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IN VITRO REGENERATION OF SUGARCANE THROUGH SOMATIC EMBRYOGENESIS

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ABSTRACT

Ganapati RK, Alam MR, Mamun MSA, Kabiraj RC, Rani R (2016) *In vitro* regeneration of sugarcane through somatic embryogenesis. *Int. J. Sustain. Crop Prod.* 11(3), 39-43.

A simple and efficient method of plant regeneration from midrib-derived callus was established in sugarcane. Explant was cultured in MS medium supplemented with 2,4-D and BA callus induction. The highest callus induction was found in MS medium supplemented with 2 mgl^{-1} 2,4-D and 0.2 mgl^{-1} BA, where 72% of explants produced callus. Two month calli were subcultured in to same medium which has shown different types and color. The highest frequency of shoot formation was obtained in MS medium supplemented with 1.0 mgl^{-1} BA and 0.5 mgl^{-1} NAA. Half strength MS supplemented with 3 mgl^{-1} NAA performed best for root induction. After acclimatization of in vitro grown plantlet, 85% were survived.

Key words: *mid rib, callus induction, plant regeneration, sugarcane*

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important cash crop in tropical and subtropical region of the world and is the major source of sugar (Guimarces and Sobral, 1998). It is clonally propagated crop and require a large amount of sett which is costly. Beside this, pathogen remains generation after generation, which affect the phenotypic expression, yield and quality of sugarcane. It is only member of the family Gramineae belong to genus Saccharum of which *in vitro* propagation is standardized and commercially viable. *In vitro* multiplication of sugarcane has received considerable attention because of its economic importance as a cash crop. It is a realistic means of large-scale production of disease-free quality planting materials. This method can play vital role for seed production for newly developed varieties in order to speed up the breeding and commercialization of Sugarcane (Feldmenn *et al.* 1994; Lal and Krishna, 1994; Lorenzo *et al.* 2001). The present study has been conducted to established a protocol for large scale production of sugarcane plantlet through somatic embryogenesis.

MATERIALS AND METHODS

The experiment was conducted at Plant Tissu Culture laboratory, Breeding Division, Bangladesh Sugarcrop Research Institute during autumn 2015. Clean and healthy tops (shoot) of 8-10 months old of five sugarcane clones Isd 20, Isd 35, I 220-92, Co 65-02 and I 214-11 were collected as plant materials. Immature leaf rolls were collected for explant preparation. Leaf roll was washed thoroughly under running tap water followed by 70% alcohol for 1 hr. The plant material was taken into laminar flow cabinet and surface sterilized with 30% Clorox for 20 minutes and rinsed 3 times with sterile double distilled water.

Media were prepared and pH was adjusted to 5.75 and autoclaved at 121ºC having pressure 15 lbs for 20 min. All cultures were incubated at 25±2ºC and kept under 16 h photoperiod of fluorescent tube light. The sterile leaf rolls were sectioned crosswise followed to longitudinal to harvest midrib(explant). Then aseptically cultured on modified MS medium (Murashige and Skoog, 1962) supplemented with different concentration of BA and 2, 4-D for callus induction. After 20 days explant with immersing calli were subculture for maturation. Callus was then transferred in MS supplemented with different concentrations and combinations of cytokinin (BA) and auxin (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 and NAA) for root initiation. These micropropagated plantlets were hardened and planted in polybags and kept inside the poly house for acclimation. Completely randomized design was used in experiment and data were statistically analyzed using Statistix 10.

RESULTS AND DISCUSSION

Explant of very young leaf placed on MS medium containing different concentration of 2,4-D and BA and incubated for 20 days. Maximum survival was observed in Isd 20 (9) followed by I 214-11(8) and most of the clone perform well for callus induction but maximum was observed in MS medium supplimented with 0.2mgl^{-1} BA and 2mgl^{-1} 2,4-D. Similar result also found by Joyce *et al.* (2010) and Głowacka *et al.* (2010). Yuan *et al.* (2009) concluded that callus was efficiently induced on medium supplemented with 2,4-D. Comparing all the condition Isd 20 perform better and 0.2mgl^{-1} BA and 2mgl^{-1} 2,4-D suitable for survival and callus induction.

Table 1. Response of varieties to different nutrient concentration for survival

BA	2,4-D	Isd 20	Isd 35	I 220-92	Co 65-02	I 214-11
0	0.5	0g	1ef	0g	1ef	1f
0	1	1f	1ef	1fg	0g	2e
0	2	2ef	3cd	2cde	2de	2e
0	4	3e	2d	1fg	2cd	3de
0.1	0.5	2ef	0f	1fg	1ef	0g
0.1	1	2ef	2d	4c	6a	5bc
0.1	2	4cd	3cd	2cde	3c	2e
0.1	4	3de	3c	3cd	2cd	6b
0.2	0.5	1f	2d	0g	1ef	2e
0.2	1	5c	5a	6b	4b	4cd
0.2	2	9a	6a	7a	5ab	8a
0.2	4	6b	5b	6ab	5ab	5bc
CV		**18.79**	**17.90**	**16.50**	**17.29**	**14.20**

Plant regeneration

Greenish cells within the callus were observed within two week of culture. Normal stems and leaves were produced from these cells. Calli exhibited shoot formation and multiplication the best when cultured on MS medium supplemented with 1 mgl^{-1} BA and 0.5 mgl^{-1} NAA. The highest number of shoot regeneration was 18.33 in Isd 20 and the most of the clones ie. Isd 35, I 220-92 and I 214-11 regenerates higher shoot (8, 11.5 and 16) in MS medium supplemented with 1 mgl^{-1} BA and 0.5 mgl^{-1} NAA except Isd 35 and Co 65-02(Table 2). Shimomae *et al.* (2013) found that the MS medium supplemented with 1 mgl^{-1} BA and 0.5 mgl^{-1} NAA suitable for shoot formation in ravenna grass a wild species of sugarcane. Mamun *et al.* (2004) studied the optimal concentrations of plant growth regulators on shoot proliferation of the sugarcane varieties Isd-28 and Isd-29 and found that 1.5 mgl^{-1} BA produced high percentage of shoot proliferation. Isd-28 and Isd-29 showed best shooting when 1.5 mgl^{-1} BA and 0.5 mgl^{-1} NAA supplemented MS medium. Yataka *et al.* (1998) reported that combinations of phytohormones often determine the course of morphogenesis such as shoot organogenesis and embryogenesis. Beside this highest shoot length (7 cm) after 30 days have been found in Isd 20 followed by Co 65-02(6 cm), Isd 35(6 cm), I 220-92(6 cm) and I 214-11(5 cm). Chattha *et al.* (2001) and Jadhav *et al.* (2001) stated similar results.

Table 2. Effect of the BA and NAA on shoot regeneration from callus

Phytohormones	Isd 20		Isd 35		I 220-92		Co 65-02		I 214-11	
BA+NAA (mg/l)	Shoot/ Callus	Shoot length (cm)	Shoot/ Callus	Shoot length (cm)	Shoot/ Callus	Shoot length (cm)	Shoot/ Callus	Shoot length (cm)	Shoot/ Callus	Shoot length (cm)
0.1 + 0.25	6ef	4bc	3e	5bc	5.3de	5ab	3.33f	6a	4.5h	5ab
0.1 + 0.5	4g	5ab	3e	6ab	6de	4bc	7.3e	5ab	6g	4bc
0.1 + 1.0	6fg	7a	4.5d	4.5cd	5de	5ab	4f	6a	6.67fg	4bc
0.5 + 0.25	8.6d	3cd	6.7c	4d	4e	4bc	4f	6a	8.2e	3cd
0.5 + 0.5	12bc	3cd	10a	5bc	9.2bc	5ab	16a	2d	11d	3de
0.5 + 1.0	7.33de	4c	8.2b	4d	6.3d	6a	12.5bc	3cd	12c	4bc
1.0 + 0.25	11c	3cd	6c	6a	8c	5ab	11cd	4cd	8ef	5a
1.0 + 0.5	18.33a	2.5d	8b	6a	11.5a	3c	14b	4bc	16a	3d
1.0 + 1.0	13b	4cd	9ab	5bc	10.3ab	3c	10d	4.5bc	14b	2e
CV	**7.98**	**15.81**	**10.64**	**10.90**	**10.82**	**13.57**	**9.33**	**12.84**	**7.18**	**13.87**

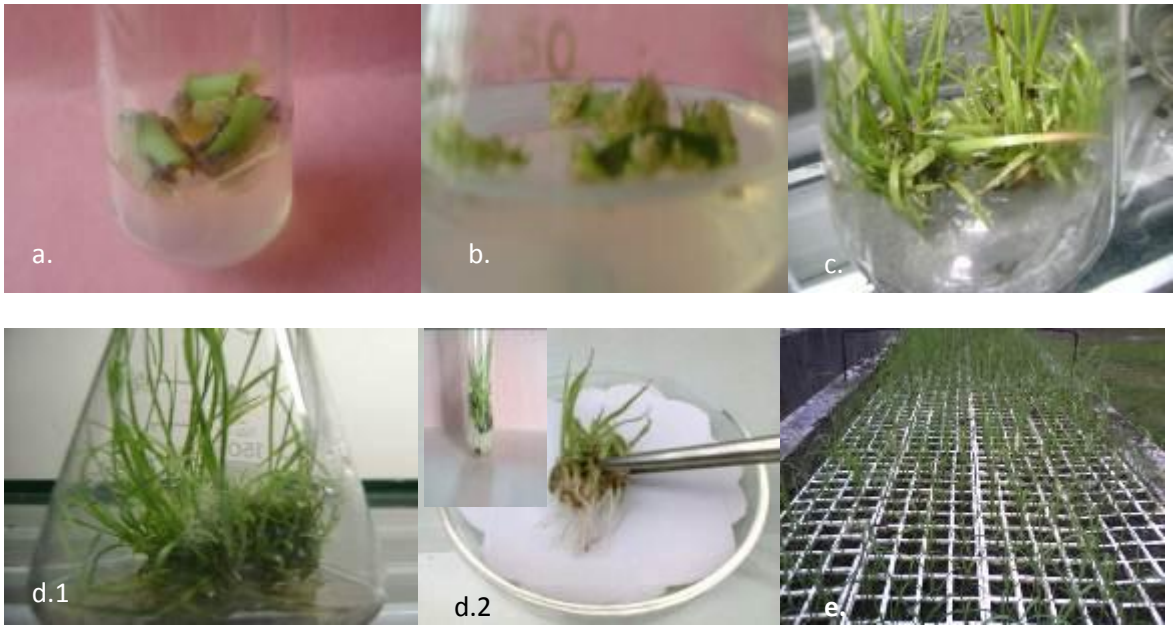

Fig. 1. a. Explant initiation b. Embryogenic callus induction c. Shoot induction d(1-2). Rooted plant e. Established plantlets on cell-u-pack

***In vitro* rooting and acclimatization**

IBA and NAA were used at different concentrations combinations to regenerate adventitious roots. Best rooting was observed in half MS medium supplemented with 3.0 mgl^{-1} NAA for % root initiation, no. of root/microshoot(13) and root length(4.2 cm) (Table 3) which take only 20 days for initiation of root primordia. All the clone performs best at half MS medium supplemented with 3.0 mgl^{-1} NAA except I 214-11. It also observed that more than 5mgl^{-1} NAA drastically reduces the root initiation. Gosal *et al.* (1998) reported that only NAA(5 mgl^{-1})and 70 gl^{-1} sucrose suited to root formation. Similar result also observed by Baksha *et al.* (2002).

Half MS medium with 3 mgl^{-1} IBA with 3% sucrose produce second most for no. of root / microshoot (number) and root length(cm) respectively for Isd 20(12.2, 3.4), I 220-92(11.4, 3.3), Co 65-02(10, 3.8) and I 214-11(8.5, 6.9). Ali and Afghan (2001) observed only 6-7 roots after 3 weeks on MS medium containing 2.0 mgl^{-1} IBA and 6% sucrose which also supported by Sabaz *et al.* (2008) and Alam *et al.* (2003). Mamun *et al.* (2004) also obtained best results of rooting on MS medium supplemented with auxins (NAA + IBA) 0.5 mgl^{-1} for each one. Lal and Singh (1994) also state that root can be easily induced on culture shoots by their transfer to another medium with or without NAA, where optimal growth were observed with 1/2 strength of MS medium.

The plantlets with well-developed shoot and roots after acclimatization were successfully transplanted in soil with 85% acclimatization of survivability potential. Thus tissue culture technique can plays an important role in this regard for supply of disease free quality planting material in a year round basis and true to true types of the mother plant.

Table 3. Effect of different auxins on root formation of the *in vitro* plantlet on half MS medium

Hormone Concentration		Isd 20			Isd 35			I 220 92			Co 65-02			I 214-11		
IBA	**NAA**	**% root initiation**	**no. of root plant^{-1}**	**Root length**	**% root initiation**	**no. of root plant^{-1}**	**Root length**	**% root initiation**	**no. of root plant^{-1}**	**Root length**	**% root initiation**	**no. of root plant^{-1}**	**Root length**	**% root initiation**	**no. of root plant^{-1}**	**Root length**
1	0	56	7.8	2.3	42	6.5	2	48	6.5	2.7	62	6.4	2.4	43	6.2	3.4
3	0	78	12.2	3.4	66	11	3.8	72	11.4	3.3	78	10	3.8	78	8.5	6.9
5	0	68	10	3.6	64	9.5	3.6	61	6	3.8	72	9.2	3	65	7.8	4.6
7	0	42	9	2.5	40	7	3.1	42	4.1	3.1	62	6.5	2.4	56	5.5	3.5
0	1	52	7	1.8	54	8.2	2.1	48	7.3	4	54	4.5	2	68	4	3.5
0	3	88	13	3.5	82	11.5	4.2	86	12	4.2	86	9.6	3.2	81	9	4.2
0	5	79	11	3.8	78	8.8	3.8	67	8	3.2	72	12	3.8	84	6.5	3.6
0	7	62	6	3.2	68	7.2	2.8	54	6.3	2.5	70	6	2.5	48	4.5	3.2
1	1	32	8.4	2.7	36	6.4	2.1	43	3.2	2.8	36	5	2.2	28	7	3.1
1	2	45	9.1	3	53	9.5	3.2	56	3	3.6	46	7	3	48	6	2.6
2	1	42	6	2.8	46	5.8	3.5	52	4	3	48	6	3.5	52	4	2.8
2	2	56	7.3	3.3	48	7.2	3.7	61	5	4.2	54	6	3.7	48	6	3.2

CONCLUSION

Callus induction was found suitable in MS medium supplemented with 0.2 mgl^{-1} BA and 2 mgl^{-1} 2,4-D. Soot induction was suitable in 1.5 mgl^{-1} BA and 0.5 mgl^{-1} NAA supplemented MS medium. Half MS medium supplemented with 3.0 mgl^{-1} NAA is the best for rooting.

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