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In vitro Mass Propagation of Sugarcane (*Saccharum officinarum* L.) var. Isd 32 through Shoot tips and Folded Leaves Culture

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Abstract: Plant tissue culture is the most commercially successful aspect of plant biotechnology, which has introduced an exciting new phase into plant propagation and breeding. This paper presents a research finding that establish an efficient regeneration method for mass propagation of sugarcane var. Isd 32 using shoot tip and folded leaf as explants. In this study, *in vitro* shoot tip and folded leaf culture techniques were used for mass propagation and quality production of sugarcane plantlets. The explants of sugarcane var. Isd 32 was cultured on MS + 1.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA for shoot tip and MS + 0.5 mg L^{-1} Kin + 5.0 mg L^{-1} NAA for folded leaf. Culture after 30 days, about 90 and 80% of the shoot tip and folded leaf explants, respectively were successfully regenerated shoots. The average number and length of shoots from shoot tip explants was observed as 17.20±1.30 and 7.20±1.40 cm, respectively. Similarly, the average number and length of shoots from folded leaf explants was observed as 20.20±2.20 and 6.70±1.20 cm, respectively. Presence of callus and morphological variants were not observed during the passage of *in vitro* culture. There is no efficient regeneration technique that had been established for mass propagation of sugarcane var. Isd 32. The proposed technique can be used to enhance mass production of sugarcane crop economically especially with the present trend of demand of sugarcane in the region.

Key words: *In vitro* mass propagation, sugarcane, Isd 32, shoot tips, folded leaves

INTRODUCTION

Sugarcane is one of the important cash crops in Bangladesh. It is the main source of sugar. It is a perennial herb and belongs to the family Gramineae. It is mainly propagated vegetatively by three budded setts. This traditional practice can result high production cost and also of large amount of sugarcane involvement. Tissue culture materials could be an alternative to overcome those variabilities and to produce uniform propagules for the cultivation. Recently, a large number of identical clones by *in vitro* culture techniques were routinely used for a wide range of plant species (Biondi, 1986). In recent decades, *in vitro* shoot tip culture for mass propagation of sugarcane was reported by many authors (Hendre *et al*., 1983; Lee, 1987; Nand and Singh, 1994). *In vitro* regeneration through leaf sheath culture was developed (Samad and Begum, 2000) and plant regeneration through callus culture was also established (Nickell, 1977). But, the nutritional requirements for *in vitro* culture vary according to genotypes as well as explant used. It is documented that commercial propagation of sugarcane through setts is slow and pathogen keep on accumulating generation after generation, which reduces the yield and quality of sugarcane. Therefore, efficient regeneration method is needed for mass propagation of this crop, which provide uniform and disease free propagules for potential and quality production of sugarcane. Thus, the study was conducted to develop an efficient protocol for the large scale *in vitro* regeneration of an elite variety Isd. 32 using shoot tip and folded leave as explants.

MATERIALS AND METHODS

Shoots containing shoot tips and folded leaves of a mature plant of sugarcane var. Isd 32 were collected from the field of Atomic Energy Research Establishment, Savar Dhaka. Shoots were washed thoroughly using household detergent, Trix for 15 min under running tap water. Subsequent sterilization was carried out in the laminar air flow cabinet under aseptic conditions. Shoots were sterilized in 0.1% mercuric chloride for 5 min. Rinsing was done 3 times with sterile distilled water. The shoot tips

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and folded leaves (Murashige and Skoog, 1962) were exposed after excision and inoculated into MS media supplemented with different concentrations of BA and Kin alone or in combination with NAA for shoot induction. Subcultures were done 15 days interval to promote multiple shoots and healthy plantlets formation. Morphologically healthy shoots were excised individually and transferred to half strength of MS media supplemented with different concentrations of IBA, IAA and NAA for root induction. The sucrose concentration was used 30 g L^{-1} and the pH of the media adjusted to 5.8 prior to autoclaving. Cultures were incubated at 26±2°C with a 16 h illumination of 21.8 μmol cm^{-2} sec^{-1} provided by cool white fluorescent tubes. The experiment was conducted with 30 explants per media combination. Data were recorded 30 days after inoculation. Observations on cultures were carried out every alternate day. The study was conducted on February 2006 in the laboratory of Plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka. The variety was collected from the Sugarcane Research Institute, Ishurdi, Bangladesh and planted in the field of Atomic Energy Research Establishment, Savar, Dhaka.

RESULTS AND DISCUSSION

Enlargement, swelling, browning and unfolding of explants were observed culture after 7 days at all media combinations investigated. Cut ends of the folded leaf explants became swollen and started to produce tiny meristems after 15 to 20 days of culture. In shoot tip explants, auxiliary shoot proliferation occurred from nodes where leaf base adjacent to the stem. But adventitious shoot proliferation was observed from cut ends and mid ribs of folded leaf explants. Shoot proliferation from shoot tip and folded leaf explants differed according to concentrations of cytokinin alone or the combinations of cytokinin and auxin used (Table 1). Among the cytokinins used, BA was found to be the best for shoot proliferation from shoot tip explants and 1.5 mg L^{-1} BA was found most optimum, in which 70% explants produced shoots. The average number of shoot produced per explants was 8.30±0.60 and the average shoot length of 6.50±0.80 cm were observed in this medium. On the other hand, explants of folded leaf did not response any in such media of cytokinin alone (Table 1).

This is obvious from the result that single hormone has no effect for *in vitro* regeneration from folded leaf explants of sugarcane cultivar. Therefore, the study suggests that combination of cytokinin and auxin is necessary for *in vitro* proliferation of folded leaf explants in *Saccharum* sp. The superiority of BA over Kin was also found among the different combinations used in shoot tip explants. Among the combinations, 1.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA was found to be best, in which 90% of the shoot tip explants induced shoots. The number of shoot produced per explant was 17.20±1.30 and the

Table 1: Effects of BA, Kin, BA + NAA and Kin +NAA on the *in vitro* shoot induction of sugarcane var. Isd 32 using shoot tip and folded leaf as explants at 30 days

Hormone supplements in mg L^{-1} added to MS	Explants responded to from shoots (%)		Average number of shoot formed/explants Mean±SE		Average length of shoot/explants (cm) Mean±SE	
	From shoot tips	From folded leaves	From shoot tips	From folded leaves	From shoot tips	From folded leaves
BA						
0.5	-	-	-	-	-	-
1.0	20	-	5.00±0.20	-	4.20±0.40	-
1.5	70	-	8.30±0.60	-	6.50±0.80	-
2.0	35	-	8.00±0.30	-	4.20±0.40	-
2.5	10	-	2.00±0.50	-	3.20±0.40	-
Kn						
0.5	10	-	4.00±0.50	-	3.25±1.30	-
1.0	15	-	6.00±0.40	-	2.20±0.40	-
1.5	5	-	4.00±0.20	-	3.30±0.60	-
2.0	5	-	2.00±0.20	-	2.50±0.40	-
2.5	-	-	-	-	-	-
BA + **NAA**						
0.5 + 0.5	20	-	2.60±0.90	-	4.40±0.80	-
1.0 + 0.5	50	20	7.00±0.20	2.50±0.50	5.30±1.10	4.20±0.40
1.5 + 0.5	90	25	17.20±1.30	5.00±0.20	7.20±1.40	4.50±1.20
2.0 + 0.5	30	10	10.00±0.40	4.00±0.30	3.20±0.40	2.20±0.20
2.5 + 0.5	-	-	-	-	-	-
Kin + NAA						
0.5 + 4	-	50	-	18.00±0.50	-	6.50±2.10
0.5 + 5	-	80	-	20.20±2.20	-	6.70±1.20
0.5 + 6	-	70	-	15.00±0.70	-	7.10±2.40
0.5 + 7	-	60	-	10.00±0.30	-	5.20±0.90
0.5 + 8	-	40	-	10.00±0.20	-	4.20±1.30

- = no response from the culture, SE = Standard Error

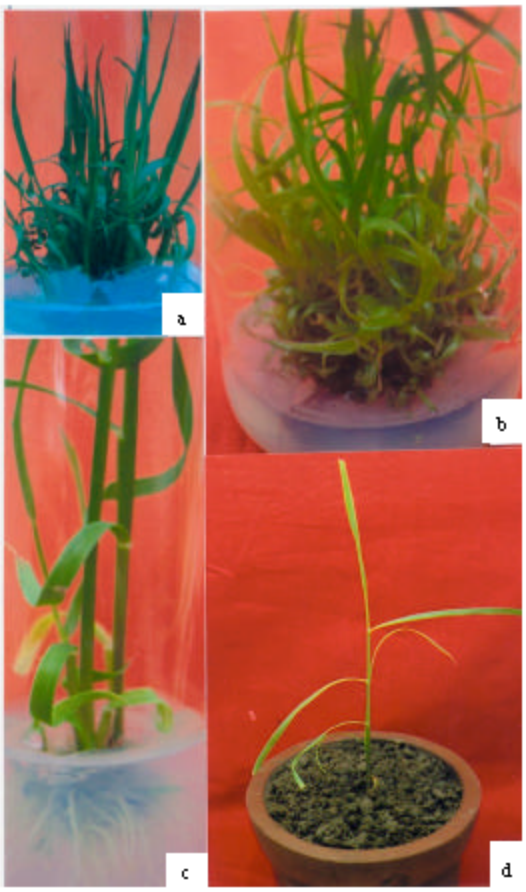

Fig. 1: *In vitro* plant regeneration through shoot tip and folded leaf culture (a) multiple shoot formation from shoot tip on MS + 1.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA (b) multiple shoot formation from folded leaf segment on MS + 0.5 mg L^{-1} Kin + 5.0 mg L^{-1} NAA (c) root induction from the shoots and (d) *in vitro* raised plant in earthen pot

average shoot length of 7.20±1.40 cm were observed in this medium (Fig. 1a). Positive effects of BA in regeneration of *Saccharum* sp. using meristem tissue as explants were reported by several authors (Paul *et al*., 1993; Tripathi *et al*., 2000). On the other hand, folded leaf explants was observed to be the best on the media combination of 0.5 mg L^{-1} Kin + 5.0 mg L^{-1} NAA, in which 80% explants induced shoots (Fig. 1b). The average number of shoot induced per explants was 20.10±2.50 and the average shoot length of 6.20±1.50 cm were observed in this medium. These results are in agreement with the previous study of sugarcane shoot tip culture in liquid modified medium of MS + 0.25 mg L^{-1} BA + 0.25 mg L^{-1} Kin as reported (Nand and Singh, 1994). These results are also similar to those obtained in auxiliary bud culture of sugarcane cultivars using the combinations of BA and Kin (Paul *et al*., 1993; Gupta and Bhatia, 2004; Sreenivasan and Sreenivasan, 1985). Good responses towards shoot formation and shoot multiplication from meristematic tissue of *Saccharum* sp. using the combination of BA and Kin was also reported (Hendre *et al*., 1983). In comparison to previous study, this study involved only 1 media combination for shoot development and multiplication. The study also involved to identify media combinations for maximum shoot proliferation with the different variety Isd 32. It is an evident in this study that folded leaf explants did not response for shooting in the media containing BA or Kin alone and shoot tip explants did not response in the media combination containing 3 mg L^{-1} or more than 3 mg L^{-1} NAA. These might be due to imbalance media composition used or due to the metabolism of toxic by-products such as polyphenol oxidases produced by the plant tissue. Tripathi *et al*. (2000) reported use of BA promoted callusing tendency of the explants of cv. Cose 95436, but this study did not show any callusing tendency of the explants during the

Table 2: Effects of IBA, IAA and NAA on half strength of MS media in root induction from *in vitro* raised shoots of sugarcane var. Isd 32 at 30 days

Different concentrations of IBA, IAA and NAA (mg L^{-1})	Shoot responded to form roots (%)	Average No. of root formed/shoot Mean±SE	Average root length/shoot (cm) Mean±SE
IBA			
0.5	10	8.00±0.20	2.00±0.20
1.0	25	8.00±0.40	5.00±1.40
1.5	30	10.00±0.60	3.00±0.80
2.0	20	10.00±1.20	3.25±0.45
2.5	15	7.00±0.90	3.00±0.60
IAA			
0.5	30	10.00±0.50	3.00±0.60
1.0	45	12.00±1.20	4.00±0.90
1.5	30	10.00±2.20	4.25±0.70
2.0	20	8.00±0.60	4.00±0.90
2.5	15	5.00±0.60	3.00±0.45
NAA			
0.5	40	10.00±0.40	4.00±0.60
1.0	45	10.00±0.60	4.25±0.90
1.5	50	12.00±1.10	5.00±1.20
2.0	65	15.00±1.30	7.00±2.20
2.5	90	18.40±2.10	7.45±1.25

SE = Standard Error

passage of *in vitro* culture. On the other hand, Sood *et al.* (2006) regenerated plants using IAA, BA and GA_3 in cv. CoJ 64. Therefore, difficulties of regeneration and non reproducibility of results might be due to the effect of genotypes. The rooting response differed according to concentration of different auxins used (Table 2). Among the auxins used, NAA was found to be the most responsive for root induction and 2.5 mg L^{-1} NAA was found most optimum, in which 90% shoots rooted within 15 days of culture. The average number of root induced per shoot was 18.40±2.10 and the average root length of 7.45±1.25 cm were observed in this medium (Fig. 1c). These observations were closely related to the previous study on root induction of Sugarcane cultivars (Nand and Singh, 1994; Paul *et al.*, 1993) and also in *Eucalyptus citriodora* (Gupta *et al.*, 1981) as using NAA as stimulant. The importance of NAA by successful induction of roots of sugarcane plants *in vitro* was also described (Anonymous, 1982; Sood *et al.*, 2006). The previous study also showed that the use of IBA or IAA was not very effective for sugarcane root induction *in vitro*. However, comparatively healthy rooted shoots were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *in vitro* rooted plantlets were then transferred to earthen pots (Fig. 1d) containing a mixture of soil and compost (2:1) and covered with transparent polyethylene lid to maintained high humidity. The polyethylene lids were removed after 7 days. The plantlets were kept in a shade and misted twice a day. About 90% of the plantlets were resumed new growth within 30 days and were transferred to the field. Out of 50 *in vitro* raised plants, 45 grew to out door environment. The protocol described in this study is reproducible and long term high frequency *in vitro* regeneration of Sugarcane var. Isd 32. Therefore, this protocol might be use for *in vitro* propagation of a wide range of sugarcane cultivars.

CONCLUSION

All the existing cultivars of sugarcane are susceptible to red rot disease that results deterioration in both yield of cane and sugar recovery. This phenomenon is occurred due to the use of traditional planting materials, sets. Adaptation of tissue culture method brought about substantial improvement in propagules free from diseases and grew vigorously, which contributes to a greater productivity. Micropropagation ensures true to type and rapid multiplication of disease free material and as such can be used as super elite seed for quick spread of new variety.

REFERENCES

Anonymous, 1982. Aseptic Bud Cultures for Safer International Exchange of Sugarcane Varieties. A Hand Book of Methods. West Indies Central Sugarcane Breeding Station, pp: 11.

Biondi, S., 1986. Practical application of *in vitro* propagation: Present situation and future prospects. Giorn. Bot. Ital., 120: 29-42.

Gupta, P.K., A.F. Mascarenhas and V. Jagannathan, 1981. Tissue culture of forest trees: Clonal multiplication of mature trees of *Eucalyptus citriodora* Hook. by tissue culture. Plant Sci. Lett., 20: 195-200.

Gupta, P.K. and A. Bhatia, 2004. Induction of efficient and reproducible plant regeneration from tissue cultures of some Indian popular sugarcane varieties. In: 5th International Plant Tissue Cult. and Biotch. Conf. 4-6 December, Dhaka, Bangladesh. Organized by BAPTC and B., pp: 17.

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

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

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

Nand, L. and H.N. Singh, 1994. Rapid clonal multiplication of sugarcane through tissue culture. Plant Tissue Cult., 4(1): 1-7.

Nickell, L.G., 1977. Crop improvement in sugarcane: Studies using *in vitro* method. Crop Sci., pp: 714-719.

Paul, W.J., Taylor and D. Snezana, 1993. Development of an *in vitro* culture technique for conservation of *Saccharum* sp. hybrid germplasm. Plant Cell Tissue Org. Cult., 34: 217-222.

Samad, M.A. and S. Begum, 2000. Somaclonal variation of nonirradiated and irradiated calli of sugarcane (*Saccharum officinarum* L.). Plant Tissue Cult., 10(1): 25-29.

Sood, N., P.K. Gupta, R.K. Srivastava and S.S. Gosal, 2006. Comparative studies on field performance of micropropagated and conventionally propagated sugarcane plants. Plant Tissue Cult. Biotech., 16(1): 25-29.

Sreenivasan, T.V. and J. Sreenivasan, 1985. *In vitro* sugarcane germplasm storage. Sugarcane, pp: 1-2.

Tripathi, J.P., S.K. Singh and S.B. Singh, 2000. High frequency plantlet regeneration through callus for improvement in sugarcane variety CoSe 95436. Plant Tissue Cult., 10(1): 89-92.