Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 3 (6): 666-669, 2004
ISSN 1682-3974

In vitro Micropropagation of Some Important Sugarcane Varieties of Bangladesh

[1]M.A. Mamun, [1]M.B.H. Sikdar, [2]Dipak Kumar Paul, [3]M. Mizanur Rahman and [3]Md. Rezuanul Islam
[1]Biotechnology and Genetic Engineering Discipline Khulna University, Khulna-9208, Bangladesh
[2]Department of Applied Nutrition and Food Science,
[3]Department of Biotechnology, Islamic University, Kushtia-7003, Bangladesh

Abstract: *In vitro* micropropagation of sugarcane variety viz., Isd-28 and Isd-29 have been studied. Nutrient media containing growth regulators enhanced callus induction, shoot differentiation and root formation *in vitro*. For covenant experiment, surface sterilization of the explants from the field grown plants was essential and sterilization with 0.1% $HgCl_2$ for 8 min was satisfactory. In this treatment, 85-90% of the leaf sheath explants was Contamination free. Among the explants cultured for proliferation fortified with 3.0 mg L^{-1} 2, 4-D explants of all the 2 varieties of sugarcane showed the best performance for the callus induction after 16-20 days of culture. The concentration of BA tested 1.5 mg L^{-1} of the cytokinin produced comparatively higher percentage of shoot proliferation. Different concentration of BA mg L^{-1} in combination with different auxin viz., IAA, IBA and NAA were tested for shoot proliferation. Isd-28 and Isd-29 showed best shooting when media were supplemented with BA 1.5 mg L^{-1}+0.5 mg L^{-1} NAA. *In vitro* proliferated shoots were rooted on MS and modified MS media. Best results of rooting ware observed on modified MS medium supplemented with auxins (NAA+IBA) 0.5 mg L^{-1} and 0.5 mg L^{-1} showed fair results. Eighty to ninety percent regenerated plantlets were viable at normal temperature with 85% humidity while transferred sterilized soil.

Key words: Auxins, cytokinin, callus induction, surface sterilization, sugarcane varieties

INTRODUCTION

Using conventional method 10 to 15 years of work is needed to complete a selection cycle and an improved variety can be planted commercially several years later when seedcanes will have been produced in Bangladesh, sugarcane is propagated by settlings. Every year a lot of sugarcane is required as sets. Thus, farmers loss their production and sugar industries also loss sugar. Moreover, due to flood, farmers need to wait for "Jow condition" (optimum soil moisture condition for ploughing) for set sowing. Sugarcane is a major agricultural crop in the tropical and subtropical regions of the world. If the seedlings are produced in a Biotechnology Laboratory as commercial basis, farmers can sow them immediately after the run-off of the floodwater[1] On the other hand, sets use for sowing can be sent to the sugar industries for sugar production time requirement and continued infection by systematic disease are serious problems to multiply an elite genotype of sugarcane in the open field[2]. The technique of plant tissue culture is being routinely used for producing large number of clonal plants by *in vitro* culture of explants from wide range of species throughout the world. It has become now a viable alternative to the conventional clonal propagation methods, therefore, the present experiment was conducted to investigate the following objective: Determination of the most suitable medium compositions for rapid response on callus induction, selection and standardization of medium compositions, growth regulators and other additives for maximizing shoot differentiation, proliferation and growth, selection of suitable auxin, it's concentration and combination for inducing roots *in vitro,* establishment of plantlets and to transfer in the soil for commercial crop production, to observe the variation of *in vitro* grown sugarcane plant for breeding program.

MATERIALS AND METHODS

The young leaf-sheath of two sugarcane varieties viz., Isd-28 and Isd-29 were used as an experimental material. The explants were collected from 2 months old field grown plants, raised from the stem cutting. These explants were surface sterilized with various percentages of mercuric chloride ($HgCl_2$) and inoculated aseptically for

Corresponding Author: Md. Rezuanul Islam, Assistant Professor, Department of Biotechnology, Islamic University, Kushtia 7003, Bangladesh
Tel: +880 62201-5 Ext: 2306 Fax: +880 71 54400 E-mail: rezwangbt@yahoo.com

in vitro regeneration on MS or modified MS medium with different concentrations and combinations of growth regulators.

Different culture media used: Media for callus induction and there multiplication, different strengths of MS media supplemented with mesoinositol as organic compound as well as different concentration of auxin (2,4-D) and coconut milk were used, shoot induction media/Media for shoot differentiation from callus tissue, MS medium supplemented with auxins (NAA, IBA), cytokinins (BA) and coconut water were used for the purpose, media for root formation *in vitro* growing shoot, modified medium supplemented with different concentrations and combinations of auxins (NAA, IBA) and Activated Charcoal (AC) were used for the rooting of the shoots.

RESULTS AND DISCUSSION

Sterilization and culture establishment: The leaf sheath from varieties Isd-28and Isd-29 were treated with $HgCl_2$ for raising aseptic cultures. The treatment with different concentration of mercuric chloride ($HgCl_2$) at different duration was applied. It was observed that cent percent contamination free cultures could be obtained when the leaf sheath explant were treated with 0.1% $HgCl_2$ for 8 to 10 min.

Callus induction: After sterilization, the leaf sheath explants were cultured on MS medium supplemented with different growth regulators. Satisfactory callus formation was noticed only with few treatments. 2,4-D was used in different concentration viz., 0.5, 1.0, 2.5, 3.0, 4.5. Among these treatment, the modified MS medium with only inositol as the organic compound and supplemented with 3 mg L^{-1}, 2,4-D and 10% coconut milk produce maximum amount of regenerative callus from the leaf sheath (Table 1). On this media the explant produced creamy white calli and the callus showed rapid growth and gradually spread on the surface of media after 4-5 weeks of callus induction. The calli were then subcultured of on the freshly prepared media of same composition to increase their volume for conducting experiment on shoot differentiation[3].

Shoot differentiation: The growth regulators only the BA as cytokinin and IBA and NAA as auxins were used. Various concentration of BA viz., 0.1, 0.5, 1.0, 2.5, 3.5 mg L^{-1} and BA+NAA or BA+IBA viz., 0.5+0.1, 0.5+0.25, 0.5+0.5, 1.5+0.1, 1.5+0.25, 1.5+0.5, 2.5+0.1, 2.5+0.25 and 2.5+0.5 mg L^{-1} were used indifferent replication. Different concentrations and combinations of growth regulators in MS media only the combination of 1.5 mg L^{-1} BA+0.5 mg L^{-1} NAA or 1.5 mg L^{-1} BA+0.5 mg L^{-1} IBA were found suitable for regeneration of shoots from the callus tissue (Table 2).

Table 1: Effect of 2, 4-D on MS basal and modified media on callus induction

	Percentage of explant with callus induction			
	MS Basal medium		Modified MS medium	
Concentration of 2, 4-D (mg L^{-1})	Isd 28	Isd 29	Isd 28	Isd 29
0.5	-	-	-	-
1.0	22	20	25	23
2.5	50	58	55	65
3.0	85	89	83	92
4.5	72	82	83	77

Isd 28 and 29 = Sugarcane variety (Ishardi 28 and Ishardi 29)

Rooting of the shoots: Usable shoots were isolated from *in vitro* proliferating cultures. Shoots were placed on modified MS medium supplemented with 0.1-2.0 mg L^{-1} of three different auxins viz., IAA, NAA and IBA. Three types of auxins, IBA and are found to be more effective. Microshoot of Isd-28 and Isd-29 showed better rooting performance. The quinces of root formation for the two varieties were 90 and 87% for NAA; 93 and 95% for IBA, respectively. On the medium with 0.5 mg L^{-1} all the auxins produced the highest number, of roots per shoot (3.12±0.8 and 3.4±0.3 for the auxins NAA+IBA and 3.5±0.1, 3.5±0.03 for the auxins IAA+IBA in Var. Isd-28 and Isd-29, respectively) as shown in Table 3.

Effect of activated charcoal on root: Use of activate charcoal with 0.5 mg L^{-1} IBA+0.5 mg L^{-1} NAA supplemented modified MS rooting medium showed better performance than those rooted on activated charcoal omitted medium.

Establishment of the plantlets: After rooting of the *in vitro* regenerated shoots they were transferred to soil. Before transplantation, the individual plantlets were taken out from the culture media and the roots of the plantlet were washed gel free by continuous flowing of tap water. Then the plantlets were transferred on to the sun-dried soil containing sand in pots. The potted plants were then watered adequately, covered with perforated polythene bag and kept in the growth chamber for 10-12 days. Among the transplanted plants 60-75% were survived and acclimatized successfully on the soil. The rest of the transplants could not survive under the *ex in vitro* due to desiccation and microbial overgrowth. The survival of the plantlets under the *ex in vitro* condition on the soil also influenced by state of root growth and general health of the plantlets. The survival rate of healthy plantlets having

Table 2: Combined effect of auxin and cytokinin on shoot differentiation and proliferation

	Isd-28			Isd-29		
Concentration of growth regulators (mg L^{-1})	Explant produced shoot (%)	No. of shoot/explant (cm±sd)	Average length explant (cm±sd)	Explant produced shoot (%)	No. of shoot/explant (cm±sd)	Average length shoot (cm±sd)
BA+NAA						
0.5+0.10	30	2.00±0.40	3.40±0.50	25	2.70±0.30	3.25±0.30
0.5+0.25	45	3.00±0.30	3.25±0.30	42	2.80±0.50	3.50±0.40
0.5+0.50	48	3.00±0.50	3.30±0.30	52	3.80±0.40	3.80±0.30
1.5+0.10	67	4.25±0.40	4.00±0.40	65	3.30±0.50	3.80±0.80
1.5+0.25	72	5.20±0.30	5.00±0.50	68	4.30±0.30	3.80±0.50
1.5+0.50	90	6.20±0.40	6.80±0.30	87	7.00±0.50	4.12±0.50
2.5+0.10	40	5.00±0.30	5.20±0.50	48	6.10±0.30	3.18±0.30
2.5+0.25	50	4.20±0.30	4.25±0.30	38	4.12±0.30	3.00±0.50
2.5+0.50	52	5.30±0.40	3.80±0.70	58	3.80±0.40	3.12±0.30
BA+IBA						
0.5+0.10	40	2.52±0.30	3.00±0.12	38	3.30±0.50	3.20±0.50
0.5+0.25	62	3.12±0.50	5.12±0.50	58	3.30±0.80	3.80±0.80
0.5+0.50	42	3.20±0.50	3.80±0.30	52	3.20±0.30	3.70±0.20
1.5+0.10	30	3.00±0.30	3.30±0.30	28	3.80±0.30	3.10±0.30
1.5+0.25	50	3.80±0.50	3.40±0.30	62	3.80±0.38	3.80±0.50
1.5+0.50	93	3.80±0.12	3.30±0.50	95	3.10±0.28	4.10±0.50
2.5+0.10	58	3.12±0.80	3.20±0.30	60	3.12±0.80	3.80±0.30
2.5+0.25	38	3.80±0.50	3.30±0.80	37	3.20±0.20	3.20±0.50
2.5+0.50	47	3.10±0.50	3.10±0.20	48	3.10±0.50	3.10±0.30

BA=Cytokinin ; NAA, IBA= Auxin; Isd 28 and 29 = Sugarcane variety (Ishardi 28 and Ishardi 29)

Table 3: Combined effect of auxins on root induction of sugarcane varieties

	Isd-28			Isd-29		
Concentration of growth regulators (mg L^{-1})	Shoot produced root (%)	No. of roots (cm±sd)	Average length of roots (cm±sd)	Shoot produced root (%)	No. of roots (cm±sd)	Average length roots (cm±sd)
0.2+0.25	36	3.02±0.10	1.3±0.5	40	3.2±0.3	1.2±0.2
0.2+0.50	42	3.02±0.20	1.3±0.3	43	3.2±0.5	1.3±0.1
0.5+0.25	68	3.30±0.30	1.8±0.3	68	3.4±0.5	1.4±0.1
0.5+0.50	92	3.12±0.80	1.8±0.8	82	3.4±0.5	1.5±0.5
IAA+IBA						
0.2+0.25	38	3.10±0.20	1.1±0.2	30	3.1±0.1	1.2±0.1
0.2+0.50	45	3.20±0.20	1.2±0.2	38	3.3±0.2	1.3±0.1
0.5+0.25	60	3.50±0.30	1.8±0.3	58	3.2±0.5	1.5±0.2
0.5+0.50	88	3.50±0.10	1.7±0.5	85	3.5±0.1	1.7±0.5

NAA, IBA, IAA= Auxin; Isd 28 and 29 = Sugarcane variety (Ishardi 28 and Ishardi 29)

1-2 cm root length was more than those of plantlets having elongated roots.

Plant tissue culture is an essential plant biotechnology offers novel approach to plant propagation, *in vitro* micromanipulation and biodiversity and germplasm conservation. It has become now an important alternative to conventional propagation and breeding programs for wide range of plant science[4,5]. After surface sterilization with $HgCl_2$ leaf sheath explants were cultured on modified MS medium containing several concentrations of auxins (2,4-D, NAA IBA) viz., 0.5, 2.5, 3.0, 4.5 mg L^{-1} to investigate that the medium callus induction frequency of the leaf sheath explant obtained callus from leaf explant on medium containing 31.7 μm of 2,4-D after 4 weeks[6,7]. Established callus on explant tissue taken from a range of 18 genetically diverse sugarcane cultivars on MS medium containing 13.4 μm of 2,4-D found suitable of 2,4-D (3-5 mg L^{-1}) for callus in Bangladeshi sugarcane varieties[8]. All these studies indicate that sugarcane explant requires higher concentration of 2,4-D for callus induction. In this regard results of the present investigation are consistent with high level of cytokine and low level of auxin was essential for differentiation of adventitious shoot in sugarcane leaf sheath callus[9]. Shoots of *in vitro* proliferating culture were isolated and individually cultured on MS medium supplemented with auxins viz., IBA and NAA for root induction. During the investigation the number of roots per shoot were highly influenced by concentration and type of auxin used. Among the different auxin supplemented media lowest rooting was obtained on half strength MS medium and highest was obtained on

modified half strength MS media. NAA was found to be comparatively more effective than IBA at different concentrations tested, The various concentrations tested 0.5 mg L^{-1} of all the auxin produce highest frequency of root formation. It is also showed that 0.5 mg L^{-1} of auxins with activated charcoal the best result. Sugarcane roots can be developed from plantlets when they are transplanted to modify MS media 0.5 mg L^{-1} IBA or 0.5 mg L^{-1} NAA+0.5 mg L^{-1} IBA[10]. The best root induction was observed MS medium supplemented with 7.4 μm IAA and 4% of sucrose[11] MS or modified MS with 1 mg L^{-1} IBA was suitable for rooting in the experiment.

REFERENCES

1. Saddia, B. and M.S. Shaheen, 1997. Tissue culture of sugarcane genotypes. J. Ani. Plant Sci., 7: 73-78.
2. Nanda and Shing, 1994. Rapid clonal multiplication of sugarcane through tissue culture. Plant Tissue Culture, 4: 1-7.
3. Rangon, V., K. Wichai and A. Supart, 1988. Production of virus-free sugarcane by tissue culture. Kasetsart J. Natl. Sci., 22: 30-36.
4. Pierik, R.L.M., 1987. In: *In vitro* Culture of Higher Plants. Martinus Nijhoff publisher, Dordreeht, pp: 1-342.
5. Rangan, T.S., 1974. Morphogenic investigation on tissue cultures of *Panicum milliaceum* Z. Pflanzen. Physiol., 72: 456-459.
6. Dhamarker, V.S., 1992. Molasses, a source of nutrients of plant tissue culture-a new focus. Bharatiya Sugar, 16: 35-37.
7. Taylor, P.W.J., H.L. Ko., S.W. Adkins, C. Rathus and R.G. Birch, 1992. Establishment of embryogenic callus and high protoplast yielding suspention cultures of sugarcane (*Saccharum* spp. hybrids). Plant Cell, Tissue and Organ Cult., 28: 69-78.
8. Begum, S., L. Hakim and M.A. Aziam, 1995. Efficient regeneration of plants from leaf base callus in Sugarcane. Plant Tissue Culture, 5: 1-5.
9. Skoog, F. and C.O. Miller, 1957. Chemical regulation of growth and organ formations in plant tissue culture *in vitro*. Symp. Soc. Exp. Bill., 11: 118-131.
10. Straus, J. and C.D. Larue, 1954. Maize endosperm tissue grown *in vitro* I. Culture requirements. Am. J. Bot., 41: 687-694.
11. Gill, DV., M.A. Gonzalez, P.J. Perez and O.F.L. Herrera, 1989. Improvement of Routine Induction in Sugarcane (hybrid *Saccharum* spp.) obtained by micro propagation. Centro-Agricola, 16: 17-23.