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Tissue Culture Variability on Callus Induction and Plant Regeneration of Five Sugarcane Varieties

N. Islam, M. A. Hossain and M. A. S. Miah
Bangladesh Sugarcane Research Institute
Ishurdi-6620, Pabna

ABSTRACT

The investigation was conducted at Biotechnology Laboratory of Bangladesh Sugarcane Research Institute (BSRI), Ishurdi , Pabna, Bangladesh to investigate the tissue culture variability on callus induction and plant regeneration of five sugarcane varieties viz., Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34 from unexpanded leaf sheath explant. Explants were inoculated on MS medium containing 2.0-4.0 mg/l of 2, 4-D for callus induction. Best results were obtained from all tested varieties at 3.0 mg/l of 2, 4-D. Combination of cytokinin (BA) with auxins (NAA or IBA) showed the best performance for shoot proliferation to that of concentration of cytokinin (BA) alone. Among different media, 1.0 mg/l BA + 0.5 mg/l NAA showed best result for multiplication of shoots. The highest number of shoot (11.8) was obtained from the variety Isd 33 followed by Isd 18 and Isd 32 whereas, the variety Isd 34 showed the lowest (9.7) performance among the five varieties. *In vitro* proliferated shoots were rooted on medium supplemented with different auxins. Best results were observed on MS medium supplemeted with 5.0 mg/l NAA for all varieties. The highest number of healthy roots (14.7) was produced in Isd 34 followed by Isd 28, Isd 18 but the lowest (7.9) number in Isd 32 when 5.0mg/l NAA supplemented in interactions of varieties and media exerted remarkable effects on shoot and root capabilities. The plantlets were successfully transferred to soil after root formation percent were survived. Results indicate varietals variability of sugarcane for on and plant regeneration through tissue culture.

Key words: Sugarcane, variability, tissue culture, callus and regeneration.

INTRODUCTION

Plant tissue culture as an essential component of plant biotechnology offers novel approaches to the production, propagation, conservation and manipulation of plants. Genetic variability develops during long term tissue cultures, called somaclonal variation, which is a valuable source of new genetic information. This method refers to heritable changes that accumulate in the callus from a somatic explant and express in the progeny of *in vitro* regeneration obtained from callus. Braun (1959) reported that *in vitro* cultured cells may be display genetic alterations. Somaclonal variation is one of the important aspects of tissue culture technology and is widely recommended for crop improvement. Now it is easy to create somaclonal variation in the plantlets of sugarcane by *in vitro* callus culture and many new characters can be identified in these plants. If any character is found to be superior to mother plant, then the somaclone with these phenotype can be released as a new variety. Initial attempts to regenerate plants through tissue culture technique were made on sugarcane by Nickell (1964) and Heinz and Mee (1969). Callus culture of sugarcane have been successfully established using shoot apices, young leaves and inflorescence as explants on

MS (Murashige and Skoog, 1962) medium containing 2,4-D and coconut milk (Nadar *et al.*, 1978; Liu, 1984; Bhansali and Sing, 1984). Precise methods of control and maintenance of plant regeneration in sugarcane callus culture have been presented by Chen *et al.* (1988). Krishnamurthi and Tlaskal (1974) isolated clones of regenerated sugarcane with resistance to Figi disease virus and downy mildew without reducing sucrose yield. Similarly, Larkin and Scowcroft (1981) has been isolated variants of Sugarcane with resistance to eye spot disease. Callus induction and plant regeneration protocol for sugarcane variety Isd 16 of Bangladesh was also reported elsewhere (Hossain *et al.*, 1996).

In Bangladesh, improvement of sugarcane using tissue culture techniques deserve due research attention. Plant regeneration protocols for different varieties of sugarcane are the prerequisite for the purpose. However, very limited efforts have been made so far. The present investigation was undertaken to evaluate the callus induction, shoot multiplication and root forming potentials of five varieties and to compare different media and select suitable media for specific variety.

MATERIALS AND METHODS

The study was conducted at Biotechnology Laboratory of Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. The young leaf-sheath of five sugarcane varieties viz., Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34 were used as experimental material.

Disease-free and actively growing top shoot of sugercane varieties viz, Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34 were collected from 3-4 months old plants as explants from experimental field of BSRI. Then the plant materials were washed thoroughly under running tap water in the laboratory to reduce dust surface contaminants. Outer whorl of leaves was removed and surface sterilization induced treatment of the materials with 1% savlon for 10 minutes with constant shaking. Then the explants were washed 3-4 times with sterilized distilled water and taken under running laminar airflow cabinet and immersed in 0.1% $HgCl_2$ into a 250 ml sterilized conical flask and shaking for 10-12 minutes. To remove traces of the sterilant, the material was then washed 4-5 times with sterile distilled water. Then the explants were prepared from the surface sterilized materials for establishing aseptic culture for callus induction and plant regeneration.

For callus induction 0.5 cm of sugarcane leaf sheath explants were aseptically cultured on MS medium with varying concentrations (2, 3 and 4 mg/l) of 2,4-D after sterilizing the plant material. The cultures were kept under dark for one week at a temperature of 25±1°C.

For evaluating the optimum growth requirements for the shoot induction, the calli were cultured on different shoot induction media viz., 1.0 mg/l BA, 1.0 mg/l BA + 0.5 mg/l NAA, 1.0 mg/l BA + 0.5 mg/l Kn. After initiation of auxiliary shoots, the whole explant was rescued aseptically on a sterile petridish and was cut into convenient sizes by a sterile scalpel and then transferred on the appropriate medium for proliferating of shoots. Then each of the microcuttings of shoot was cultured on freshly prepared MS medium supplemented with IBA, NAA and IAA at the concentration of 5.0 mg/l.

Unless mentioned specially, all cultures were grown in an air-conditioned culture room illuminated by 40W white florescent tube light. The temperature of the culture room was maintained at 25±1°C and light intensity varied from 2000-3000 lux provided by cool-white florescent tubular lamp.

RESULTS AND DISCUSSION

The techniques for *in vitro* plantlet regeneration following callus induction, shoots regeneration, multiplication and rooting of the shoots have been established very carefully using five varieties of sugarcane viz., Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34. Results of the investigation are presented and discussed bellow.

Callus Induction

The surface sterilized leaf sheath explants of sugarcane varieties, Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34 were inoculated on MS medium supplemented with different concentrations of 2,4-D. Callus induction was observed after 10-15 days of culture and calli grew vigorously until nutrient of the medium became exhausted. Best callus induction was observed in MS medium containing 3.0 mg/l 2,4-D for all tested varieties (Table 1). The percentage of callus induction from leaf sheath was 100, 90, 90, 90 and 100 for Isd 18, Isd 28, Isd 32, Isd 33 and Isd 34 cultivars respectively on 3.0 mg/l concentration of 2,4-D. It was observed that callus induction percentage increased up to 2,4-D 3.0 mg/l but in case of 4.0 mg/l this become decreased which indicating that beyond certain level of 2, 4-D has an adverse effect. Callus formation from the explants of all five varieties was vigorous and expected on 3.0 mg/l concentration of 2, 4-D, but at 4.0 mg/l of 2,4-D the callus induction of Isd 32 was not in the expected rate (Table 1). Moreover, Isd 28 showed better results at 2.0 mg/l concentration of 2,4-D. Many workers found that 2,4-D was the best auxin for callus induction as in monocotyledons and even in dicotyledons (Evans *et al.*,1981; Jaiswal and Narayan, 1985; Begum *et al.*, 1995; Nadar *et al.*, 1978; Liu, 1984; Chen *et al.*, 1988 and Lal and Singh, 1991). In this investigation, the best callus was observed at 3.0 mg/l of 2,4-D for all varieties. These findings corroborated with report of Begum *et al.* (1995) for callus formation using medium supplemented with 3.0-5.0 mg/l of 2,4-D in sugarcane varieties of Bangladesh. Similar response was reported by Barba *et al.* (1977) and Mannan and Amin (1999). There are many previous reports indicating the best callus induction at 3.0 mg/l of 2,4-D (Alam *et al.*, 2003; Karim *et al.*, 2002 and Rahman *et al.*, 2001).

Above all, the efficiency of callus induction from the leaf sheath explants of all five varieties was best at the 3.0 mg/l concentration of 2,4-D.

Shoot Proliferation from Callus

The calli of five varieties of sugarcane were transferred to four different media for differentiation and proliferation of shoots. It was observed that number of shoot production per callus, number of usable shoots per culture and shoot length were differed significantly among the varieties (Table 2). Variety Isd 33 showed the highest performances for shoot production followed by varieties Isd 18 and Isd 32 with statistically similar results. Performances among four media of shoot production were significantly differed for number of shoots per culture and shoot length, however media showed insignificant differences for number of usable shoot per culture (Table 3). The media

which contained BA 1.0 mg/l + NAA 0.5 mg/l showed the best performance for number of shoot production per culture followed by media contained BA 1.0 mg/l + IBA 0.5 mg/l. In case of shoot length, the media which contained BA 1.0 mg/l alone showed the best performance followed by media containing BA 1.0 mg/l + NAA 0.5 mg/l. Interactions between varieties and media were significantly differed for number of shoots per culture, number of usable shoots per culture and shoot length (Table 4). Variety lsd 18 produced the highest number of shoots per culture (13.8) on media supplemented with BA 1.0 mg/l + NAA 0.5 mg/l followed by variety lsd 33 (13.7) on media containing BA 1.0 mg/l + NAA 0.5 mg/l. Number of usable shoots per culture was highest in variety lsd 33 on media BA 1.0 mg/l + IBA 0.5 mg/l. In case of shoot length, performance of variety lsd 32 was the best on media BA 1.0 mg/l + Kn 0.5 mg/l followed by varieties lsd 28 and lsd 32 on media containing BA 1.0 mg/l alone and BA 1.0 mg/l + NAA 0.5 mg/l respectively. Different varieties showed different performances on different media possibly due to different hormonal composition in different varieties. Therefore, the results revealed that varietal differences were prominent over media composition.

Rooting of the *in vitro* regenerated shoots

In vitro grown proliferating microshoots were isolated and cultured on MS medium suplemented with three different auxins such as NAA, IBA and IAA at the concentration of 5.0 mg/l for root formation to establish themselves in the field. Effects of root formation for varieties, auxins and their interactions present in the Tables 5, 6 and 7. The best root forming performance showed by variety lsd 34 (14.7) followed by lsd 28, lsd 33 and lsd 18 (Table 5). Auxin NAA showed the best performance for root formation (Table 6). In case of interactions effects variety lsd 34 produced the highest number of roots (24.4) cultured on NAA containing media followed by lsd 33 and lsd 18. Root length was the highest in variety lsd 34 cultured on IBA or NAA containing media followed by lsd 28 cultured on NAA containing media. Auxin (NAA) showed best performance for *in vitro* root production of sugarcane. Nadar and Heinz (1977) preferred auxin (NAA) for root initiation and NAA 5.0 mg/l showed best performance in the production of roots (Karim *et al.*, 2002). Lal and Singh (1994) reported that roots can be easily induced on cultured shoots by their transfer to another medium with or without NAA, where optimal growth were observed with a half strength modified MS medium. Alam *et al.* (2003) observed best rooting on MS medium supplemented with NAA. However, IBA formed root with vigorous growth. Growth regulator IBA was found comparatively less effective than NAA for producing roots for the all varieties. Rongrong *et al.* (1988) reported that MS medium with 1.0 mg/l IBA was suitable for rooting in their experiment. Sumana *et al.* (1992) also developed sugarcane roots from plantlets when they are transplanted to MS medium with 2.0 mg/l of IBA. Therefore, medium supplemented with 5.0 mg/l NAA was proved to be more effective than any other auxins for the root formation on sugarcane microshoots.

Table 1. Effects of different concentrations of 2,4-D on callus induction from leaf sheath explants of sugarcane. Data were recorded after three weeks of culture on MS medium.

Growth regulators 2,4-D (mg/l)	Isd 18		Isd 28		Isd 32		Isd 33		Isd 34	
	No. of explants showed callusing	Percentage of explants with callus induction	No. of explants showed callusing	Percentage of explants with callus induction	No. of explants showed callusing	Percentage of explants with callus induction	No. of explants showed callusing	Percentage of explants with callus induction	No. of explants showed callusing	Percentage of explants with callus induction
2	5	50	8	80	7	70	6	60	4	40
3	10	100	9	90	9	90	9	90	10	100
4	6	60	6	60	4	40	8	80	6	60

Table 2. Varietal differences in shoot formation capacity of five sugarcane varieties (Values are means from 4 media x 10 replications).

Varieties	Number of shoots per culture	Number of usable shoots per culture	Shoot length (cm)
Isd 18	11.3ab*	4.7ab	5.5ab
Isd 28	9.9b	4.1bc	5.7ab
Isd 32	10.4ab	4.3abc	5.9a
Isd 33	11.8a	5.1a	5.4ab
Isd 34	9.7b	3.8c	5.0b

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

Table 3. Effects of different media on shoot formation of five sugarcane varieties (Values are means from 5 varieties X 10 replications).

Media	Number of shoots per culture	Number of usable shoots per culture	Shoot length (cm)
$BA_{1.0}$**	10.0b*	4.4	6.0a
$BA_{1.0}+NAA_{0.5}$	11.9a	4.6	5.6a
$BA_{1.0} + IBA_{0.5}$	10.6ab	4.4	5.0b
$BA_{1.0} + Kn_{0.5}$	9.9b	4.2	5.4ab

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

** Figures as subscript denote (mg/l)

Table 4. Interaction effect between varieties and media on shoot formation (Values are means from 10 replications).

Combination of varieties and media			Number of shoots per culture	Number of usable shoots per culture	Shoot length (cm)
Isd 18	:	$BA_{1.0}$**	10.5abcd*	5.1abc	6.3ab
		$BA_{1.0}$+$NAA_{0.5}$	13.8a	5.2abc	5.8abcd
		$BA_{1.0}$ + $IBA_{0.5}$	12.1abc	4.8abcd	5.3abcd
		$BA_{1.0}$ + $Kn_{0.5}$	8.6cd	3.7bcd	4.8bcd
Isd 28	:	$BA_{1.0}$	10.1cd	4.1abcd	6.4ab
		$BA_{1.0}$+$NAA_{0.5}$	10.2bcd	4.4abcd	5.3abcd
		$BA_{1.0}$ + $IBA_{0.5}$	8.6cd	3.4cd	5.7abcd
		$BA_{1.0}$ + $Kn_{0.5}$	10.6abcd	4.6abcd	5.5abcd
Isd 32	:	$BA_{1.0}$	9.4cd	4.0abcd	6.0abcd
		$BA_{1.0}$+$NAA_{0.5}$	10.7abcd	4.2abcd	6.4ab
		$BA_{1.0}$ + $IBA_{0.5}$	10.8abcd	4.5abcd	4.5cd
		$BA_{1.0}$ + $Kn_{0.5}$	10.6abcd	4.5abcd	6.8a
Isd 33	:	$BA_{1.0}$	10.8abcd	4.3abcd	5.5abcd
		$BA_{1.0}$+$NAA_{0.5}$	13.7ab	5.4ab	5.7abcd
		$BA_{1.0}$ + $IBA_{0.5}$	11.3abcd	5.6a	4.5cd
		$BA_{1.0}$ + $Kn_{0.5}$	11.4abcd	4.9abcd	5.8abcd
Isd 34	:	$BA_{1.0}$	9.1cd	4.3abcd	6.1abc
		$BA_{1.0}$+$NAA_{0.5}$	11.0abcd	4.0abcd	5.0abcd
		$BA_{1.0}$ + $IBA_{0.5}$	10.4abcd	3.8abcd	4.7bcd
		$BA_{1.0}$ + $Kn_{0.5}$	8.2d	3.1d	4.2d

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

** Figures as subscript denote (mg/l)

Table 5. Varietal differences in root formation capacity in regenerated shoots of five sugarcane varieties (Values are means from 3 media x 5 replications).

Varieties	Number of roots per shoot	Root length (cm)
Isd 18	10.6ab*	1.5c
Isd 28	12.9a	1.8ab
Isd 32	7.9b	1.4c
Isd 33	11.4ab	1.6bc
Isd 34	14.7a	1.9a

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

Table 6. Effect of auxins on root formation in regenerated shoots of five sugarcane varieties (Values are means from 5 varieties x 5 replications).

Rooting media	Number of roots per shoot	Root length (cm)
$NAA_{5.0}$**	18.7a*	1.9a
$IBA_{5.0}$	9.6b	1.8a
$IAA_{5.0}$	6.2c	1.2b

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

** Figures as subscript denote (mg/l)

Table 7. Interaction effect between varieties and media on root formation of five sugarcane varieties (Values are means from 5 replications).

Combination of varieties and rooting media			Number of roots per shoot	Root length (cm)
Isd 18	:	$NAA_{5.0}$ **	16.6bc	1.4de
		$IBA_{5.0}$	9.2cd	1.7bcd
		$IAA_{5.0}$	6.0d	1.3de
Isd 28	:	$NAA_{5.0}$	19.6ab	2.1ab
		$IBA_{5.0}$	11.6cd	2.0ab
		$IAA_{5.0}$	7.4d	1.2de
Isd 32	:	$NAA_{5.0}$	12.0cd	1.6bcd
		$IBA_{5.0}$	7.6d	1.5de
		$IAA_{5.0}$	4.0d	1.1e
Isd 33	:	$NAA_{5.0}$	20.8ab	2.0abc
		$IBA_{5.0}$	8.4d	1.5cde
		$IAA_{5.0}$	5.0d	1.2de
Isd 34	:	$NAA_{5.0}$	24.4a	2.2a
		$IBA_{5.0}$	11.4cd	2.2a
		$IAA_{5.0}$	8.4d	1.2e

*Figures within column followed by different letters are significantly different by DMRT at p=0.05.

** Figures as subscript denote (mg/l)

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