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In vitro Shoot Tip Culture of Sugar-cane (*Saccharum officinarum*) Variety Isd 28

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Abstract: Multiple shoots were obtained from shoot tip explant of sugar-cane (*Saccharum officinarum)* cultured on MS medium supplemented with BAP (0.5-2.0 mg l^{-1}), Kn (0.1-0.5 mg l^{-1}) and IBA (0.1-0.5 mg l^{-1}). Roots were induced in *in vitro* regenerated shoots on half MS medium supplemented with 5.0 mg l^{-1} NAA, IBA and IAA. Plant regeneration from shoot tip was the highest on MS medium supplemented with BAP 2.0 mg l^{-1} and IBA 0.5 mg l^{-1}. The reported experimental findings present a method of plant regeneration of sugar-cane variety Isd 28 through shoot tip culture. The plantlets were successfully transferred to soil and the percentage of survivability under *ex vitro* condition was 70.

Key words: Sugar-cane, shoot tip, regeneration, *in vitro*, micro propagation

Introduction

Sugar-cane is a member of the genus *Saccharum* from family Gramineae. Varieties of sugar-cane are highly heterogeneous and generally multiplied vegetatively by stem cutting. Time required and continuous contamination by systemic diseases are the serious problems to multiply an elite genotype of sugar-cane in the open field (Nand and Singh, 1994). Tissue culture of sugar-cane has received considerable research attention because of its economic importance as a cash crop. Plant regeneration through tissue culture technique would be a viable alternative for improving the quality and production of sugar-cane. Initial attempts to regenerate plants through *in vitro* technique were made on sugar-cane by Nickell (1964) and Heinz and Mee (1969). Protocols for *in vitro* plant regeneration of sugar-cane through callus culture, axillary bud and shoot tip culture have been developed by many authors (Barba *et al*., 1978; Sauvaire and Glazy, 1978; Heinz *et al*., 1977; Lee, 1987). Induction of callus and regeneration of plants using sugar-cane varieties of Bangladesh were reported elsewhere (Islam *et al*., 1982; Hossain *et al*., 1993; Karim *et al*., 2002). However, reports are scarce on shoot tip culture in sugar-cane varieties of Bangladesh. Multiplication and germplasm preservation of sugar-cane is possible and for this purpose shoot tip has a greater potentiality. Therefore, the present investigation has been undertaken to establish plant regeneration protocol through shoot tip culture in sugar-cane using variety Isd 28.

Materials and Methods

The experiment was conducted at Biotechnology Laboratory in Bangladesh Sugar-cane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during the period of 2000-2001. The plants of sugar-cane variety Isd 28 developed by BSRI, Bangladesh were used as experimental material. Shoot tips were collected from juvenile sugar-cane plants (3-4 months age) and were used as explants. Sterilization of explants was carried out using 0.1% $HgCl_2$ after washing thoroughly under tap water for 7-10 min. Subsequently the explants were washed gently with sterile DDH_2O (double distilled water) in aseptic condition under laminar flow hood. Shoot tips of 2-4 mm were excised and placed on MS (Murashige and Skoog, 1962) medium supplemented with different combinations of auxin and cytokinin to identify the appropriate media combinations for regeneration of sugar-cane through shoot tip culture. Media were consisted of 3% sucrose, 0.6% agar, pH was adjusted to 5.7 before addition of agar and autoclaved at 120°C for 15 min. Explants were incubated at 25±2°C under 16 h photoperiod regime. The experiments were replicated two times and the means and standard errors of the results were calculated.

Results and Discussion

In experiment reported here, different concentrations and combinations of auxin and cytokinin were used in MS medium for multiple shoot regeneration from shoot tip of sugar-cane. Yutaka *et al.* (1998) reported that combinations of phytohormones often determine the course of morphogenesis e.g. shoot organogenesis or embryogenesis. For multiple shoot regeneration, shoot tips were remarkably influenced by types and concentrations of the auxins and cytokinins used. The cytokinin BAP was more effective than Kn and IBA for shoot formation. Low auxin and high cytokinin supplementation in medium favoured the induction of multiple shoot regeneration. Various combinations of BAP with IBA or Kn were tried. The maximum response for multiple shoot initiation were found when explants were cultured on MS medium supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} IBA, 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} IBA and 1.0 mg l^{-1} + 0.5 mg l^{-1} Kn. On these media 70-75% explants produced 2-6 shoots from a single shoot tips within 2-3 weeks (Fig. 1A-C). Skirvin (1984) has revealed the effects of different hormone combinations on proliferation and elongation. Number of explants cultured and their responses to shoot regeneration and growth were recorded (Table 1). The highest shoot length was 4.5±0.01 cm followed by 4±0.35 and 4±0.21 cm. The regenerated shoots were multiplied manifolds when they were sub-cultured in the same medium within three weeks.

The regenerated shoots were devoid of roots. So, for root induction the shoots were excised separately and placed on rooting media. The same concentration (5 mg l^{-1}) of IAA, NAA or IBA were used alone in half MS medium for healthy and profuse root induction. Rooting of shoots may be affected by pH, auxin level and nutrient concentration of the root induction media (Dannis and James, 1993). Best response was observed when 5 mg l^{-1} of NAA was used in half MS medium (Table 2 and Fig. 1D). Root developed in IAA or IBA containing medium was poor in quality. These results agree well with the previous findings of Nadar and Heinz (1977), who reported that preferred auxin

Table 1: Effects of auxin and cytokinin in MS medium on shoot regeneration from shoot tips of Sugar-cane variety Isd 28. There were 18-20 explants for each treatment and data (X ± SE) were recorded after 2-3 weeks of culture

Hormonal concentrations (mg l^{-1})	(%)	No. of explant Inoculated	No. of explant regenerated	Average No. of initial shoot	Days to shoot	Average length of initiation	Shoot regeneration shoots (cm)
BAP	0.5	20	10	1*±0.2**	12	3.0*±0.01**	50
	1.0	20	12	1±0.4	12	3.0±0.15	60
	2.0	20	10	1±0.5	12	3.5±0.13	50
BAP+Kn	0.5+0.1	20	12	2±0.2	12	2.0±0.15	60
	0.5+0.2	20	12	2±0.4	12	3.5±0.15	60
	0.5+0.5	20	11	3±0.5	12	3.5±0.11	60
	1.0+0.1	20	11	3±0.5	12	3.5±0.23	55
	1.0+0.2	18	12	3±0.2	13	3.5±0.21	66
	1.0+0.5	18	13	4±0.4	10	4.0±0.15	72
	2.0+0.1	20	12	3±0.5	12	3.0±0.31	60
	2.0+0.2	20	12	3±0.2	12	3.5±0.12	60
	2.0+0.5	20	13	5±0.7	10	4.0±0.12	65
BAP+IBA	0.5+0.1	20	11	2±0.2	12	4.0±0.12	55
	0.5+0.2	18	10	2±0.5	11	4.0±0.16	55
	0.5+0.5	18	10	3±0.3	12	3.5±0.12	55
	1.0+0.1	20	12	3±0.7	12	4.0±0.15	60
	1.0+0.2	20	11	3±0.5	12	3.5±0.11	55
	1.0+0.5	20	14	5±0.2	10	4.0±0.21	70
	2.0+0.1	20	12	3±0.2	12	3.5±0.12	60
	2.0+0.2	20	11	3±0.4	11	4.0±0.35	55
	2.0+0.5	20	15	5±0.5	10	4.5±0.01	75

6- Benzylaminopurine (BAP), Kinetin (Kn), Indole-3- butyric acid (IBA) *Mean value.**Standard error.

Table 2: Effects of different levels of IBA, NAA and IAA on root formation in Sugar-cane variety Isd 28. There were 18-20 explants for each treatment and data (X ± SE) were recorded after 2-3 weeks of culture

Treatments	Average number of roots/shoot	Average length of root (cm)	Days to root initiation	Percentage of shoot rooted
½ MS +5.0 mg l^{-1} IBA	12*±0.2**	3*±0.1**	10	80
½ MS +5.0 mg l^{-1} NAA	15±0.5	4±0.5	7	85
½ MS +5.0 mg l^{-1} IAA	8±0.3	1±0.5	10	70

Indole-3- acetic acid (IAA),1-naphthaleneacetic acid (NAA), Indole-3- butyric acid(IBA)
*Mean Value.**Standard Error.

for root initiation was NAA. The proper stage of root development was another criterion for selecting plantlets to be transferred to the soil. *In vitro* regenerated plantlets were transferred to small pots containing mixture of soil and sand (2:1) for future establishment (Fig. 1E). In general it has been reported that plants regenerated from meristem (shoot tips) are very similar both phenotypically and genotypically to the mother plants (Amato, 1977). Grisham and Bourg (1989) compared two

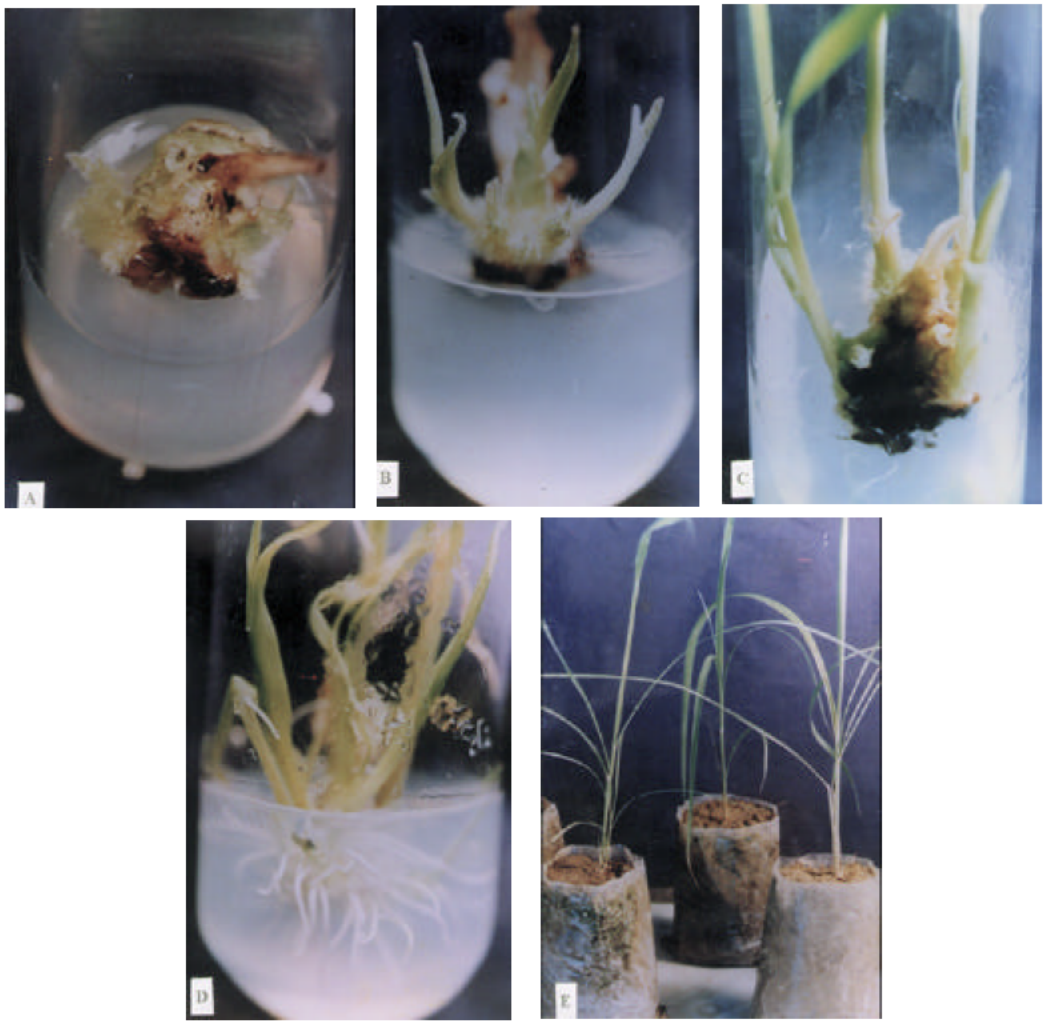

Fig. 1(A-E): Different stages of *in vitro* derived regenerated plantlets from shoot tip culture of sugar-cane.

A: Initiation of axillary shoots on MS medium with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} Kn.

B-C: Development and multiple shoot formation on MS medium with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} IBA after two-three weeks of culture.

D: Root formation on half strength MS medium with 5.0 mg l^{-1} NAA after four weeks of culture.

E: *In vitro* raised plantlets into soil in polythene bags.

micro propagation methods for the cultivars CP65-357 and CP70-321 and found that shoot tip culture was better than leaf roll culture for plant production. The rapid regeneration and germplasm preservation of elite sugar-cane variety is possible and for this purpose shoot tip has greater potential towards the multiple shoot regeneration. Present report showed that among the media, MS medium containing 2.0 mg l^{-1} BAP+0.5 mg l^{-1} IBA, 1.0 mg l^{-1} BAP+ 0.5 mg l^{-1} IBA and 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} Kn was more effective for multiple shoot formation. Furthermore, it has been demonstrated that for shoot regeneration the combination of auxin and cytokinin was essential. Sugar-cane variety Isd 28 displayed an excellent regeneration capacity. This study of micro propagation has given a rapid technology compared with conventional technique for multiplication and germplasm preservation of elite sugar-cane varieties. Therefore, shoot tip culture offers a definite scope for further improvement of this well adapted genotype through gene manipulation using other biotechnological techniques.

References

Amato, D., 1977. Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Reinert, J. and Bajaj, Y.P.S. (Eds.). Springer-Verlag, Berlin, pp: 343-357.

Barba, R.C., A.B. Zamora., A.K. Malion and C.K. Linga, 1978. Sugar-cane tissue culture research. Proc. Int. Soc. Sugar-cane Technol., 16 :1843-1863.

Dannis, P.S. and F.H. James, 1993. Growth of rooted "Galla" apple micro cutting *in vitro* as influenced by initial adventitious shoot count. Hort. Sci., 18: 664-666.

Grisham, M.P. and D. Bourg, 1989. Efficiency of in vitro propagation of sugar-cane plants by direct regeneration from leaf tissue and by shoot tip culture. J. American Soc. Sugar-cane Technologists. No. 6 pp: 97-102.

Heinz, D.J. and W.P. Mee, 1969. Differentiation from callus tissue of Saccharum species. Crop Sci., 9: 346-348.

Heinz, D.J., M. Krishnamurti, L.G. Nickell and A. Maretzki, 1977. Cell tissue and organ culture in sugar-cane improvement. In: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. (Eds. Reinert J. and Bajaj Y.P.S.), Springer, Berlin, Heidelburg, New York, pp: 3-17.

Hossain, M.A., S. Begum, M.A.S. Miah, M.J. Uddin, and A.J. Miah, 1993. Induction of callus and regeneration of plants in Sugar-cane (Abstract). Proceed. Int. Conf. Plant Tissue Culture. December 19-21, Dhaka, Bangladesh, pp : 41.

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

Karim, M. Z., R. Alam, R. Baksha, S. K. Paul, M. A. Hossain and A. B. M. M. Rahman, 2002. *In vitro* clonal propagation of sugar-cane (*Saccharum officinarum*) variety Isd 31. Pak. J. Biol. Sci., 5: 659-661.

Lee, T.S.G., 1987. Micro propagation of sugar-cane (*Saccharum* spp.). Plant Cell, Tissue Organ Cult., 10: 47-55.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Pl. Physiol., 9: 473-497.

Nadar, H.M. and D.J. Heinz, 1977. Root and shoot development from sugar-cane callus tissue. Crop Sci., 17: 814-816.

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

Nickell, L.G., 1964. Tissue and Cell Culture of sugar-cane: an other research tool. Hawaii Planters Records., 57: 223-229.

Skirvin, R.M., 1984. Stone fruits. In: Handbook of Plant Cell Cultures. Ammirato P.V., Evans D.A., Sharp W.R. and Yamado Y., (Eds.). Macaillan Pub. Co., New York, pp: 402-452.

Sauvaire, D. and R. Glazy, 1978. Multiplication vegetative de canne a Sucre (*Saccharum* sp.) par bouturage *in vitro*. CR Acad Sc. Paris, Seri D: 467-470.

Yutaka, T., Y. Tomohiro., M. Toshikazu., and O. Takeshi, 1998. Plant regeneration via shoot organogenesis from cotyledons in two wild *Cucumis* species, *C. figarei* and *C. metuliferus*. JARQ., 32: 281-286.