Bangladesh J. Sugarcane, **21 : 89-92** *July, 1999*

Effect of IBA, Kn and GA_3 for *In Vitro* Clonal Propagation of Sugarcane

Sk. A. Mannan and M.N. Amin[1]
Bangladesh Sugarcane Research Institute,
Ishurdi, Pabna, Bangladesh.

Received on 10.2.98

ABSTRACT

For *in vitro* clonal propagation, shoot tips of the clones Isd 16 and I 327-86 were cultured on modified Murashige and Skoogs' medium supplemented with different concentrations and combinations of growth regulations. When the explants were cultured with only cytokinin failed to give satisfactory proliferation and elongation of axillary shoots. Similarly the explants were cultured with both cytokinin and auxin combinations showed elongation of some shoots but unusable in size. After, six weeks of culture the optimum elongation of axillary shoots with uniform characteristics were obtained on the medium supplement with 1.0 mg / litre Kn +1.0 mg / litre IBA + 0.5 mg / litre GA_3.

Key words : Clonal propagation, auxin and cytokinin, tissue culture of sugarcane.

INTRODUCTION

The potential benefit of tissue culture is that it offers a methodology through micropropagation to obtain quality propagule at a faster rate in short period of time. The new varieties of sugarcane can not be multiplied fast because of slow rate of multiplication by conventional means (Sreenivasan and Sreenivasan, 1993).

The conventional vegetative seed multiplication rate of 8-10 cycles usually require 10-12 years for commercial utilization of new sugarcane variety. Therefore, it is quite important that a fast seed multiplication technique is to be developed so that an improved variety can be multiplied rapidly and commercially exploited quickly. Clonal propagation through *in vitro* culture can enhance multiplication many folds (Sauvaire and Galzy, 1978; Hendre *et al.* 1983; Lee, 1987). Multiplication through micropropagation of sugarcane plant cane be done through axillary shoot proliferation, adventitous shoot formation and somatic embryogenesis.

Micropropagation using axillary shoot proliferation from shoot tip culture is the most desirable and safe as micropropagules to minimise genetics variation. For large scale production it involves the four stages viz. culture establishment, shoot multiplication, rooting and hardening of propagule.

Therefore, the present investigation was undertaken to develop optimum media for *in vitro* clonal propagation of plants from the explants of the clones Isd 16 and I 327-86.

MATERIALS AND METHODS

The source of explants, the clones, the Isd 16 and I 327-86 as they were grown in the experimental farm of Bangladesh Sugarcane Research Institute, Ishurdi, Pabna. The shoot tip or meristematic zones were used as experiment materials. The shoot tips were collected from the 3-6 months old plant grown in the field.

---

[1] Professor, Department of Botany, Rajshahi University

The first step of sterilization was the treatment of the plant material with savlon for 5-10 minutes followed by second washing with tap water to make the material free from savlon foam. The explant were then taken under laminar flow hood, rinsed with 70% ethanol and surface sterilized with 0.1 % $HgCl_2$ for different durations. The shoot tips (3-5 mm) were finally prepared for culturing of the explants.

The culture media described by Murashige and Skoog (1962) was supplemented with cytokinin, auxins and gibberellic acid in different concentrations and combinations were used for the growth and multiplication of axilliary shoot from the cultures of shoot tip / meristematic zone. Normally cultures were grown on media having 3% carbon source (Sucrose), 0.6% gelling agent (agar) and pH was set at 5.7 with 0.1N NaOH.

RESULTS AND DISCUSSION

Effect of cytokinin : Surface sterilized shoot tips were cultured on modified MS media supplement with different concentration of cytokinin at doses of 0.5, 1.0, 2.0 and 5.0 mg / litre (Table 1). After, 6 weeks of culture, media supplement with different doses followed to induce proliferation of axillary shoot from the explant. Treatment with cytokinin alone failed to induce proliferation of axillary shoot from the explant.

Table 1. Effect of cytokinin and auxin of direct regeneration from the shoot tip cultured on modified MS medium.

Homonal supplement (mg/litre)	lsd 16			I 327-86		
	Percent of explant responded	Usable shoot per explant	Length of usable shoot (cm)	Percent of explant responded	Usable shoot per explant	Length of usable shoot (cm)
BA						
0.5	54	-	-	50	-	-
1.0	58	-	-	60	-	-
2.0	50	-	-	46	-	-
5.0	40	-	-	36	-	-
Kn						
0.5	42	-	-	46	-	-
1.0	75	-	-	73	-	-
2.0	58	-	-	60	-	-
5.0	42	-	-	40	-	-
BA+IBA						
0.5+0.1	-	-	-	-	-	-
1.0+0.1	-	-	-	-	-	-
2.0+0.1	-	-	-	-	-	-
5.0+0.1	-	-	-	-	-	-
Kn+IBA	-	-	-	-	-	-
0.5+0.1	-	-	-	-	-	-
+0.5	-	-	-	-	-	-
+1.0	-	-	-	-	-	-
1.0+0.1	-	-	-	-	-	-
+0.5	22	1	0.40	28	1	0.45
+1.0	50	2	0.60	45	2	0.67
2.0+0.1	-	-	-	-	-	-
5.0+0.1	-	-	-	-	-	-

Here the response means elongation and /or enlargement of the explant.

Effect of cytokinin and auxin : Surface sterilized shoot tips were also cultured on medium supplement with cytokinins BA and Kn at concentrations of 0.5, 1.0, 2.0 and 5.0 mg / litre with the auxin, IBA at concentrations 0.1, 0.5 and 1.0 mg/ litre (Table 1). After, 6 weeks of culture, the axillary shoots that produced are very slow growing and fasciated to an abnormal size (Figure 1A). These shoots were unsuable for further multiplication studies.

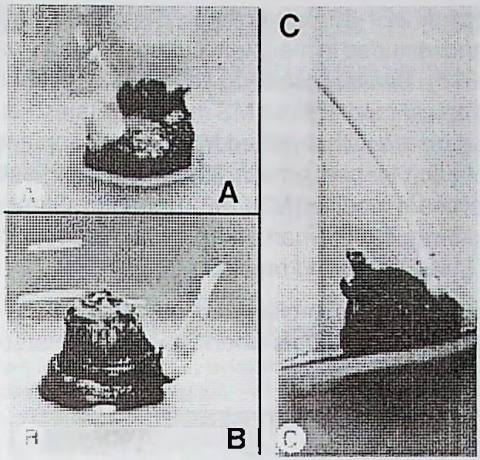

Figure 1. Effect of gibberellic acid with cytokinin plus auxin on shoot proliferation.
A. Six weeks old culture of Isd 16;
B. Six weeks old culture of I 327-86;
C. Eight weeks sub-culture of Isd 16.

Effect of gibberellic acid with cytokinin and auxin : From the foregoing experiments it is evident that no satisfactory response was obtained with the cytokinin and auxin treatments. For better regeneration, the explants were cultured on medium supplement with IBA, Kn and GA_3 (Table 2).

Table 2. Effect of gibberellic acid, IBA and Kn on direct regeneration from the shoot tip cultured.

Homonal Supplement mg/litre	Isd 16			I 327-86		
	Percent of explant responded	Usable shoot per explant	Length of usable shoot (cm)	Percent of explant responded	Usable shoot per explant	Length of usable shoot (cm)
Kn+IBA+GA_3	-	-	-	-	-	-
0.5+0.1+0.1	-	-	-	-	-	-
1.0+0.1+0.1	-	-	-	-	-	-
+.5	-	-	-	-	-	-
+1.0	-	-	-	-	-	-
+0.5+0.1	-	-	-	-	-	-
+0.5	20	2	-	1.4	20	1.0
+1.0	-	-	-	-	-	-
+1.0+0.1	-	-	-	-	-	-
+0.5	54	3-4	3.1	54	1-3	2.6
+1.0	27	2-3	2.0	53	1-2	1.3
2.0+0.1+0.1	-	-	-	-	-	-
5.0+0.1+0.1	-	-	-	-	-	-

Here the response means elongation and / or enlargement of the explant.

After, 6 weeks of culturing the best response was obtained on the medium supplement with 1.0, 1.0 and 0.5 mg / litre of Kn, IBA and GA_3, respectively. In this growth combination 54% explants of Isd 16 produced 3-4 shoot per explants; whereas 50% explants of I 327-86 produced 1-3 shoots per explants (Figure B,C). On average, length of the usable shoots were

3.1 and 2.6 cm in lsd 16 and I 327-86, respectively. Sugarcane apical meristem was used to obtain virus free plants and included in meristem culture procedures (Hyman and Mori, 1967; Mori and Hosokaman 1977). Similar result was reported by (Hendre *et al.* 1983 and Jimnez *et al.*, 1991) by culturing apical meristem on MS medium. Rajesh *et al.* (1994) studied *in vitro* clonal propagation of sugarcane with modified MS media supplemented with IAA, BAP and Kn having 0.5 mg/ litre of each for best growth. Nand Lal (1992) established shoot tip culture by putting sterilized shoot tips in modified MS (1962) medium. Other worksers like (Grishaman and Bourg, 1989 Lal and Sing, 1990; Dhamarkar, 1992) also reported that shoot tip produce plantlets *in vitro* on MS (1962) modified media.

However, the axillary shoots proliferated on the primary explants were found to produce any axillary or adventitous shoots when they were isolated from the primary explant and subcultured.

REFERENCES

Dhamarker, V.S. 1992. Molasses. A source of nutrient for *in vitro* sugarcane culture. *Sugarcane*, **4** :14-15.

Grishaman, M.P. and Bourg, D. 1989. Efficency of in vitro propagation of sugarcane plants by direct regeneration from leaf tissue and by shoot tip culture. *J. Amer. Soc. Sugar. Tech.*, **9** : 97-102.

Hendre, R.R.; Lyer, R.S.; Kotwal, M.; Khuspe, S.S. and Masearenhas, A.F. 1983. Rapid multiplication of sugarcane by tissue culture. *Sugarcane*, 1 : 5-8.

Hyman, E. and Mori, K. 1967. Obtain healty plants from mosaic infected sugarcane by means of tissue culture. *Ann Phytopathol. Soc. Japan*, **33** : 102.

Jimenez, G.E.; Perz, P.J.; Herrera, O.; Farrili, I. and Velazeo, O. 1991. in vitro microprogation in sugarcane (hybrid S sp.). *Breed. Abst.*, **61** (3) : 337.

Lee, T.G.S. 1987. Micropropagation of sugarcane (*Saccharum* sp.). *Plant Cell. Tissue and Organ Culture*, **10** : 47-55.

Lal, N. and Sing, H.N. 1990. Rapid clonal multiplication of sugarcane through tissue culture (manuscript submitted to Tropical Agriculture).

Mori, K. and Hosokaman, D. 1977. Localization of viruses in apical meristem and production of virus-free plants by means of meristem and tissue culture. *Acta. Hortic.* (The Hague), **78** : 389-396.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, **15** : 473-497.

Nand Lal. 1992. Assesment of IAA, IBA and NAA for *in vitro* rooting and plant growth in sugarcane. *Indian Sugar*, **42** : 205-208.

Rajesh, S.; Khan, A.Q. and Gang, G.K. 1994. *In vitro* clonal propagation of sugarcane : Optimization of media and hardening of plants. *Indian Sugar* , **44** (2) : 113-116.

Sauvaire, D. and Galzy, R. 1978. Multiplication of vegetative dela Cannal a sourse par bounturage *in vitro*. CRACad D Sci. Ser. 111, **287** : 467-470.

Sreenivasan, T.V. and Sreenivasan, J. 1993. Micropropagation of sugarcane varieties for increasing cane yield (personal communication).