MICROPROPAGATION OF SUGARCANE THROUGH LEAF SHEATH CULTURE

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ABSTRACT

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An experiment was conducted to develop an efficient protocol for micropropagation of sugarcane using leaf sheath culture method of three sugarcane varieties viz. Isd16, Isd36 and Isd37 in the germplasm laboratory of breeding division, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna. Leaf sheath explants were used for callus formation followed by shoot initiation. Basic medium Murashige and Skooge (MS) supplemented with different concentration of auxin (NAA and 2, 4-D) were used to observe their effects on callus formation. Best results were observed from the varieties Isd36 and Isd37 at 4.0 mg/l of 2, 4-D and the variety Isd16 at 3.0 mg/l of 2, 4-D for callus induction. Different combinations of BAP with NAA or IBA supplemented with MS medium were used for shoot initiation and multiplication. 1.0 mg/l BAP + 0.5 mg/l NAA showed the best result for induction and multiplication of shoot. MS medium supplemented with different concentrations of auxin (NAA and IBA) were used alone for *in vitro* root formation from proliferated shoots. NAA showed better performance than IBA for root initiation. Best result of root formation was observed on MS medium supplemented with 5.0 mg/l of NAA. The variety Isd37 produced the highest number of roots (13.47) at 5.0 mg/l of NAA. The plantlets were successfully transferred to soil with eighty to ninety percent survivability at normal temperature with 85% humidity.

Key words: *Sugarcane, micropropagation, leaf sheath, explant*

INTRODUCTION

Micropropagation has become an attractive and powerful tool in the research field throughout the world, especially in the area of large scale clonal propagation, crop improvement through genetic manipulation, conservation of plant genetic resources and valuable germplasm. In Bangladesh, sugarcane is propagated by settlings. Every year a lot of sugarcane is required as sets. Thus, farmers loss their production and sugar industries also loss sugar. Moreover, due to flood, farmers need to wait for "Jow condition" (Optimum soil moisture condition for ploughing) for set sowing. Delayed sowing hampers the production of sugar during maturation. If the seedlings are produced in a Biotechnology Laboratory as commercial basis, farmers can sow them immediately after the run-off of the floodwater. On the other hand, sets use for sowing can be sent to the sugar industries for sugar production. Time requirement and continued infection by systematic disease are serious problems to multiply an elite genotype of sugarcane in the open field (Lal and Singh, 1994). The technique of micropropagation is being routinely used for producing large number of clonal plants by *in vitro* culture of explants from wide range of species throughout the world. It has become now a viable alternative to the conventional clonal propagation methods. The sugarcane breeding progrmme has been under serious problem due to lack of suitable multiplication procedure (Lal and Sing, 1994). Using conventional method usually 10-15 years of work is needed to complete a selection cycle and an improved variety can be planted commercially several years later when enough seed canes will have been produced. Although, sugarcane is one of the most important industrial crops, very limited effort have been made on tissue culture and *in-vitro* propagation for variety development and rapid multiplication in Bangladesh. Therefore, this investigation was undertaken to establish the *in vitro* regeneration and rapid propagation techniques of field grown sugarcane. Barba and Nickel (1969) and Heinz and Mee (1969) demonstrated that plantlets could be developed from sugarcane callus culture.

MATERIALS AND METHODS

Leaf sheath of three different varieties of sugarcane viz, Isd16, Isd36 and Isd37 were collected from 3-4 months old field grown plant at experimental farm, Bangladesh Sugarcane Research Institute, Ishurdi, Pabna. The explants were then treated with 1% savlone for five minutes with constant shaking and washed 3-4 times with distilled water for surface sterilization. Surface disinfestation was done with 0.1% $HgCl_2$ for 12 minutes. After sterilization explants were sliced into 0.5-1.0cm aseptically under laminar air-flow cabinet and culture on modified MS medium (Murashige and Skoog's, 1962) supplemented with different concentration of auxin (2, 4-D and NAA). After 3-4 weeks of culture, calli were transferred to MS medium supplemented with different combination of BA with NAA or IBA were used for shoot formation. Rooting of the shoots (2-3 cm long) was done on MS medium supplemented with different concentration of IBA or NAA (Table-3). Callus and shoot proliferation were established on solid media and then after formation of multiple shoots these were shifted in liquid media for further multiplication of shoot. MS media was supplemented with 30 gm sucrose. The P^H of media was adjusted 5.7 to 5.8. Media was measured 10 ml was poured in the test tubes. The media was

autoclaved at 121^0C for 20 minutes. Culture were kept at 25 ± 1^0 C with 16 hours photoperiod. After establishment of full-fledged plant, these were shifted in green house for acclimatization. Different combinations of compost were used with or without autoclaving for hardening. Then these were shifted in trial farm for further growth. Statistical analysis was performed by using a computer programme MStat-C for comparison of treatments means at 5 % probability level.

RESULTS AND DISCUSSION

Effect of different concentration of auxin (NAA or 2, 4-D) for callus induction was observed in this study, 2, 4-D showed better performance than NAA for callus initiation. The highest percent of callus was obtained in modified MS medium supplemented with 4.0 mg/l 2, 4-D. It showed Table-1 that the callus development was increased as the concentration of 2, 4-D was increased in the modified MS medium. Best results were observed from the variety Isd36 and Isd37 at 4.0 mg/l of 2, 4- D and the variety Isd16 at 3.0 mg/l of 2,4-D for callus induction. Similar observation was reported by Barba *et al.*, (1977) and Mannan and Amin (1999). Barba *et al*., also stated that callus formed at lower concentration of 2, 4-D was whitish and loose, while at higher concentration it was brownish. After establishment of callus from leaf sheath, various dozes and combination of the growth regulators viz. BA (0.5, 1.0 & 2.0 mg/l) and NAA & IBA (0.1, 0.2 & 0.5 mg/l) were used in different combination for shoot induction. Among the combination, 1.0 mg/l BA + 0.5 mg/l NAA and 1.0 mg/l BA + 0.5 mg/l IBA were found to show satisfactory performance for all verities regarding regeneration of shoot from the callus tissue (Table-2). Shoot differentiation from the callus tissue was demonstrated by Heinz and Mee (1969); Barba and Nickel (1969); Mannan and Amin (1999). Different concentrations of NAA (0.5 ~ 5.0 mg/l) and IBA (0.5 ~ 5.0 mg/l) in MS media were used for root induction.

Table –1: Effect of different concentration of auxin-NAA & 2, 4-D on callus induction from the leaf sheath explants of different sugarcane varieties after 3-4 weeks of inoculation

Hormonal Concentration mg/l	% of explants formed in callus					
	Isd16		Isd36		Isd37	
	NAA	2,4-D	NAA	2,4-D	NAA	2,4-D
1.0	0.00 j	71.11 f	0.00 j	75.55 ef	0.00 j	75.55 ef
2.0	11.11 hi	84.45 bc	15.55 gh	82.22 bcd	20.00 g	82.22 bc
3.0	20.00 g	95.55 a	889 j	86.67 b	11.00 hi	84.45 bc
4.0	0.00 j	82.22 bcd	0.00 j	97.78 a	0.00 j	97.78 a
5.0	0.00 j	71.11 f	0.00 j	77.78 de	0.00 j	80.00 cde

Note: NAA (Naphthalene acetic acid); 2,4-D (2,4 diclorophenoxy acetic acid)

Table -2: Effect of different combination phytohormones on shoot regeneration from callus tissue of different sugarcane varieties after 3-4 weeks of inoculation

Hormonal Concentration & Combination(mg/l)	Isd16		Isd36		Isd37	
	No. of shoot /culture	Length (cm)	No. of shoot /culture	Length (cm)	No. of shoot /culture	Length (cm)
$B_{0.5}$ $NA_{0.1}$	6.43 jkl	3.50 pqr	6.70 ukl	3.50 pqr	7.27 ghu	3.53 pqr
$B_{0.5}$ $NA_{0.2}$	6.57 jkl	4.00 jkl	7.73 ghu	3.90 klm	8.20 fgh	3.67 nop
$B_{0.5}$ $NA_{0.5}$	7.23 ghu	4.53 be	8.17 fgh	4.23 ghi	8.60 efg	3.77 mno
$B_{1.0}$ $NA_{0.1}$	8.33 efg	4.30 fgh	9.73 de	4.43 ef	9.40def	4.40 efg
$B_{1.0}$ $NA_{0.2}$	10.60 cd	4.90 c	8.00 fgh	4.97 bc	11.73 bc	4.90 c
$B_{1.0}$ $NA_{0.5}$	13.80 a	5.13 ab	12.73 ab	5.27 a	13.17 a	5.13 ab
$B_{2.0}$ $NA_{0.1}$	5.80 mno	3.17 st	5.93 mno	2.63 v	6.13 lmn	3.10 st
$B_{2.0}$ $NA_{0.2}$	4.43 s	2.53 v	4.80 rs	2.47 vw	5.60 nop	2.53 v
$B_{2.0}$ $NA_{0.5}$	4.83 rs	2.30 wx	4.77 rs	2.23 x	5.00 qrs	2.63 v
$B_{0.5}$ $IB_{0.1}$	5.27 pqr	2.17 x	5.30 pqr	2.20 x	5.63 nop	3.20 s
$B_{0.5}$ $IB_{0.2}$	6.57 jkl	3.17 st	6.97 huk	3,07 stu	6.97 huk	3.47 qr
$B_{0.5}$ $IB_{0.5}$	5.13 pqr	2.90 u	4.50 s	3.00 tu	4.70 rs	2.60 v
$B_{1.0}$ $IB_{0.1}$	7.30 ghu	3.13 st	6.27 klm	4.03 jk	5.77 mno	4.07 uk
$B_{1.0}$ $IB_{0.2}$	8.53 efg	3.43 r	8.17 fgh	3.63 opq	7.60 dhu	4.53 de
$B_{1.0}$ $IB_{0.5}$	11.43 bc	4.70 d	10.50 cd	3.83 lmn	10.63 cd	5.10 ab
$B_{2.0}$ $IB_{0.1}$	6.17 klm	4.37 efg	6.10 lmn	4.10 u	6.13 lmn	3.13st
$B_{2.0}$ $IB_{0.2}$	7.53 ghu	4.10 u	7.03 ghu	4.50 e	7.07 ghu	4.13 hu
$B_{2.0}$ $IB_{0.5}$	5.47 opq	3.63 opq	6.53 jkl	3.10 st	5.20 pqr	3.53 pqr

Note: BA (6 Benzyl aminopurin); IBA (Indol 3 butyric acid)

It was evident from Table -3 that nearly all varieties showed better response on MS media with 5.0 mg/l NAA. Profuse rooting was observed in all varieties after 14 days. Higher doses of auxin especially NAA is required for efficient root development in sugarcane (Nadgauda, 2002). Lal (1992) and Cooke *et al.*, (2002) also reported that NAA is most suitable auxin for rooting particularly in sugarcane. After 3–4 weeks, vigorous rooting was observed, after full establishment of roots (6 to 7 roots and 1 to 1.5 inches long), the plantlets were transferred to the poly bags filled with sterilized sandy loam soil and then the poly bags were placed in a glasshouse with controlled environment maintaining 30^0 C temperature as well as 95% humidity for acclimatization and after 6-8 weeks they were transplanted in the soil. Micropropagation technology was viable method for disease free, true to type and rapid propagation of released varieties or promising clones.

Table –3: Effect of different concentrations of phytohormones on root initiation from the regenerated shoot after 3-4 weeks of inoculation

Media + Hormone mg/l	Isd16		Isd36		Isd37	
	No. of root/shoot	Root length (cm)	No. of root/shoot	Root length (cm)	No. of root/shoot	Root length (cm)
MS + NAA 0.5	5.17 o	1.43 fg	5.07 o	1.47 efg	5.57 n	1.63 d
+ NAA 1.0	8.47 k	2.03 ab	8.10 e	2.03 ab	9.97 h	2.13 a
+ NAA 3.0	11.53 e	1.83 c	10.90 f	1.93 bc	12.17 d	1.97 b
+ NAA 5.0	12.83 b	1.53 def	12.53 c	1.40 g	13.47 a	1.60 d
+ IBA 0.5	4.53 p	1.37 g	4.27 q	1.37 g	4.57 p	1.33 g
+ IBA 1.0	9.30 i	1.57 de	7.27 m	1.53 def	9.63 i	1.43 fg
+ IBA 3.0	10.53 g	1.37 g	9.53 i	1.37 g	10.67 g	1.60 d
+ IBA 5.0	10.97 f	1.13 h	10.57 g	1.03 h	11.37 e	1.13 h

Note: MS = Murashige & Skoog's (1962) medium

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