

IN VITRO REGENERATION OF PROMISING SUGARCANE VARIETIES OF BANGLADESH**S.M.M. Rahman^a, M.A. Mamun^a, M.Z. Karim^b and A.B.M.M. Rahman^b**^a Biotechnology Discipline Khulna University, Khulna-9208, Bangladesh^b Bangladesh Sugarcane Research Institute, Ishurdi-5205, Bangladesh

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Abstract: *In vitro* regeneration of sugarcane varieties viz. Isd-20, Isd-30 and Isd-2/54 has been studied. Nutrient media containing growth regulators enhanced callus induction, shoot differentiation and root formation *in vitro*. All the three varieties cultured fortified with 3.0 mg/l 2,4-D, showed the best performance in the callus induction on modified MS media. In shoot induction, effectiveness of cytokinin (BA 1.5 mg/l) along with either 0.25 mg/l NAA or IBA was proved to be superior to other concentrations. Microshoots were rooted on MS and modified MS media. The striking number of roots was observed on modified MS medium supplemented with NAA 0.5 mg/l + 0.5 mg/l IBA and IBA 0.5 mg/l + IAA 0.5 mg/l. Eighty to ninety per cent regenerated plantlets were viable with 85% relative humidity while transferred to sterilized soil.

Key words: Sugarcane, Surface sterilization, Auxins, Cytokinin and Callus

Introduction

The sugarcane is a major industrial crop in the tropical and subtropical regions of the world. It belongs to the genus *Saccharum* in the family Poaceae (Kochhar, 1998). The genus contains three cultivated (*S. officinarum* L., *S. sinensis* and *S. barberi* L.) and two wild (*S. robustum* L. and *S. spontanium*) species (Kochhar, 1998). Chromosome numbers most commonly found are *S. officinarum* (2n=80), *S. barberi* (2n=82 to 124), *S. sinensis* (2n=116 to 118), *S. spontaneum* (2n=40 to 124) and *S. robustum* (2n=80) (Dutt and Rao, 1935; Li *et al.*, 1948, 1954).

Conventional method generally needs 10 to 15 years of breeding work to complete a selection cycle and an improved variety can be planted commercially several years later when seedcane will have been produced. In Bangladesh, farmers use three budded sets for sugarcane cultivation. It needs 7-10 tons of cane stalks per hectare as seed material for plantation. Nationally about 10 lakh tons of cane are being used every year as seed material (Miah *et al.*, 1996). It is a high figure for a poor country. Besides due to low ambient temperature during winter months as well as effect of apical dominance, satisfactory germination is not achieved in three budded sets planted in the trench. Moreover, due to flood, farmers need to wait for "Jow condition" (optimum soil moisture condition for planting). Delayed sowing may reduce the production of sugar during maturation as because inversion of sucrose by an enzyme, invertase, which reside in green tops as well as cane stalks (Miah *et al.*, 1996). If the plantlets are produced *in vitro* as commercial basis, farmers can transplant them immediately after receding the floodwater. On the other hand, sets use for sowing can be sent to the sugar industry for sugar production.

Infection by systemic diseases is serious problem to multiply the elite genotype of sugarcane in the open field (Nandon and Singh, 1994). The techniques of plant tissue culture are being routinely used for producing large number of clonal plants and have become a viable alternative to the conventional clonal propagation. Cell and tissue culture techniques also may produce mutant and thus has spectacular impact on agriculture (Pierik, 1987).

Therefore, the present experiment was conducted to investigate the following objectives: determination of the most suitable medium for maximum callusing; selection of suitable combination and optimization of the concentration of growth regulator(s) for shoot proliferation and subsequent rooting.

Materials and Methods

Plant Materials: The experiment was conducted at Biotechnology Laboratory of Bangladesh Sugarcane Research Institute, Ishurdi, Pabna. The young leaf-sheath of three sugarcane varieties viz. Isd-20, Isd-30 and Isd-2/54 (Table 1) was used as experimental material. The explants were collected from two months old field grown setting and were treated with the following concentrations of HgCl₂-0.01%, 0.025%, 0.05%, 0.1%, 0.25% and 0.5% for surface sterilization

* Corresponding Author: Tel.: 88-041-721791, 720171; Fax: 88-041-731244 e-mail: ku@bdonline.com.

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Preparation of culture media: MS (Murashige and Skoog, 1962) medium or modified MS medium supplemented with mesoinositol and coconut milk containing different concentrations of 2,4-D were prepared for callus induction.

Table 1. Important morphological characters of the experimental materials (Khan and Podder, 1982).

Variety	Parentage	Leaf			Cane			
		Lamina	Sheath	Arrangement	General	Color	Internodes	Buds
Isd-20	Co. 1148 X I1354/77	Light green, medium length, leaf edges serrated	Green with purple blotches in the older leaves but younger ones are full with wax deposit	Medium in Number, Alternatively Arranged	Medium thick round ring hadr pipe present	Greenish yellow with yellow color near bud	Cylindrical internodes, bud groove prominent narrow growth ring	Medium to large triangular greenish color, prominent germ pore
Isd-30	I-28-76 X I1147-77	Light green, medium length, medium width	Greenish with few purple blotches	Profuse, Closely Arranged	Medium thick, straight round in cross section, rind, hard pipe present	Greenish yellow with yellowish color	Cylindrical internodes, straight. Splits absent, leaf scar prominent	Small site, ovate shape and upper end of ends touches the growth ring
Isd-2/54	Co. 644 X Co. 381	Light green, narrow, and medium length	Light green to purplish blotches, whitish bloom, leaves partially dry	Scantly, Sparsely Arranged	Medium canes, round in cross section, internal tissue whitish yellow ring,	Yellowish green with yellowish area near the bud	Clendrical with slight bulring above the growth ring opposite the growth bud	Medium size and plump, ovate in shape, cushion well developed and upper end of the bud crosses the growth ring

Co: Coimbatore, India; I: Ishurdi, Bangladesh.

For shoot and root differentiation, MS media supplemented with single or combination of cytokinin (BA) and auxin (NAA, IBA) containing coconut milk were used. Half strength MS media containing auxin (NAA, IBA and IAA) and with or without activated charcoal were prepared for root induction.

Data analysis: Data were recorded after 4 to 6 weeks for each culture. Data were analyzed and standard error was calculated according to Mian and Mian (1984).

Results and Discussion

Surface sterilization and culture establishment: Regeneration of plants following callus formation was obtained from three commercial sugarcane cultivars. Data on percentage of aseptic culture were recorded after 10 days of culture. Eighty percent contamination free explants remain green when treated with 0.10% HgCl₂ 10 min. High concentration (0.25% and 0.50%) of HgCl₂ treatment obtained cent percent contamination free, but explants turned brown and failed to show any response probably due to toxic effect of HgCl₂. On the other hand low concentrations and short duration of treatment were not suitable for surface sterilization (Data not shown). In this study, surface sterilization with 0.10% HgCl₂ for 10 min found suitable for aseptic culture for leaf-sheath explant.

Effect of auxin on callus induction: Among the treatments tested, the modified MS medium supplemented with 3.0 mg/l 2,4-D and 10% coconut water produced maximum amount of creamy white callus for Isd-20 and Isd-30 and greenish white callus for Isd-2/54. The calli were sub-cultured on the freshly prepared media of similar composition. At this time, callus tissue secreted phenolic compounds and the media turned brown within three to four days, therefore, this was minimized by transferring the callus to freshly prepared medium. Relyable percent explant obtained callus on MS medium supplemented with 3.0 mg/l and 4.5 mg/l 2,4-D respectively (Table 2).

A combination of the auxin (2,4-D) and coconut water was highly effective in callus formation. Coconut water contains a compound that is analogous to kinetin. Letham (1974) showed that mature coconuts contain 9- β -D-riboflurasylyzeatin (a cytokinin) and in principle, cytokinin can be used in the place of coconut milk. After 4-5 weeks the calli were transferred onto MS media for shoot differentiation

Table 2. Effect of 2,4-D on MS Basal and modified media on callus induction.

Concentration of 2,4-D (mg/l)	Percentage of explant with callus induction					
	MS Basal medium			Modified MS medium		
	Isd-20	Isd-30	Isd-2/54	Isd-20	Isd-30	Isd-2/54
0.5	-	12	-	-	-	-
1.0	20	22	-	21	16	23
2.5	47	34	40	33	33	37
3.0	45	67	72	90	94	90
4.5	67	59	70	85	78	80

Shoot differentiation: MS medium supplemented with different concentrations and combinations of growth regulators were tested. Among them BA 1.5 + NAA 0.25 mg/l and BA 1.5 +IBA 0.25 mg/l produced the highest number of usable shoots (8 fold for both Isd-20 and Isd-30 and 5 fold for Isd-2/54). But the highest percentage with less number of usable shoots were obtained at the concentration of 0.50 mg/l for both NAA and IBA along with 1.5 mg/l BA. When the auxin concentration was high, a large volume of callus was obtained even in presence of high concentration of cytokinin. A suitable range of auxin was found 0.1 to 0.5 mg/l in presence of moderately high concentration (0.5-1.5 mg/l) of cytokinin. This results support the statement of Pierik (1987) that adventitious shoot can appear in callus tissue in presence of high cytokinin and low auxin concentration.

Table 3. Combined effect of auxin and cytokinin on shoot differentiation and proliferation.

Conc. of growth regulators	Isd-20			Isd-30			Isd-2/54		
	% explant produced shoot	No. of shoot/explant (cm±sd)	Average length of shoot (cm±sd)	% explant produced shoot	No. shoot/explant (cm±sd)	Average length of shoot (cm±sd)	% explant produced shoot	No. of shoot/explant (cm±sd)	Average length of shoot (cm±sd)
BA+NAA									
0.5+0.10	27	2.5±0.5	3.3±0.1	45	2.5±0.8	3.5±0.4	58	3.0±0.3	4.4±0.3
0.5+0.25	45	3.8±0.4	3.3±0.2	60	3.1±0.1	3.4±0.5	50	3.0±0.3	3.2±0.2
0.5+0.50	50	3.5±0.4	2.8±0.3	38	3.2±0.2	3.3±0.3	20	3.2±0.5	3.1±0.3
1.5+0.10	70	3.5±0.5	3.2±0.5	20	3.3±0.3	3.2±0.5	20	3.8±0.5	3.8±0.3
1.5+0.25	52	8.5±0.4	4.2±0.4	75	8.0±0.3	3.1±0.5	80	5.0±0.3	5.5±0.1
1.5+0.50	100	6.5±0.3	3.8±0.5	95	6.0±0.4	3.7±0.3	100	5.0±0.4	2.5±0.4
2.5+0.10	50	4.7±0.1	3.8±0.4	60	3.0±0.5	3.1±0.5	45	4.1±0.3	2.8±0.5
2.5+0.25	58	5.5±0.2	3.2±0.5	55	3.0±0.4	3.2±0.5	60	3.2±0.3	4.2±0.2
2.5+0.50	45	5.8±0.5	3.9±0.3	48	3.2±0.2	3.1±0.4	28	2.8±0.5	3.5±0.1
BA-IBA									
0.5+0.10	40	2.5±0.8	3.5±0.4	45	2.5±0.5	4.4±0.3	48	3.0±0.3	3.3±0.1
0.5+0.25	60	3.1±0.1	3.4±0.5	65	3.8±0.4	3.2±0.2	63	3.0±0.3	3.3±0.2
0.5+0.50	25	3.2±0.2	3.3±0.3	38	3.5±0.4	3.1±0.3	39	3.2±0.5	2.8±0.3
1.5+0.10	20	3.3±0.3	3.2±0.5	25	3.5±0.5	3.8±0.3	25	3.8±0.5	3.2±0.5
1.5+0.25	50	8.0±0.3	3.1±0.5	60	8.5±0.4	5.5±0.1	68	5.0±0.3	4.2±0.4
1.5+0.50	70	6.0±0.4	3.7±0.3	80	6.5±0.3	2.5±0.4	79	5.0±0.4	3.8±0.5
2.5+0.10	52	3.0±0.5	3.1±0.5	51	4.7±0.1	2.8±0.5	53	4.1±0.3	3.8±0.4
2.5+0.25	30	3.0±0.4	3.2±0.5	38	5.5±0.2	4.2±0.2	36	3.2±0.3	3.2±0.5
2.5+0.50	48	3.2±0.2	3.1±0.4	42	5.8±0.5	3.5±0.1	46	2.8±0.5	3.9±0.3

Rooting of the shoots: Combinations of auxin (NAA, IBA and IAA) ranging from 1.0 to 1.5 mg/l (Table 4). High concentration of auxin produced large volume of callus in spite of root induction. Both the combinations were found suitable at moderately low concentration. The highest percentage of root formation for the three varieties was 85% (Isd-20), 90% (Isd-30) and 87% (Isd-2/54) respectively at 0.5 +0.5 mg/l of NAA+IBA. On the other hand Isd-20 showed 89% rooting at the concentration of 0.5+0.5 mg/l of IBA+IAA, whereas, Isd-30 and Isd 2/54 showed 88% and 85% at the concentration of 0.25 IBA+ 0.25 IAA mg/l (Table 4). The different

results occurred probably due to genotypic variation among varieties. The other concentrations produced lower percentage of root; less number of root per shoot. The result showed that NAA + IBA and IBA + IAA at 0.2 + 0.25 mg/l and 1.0 + 0.5 mg/l were fair for rooting. Activated charcoal also found suitable for rooting. Medium without activated charcoal turned slightly brown after three weeks that had a negative effect on root formation as well as root growth. These phenomena were not found in the presence of activated charcoal as because it absorb the toxic compounds (Peirik, 1987) and abscisic acid secreted by plant (Johansson, 1983).

Table 4. Combined effect of auxins on root induction.

Conc. of growth regulators (mg/l)	Isd-20			Isd-30			Isd-2/54		
	% shoot produced root	No. roots/shoot (cm±sd)	Ave. length of roots (cm±sd)	% shoot produced root	No. roots per shoot (cm±sd)	Ave. length of roots (cm±sd)	% shoot produced root	No. roots/shoot (cm±sd)	Ave. length roots (cm±sd)
NAA+IBA									
0.1+0.25	-	-	-	-	-	-	-	-	-
0.1+0.50	-	-	-	-	-	-	-	-	-
0.2+0.25	35	3.0±0.1	1.5±0.1	30	3.0±0.1	1.5±0.2	40	3.1±0.3	1.8±0.2
0.2+0.50	40	3.2±0.2	1.3±0.2	45	3.2±0.4	1.7±0.5	45	3.4±0.2	1.3±0.3
0.5+0.25	45	3.0±0.2	1.5±0.4	50	2.8±0.1	1.8±0.3	50	3.3±0.2	1.5±0.5
0.5+0.50	85	3.5±0.5	1.3±0.3	90	3.5±0.2	1.4±0.1	87	3.4±0.5	1.6±0.1
1.0+0.50	25	3.5±0.1	1.4±0.3	-	-	-	25	3.3±0.1	1.5±0.5
1.0+1.0	-	-	-	-	-	-	-	-	-
1.0+1.5	-	-	-	-	-	-	-	-	-
1.5+1.5	-	-	-	-	-	-	-	-	-
IBA+IAA									
0.1+0.25	-	-	-	-	-	-	-	-	-
0.1+0.50	-	-	-	-	-	-	-	-	-
0.2+0.25	45	3.5±0.2	1.3±0.3	40	3.4±0.4	1.8±0.1	40	3.4±0.2	1.7±0.1
0.2+0.50	40	3.5±0.1	1.5±0.4	62	3.4±0.1	1.2±0.3	35	3.5±0.3	1.3±0.2
0.5+0.25	50	3.4±0.2	1.3±0.1	88	3.2±0.1	1.3±0.4	85	3.2±0.5	1.2±0.4
0.5+0.50	89	3.5±0.1	1.3±0.4	60	3.3±0.2	1.5±0.4	50	3.4±0.1	1.4±0.3
1.0+0.50	25	2.3±0.5	1.5±0.5	30	3.5±0.3	1.4±0.5	32	3.3±0.2	1.4±0.5
1.0+1.0	-	-	-	-	-	-	-	-	-
1.0+1.5	-	-	-	-	-	-	-	-	-
1.5+1.5	-	-	-	-	-	-	-	-	-

Establishment of plantlets: After rooting the plantlets were transferred to sun dried soil containing 50% sand in pots. The potted plants were then irrigated adequately, covered with perforated polyethylene bag to maintain high humidity and kept at controlled temperature for 10 to 12 days. Among the transplanted plants 70% were survived in the soil. The rest of the plantlets could not survive due to desiccation, physiological abnormality and microbial contamination. *In vitro* regenerated plants generally show some structure and physiological abnormalities i.e. survivability partially depends on photosynthetic efficiency, state of root growth and good health.

Conclusion

Eighty percent aseptic cultures were obtained when treated with 0.10% HgCl₂ for 10 min. The modified MS medium supplemented with 3.0 mg/l 2,4-D and 10% coconut water produced maximum amount of creamy white callus for Isd-20 and Isd-30 and greenish white callus for Isd-2/54. BA 1.5 + NAA 0.25 mg/l and BA 1.5 + IBA 0.25 mg/l produced highest number of shoots from the callus tissue. The concentration of NAA + IBA and IBA + IAA at 0.2 + 0.25 mg/l and 1.0 + 0.5 mg/l were found suitable for rooting. Activated charcoal also enhanced root formation.

References

- Dutt, N.L. and Rao, S. 1935. Observations on the cytology of sugarcane. *Indian Journal of Agricultural Science*, 3: 37-56.
- Johansson, 1983. Plant Physiology, 49: 397-403. In R.L.M. Pierik 1987. *In vitro culture of higher plants*. Martinus Nijhoff Publishers, (ed.), 1987. Dordrecht, The Netherlands, pp. 1-343.
- Kochhar, S.L., 1998. *Economic botany in the tropics*. Second edition. Macmillan India Ltd. pp. 1-476.
- Latham, D.S., 1974. Regulators in cell division in plant tissues. The cytokinins of coconut milk. *Plant Physiology*, 32: 66-70.

- Li, H.W., Ma, T.H. and Shang, K.C., 1954. Cytological study of sugarcane and its relatives. *Taiwan Sugar*. 1(4): 13-24.
- Li, H.W., Loh, C.S. and Lee, C. L., 1948. Cytological study on sugarcane and its relatives. I. Hybrids between *Saccharum officinarum*, *Miscanthus japonicus* and *Saccharum spontaneum*. *Academic Society of Botany Bulletin*, 2: 147-160.
- Miah, S.M.A., Chowdhuty, M.K.H., Alam, M.R. and Ali, S., 1996. Potential of bud chips as planting materials. *Ann. Rep. Bangladesh Sugarcane Research Institute*. 72 pp.
- Mian, M.A. and Mian, M.A. 1984. *An Introduction to Statistics*. Ideal Library, Dhaka, Bangladesh, 76 pp.
- Muraghige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology*, 15: 473-497.
- Nandan and Singh, 1994. Rapid clonal multiplication of sugarcane through tissue culture. *Plant Tissue Culture*. 4:1-7.
- Pierik, R.L.M., 1987. *In vitro culture of higher plants*. Martinus Nijhoff Publishers. Dordrecht, The Netherlands. pp. 1-343.