OnLine Journal of Biological Sciences 2 (10): 682-685, 2002
ISSN 1608-4127

Micropropagation of Two Sugarcane (*Saccharum officinarum*) Varieties from Callus Culture

M.Z. Karim, M.N. Amin, M.A. Hossain, S. Islam, Faruk Hossin and R. Alam
Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: Protocol for induction of callus and regeneration response of two sugarcane varieties (Isd-16, Isd-28) was established through callus culture using leaf sheath. Multiple shoot regeneration at various frequencies was observed using different concentrations and combinations of growth regulators. The highest percentage of callus induction was observed in the medium containing 3.0 mg l^{-1} 2,4-D with 10% coconut water (CM). The best response in terms of multiple shoot formation was observed that on MS medium supplemented with BAP 1.0 mg l^{-1} + IBA 0.5 mg l^{-1}. NAA (3.0 mg l^{-1}) was found effective in the production of roots. The variety Isd-16 showed better response than the variety Isd-28 towards shoot multiplication. Seventy percent of the plantlets produced from *in vitro* culture method survived in the *ex vitro* condition.

Key words: Callus culture, regeneration, *In vitro*, micropropagation, *Saccharum officinarum*

Introduction

Sugarcane (*Saccharum officinarum*) is a major agricultural crop in tropical and subtropical regions of the world and important export product in many developing countries (Heinz *et al*., 1977). It is a member of the family Gramineae and belongs to the genus *Saccharum*. Sugarcane varieties are highly heterogeneous and generally multiplied vegetatively by stem cutting. Lack of suitable multiplication procedure has long been serious problem in sugarcane breeding programme (Nondan and Singh, 1994). Plant tissue culture offers the best methodology through micropropagation of sugarcane for quality and phytosanitary planting material at a faster rate in a shorter period of time. It has become now a viable alternative to the conventional clonal propagation methods. There are many reports on tissue culture and plant regeneration of sugarcane from different countries. Initial attempts to regenerate plants through *in vitro* 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 young inflorescences as explant on MS medium containing 2,4-D and coconut milk (CM) (Heinz *et al*., 1977; Nadar *et al*.,1978; Liu, 1984; Bhansali and Singh, 1984).

Therefore, this study was undertaken to establish a protocol for large scale propagation of sugarcane variety Isd-28 and Isd-16 of Bangladesh through callus culture using leaf sheath explants.

Materials and Methods

The experiment was conducted at Plant Tissue Culture Laboratory, Department of Botany, University of Rajshahi, Bangladesh during the period of 1998 to 1999. The genotypes Isd-16 and Isd-28 are the experimental varieties. Leaf sheath explants of two varieties (Isd-16, Isd-28) were collected from 3-4 months old grown plant from BSRI (Bangladesh Sugarcane Research Institute, Ishurdi, Pabna) field and they were washed thoroughly under running tap and distilled water. The material was then taken into laminar flow cabinet and surface sterilized with 0.1% $HgCl_2$ for different duration of time. The explants were then aseptically cultured on modified MS medium (Murashige and Skoog, 1962) as recommended by Heinz and Mee (1969) supplemented with 3.0 mg l^{-1} 2,4-D and coconut milk (CM) 10% for callus induction. Media were consisted of 3% sucrose, 0.6% agar, pH was adjusted to 5.7 and autoclaved at 121°C for 20 min. All the culture were incubated at 25 ± 2°C and kept under 14 h photoperiod of fluorescent tube light.

Results and Discussion

Callus induction was observed within two weeks time after inculation from the leaf sheath explants on modified MS medium containing different concentrations of IBA, NAA and 2,4-D (0.5-5.0 mg l^{-1}). Though in all concentrations the callus induction was triggered, best callus induction was observed at 3.0 mg l^{-1} 2,4-D with 10 % coconut milk (CM) for both the varieties (Fig.1A_1, A_2). On this media composition the explant produced creamy white callus for variety Isd-28 and greenish white callus for variety Isd-16. The percentage of callus induction was 90 and 100 respectively (Table 1). Begum *et al*. (1995) found 3-5 mg l^{-1} of 2,4-D produced highest percentage of callus in Bangladeshi sugarcane varieties (viz. Nagarbari, L. jaba, Isd-16, Isd-20 and Clone I.123). Barba *et al*. (1997) also reported that 0.5-5.0 mg l^{-1} 2, 4-D showed callus induction from leaf tissue on MS medium. The concentrations NAA at 2.0 and 3.0 mg l^{-1} produced small amount of callus, but IBA showed not remarkable callusing. The variety Isd-16 and Isd-28 produced poor callus in lower concentrations of 2, 4-D and did not at all 0.5 mg l^{-1}.

All these studies indicate that sugarcane explant requires higher concentrations of 2,4-D for callus induction. Various concentrations of cytokinin (BAP, Kn) and auxins, (IBA, NAA) were used in different combinations for shoot regeneration. During this investigation shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment. Among different concentrations and combinations for shoot multiplication, best performance was showed on MS medium supplemented with BAP 1.0 mg l^{-1} + IBA0.5 mg l^{-1} (Table 2). On this combination the percentage of explant produced shoots was 92 and 100 for both the varieties respectively. The number of usable shoots, average length of the shoots was 12, 15 and 4.5, 5.2 cm per culture of the variety Isd-28 and Isd-16 (Fig.1 B_1, B_2). Islam *et al*. (1982) also reported the positive effects of BAP + IBA combination on shoot formation in sugarcane. It was also observed that BAP + NAA combination showed effective result. However, a high level of cytokinin and a low level of auxin were essential for differentiation of adventitious shoot in sugarcane leaf sheath callus. Different types of auxins were used at different concentrations and combinations to regenerate adventitious roots. Among different concentrations and combinations of auxins, NAA and IBA was found to be comparatively better response than IAA for producing roots. NAA + IBA combination showed positive result. Best rooting was observed ½ strength of MS medium supplemented with 3 mg l^{-1} NAA (Table 3) and the highest number roots per microshoots were 11 and 16 for both the varieties (Fig.1C_1, C_2). Micro-shoots of variety-16 showed better rooting performance than that of the variety Isd-28. According to Lal and Sing (1994) root can be easily induced on cultured shoots by their transfer to another medium with or without NAA, where optimal growth were observed with

Corresponding author: Mr. M.Z. Karim, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Table 1: Effect of different concentrations, auxin and 2,4-D on callus induction from leaf sheath explants of two sugarcane varieties

Hormonal supplement (mg l^{-1})	Variety lsd-28		Variety lsd-16	
	No. of explant showed callusing	% of explant with callus induction	No. of explant showed callusing	% of explant with callus induction
IBA				
0.5	-	-	-	-
1.0	-	-	-	-
2.0	-	-	-	-
3.0	-	-	-	-
4.0	-	-	-	-
5.0	-	-	-	-
NAA				
0.5	-	-	-	-
1.0	-	-	-	-
2.0	2	10	2	10
3.0	3	15	6	30
4.0	-	-	-	-
5.0	-	-	-	-
2,4-D				
0.5	4	20	6	30
1.0	10	50	8	40
2.0	14	70	12	60
3.0	18	90	20	100
4.0	8	40	14	70
5.0	-	-	-	-

"-" = No callusing; Poor callusing = 20-50%; Considerable callusing = 51-85%; Intensive callusing = 86-100%.

Table 2: Effect of the cytokinin (BAP, Kn) and the auxin (IBA, NAA) at different concentrations and combinations in MS medium on shoot regeneration from the callus tissue of sugarcane variety lsd-16 and lsd-28

Hormonal supplement mg l^{-1}	Variety lsd-28			Variety lsd-16		
	% of explant produced shoots	No. of shoots per explant	Average length of the usual shoot	% of explant produced shoots	No. of shoots per explant	Average length of the usual shoot
BAP						
0.5	14	3	3.5	20	3	3.9
1.0	60	6	3.9	75	7	3.2
2.0	49	5	3.7	55	4	3.0
5.0	20	6	2.5	40	2	2.0
Kn						
0.5	12	2	3.1	13	2	3.2
1.0	42	3	2.9	45	4	3.0
2.0	30	2	3.5	33	3	3.6
5.0	17	1	2.0	20	2	2.3
BAP+IBA						
0.5+0.1	40	3	2.2	50	3	3.3
+0.2	65	4	3.1	66	7	3.4
+0.5	25	6	3.0	40	9	2.0
+1.0	15	6	2.8	25	10	2.5
1.0+0.1	50	8	3.2	65	12	3.0
+0.2	69	9	3.5	75	11	4.5
+0.5	92	12	4.5	100	15	5.2
+1.0	25	10	4.0	36	6	2.5
2.0+0.1	45	7	3.7	47	4	2.8
+0.2	61	3	2.9	67	3	4.2
+0.5	40	5	2.1	53	3	3.5
+1.0	3	4	2.0	45	2	2.0
BAP+NAA						
0.5+0.1	45	2	2.0	55	2	4.2
+0.2	50	3	4.5	60	3	3.5
+0.5	57	4	4.2	65	5	4.2
+1.0	60	4	3.0	70	7	4.4
1.0+0.1	67	6	4.3	75	8	5.6
+0.2	75	4	5.5	85	4	3.5
+0.5	89	7	8.5	95	6	3.9
+1.0	40	3	2.5	50	3	2.0
2.0+0.1	35	5	2.3	40	3	2.4
+0.2	33	3	3.0	35	2	2.0
+0.5	25	2	2.0	33	2	2.5
+1.0	36	6	4.0	30	1	2.0

BAP = 6-Benzyl amino purine; Kn = Kinetin; IBA = Indole-3-butyric acid; NAA = α-naphthalene acetic acid; IAA = Indole-3-acetic acid

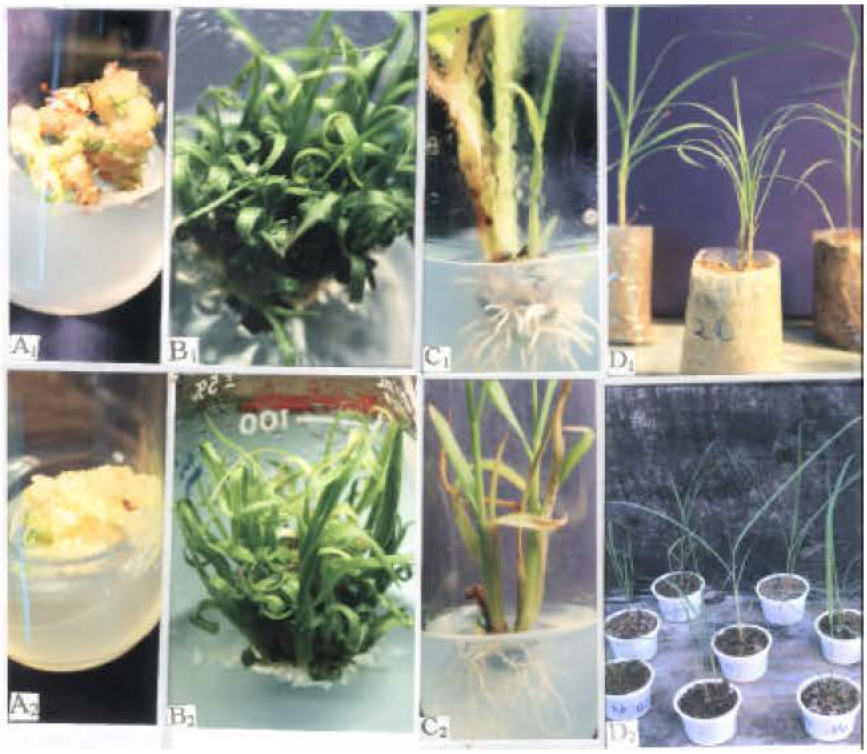

Fig.1 A_1-D_2: Regeneration of plantlets *in vitro* from leaf sheath explant of two sugarcane varieties
A_1-A_2: Callus induction from leaf sheath explant on MS+3.0 mg l^{-1} 2,4-D+10% CM of variety lsd-16 and lsd-28
B_1-B_2: Development of multiple shoots on MS medium containing 1.0 mg l^{-1} BAP+0.5mg l^{-1} IBA of variety lsd-16 and lsd-28
C_1-C_2: Root formation in regenerated shoots in ½ strength MS+3.0 mg l^{-1} NAA variety lsd-16 and lsd-28
D_1-D_2: Hardened plantlets established in the soil of variety lsd-16 and lsd-28

Table 3: Effect of the different auxins on formation of root on the *in vitro* grown micro-shoots cultured on MMS_2 medium

	Variety lsd- 28				Variety lsd -16			
Auxin supplements (mg l^{-1})	% of micro shoots rooted	No. of root per microshoots	Average length of roots (cm)	Days to emergence of roots	% of micro shoots rooted	No. of root per micro shoots	Average length of roots (cm)	Days to emergence of roots
IBA								
0.5	25	4	1.9	15-20	35	7	1.7	15-20
1	60	7	2.1	11-14	66	9	2.5	10-12
3	82	10	3.4	10-12	87	11	3.8	10-12
5	72	8	2.5	10-12	78	8	3.6	10-12
7	46	5	1.8	10-15	50	6	2.1	15-20
NAA								
0.5	20	7	0.9	12-15	40	9	1.0	10-12
1	65	7	1.5	10-12	65	10	1.5	15-18
3	85	11	4.0	9-12	97	16	5.0	9-12
5	79	8	3.4	10-12	82	8	4.1	10-13
7	55	5	2.0	10-15	57	7	3.9	10-15
IAA								
0.5	-	-	-	-	-	-	-	-
1	20	3	0.8	10-17	22	5	1.5	10-15
3	50	5	1.5	12-15	53	8	1.6	10-12
5	25	2	2.5	10-15	28	4	2.3	10-17
7	-	-	-	-	-	-	-	-
NAA + IBA								
0.5 + 0.5	-	-	-	-	-	-	-	-
+ 1.0	40	5	2.3	10-17	43	5	2.3	10-18
1.0 + 0.5	52	7	3.2	10-15	54	6	2.5	10-15
+ 1.0	60	7	2.5	10-12	65	8	3.8	10-12
3.0 + 0.5	75	10	3.5	12-14	79	11	3.6	11-15
+ 1.0	82	11	3.9	10-15	86	13	4.0	12-14
5.0 + 0.5	50	6	1.9	10-12	55	7	2.1	10-12
+ 1.0	60	7	1.4	15-17	66	7	2.5	10-15
7.0 + 0.5	48	5	1.2	10-17	52	6	1.6	10-17
+ 1.0	-	-	-	-	-	-	-	-

a half strength modified MS medium. The plantlets with well developed roots were successfully transplanted in soil and the percentage of survivability was 70 (Fig. 1 D_1, D_2).

It is hard to release a new variety of sugarcane by the conventional breeding for difficulting genetic behaviour of the cane. Moreover, it takes long time to release a stable variety. The tissue culture technique can play an important role in this regard. It is easy to create somaclonal variation in the tissues of sugarcane by *in vitro* callus culture. The plants, which are regenerated from the callus, are not true-to-true types for their chromosomal aberrations. Many new characters have been identified in these plants. If any character is found to be superior to mother plant, then the somaclone with this phenotype can be released as a new variety.

References

Barba, R.C., A.B. Zomora, A.K. Mallion and C.K. Linga, 1997. Sugarcane tissue culture research proc. ISSC T., 16: 1843-1864.

Begum, S., L. Hakim and M.A. Azam, 1995. Efficient regeneration of plants from leaf base callus in sugarcane. Plant Tissue Cult., 5:1-5.

Bhansali, R.R. and K. Singh, 1984. Callus and shoot formation from leaf of sugarcane in tissue culture. Phytomorphol., pp: 167-170.

Heinz, D.J. and G.W. Mee, 1969. Plant differentiation from callus tissue of *Saccharum* species. Crop Sci., 9: 346-348.

Heinz, D.J., M. Krisnamurti, L.G. Nickell and A. Maretzki, 1977. Cell tissue and organ culture in sugarcane improvement. In: Applied and Fundamental Aspects of Plant Cell and Organ Culture. (Eds. Reinert, J. and Y.P.S. Bajaj), Springer, Berlin, Heidelburg, New York.

Islam, A.S., H.A. Begum and M.M. Haque, 1982. Studies on regeneration of *Saccharum officinarum* for disease resistant varieties. Proc. Int. Cong. Plant Tissue and Cell Culture, 5:709-710.

Lal, N. and H.N. Sing, 1994. Rapid clonal multiplication of sugarcane through tissue culture: Plant Tissue Cult., 4:1-7.

Liu, M.C., 1984. Hand Book of Plant Cell Culture: Crop Species, 2:572-605. McGraw-Hill, New York.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and biossays with tobacco cultures. Physiol. Plant, 15: 473-497.

Nondan and Singh, 1994. Rapid clonal multiplication of sugarcane through tissue culture, Plant Tissue Cult., 4:1-7.

Nickell, L.G., 1964. Tissue culture of sugarcane, another research tool. Hawaiian Planters Res., 57: 223-229.

Nadar, H.M., S. Soepraptop, D.J. Heinz and S.L. Ldd, 1978. Fine structure of sugarcane (*Saccharum* spp.) callus and the role of auxin in embryogenesis. Crop Sci., 18:210-216.