Pak. J. Biotechnol. 10 (1) 21-25 (2013) ISSN 1812-1837

COMPARISON OF DIFFERENT DOSES OF PLANT GROWTH HORMONES ON CALLUS INDUCTION AND REGENERATION IN SUGARCANE

Simair A. Altaf**+, Mangrio G. Sughra*, Wain M. Shahbaz*, Thebo K. Nasreen***, Mangrio Sher M.*** and Dahot M. Umar**

*Department of Biotechnology, Sindh Agriculture University, Tandojam, Sindh, Pakistan. **Institute of Biotechnology & Genetic Engineering and ***Institute of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan.
Email+: altafsimair@hotmail.com

ABSTRACT

Sheath rolls of three sugarcane cultivars (BL-4, Gulabi-95 and NIA-2004) were grown for callus induction and subsequent *in vitro* plant regeneration on basic medium containing Murashige and Skoog (MS) supplementd with different concentrations of 2, 4-D for callus induction. Media comprised of MS+BAP and MS+NAA+Kin with various concentrations for shoot induction and root induction respectively. Maximum callus was obtained with MS+5.0 mg/L 2,4-D. Shoot induction was best at MS+2.0 mg/L BAP and best root induction was observed at MS+NAA at 2.0 mg/l + 1.25 mg/L Kinetin.

Key words: Sugarcane, Callus, 2,4-D, BAP, NAA, Shoot, roods

INTRODUCTION

Sugarcane *(Saccharum officinarum L.)* 2n=80 to 205 is one of six species of a tall tropical southeast Asian grass (Family Poaceae) having stout fibrous jointed stalks whose sap at one time was the primary source of sugar (Purseglove, 1979). Sugar cane is composed of six species of the genus *Saccharum,* in tribe *Andropogoneae*. All commercial canes grown today are inter-specific hybrids (Wrigley, 1982). Sugarcane accounts for approximately 70% of the world's sugar and is an economically important cash crop (Chatenet *et al.,* 2001). Sugarcane is an established agricultural crop and primarily grown as source of sugar due to the safe industrial uses for cheapest domestic products (Junkins, 1966). The tissue culture techniques can play an important role in the varietal improvement. Callus culture of sugarcane has been successfully established using shoot apices, young leaves and young inflorescence as explants (Bhansali and Singh, 1984, Khan et al., 2004). The purpose of this study was to compare different doses of growth regulators for their ability

to induce callus and regeneration in sugarcane.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory, Department of Biotechnology, Sindh Agriculture University, Tando Jam. Three varieties of sugarcane (*Saccharum officinarum*) viz. BL-4, Gulabi-95 and NIA-2004 were used for tissue culture. The surface sterilization of explants material comprised of leaf sheath rolls used for callus induction was carried out in 10% solution of Sodium Hypochlorite (NaOCl) for ten minutes. 2-3 drops of Tween-20 were added as wetting agent and for breaking surface tension. Then, the explants were rinsed three times with sterilized distilled water for five minutes in the laminar air-flow cabinet under aseptic conditions, for the removal of any traces of disinfectant. The explants were then, cultured on basic medium (Murasige and Skoog, 1962) supplemented with the auxin 2,4-D (2,4 dichloro-phenoxyacetic acid) at three levels (2.0, 3.0 and 5.0 mg/l) for callus induction. The cultures were, then, kept in the 16/8-hour photoperiod at 25±2ºC and a light intensity of 2000-2500 lux. Then the calli were transferred to shooting medium which comprised of MS + BAP (2.0, 2.5 and 3.0 mg/l) along with 0.8% Difco Bacto agar as gelling agent. After that young shootlets were transferred to rooting medium containing MS + NAA (1.0, 1.5 and 2.0 mg/l) + Kin (1.25 mg/l) with pH adjusted 5.7 and temperature was maintained at 28±2°C. The cultures were kept under complete darkness and data was recorded for regeneration of plant from callus and number of shoots on weekly basis.

RESULTS AND DISCUSSION

In the present study, sterilized sheath rolls of the innermost whorls of sugarcane, were cultured on the MS medium supplemented with different concentrations of 2,4-D. After about 2-3 weeks, callus initiated from the explants was yellowish brown in colour and results obtained at this stage are presented in Table-1.

The statistical analysis of variance for callus initiation of sugarcane showed that the varieties with different 2, 4-D concentrations and their interaction were highly significant at 1% probability level. It is clear from the results that an increase in 2, 4-D concentration inversely affected the number of days to callusing.

Table-1: Callus induction in 3 varieties under different concentrations of 2,4-D

M.S+ 2,4-D (mg/l)	Explants	Days taken to callus induction			% of regenerable callus			Callus diameter (cm)		
		BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004
2.0	100	15.00	17.00	17.00	80.00	72.00	62.00	3.00	2.90	2.80
3.0	100	14.00	16.00	17.00	84.00	78.00	70.00	3.10	3.00	2.90
5.0	100	13.00	15.00	16.00	88.00	82.00	84.00	3.30	3.20	3.00

Each value is an average of three parallel replicates.

The results revealed that BL-4 took minimum (13.00) days to initiate callus followed by Gulabi-95 (15.00) at 5 mg/l 2, 4-D. The observations of Khalida *et al.* (2003) are similar to the present study who reported that the number of days taken for callus initiation, was lowest with application of 2,4-D at 5 mg/l and highest with 1 mg/l 2,4-D.

Table-2: Shoot induction in the three varieties under different concentrations of BAP

M+S BAP (mg/l)	Cultures	Days taken to Shoot induction			Number of Shootlets per bottle			Length of Shootlets (cm)		
		BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004
2.0	100	12.00	14.00	13.00	4.00	3.80	3.60	3.96	3.43	3.48
2.5	100	13.00	15.00	16.00	3.80	3.20	2.60	3.57	3.41	3.28
3.0	100	14.00	16.00	17.00	3.50	2.40	2.20	3.43	3.27	3.18

Each value is an average of three parallel replicates.

The statistical analysis of variance for days to shoot induction, number of shootlets per bottle and length of shootlets of sugarcane showed that the varieties, with different BAP concentrations and their interaction were highly significant at 1% probability level. It is clear from the results that an increase in BAP concentration caused an increase in days taken to shoot induction, but decreased the number and length of shoots. Table-2 shows that the best rooting dose, hence, was 2.0 mg/l BAP at which BL-4 took minimum days for shoot induction (12.00) and also more number of shoots (4.00) along with longer shoots (3.96cm) followed by Gulabi-95 (14.00,3.80 and 3.43cm respectively). Mangrio *et al.,* (2005) also reported similar results for all these parameters.

The statistical analysis of variance for days to shoot induction, number of roots per shootlet and length of roots of sugarcane showed that the varieties, with different BAP concentrations and their interaction were highly significant at 1% probability level.

Table-3: Root induction in the three varieties under different concentrations of NAA+Kin

M.S NAA +Kin (mg/l)	Cultures	Days taken to Root induction			Number of Roots per shootlet			Length of Roots (cm)		
		BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004	BL-4	Gulabi -95	NIA-2004
1.0+1.25	100	6.00	7.00	8.00	5.80	4.47	2.80	1.17	1.03	1.10
1.5+1.25	100	7.00	8.00	8.00	5.40	4.40	3.40	1.20	1.00	0.90
2.0+1.25	100	8.00	9.00	7.00	4.80	3.60	4.80	0.90	0.80	0.70

Each value is an average of three parallel replicates.

It is clear from the results given in Table-3 that an increase in NAA + Kin concentration caused an increase in days taken to root induction and the number of roots, but decreased the length of roots. The best rooting dose, hence, was 2.0mg/l NAA + 1.25 mg/l Kin on which BL-4 took minimum days for root induction (6.00) and also more number of roots (5.80) along with longer roots (1.17cm) followed by Gulabi-95 (7.00,4.47 and 1.03cm respectively). Jadhav *et al.,* (2001) also observed similar results for root induction.

CONCLUSION

In the present study we have tried to optimize and improve the mass propagation protocol of these three varieties and studied their nutritional aspects of for callus culture and regeneration. Study revels that among tested varieties, BL-4 proved superior to the other two varieties in all callusing, shooting and rooting parameters. It was also concluded that 2, 4-D at 5.0 mg/l was better for callusing phase while BAP at 2.0mg/L was superior at the shooting phase and NAA at 2.0 mg/l + 1.25 mg/l Kin produced better results for the rooting phase.

REFERENCES

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

Jadhav, A.B., E.R. Vaidya, V.B. Aher and A.M. Pawar, *In vitro* multiplication of CO-86032 sugarcane (S. officinarum) hybrid. Indian J. Agri. Sci. 71: 113-115 (2001).

Jenkins,G.H., Introduction to cane sugar technology. Elsevier Publishing Co., New Yark. Pp. 478 (1966).

Kale, V. P., T. V. Bruno and S.V. Bhagade, Studies on callus initiation and plantlet regeneration in sugarcane (*Saccharum* spp.). Indian Journal of Genetics and Plant Breeding 64(2): 165-166 (2004).

Khalida, P., H. Makhdoom, M.T. Pervaiz, M.T.H. Shahid, J. Ahmed, S. Akhtar and M.K. Tanveer, Factors affecting callogenesis and plant differentiation in different genotypes of *Saccharum officinarum*. In: Proc. 4th workshop on R&D activities on sugar crops in Pakistan. October 6-7, at SSRI, Jhang, Pp.10-15 (2003).

Khan,I.A., A.Khatri, G.S.Nizamani,M. A. Siddiqui, M.H. Khanzada, N. A.Dahar,N.Seema and M.H.Naqvi, *In vitro* studies in sugarcane.*Pak. J. Biotech*. 1: 6-10 (2004).

Mangrio,G.S. and Z.Brohi, Regene ration of sugarcane plantlets via embryogenic callus. Indus Journal of Plant Sciences 4(2): 181-184 (2005).

Mohan, M.M. and S. M. Ibrahim, Callus induction from spindle leaf explants of sugarcane. Research on Crops 7(1): 301-303 (2006).

Murashige, T. and F. Skoog, Arevised medium for rapid growth and biossays with tobacco cultures. Physiology Plantarum 15: 473-497 (1962).

Purseglove,J. W., Tropical crops: monocotyledons. Longman Group Ltd., London, Pp.607 (1979).

Wrigly, G., Tropical agriculture: the development of production. Fourth Ed. Longman Inc. New Yark Pp. 496 (1982).