International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 1465-1467

Received: 28-09-2019
Accepted: 30-10-2019

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Effect of different 2,4-D concentration on callus induction of sugarcane varieties

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Abstract
An efficient protocol for callus induction and response of callus to different concentrations of 2,4-D of two sugarcane varieties CoN 13073 and CoN 09072 has been developed and reported here. Leaf whorl and meristem taken as an explant portions for callus induction in both the varieties. % The results showed that leaf whorl was found suitable for quick callus induction in both the varieties. Both the explant portions exhibited differential performance towards 2,4-D concentrations for callus induction. Earlier callus induction was observed in variety CoN 13073 where leaf whorl was an explant portion. At 0 level of 2,4-D concentration poor and moderate callus induction was observed in both the varieties. At 3 mg/l 2,4-D concentration, profuse and good quality of callus was observed in both leaf whorl and meristem, with the increase in concentration of 2,4-D above 4 mg/l quantity and quality of callus was reduced among the varieties.

Keywords: Sugarcane, 2,4-D, callus induction, somaclonal variation

Introduction
Sugarcane (*Saccharum* spp. Complex) is important cash crop of India, belongs to poaceae family and genus *Saccharum*. It is a highly polyploidy species with complex genome, with variable chromosome number (2n = 40 to 128). Sugarcane is produced in both tropical and subtropical regions of world. Sugarcane plays a major role in the economy of sugarcane growing areas and hence improving sugarcane production will greatly help in economic prosperity of the farmers and other associated industries with sugarcane cultivation. Due to a decline in sugarcane productivity with an increase in stress conditions and other environmental factors associated with sugarcane cultivation (Virupakshi *et al.*, 2002) [19]. attempts have been made during the recent years to improve sugarcane plants by using tissue culture techniques. Plant tissue culture is a multi-dimensional field that offers exciting prospects for crop productivity and improvement (Jain, 2001) [8]. The main goal of acquiring tissue culture is to ensure healthy, high levels of plant regeneration and rapid multiplication. Barba and Nickel (1969) [2]. who first independently demonstrated that plantlets could be developed from sugarcane callus cultures, and from the published results, it is evident that every part of the sugarcane plant is capable of producing callus (Liu, 1993). Callus cultures are considered as "tissue culture breeding" because of its tremendous potential for creating genetic variability. One of the most important element in callus induction is 2,4-D. 2,4-D is known to cause genetic changes in regenerative plants, especially chromosomal deviations, or in other words somaclonal variation in plants as regenerating from callus (Benlioglu and Ozgen, 2014) [4]. Present investigation was carried out to check the effect of different 2,4-D concentrations callus induction of two sugarcane varieties (CoN 13073 and C0N 09072) was studied to evaluate somaclonal variants at sugarcane tissue culture laboratory, Main Sugarcane Research Station, Navsari Agricultural University, Navsari.

Materials and Metods
The commercial cultivars of sugarcane CoN 13073 and CoN 09072 grown in Gujarat were used as the source of explants in this experiment. The explants were obtained from Main Sugarcane Research Station, Navsari Agricultural University, Navsari. The direct leaf whorl and meristem of sugarcane were used as explants and these explants were true to type, visually healthy and disease free. Disease-free and actively growing cane tops were selected from five months old sugarcane crop as an explant. 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 for about five to six minutes in a sterile 1-liter 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 four to five 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 three to four times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex was done by carefully removing the two to three outer whorls of the developing leaves with the help of a sterile sharp blade. The second innermost whorls of developing leave cut in to small pieces of approximately one-centimeter 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 in different treatments.

Results and Discussion

1. Number of days required for callus induction from leaf whorl and meristem

In variety CoN 13073, MS supplemented with NAA 2 mg/l + 3 mg/l 2,4-D in treatment T_4 registered minimum number of days (12.24) for callus induction followed by MS + 4 mg/l 2,4-D in treatment T_5 (14.62) and MS + 2 mg/l 2,4-D in treatment T_3 (15.65) from leaf whorl taken as explant. On the other hand, MS medium supplemented with 3 mg/l 2,4-D in treatment T_4 registered minimum number of days (17.14) in callus induction followed by MS + 2 mg/l 2,4-D in treatment T_2 (17.42) from meristem taken as explant. The MS medium supplemented only with 2 mg/l NAA in treatment T_1 registered maximum number of days (23.52 and 22.54) for callus induction from meristem and leaf whorl taken as an explant, respectively.

In variety CoN 09072, MS supplemented with 3mg/l 2,4-D in treatment T_4 registered minimum number of days (18.42) for callus induction followed by MS + 4 mg/l 2,4-D in treatment T_5 (20.15) from leaf whorl taken as explant. On the other hand, MS medium supplemented only with NAA 2 mg/l in treatment T_1 registered minimum number of days (20.18) for callus induction followed by MS + 4 mg/l 2,4-D in treatment T_5 (21.16) from meristem taken as an explants. MS medium supplemented with 5 mg/l 2,4-D in treatment T_6 registered maximum number of days (28.32) for callus induction where meristem taken as an explants.

The MS medium supplemented with 3 mg/l 2,4-D taken minimum number of days for callus induction followed by MS medium supplemented with 4 mg/l 2,4-D from both the expants in both the varieties, similar results were reported by Tahir *et al.* (2011) [18]. Mali *et al.* (2015) [12]. and Patel *et al.* (2015) [14]. In variety CoN 13073 minimum number of days for callusing exhibited in 12.24 days. Similar findings were reported by Lago and Barreto (1987) [10]. and they observed in 14 days for callusing, the results were illustrated and demonstrated that the difference in callusing was due to genotypic variations, explant portion selected and plant growth regulators. Earlier callus induction was observed in variety CoN 13073 where leaf whorl was an explant portion. Both the explant portions exhibited differential performance towards 2,4-D Concentrations for callus induction. Leaf whorl was found suitable for quick callus induction in both the varieties similar results were reported by Tahir *et al.* (2011) [18]. Gadakh (2014) [5]. and Patel *et al.* (2015) [14].

Table 1: Response of different 2,4-D concentration on number of days to callus induction and callus induction per cent in sugarcane varieties CoN 13073 and CoN 09072

No	No of days required for callus induction				Callus induction per cent			
	Variety 13073		Variety 09072		Variety 13073		Variety 09072	
	Leaf whorl	Meristem	Leaf whorl	Meristem	Leaf whorl	Meristm	Leaf whorl	Meristem
T_1	22.54	23.52	22.52	20.18	64.38	50.71	56.44	45.74
T_2	18.44	17.42	20.16	26.44	67.16	52.36	60.16	46.18
T_3	15.65	19.24	21.56	24.38	76.44	56.41	58.88	51.36
T_4	12.24	17.14	18.42	22.74	84.61	63.14	62.36	54.64
T_5	14.62	19.56	20.15	21.16	80.58	63.09	56.74	50.86
T_6	21.51	22.14	24.37	28.32	61.16	51.14	50.15	44.72
SEm	0.45	0.29	0.56	0.33	0.47	0.38	0.59	0.62
CV	1.40	0.91	1.74	1.04	1.46	1.17	1.82	1.92
CD	4.51	2.58	4.61	2.45	1.14	1.17	1.79	2.20

T_1 = MS + NAA (2mg/l) + 0 mg/l 2,4-D, T_2 = MS + NAA (2 mg/l) +1 mg/l 2,4-D,
T_2 = MS + NAA (2 mg/l) +2 mg/l 2,4-D, T_3 = MS + NAA (2 mg/l) + 3 mg/l 2,4-D,
T_5 = MS + NAA (2 mg/l) + 4 mg/l 2,4-D, T_6 = MS + NAA (2mg/l) + 3 mg/l 2,4-D

2. Callus induction per cent

Variety CoN 13073 supplemented with MS + 3 mg/l 2,4-D in treatment T_4 registered maximum callus induction per cent (84.61) followed by MS + 4 mg/l 2,4-D in treatment T_5 (80.58) leaf whorl taken as an explants. On the other hand, MS medium supplemented with 3 mg/l 2,4-D in treatment T_4 registered maximum callus induction per cent (63.14) followed by MS + 4 mg/l 2,4-D in treatment T_5 (63.09) from meristem taken as an explant. The MS medium supplemented with 5 mg/l 2,4-D in treatment T_6 and MS medium without 2,4-D in treatment T_1 registered minimum callus induction per cent (61.16 and 50. 71) from leaf whorl and meristem taken as an explants respectively.

In variety CoN 09072 MS medium supplemented with 3 mg/l 2,4-D in treatment T_4 registered maximum callus induction per cent (62.36) followed by MS + 1 mg/l 2,4-D in treatment T_2 (60.16) from leaf whorl taken as explants. On other hand, MS medium supplemented with 3 mg/l 2,4-D in treatment T4registered maximum callus induction per cent (54.64) followed by MS + 2 mg/l 2,4-D in treatment T3(51.36) from meristem taken as an explant. The MS medium supplemented with 5 mg/l 2,4-D in treatment T6 registered minimum callus induction per cent (50.15 and 44.72) from leaf whorl and meristem taken as an explants.

Callus induction per cent was varied in different explants with different levels of 2,4-D in both varieties (Bahera and Sahoo,

2009) [3]. Maximum callus induction per cent was observed in MS medium supplemented with 3 mg/l 2,4-D (Ramanand *et al.* 2006) [15]. Followed by MS medium supplemented with 4 mg/l 2,4-D from both the explants in both the varieties. Similar results were observed by Gandonou *et al.*, 2005 [6]. Ather *et al.*, 2009 [1]. Gopitha *et al*., 2010 [7]. Satpal *et al*., 2011 [17]. Mali *et al*., 2015 [12]. and Reena *et al*., 2017 [16]. The MS medium supplemented with 5 mg/l 2,4-D showed minimum callus induction in both explants in both varieties. It is interesting to note that high callus induction per cent was observed in variety CoN 13073 compare to CoN 09072. In both the explants portion higher concentration of 2,4-D 5mg/l resulted poor callus induction

3. Response of different 2,4-D concentration on quality of callus induction

Results showed that, both the explants *i.e.* leaf whorl and meristem were given good and very good callusing when MS medium supplemented with 3 mg/l 2, 4-D and 4 mg/l 2, 4- D, respectively. At 3 mg/l 2,4-D concentrations, the profuse and good quality callus observed in both leaf whorl and meristem. These findings are close to the Khan *et al*., (2008) [9]. Tahir *et al*., (2011) [18]. Patel *et al.*, (2011) [13]. and Reena *et al*., (2017) [16]. Poor quality callus formation was resulted with the higher and lower concentration of 2,4-D *i.e.* 1 mg/l and 5 mg/l 2,4-D.

Table 2: Response of different 2,4-D concentration on quality of callus induction in sugarcane varieties CoN 13073 and CoN 0072

No	Treatment MS + NAA (2 mg/l) + 2,4-D	Response of different 2,4-D concentration on quality of callus induction			
		CoN 13073		CoN 09072	
		Leaf whorl	Meristem	Leaf whorl	Meristem
T_1	2,4-D (0 mg/l)	+	-	-	-
T_2	2,4-D (1 mg/l)	+	-	+	+
T_3	2,4-D (2 mg/l)	++	++	+	+
T_4	2,4-D (3 mg/l)	+++	+++	+++	++
T_5	2,4-D (4 mg/l)	+++	+++	++	++
T_6	2,4-D (5 mg/l)	+	+	-	-
+++ Very good		++ Good			
+ Moderate		- Poor callus			

Conclusion

From the study it is concluded that sugarcane mainly depends on the concentrations and combination of growth regulators irrespective of the variety. Earlier callus induction was observed in variety CoN 13073 where leaf whorl was an explant portion. Both the explant portions exhibited differential performance towards 2,4-D Concentrations for callus induction. It is interesting to note that high callus induction per cent was observed in variety CoN 13073 compare to CoN 09072. With the increase in concentration of 2,4-D above 4mg/l quantity and quality of callus was reduced in both the varieties.

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