarcane Research Station, Navsari Agricultural University, Navsari, Gujarat during 2014-2015. The genotypes Co 86032 and CoM 0265 are the experimental varieties. Meristem and leaf whorl explants of these two varieties (Co 86032 and CoM 0265) were collected from 5 month old grown plant from Main Sugarcane Research Station field. Cane tops with the growing apices were cut approximately 10 cm long and washed thoroughly in running tap water for 30 minutes. Outer sheaths of cane tops were removed by wiping the sheath with rectified spirit. The shoots were then washed with soap water (2 drops of Labonin into 250 ml of water) for about 5 to 6 minutes in a sterile 1 litre conical flask, followed by cleaning the materials with distilled water. The shoots were rinsed in 5 per cent sodium hypochlorite for 10 minutes. Then

shoots were thoroughly rinsed in 70 per cent ethanol for 30 seconds followed by sterilize double distilled water for 4-5 times till ethanol was completely washed out from the surface of material. Surface sterilization was performed by using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Then the container was taken to a laminar clean air station. They were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex was done by carefully removing the 2-3 outer whorls of the developing leaves with the help of a sterile sharp blade. The second innermost and inner most whorls of developing leave cut in to small pieces of approximately one centimetre length with the help of a sterile sharp blade and utilized as explant for callus induction on MS medium supplemented with different concentrations of 2,4-D.

Result and Discussion

1 Number of days required for callus induction (Table 1)

MS medium supplemented with 4 mg/l 2, 4- D showed minimum number of days (13.67 days in Co 86032 and 21.33 days in CoM 0265) for induction of callus from leaf whorl taken as an explant. On the other hand, MS medium supplemented with 4 mg/l 2, 4- D registered minimum number of days (13.67 days in Co 86032 and 20.33 days in CoM 0265) for callus induction from meristem taken as an explant. Similar results was found by [Ramanand *et al.* (2006), Behera and Sahoo (2009) and Tahir *et al.* (2011)] [20, 4, 21]. From meristem taken as an explant. However, Lago and Barreto (1987) [13]. Observed callusing in 14 days and the differences in the results may be ascribed to the genotypic differences, plant growth regulators or the explant used.

Overall, the MS medium supplemented with 4 mg/l 2, 4- D registered minimum number of days for callus induction in Co 86032 (Leaf whorl=13.67, Meristem= 13.67) and CoM 0265 (Leaf whorl=21.33, Meristem= 20.33). But CoM 0265 taken more days for callus induction as compare to Co 86032 in both the explants, while no callus induction was observed in control in both genotypes.

Table 1: Days to callus induction and callus induction percentage (%) of sugarcane Cv Co 86032 and CoM 0265 as influenced by different concentrations of 2, 4-D.

Tr. No.	Concentrations of 2, 4-D (mg/l)	No. of Days required for callus induction		Callus induction %		No. of Days required for callus induction		Callus induction %	
		Co 86032				CoM 0265			
		Leaf whorl	Meristem	Leaf whorl	Meristem	Leaf whorl	Meristem	Leaf whorl	Meristem
T_1	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2	1	20.33	20.67	54.67	60.67	26.33	26.67	49.33	54.00
T_3	2	16.67	18.33	71.67	74.33	24.33	24.33	66.67	67.33
T_4	3	14.33	15.33	81.33	82.67	22.67	21.67	74.67	74.33
T_5	4	13.67	13.67	83.00	84.33	21.33	20.33	76.00	75.67
T_6	5	21.67	21.33	50.67	53.67	25.67	24.67	47.67	43.00
SEM ±	Explant	0.124		0.236		0.124		0.283	
	Treatment	0.176		0.333		0.176		0.401	
	Explant × Treatment	0.304		0.577		0.304		0.694	
CD at 5 %	Explant	0.363		0.688		0.363		0.827	
	Treatment	0.513		0.973		0.513		1.169	
	Explant × Treatment	0.888		1.685		0.888		2.025	
CV %		3.59		1.72		2.65		2.31	

2 Callus induction (%) (Table 1)

Callus induction per cent was varied in different explants with different levels of 2, 4-D in both the genotypes [Behera and Sahoo (2009)] [4]. MS medium supplemented with 4 mg/l 2, 4-D showed maximum callus induction % (83.00% in Co 86032 and 76.00% in CoM 0265) from leaf whorl taken as an explant. On the other hand, MS medium supplemented with 4 mg/l 2, 4- D maximum callus induction % (82.67% in Co 86032 and 75.67% in CoM 0265) for callus induction from meristem taken as an explant.

Maximum callus induction per cent was observed in MS medium supplemented with 4 mg/ l of 2, 4-D [Ramanand *et al.* (2006)] followed by MS medium supplemented with 3 mg/l 2, 4-D from both the explants in both the genotypes. Similar results were observed by [Karim *et al.* (2002), Gandonou *et al.* (2005a), Gandonou *et al.* (2005b), Ather *et al* (2009), Gopitha *et al.* (2010), Satpal *et al.* (2011) and Mali *et al* (2015)] [11, 8, 9, 3, 10, 17, 14]. Overall, the MS medium supplemented with 4 mg/l 2, 4- D registered maximum callus induction per cent in Co 86032 (Leaf whorl=83.00%, Meristem= 84.33%) and in CoM 0265 (Leaf whorl=76.00%, Meristem=74.67%). But genotype CoM 0265 was given lesser amount of callus induction percentage as compare to Co 86032 in both the explants, while no callus induction was observed in control in both genotypes. It is interesting to note that callus induction per cent in both explants was higher in Co 86032 compare to CoM 0265. Both the explants were supplemented with only MS medium (control), there was no callus induction observed in both the genotypes. Similar results were observed by [Tahir *et al.* (2011), Gopitha *et al.* (2010)] [21, 10].

3 Response of different 2-4, D concentrations on callus Induction (Table 2)

No callus induction was observed when leaf whorl as well as meristem supplemented with only MS medium (Control). The callus induction was increased with increased in the concentrations of 2, 4-D up to the level of 4 mg/l 2, 4-D supplemented with MS medium. The profuse and good quality callus was observed in leaf whorl as well as in meristem when it was supplemented MS + 3 mg/l 2, 4-D and 4mg/l 2, 4-D in both the genotypes. The quantity as well as quality should be decreased when both the explants were supplemented with MS + 5mg/l 2, 4-D in both the genotypes. These findings are close to the [Tahir *et al.* (2011), Karim *et al.* (2002), Khan *et al.* (2008), Patel (2012), and Yadav and Ahmad (2013)] [21, 11, 12, 16, 22]. The quantity as well as quality of callus decreased when both the explants were

supplemented with MS + 5 mg/l 2, 4-D. Similar results were also found by Karim *et al.* (2002) [11].

Table 2: response of different 2, 4-d concentration on callus induction in sugarcane cv. co 86032 and com 0265.

Tr. No	Concentrations of 2, 4-D (mg/l)	Co 86032		CoM 0265	
		Leaf whorl	Meristem	Leaf whorl	Meristem
T_1	Control	-	-	-	-
T_2	1	+	++	+	+
T_3	2	++	++	++	++
T_4	3	+++	+++	+++	+++
T_5	4	+++	+++	+++	+++
T_6	5	+	+	+	+

(-) No callusing, (+) poor callusing, (++) good callusing and (+++) very good callusing

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