Research Article

Impact of cefotaxime on somatic embryogenesis and shoot regeneration in Sugarcane

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ABSTRACT

A cephalosporin antibiotic, cefotaxime (Omnatax^{TM}) promoted somatic embryogenesis and subsequent shoot regeneration *in vitro* from spindle in sugarcane irrespective of the genotypes as (CoJ 83, CoJ 88 and CoJ 64) culturered on MS medium with 2,4-D (2.5 mgl^{-1}) and kinetin (0.5 mgl^{-1}). Seven different concentrations of cefotaxime (100, 200, 300, 400, 500, 600 and 700 mgl^{-1}) were tested to find the optimal concentration of cefotaxime for somatic embryogenesis from callus cultures. Among the three varieties, calli of variety CoJ 83 incubated on MS medium with 2,4-D (2.5 mgl^{-1}) + kinetin (0.5 mgl^{-1}) + cefotaxime (500 mgl^{-1}) exhibited maximum somatic embryogenesis. To improve shoot regeneration, the callus was transferred to MS medium with BAP (0.5 mgl^{-1}) + kinetin (0.5 mgl^{-1}) in combination with different levels of cefotaxime. Highest frequency of shoot regeneration was observed in callus of CoJ 83 in the presence of 500 mgl^{-1} cefotaxime. The plantlets could be successfully hardened in polybags and transferred to soil, where they exhibited normal growth. Our results convincingly demonstrated that cefotaxime improves somatic embryogenesis from spindle and regeneration from embryogenic calli of sugarcane and hence can be strongly recommended for rapid and large scale multiplication of sugarcane. **[Physiol. Mol. Biol. Plants 2009; 15(3) : 257-265]** *E-mail : pradeepgkp17@yahoo.co.in*

Key words : *Saccharum officinarum L., leaf segments, callus, plant regeneration, antibiotic.*

Abbreviations : *BAP: 6-benzylaminopurine, 2,4-D: 2,4-Ddichlorophenoxyacetic acid, kinetin: 6-furfurylaminopurine, MS: Murashige and Skoog.*

INTRODUCTION

The antibiotic cefotaxime (Omnatax^{TM}, $C_{16}H_{16}N_5O_7S_2Na$) has been reported to enhance the frequency of somatic embryogenesis and subsequent plant regeneration in barley (Mathias and Mukasa, 1987), wheat (Borrelli *et al.,* 1992), *Dianthus* (Nakano and Mii, 1993), pearl millet (Pius *et al.,* 1993), apple (Yepes *et al.,* 1994), sorghum (Rao *et al.,* 1995), *Vitis vinifera* L. (Torregorsa *et al.,* 2000), papaya (Yu *et al.,* 2001), maize (Danilova *et al.,* 2004), tea (Aoshima 2005), and rice (Grewal *et al.,* 2006). Recently it has been reported that cefotaxime also improved the elongation and multiplication rate in sugarcane (Kaur *et al.*, 2008). Sugarcane (*Saccharum officinarum* L. 2n = 80 to 205) belonging to family Poaceae, is one of the oldest sugar crops known to man from times immemorial. Unlike other crops, sugarcane has unique agro-industrial applications. It provides raw material to different industries to produce sugar, jaggery, khandsari, and a range of agro-industrial co-products such as alcohol, paper, a variety of chemicals, cattle feed and electricity. Due to its global importance, extensive research has been conducted on conventional breeding of sugarcane, however genetic complexity, low fertility and large genotype & environment interactions make traditional breeding and genetic studies arduous in sugarcane. Further, conventional propagation rate of sugarcane is 1 to 10 in one year, which is very less. Also, during vegetative propagation, the pathogens keep on accumulating generation after generation, which ultimately results in the decline of a variety. In this regard, biotechnological approaches involving cell and tissue culture and genetic transformation can play an important role in the genetic improvement of sugarcane (Chahal and Gosal, 2002). The utilization of these techniques depends largely on the ability of cell cultures

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to differentiate and regenerate. Sugarcane tissue culture was first initiated in Hawaii in 1961. Subsequently, several protocols for induction of somatic embryogenesis from callus cultures have been developed. Somatic embryogenesis in sugarcane has been reported from cultures of young leaves (Ho and Vasil, 1983; Brisibe *et al.,* 1994), and apical meristems (Ahloowalia and Maretzki, 1983). Greater is the degree of somatic embryogenesis in cell or callus cultures; greater is the frequency of shoot regeneration.

Hence, in the present study, an attempt was made to improve the frequency of somatic embryogenesis and subsequent plant regeneration in callus cultures of three commercial varieties of sugarcane through the addition of cefotaxime in the callus proliferation and regeneration medium.

MATERIALS AND METHODS

Callus induction and sub-culturing

Apical stem portions were excised from actively growing healthy plants of three commercial varieties of sugarcane viz., CoJ 64, CoJ 83 and CoJ 88. These were surface sterilized with $HgCl_2$ (0.1 %) for 10 min and aseptically cultured on MS (Murashige and Skoog, 1962) medium supplemented with 2,4-D (4.0 mgl^{-1}) and kinetin (0.5 mgl^{-1}). Primary calli thus formed were sub-cultured on eight different media based on MS salts supplemented with 2,4-D (2.5 mgl^{-1}), kinetin (0.5 mgl^{-1}) that acted as control (S1 without cefotaxime) and 7 different concentrations of cefotaxime, which were designated as S2 (S1 + 100 mgl^{-1}), S3 (S1 + 200 mgl^{-1}), S4 (S1 + 300 mgl^{-1}), S5 (S1 + 400 mgl^{-1}), S6 (S1 + 500 mgl^{-1}), S7 (S1 + 600 mgl^{-1}) and S8 (S1 + 700 mgl^{-1}). Cefotaxime (Omnatax™) was dissolved in sterile water and sterilized by passing through membrane filter (0.2 mm), and added into molten medium before solidification. The callus cultures were incubated at 25 ± 2 °C and 70-80 % humidity under total dark conditions.

Shoot regeneration

After 2 sub-culture cycles (one cycle consisted of 2 weeks), embryogenic calli from control and cefotaxime containing media were transferred to eight different regeneration media based on MS salts supplemented with BAP (0.5 mgl^{-1}), kinetin (0.5 mgl^{-1}) that acted as control (M1), and seven different concentrations of cefotaxime ranging from 100 to 700 mgl^{-1} (M2-M8). The cultures were exposed to normal tissue culture growth with an illumination of 16 h.

Rooting and hardening

For induction of roots, shoots were transferred to MS + NAA (3.0 mgl^{-1}) + IBA (2.0 mgl^{-1}) + sucrose (70 g/l) medium. Plantlets with well developed root system were taken out from the test tubes and thoroughly washed under running tap water. Thereafter, the plantlets were hardened by keeping them on moist cotton in open test tubes for a week in the incubation room. Hardened plants were transferred to soil in polybags and kept in the glass house.

Statistical analysis

Statistical analysis was done according to CPCS-1 package using factorial CRD design. CD values were calculated at 5 % level of significance and the interpretations were made accordingly.

RESULTS

Callus induction

In all the three sugarcane varieties, initiation of callus from cut ends of cultured leaf segments was observed after 12-15 days of incubation (Fig. 1A). The variety CoJ 83 exhibited complete callusing within 30 days, whereas, CoJ 88 and CoJ 64 took almost 40 days for callus formation. Highest callus induction (94.73 %) (Fig. 1B) and its subsequent proliferation was observed in CoJ 83, followed by CoJ 88 (85.29 %) and CoJ 64 (82.54 %).

Somatic embryogenesis

After 4 weeks of incubation of explants, primary calli were excised and transferred to control medium and media containing different concentrations of cefotaxime (Table 1). Secondary calli of the three sugarcane varieties thus formed exhibited varied degree of somatic embryogenesis that was evident from the nodular regions on the growing calli. Per cent somatic embryogenesis in calli of variety CoJ 83 cultured on S1, S2, S3, S4, S5, S6, S7 and S8 medium was 68.28, 71.08, 73.13, 78.06, 80.49, 92.13, 82.42 and 65.88, respectively (Table 1) (Figs. C-F). Similar trend of per cent somatic embryogenesis was observed in other two varieties as well, which revealed that the number of somatic embryos increased with increasing the concentration of cefotaxime upto 500 mgl^{-1}, after which it started decreasing. At higher concentration of the antibiotic (500 mgl^{-1}), frequency of somatic embryogenesis in callus cultures of CoJ 88 and CoJ 64 was maximum i.e. 85.33 and 82.81 per cent, as

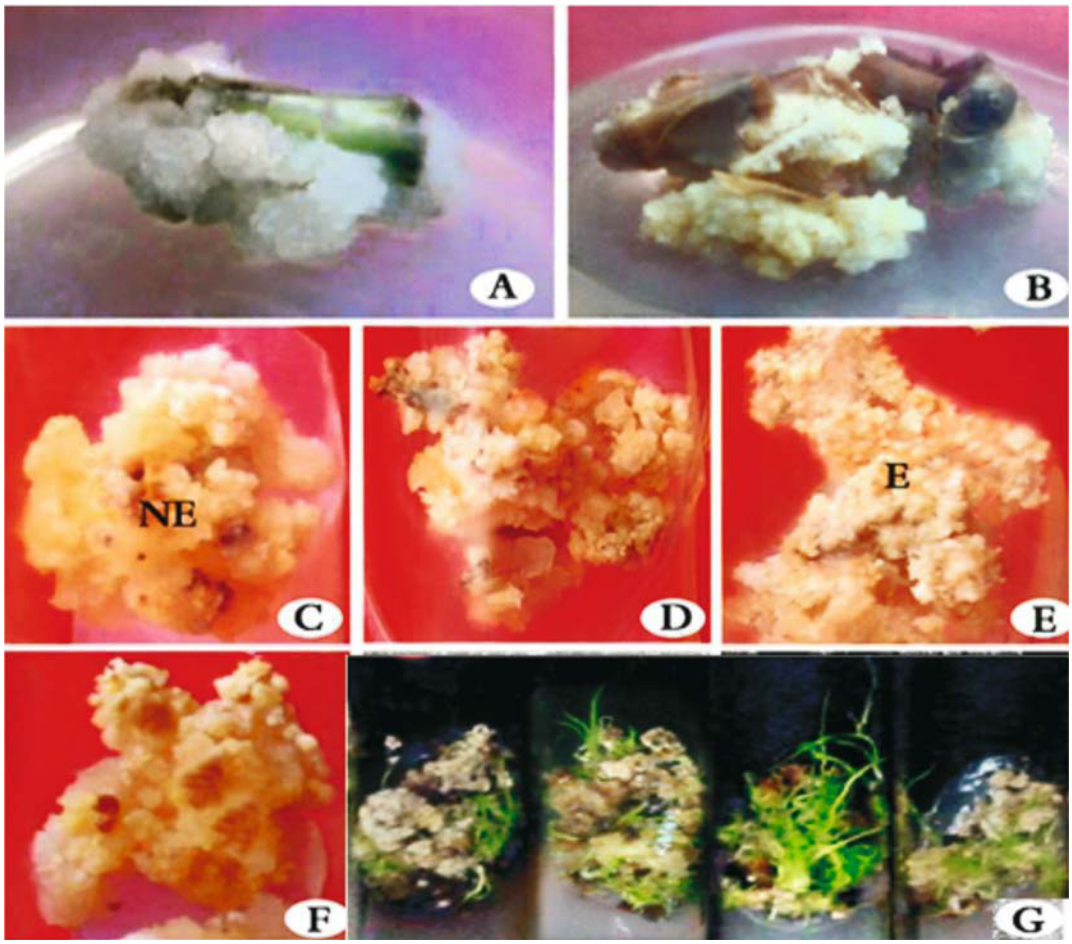
Table 1. Effect of Cefotaxime (Omnatex)^{TM} on Somatic Embryogenesis in Sugarcane Calli

Medium	Genotype	No. of calli cultured				Mean no. of calli cultured	No. of embryogenic calli				Mean no. of embryogenic calli	% somatic embryogenesis				Mean somatic embryogenesis	Arc Sine Transformed Mean
		R1	R2	R3	R4		R1	R2	R3	R4		R1	R2	R3	R4		
S1	Co J 83	53	51	46	52	50.50±3.11 (202)	36	34	31	37	34.50±2.65 (138)	67.92	66.67	67.39	71.15	68.28±1.98	55.71
	Co J 88	45	42	53	55	48.75±6.24 (195)	27	25	32	32	29.00±3.56 (116)	60.00	59.52	60.38	58.18	59.52±0.96	50.46
	Co J 64	52	55	50	48	51.25±2.99 (205)	29	28	26	26	27.25±1.50 (109)	55.77	50.91	52.00	54.17	53.21±2.18	46.82
S2	Co J 83	52	55	50	54	52.75±2.22 (211)	36	40	36	38	37.50±1.91 (150)	69.23	72.73	72.00	70.37	71.08±1.58	57.45
	Co J 88	50	54	53	50	51.75±2.06 (207)	34	33	34	31	33.00±1.41 (132)	68.00	61.11	64.15	62.00	63.82±3.07	53.01
	Co J 64	53	50	52	54	52.25±1.71 (209)	30	28	30	30	29.50±1.00 (118)	56.60	56.00	57.69	55.56	56.46±0.93	48.69
S3	Co J 83	52	55	50	51	52.00±2.16 (208)	37	39	37	39	38.00±1.15 (152)	71.15	70.91	74.00	76.47	73.13±2.63	58.77
	Co J 88	50	55	50	53	52.00±2.45 (208)	35	35	34	33	34.25±0.96 (137)	70.00	63.64	68.00	62.26	65.98±3.63	54.31
	Co J 64	55	53	53	51	53.00±1.63 (212)	30	30	31	30	30.25±0.50 (121)	54.55	56.60	58.49	58.82	57.12±1.97	49.07
S4	Co J 83	54	53	52	50	52.25±1.71 (209)	40	42	40	41	40.75±0.96 (163)	74.07	79.25	76.92	82.00	78.06±3.37	62.09
	Co J 88	53	52	50	51	51.50±1.29 (206)	39	37	36	35	36.75±1.71 (147)	73.58	71.15	72.00	68.63	71.34±2.07	57.62
	Co J 6′	45	42	40	42	42.25±2.06 (169)	30	29	28	30	29.25±0.96 (117)	66.67	69.05	70.00	71.43	69.29±2.00	56.32
S5	Co J 83	45	42	46	40	43.25±2.75 (173)	34	34	37	34	34.75±1.50 (139)	75.56	80.95	80.43	85.00	80.49±3.87	63.83
	Co J 88	45	48	45	46	46.00±1.41 (184)	34	36	33	31	33.50±2.08 (134)	75.56	75.00	73.33	67.39	72.82±3.74	58.59

Table 1. Continued .....

Medium	Genotype	No. of calli cultured				Mean no. of calli cultured	No. of embryogenic calli				Mean no. of embryogenic calli	% somatic embryogenesis				Mean somatic embryogenesis	Arc Sine Transformed Mean
		R1	R2	R3	R4		R1	R2	R3	R4		R1	R2	R3	R4		
	Co J 64	40	44	40	42	43.50±1.91 (166)	29	30	29	29	29.25±0.50 (117)	72.50	68.18	72.50	69.05	70.56±2.27	57.12
S6	Co J 83	51	55	44	42	48.00±6.06 (192)	50	49	40	38	44.25±6.13 (177)	98.04	89.09	90.91	90.48	92.13±4.02	74.25
	Co J 88	50	48	46	46	47.50±1.91 (190)	41	41	40	40	40.50±0.58 (162)	82.00	85.42	86.96	86.96	85.33±2.34	67.49
	Co J 64	40	44	46	44	43.50±2.52 (174)	34	36	38	36	36.00±1.63 (144)	85.00	81.82	82.61	81.82	82.81±1.51	65.49
S7	Co J 83	50	53	50	52	51.25±1.50 (205)	40	43	41	45	42.25±2.22 (169)	80.00	81.13	82.00	86.54	82.42±2.87	65.23
	Co J 88	50	54	50	52	51.50±1.91 (206)	39	40	39	42	40.00±1.41 (160)	78.00	74.07	78.00	80.77	77.71±2.75	61.83
	Co J 64	45	48	46	45	46.00±1.41 (184)	36	34	33	33	34.00±1.41 (136)	80.00	70.83	71.73	73.33	73.97±1.51	59.36
S8	Co J 83	52	50	52	51	51.25±0.96 (205)	35	34	32	34	33.75±1.26 (135)	67.31	68.00	61.54	66.67	65.88±2.94	54.24
	Co J 88	45	40	41	42	42.00±2.16 (168)	26	23	24	23	24.00±1.41 (96)	57.78	57.50	58.54	54.76	57.14±1.65	49.08
	Co J 64	53	50	52	50	51.25±1.50 (205)	23	24	26	24	24.25±1.26 (97)	43.40	48.00	50.00	48.00	47.35±2.80	43.46

S1 (Control) = MS ± 2,4-D (2.5 mg/l) ± Kinetin (0.5 mg/l) ± Inositol (100 mg/l) ± Proline (560 mg/l) ± Sucrose (30 g/l) ± Agar-Agar (0.8 % w/v)
S2 (S1 ± Cefotaxime 100 mg/l), S3 (S1 ± Cefotaxime 200 mg/l), S4 (S1 ± Cefotaxime 300 mg/l), S5 (S1 ± Cefotaxime 400 mg/l), S6 (S1 ± Cefotaxime 500 mg/l), S7 (S1 ± Cefotaxime 600 mg/l), S8 (S1 ± Cefotaxime 700 mg/l)
Values in parenthesis are total number of calli
CD (5 %) for media = 1.62
CD (5 %) for variety = NS
CD (5 %) for media × variety = 3.49



compared to 59.52 and 53.21 per cent, respectively in control (Table 1). It was interesting to note that the use of cefotaxime not only improved somatic embryogenesis, but also reduced rhizogenic sectors in all the three varieties as compared to condrol (Fig. 1E). Statistical analysis showed that media mean squares were significant (CD = 1.62) and variety mean squares were non-significant (CD = NS), whereas, interaction between variety and media was significant (CD = 3.49) at 5 % level of significance. This revealed that varieties showed a significant difference with respect to somatic embryogenesis upon culture of their explants on different media.

Shoot regeneration

Calli sub-cultured on different media were transferred to eight different regeneration media (M1-M8) (Table 2). In general, two types of responses were observed. Small callus clusters upto 10 mm^2 size exhibited islets formation within 15 days of transfer to regeneration medium and produced multiple shoots after 30 days of incubation. Calli larger than 25 mm^2 size did not regenerate and became rhizogenic and necrotic. Among the three sugarcane varieties, highest shoot regeneration from control callus was observed in variety CoJ 83 (57.79 %), followed by CoJ 88 (47.31 %) and CoJ 64 (44.32 %) (Table 2). On the other hand, embryogenic calli cultured on containing cefotaxime, exhibited a higher frequency of shoot regeneration én all the three sugarcane varieties. The frequency of regeneration increased with increasing the concentration of cefotaxime and was maximum on medium containing 500 mgl^{-1} cefotaxime, i.e. 75.16 %, 65.82 % and 60.57 % in CoJ 83, CoJ 88 and CoJ 64, respectively, but further increase in antibiotic concentration inhibited per cent shoot regeneration (Fig. 1). In the calli grown on cefotaxime containing medium (500 mgl^{-1}), hardly any callus was detectable between the shoots after 1 month of culturing as compared to control that showed a considerable amount of undifferentiated callus on regeneration medium (Fig. 1).

Table 2. Plant regeneration from embryonic calli of sugarcane on regeneration medium {MS ± BAP (0.5 mg/l ± Sucrose (30 g/l) ± Inositol (100 mg/l) ± Proline (100 mg/l) ± Agar (0.8% w/v)}

Medium	Genotype	No. of calli cultured				Mean no. of calli cultured	No. of embryogenic calli				Mean no. of embryogenic calli	% somatic embryogenesis				Mean somatic embryogenesis	Arc Sine Transformed Mean
		R1	R2	R3	R4		R1	R2	R3	R4		R1	R2	R3	R4		
M1	Co J 83	35	33	30	35	33.25±3.11(133)	20	20	17	20	19±1.75(77)	57.50	60.53	55.36	57.78	57.79±2.12	49.46
	Co J 88	26	24	31	30	27.75±6.24(111)	13	11	14	15	13±1.67(53)	48.65	46.15	44.44	50.00	47.31±2.49	49.94
	Co J 64	28	27	25	24	26.00±2.99(104)	13	11	11	11	12±0.83(46)	45.24	42.50	42.86	46.67	44.32±1.98	51.18
M2	Co J 83	36	37	35	36	36.00±2.22(144)	21	23	19	22	21±1.62(84)	57.50	61.90	54.55	60.47	58.60±3.27	54.28
	Co J 88	32	32	32	30	31.50±2.06(126)	16	16	15	15	15±0.72(62)	51.11	48.78	47.62	48.78	49.07±1.47	55.24
	Co J 64	29	27	27	29	28.00±1.71(112)	14	12	11	13	13±1.27(50)	47.62	45.00	40.91	46.51	45.01±2.94	60.09
M3	Co J 83	35	38	35	37	36.25±2.16(145)	21	24	19	23	22±2.37(88)	60.00	64.44	55.00	63.41	60.71±4.26	57.10
	Co J 88	34	33	32	31	32.50±2.45(130)	19	16	17	16	17±1.33(67)	54.76	47.73	52.50	51.16	51.54±2.94	53.83
	Co J 64	28	28	29	29	28.50±1.63(114)	13	14	13	15	14±0.74(54)	47.73	48.84	43.90	50.00	47.62±2.64	43.43
M4	Co J 83	37	40	37	38	38.00±1.71(152)	25	28	19	29	25±4.39(100)	66.67	70.00	51.22	75.00	65.72±10.26	4.45
	Co J 88	36	36	35	33	35.00±1.29(140)	20	19	20	20	20±0.61(80)	55.00	53.33	58.54	61.90	57.19±3.82	45.86
	Co J 64	27	27	25	28	26.75±2.06(107)	13	14	12	15	13±1.37(54)	46.51	53.66	47.62	52.27	50.02±3.48	49.12
M5	Co J 83	32	33	36	32	33.25±2.75(133)	22	24	25	20	22±2.15(90)	68.89	71.43	68.29	61.36	67.49±4.31	50.11
	Co J 88	30	35	30	29	31.00±1.41(124)	17	20	17	19	18±1.38(73)	57.78	56.10	55.81	65.85	58.89±4.73	54.23
	Co J 64	26	28	26	25	26.25±1.91(105)	14	14	14	12	13±0.88(54)	52.38	51.11	52.50	48.84	51.21±1.70	52.29
M6	Co J 83	45	45	38	35	40.75±6.06(163)	35	34	28	26	31±4.13(123)	77.27	74.47	73.91	75.00	75.16±1.47	48.32
	Co J 88	32	40	37	37	36.50±1.91(146)	21	25	27	23	24±2.51(96)	65.12	63.41	71.79	62.96	65.82±4.09	41.71
	Co J 64	31	33	36	35	33.75±2.52(135)	19	20	21	22	20±1.11(82)	61.36	60.87	58.49	61.54	60.57±1.41	42.11
M7	Co J 83	46	41	40	42	42.25±1.50(169)	33	27	28	32	30±3.20(119)	72.73	65.12	69.05	75.00	70.47±4.33	43.61
	Co J 88	37	38	37	40	38.00±1.91(152)	22	23	23	27	24±1.96(95)	60.00	60.98	62.79	66.67	62.61±2.94	44.99
	Co J 64	35	32	32	30	32.25±1.41(129)	20	18	17	18	18±1.32(73)	57.50	54.76	53.66	60.00	56.48±2.85	45.67

Table 2. Continued .....

Medium	Genotype	No. of calli cultured				Mean no. of calli cultured	No. of embryogenic calli				Mean no. of embryogenic calli	% somatic embryogenesis				Mean somatic embryogenesis	Arc Sine Transformed Mean
		R1	R2	R3	R4		R1	R2	R3	R4		R1	R2	R3	R4		
M8	Co J 83	33	31	31	31	31.50±0.96(126)	23	19	22	18	21±2.23(82)	70.00	60.98	70.00	59.52	65.12±5.66	51.08
	Co J 88	24	21	22	20	21.75±2.16(87)	13	12	12	12	12±0.71(48)	54.76	55.00	53.49	60.00	55.81±2.87	48.70
	Co J 64	22	21	23	22	22.00±1.50(88)	10	11	12	11	11±0.55(44)	46.67	53.66	50.00	48.84	49.79±2.93	44.86

M1 Control) = MS ± BAP (0.5 mg/l) ± Kinetin (0.5 mg/l) ± Inositol (100 mg/l) ± Sucrose (30 g/l) ± Agar-Agar (0.8 % w/v)
M2 (M1 ± Cefotaxime 100 mg/l), M3 (M1 ± Cefotaxime 200 mg/l), M4 (M1 ± Cefotaxime 300 mg/l), M5 (M1 ± Cefotaxime 400 mg/l), M6 (M1 ± Cefotaxime 500 mg/l), M7 (M1 ± Cefotaxime 600 mg/l), M8 (M1 ± Cefotaxime 700 mg/l)
Values in parenthesis are total number of calli
Values in parenthesis are total number of calli CD (5 %) for media = 1.84CD (5 %) for variety = NSCD (5 %) for media × variety = 3.20

Statistical analysis showed that media mean squares were significant (CD = 1.84), variety mean squares were non-significant (CD = NS) and interaction between variety and media was significant (CD = 3.20) at 5 % level of significance. This implicated that varieties exhibited a significant difference with respect to shoot regeneration on different media.

Rooting and hardening

After root formation on MS + NAA (3.0 mgl^{-1}) + IBA (2.0 mgl^{-1}) + sucrose (70 g/l) medium plantlets with well-developed shoot and root system were removed from test tubes and hardened by placing on moist cotton in open test tubes. During the hardening process, there was very less mortality in the plants regenerated from cefotaxime treated callus. Upon transfer to soil, nearly 90 % plants survived. In the glass house, plants exhibited normal growth.

DISCUSSION

The results clearly indicate that the inclusion of antibiotic cefotaxime at an optimum concentration (500 mgl^{-1}) in the culture medium promotes somatic embryogenesis and subsequent shoot regeneration in callus cultures of sugarcane to the maximum extent as compared to control. Similarly, (Yepes and Aldwinckle, 1994) observed higher number of shoots per culture with 200 mg/l cefotaxime as compared to control but at the same time higher concentration cefotaxime reduce the elongation and multiplication rate. Increase in number of microtillers may be associated with inhibition of ethylene production in the cultures by cefotaxime, which is positively correlated with plantlet differentiation (Pius *et al.,* 1993). Although addition of antibiotics into cell culture media, particularly at bacteriostatic or bactericidal concentrations, frequently results in inhibition of plant cell growth and development, however, improved *in vitro* responses in the presence of antibiotic have also been described (Mathias and Boyd, 1986; Rao *et al.*, 1995; Grewal *et al*., 2006). Among antibiotics, the beta-lactams (penicillins and cephalosporins) have been reported to 'potentiate' the growth in indica-type basmati rice cultures (Grewal *et al.,* 2006). Likewise, cefotaxime, and to a lesser extent carbenicillin, have been observed to have a significant positive effect on *in vitro* response of wheat callus (Mathias and Boyd, 1986). Our report describes similar effect of cefotaxime on callus growth and shoots regeneration in sugarcane. While carrying out genetic transformation, use of antibiotics such as carbenicillin to eliminate

Agrobacterium, and use of kanamycin or hygromycin as selective agents, reduce both embryogenesis and plant regeneration, leading to a very low frequency of transformation (Arencibia *et al.,* 1998; Bower and Birch, 1992). We have found that the use of antibiotic cefotaxime (Omnatax^{TM}, 500 mgl^{-1}) which is commonly recommended for human injections, promoted somatic embryogenesis and subsequent plant regeneration in callus cultures of three sugarcane varieties. Above this concentration, i.e. 500 mgl^{-1} the antibiotic stopped acting as growth promoter and per cent somatic embryogenesis decreased. It was interesting to note that sub-cultured calli, after 2 weeks of incubation, exhibited two types of phenotypes. Calli with a dry, compact and nodular appearance exhibited numerous globular structures and were called embryogenic (E) (Fig. 1E). Translucent and somewhat slimy callus never formed embryos and was called non-embryogenic (NE) (Fig. lC) as described earlier by Naboras *et al.* (1983). Embryogenic calli were characterized on the basis of their texture, colour and regeneration. A direction of root and shoot axis was indicative of regeneration via somatic embryogenesis, which is the preferred mode of differentiation (Lorz *et al.,* 1998). Somatic embryoids like their zygotic counter parts arise from single cells and thus the problem of possible genetic chimerism arising in the developmental mode is minimized. It was interesting to note that addition of cefotaxime (500 mgl^{-1}) in MS medium with 2,4-D (2.5 mgl^{-1}) and kinetin (0.5 mgl^{-1}) played an appreciable role in improving somatic embryogenesis in all the three varieties. This is reflected in the percentage of cultures showing shoot regeneration because the embryogenic calli usually regenerate into plantlets.

Furthure an obvious explanation for the activity of cefotaxime in culture is that the molecule mimics a plant growth regulator. The possibility that cell metabolism converts cefotaxime to a compound with growth regulator activity has been discussed by Mathias and Boyd (1986). The addition of cefotaxime in the culture medium also boosted up the photosynthetic machinery of sugarcane plants Zaghmout and Torello (1992). Several reports concluded that cefotaxime might be killing endophytic bacteria, that finally result in disease-free and vigorously growing plant cultures As a matter of fact, cefotaxime is a b-lactam antibiotic that inhibits cell wall synthesis in dividing bacterial cells and results in cell lysis (Selwyn, 1980; Leifert and Waites, 1990).

In conclusion, it is possible to obtain a consistently high frequency of somatic embryogenesis and plant regeneration from sugarcane callus cultures using 500 mgl^{-1} cefotaxime in the culture medium. Thus, during micropropagation, induction of somaclonal variation in callus cultures and genetic transformation of sugarcane, cefotaxime can augment somatic embryogenesis and plant regeneration of green shoots to a significant extent and it can be routinely used in sugarcane tissue culture.

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