

Research Article

Effect of Auxin and Cytokinin for Shoot Regeneration from *In vitro* Culture of Sugarcane

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Abstract

The present investigation was carried out at Tissue Culture Laboratory of Breeding Division of Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna on *in vitro* shoot regeneration of sugarcane callus during the period of 2011. The regeneration of sugarcane varieties with supplementation of cytokinin (1mg/l of BA) and auxin IBA and IAA 0.25mg/l, 0.50mg/l and 0.75 mg/l respectively, were used in sugarcane on defined MS media for shoot induction. The shortest days to shoot initiation and the highest number of shoot per culture were found in Isd 40. The highest shoot length was observed in Isd 37. The concentration of 1mg/l of BA and 0.50 mg/l of IBA showed the highest number shoots per culture and the highest shoot length. The combination of 1.0 mg/l BA and 0.5 mg/l of IBA produced the maximum number of shoots per culture and shoot length was noticed in Isd 40 and Isd 37 respectively. Between two types of auxin, IBA was found more effective than IAA at different concentrations tested for producing shoots.

INTRODUCTION

Sugarcane is one of the economically important food-cum cash crops and widely cultivated in tropical subtropical of the world for sugar and bio-ethanol production in a total of 19.4 million hectares as a single crop [1,2]. The present requirement of sugar and gur in the country is 1.0 million and 0.8 million tons, respectively. In Bangladesh sugarcane occupies nearly 0.19 million hectares of land only 1.70 % of the total cultivated land under wide ranges of agro-ecological condition [3] and average yield of cane is 45 t ha⁻¹ under rain fed condition and average recovery of sugar is around 8.3 to 8.5 per cent. In order to meet the demands it is urgent to increase cane productivity without the expansion of area.

Plant tissue culture technique would be a better alternative for improving quality and production [4]. Micro-propagation method is an effective method for rapid propagation of sugarcane. 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. Tissue culture techniques play an important role in the genetic improvement of vegetatively propagated crops like sugarcane [5-7]. The *in vitro* plant regeneration from callus capable of producing somaclonal variants for different traits like high yield, more sugar recovery, disease resistance, drought tolerance and early maturity etc.

Rapid callus formation has been obtained mostly from young leaf sheath [8,9]. To promote regeneration, callus was transferred to medium with different growth hormones [10]. This technique has been developed as a breeding tool for improving the quality and production of vegetatively propagated crops such as sugarcane [12]. Regeneration potential was specific and genotype dependent phenomenon. The present study is the regeneration potential of sugarcane varieties with supplementation of cytokinin (BA) and auxin (IBA and IAA) in sugarcane on defined MS media for shoot induction.

MATERIALS AND METHODS

Five hundred callus of five sugarcane varieties Viz: Isd 2/54, LJ-C, Isd 17, Isd 37 and Isd 40 were used as experimental materials in the study. The developed callus was aseptically transferred into fresh medium containing the different hormonal treatments (viz., BA 1.0 and IBA and IAA 0.25, 0.50 and 0.75) mg/l for proliferation and development of shoots. All these operations were carried out under aseptic conditions and cultures were incubated at 28 ± 2°C with a 16 hours photoperiod. To maintain and ensure aseptic condition precautions were taken in every step of works. All inoculation and aseptic manipulation were carried out by using a laminar air flow cabinet. Hands were properly washed with soap before starting work in laminar airflow cabinet. During the operation hands were rubbed with 70% ethyl alcohol frequency with cotton and wiped cabinet base for maintaining clean condition. Successful shoot formation (Figure 1,2) became

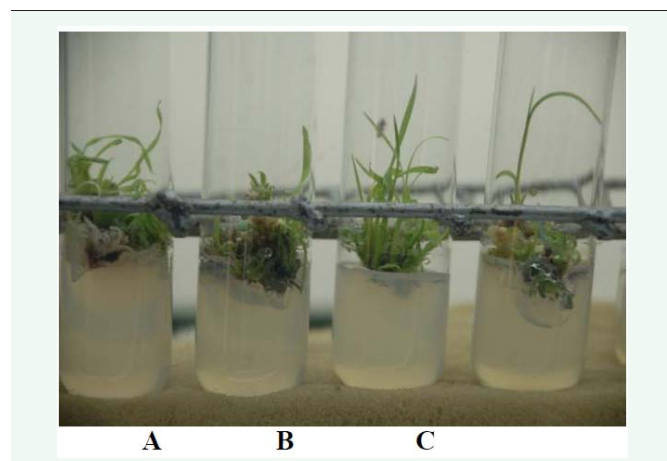


Figure 1 Shoot formation on shooting media of BA1.0 mg/l + IBA0.50 A) Isd 2-54, B) LJ-C, C) Isd 17.



Figure 2 Vigorous shoot formation on shooting media of BA1.0 mg/l + IBA0.50 mg/l A) Isd 37 and B) Isd 40.

evident when small green fresh leaves began to emerge. It is the first sign of regeneration. These tiny leaves when developed in their actual shape were sub cultured in fresh medium containing the best combination of BA and IAA at different concentrations showing better performance. Subculture was carried out regularly at an interval of 2-3 weeks. Data were recorded on days to shoot initiation, number of shoots per culture and shoot length (cm). The collected data were analyzed statistically following the analysis of variance (ANOVA) technique and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C [11].

RESULTS AND DISCUSSION

Varietal response to application of BA and IBA on shoot regeneration

Varietal response to media supplemented with cytokinin and auxin (BA and IBA) on shoot initiation from culture of sugarcane are shown in (Table 1).

The days to shoot initiation ranged from 11.67 to 14.00 which showed significant variation among the varieties (Table 1). The highest days to shoot initiation (14.0) were found in LJ-C. The

early days to shoot initiation (11.67) were found in Isd 40. The maximum number of shoot per culture (3.95) and the minimum shoot length (1.51cm) was exhibited in Isd 40. The lowest number of shoot per culture (3.02) and the highest shoot length (2.58cm) was observed in Isd 37. The finding of the present study was agreement with the findings of Alam et al., for sugarcane variety Isd 18.

Hormonal effect of BA and IBA on shoot initiation

Effects of different concentrations of hormones (BA and IBA) on shoot initiation from callus are shown in Table 2. The shortest days to shoots initiation (10.40) were found in concentration of 1mg/l of BA and 0.25mg/l of IBA which is always suitable for shoot induction. Different concentrations of BA and IBA combination, 1.0 mg/l BA and 0.5 mg/l of IBA producing the highest number of shoot per culture (5.48) and the highest shoot length (2.50cm). The doses of 1mg/l of BA and 0.75 mg/l of IBA showed the minimum number of shoot per culture (2.13) and shoot length (1.23cm). The finding of the present study was agreement with the findings of [12,13,4] for sugarcane variety Isd 18 at the combination of 1.0 mg/l BA + 0.5 mg/l IBA. It seemed that the number of shoots per culture and shoot length was intensively related.

Interaction effect of varieties and doses of hormone (BA and IBA)

1 mg/l of BA with different concentration of IBA (0.25, 0.50 and 0.75 mg/l) was used for shoot initiation, number of shoots per culture and shoot length. Results of interaction effect of variety and doses of hormones (cytokinin and auxin) on regeneration of shoot from callus of sugarcane are shown in (Table 3).

The days to shoot initiation ranged from 9.0 to 17.0 which caused significant variation among the concentrations of BA and IBA (Table 3). The early days to shoots initiation (9.0) was found in Isd 2-54, Isd 17 and Isd 40 on shooting media supplemented with 1mg /l of BA and 0.25 mg/l of IBA.

Table 1: Varietal response to application of cytokinin and auxin (BA and IBA) on shoot initiation from culture of sugarcane.

Varieties	Days to shoot initiation	No. of shoot / culture	Shoot length (cm)
Isd 2-54	12.00	3.73	1.55
LJ-C	14.00	3.17	1.61
Isd 17	12.67	3.22	1.56
Isd 37	13.33	3.02	2.58
Isd 40	11.67	3.95	1.51
LSD (5%)	0.824	0.129	0.410

Table 2: Effects of different concentrations of hormones (BA and IBA) on shoot initiation from callus of sugarcane.

Cytokinin and auxin (mg/l)	Days to shoot initiation	No. of shoot / culture	Shoot length (cm)
BA1.0 + IBA 0.25	10.40	2.65	1.56
BA1.0 + IBA 0.50	12.40	5.48	2.50
BA1.0 + IBA 0.75	15.40	2.13	1.23
LSD (5%)	0.638	0.100	0.318

The hormonal concentration of 1.0 mg/l BA and 0.5 mg/l of IBA produced the highest number of shoots per culture (6.73) and the highest shoot length (3.71cm) were noticed in Isd 40 and Isd 37 respectively. Among the varieties, the highest number of shoot per culture (2.46) and the highest shoot length (1.84cm) were found in Isd 2-54 and Isd 17 respectively. The lowest number of shoot per culture (1.66) and shoot length (1.52 cm) were showed in Isd 17 and Isd 40 respectively. The result of the present study was comparable to those report by [12,13,4] for sugarcane variety Isd 18 at the combination of 1.0 mg/l BA + 0.5 mg/l IBA. for sugarcane variety Isd 18 at the combination of 1.0 mg/l BA and 0.50 mg/l of IBA.

Varietal response to media supplemented with different cytokinin and auxin (BA and IAA) on shoot initiation from *in vitro* callus culture of sugarcane are shown in (Table 4).

The days to shoot initiation ranged from 13.33 to 15.00 which showed significant variation among the varieties (Table 4). The highest days to shoot initiation (15) were found in variety Isd 37. The shortest days to shoot initiation (13.33) were found in Isd 2-54, LJ-C and Isd 40. Among the varieties, the highest number of shoots per culture (2.46) and the highest shoot length (1.84cm) were found in Isd 2-54 and Isd 17 respectively. The lowest number of shoots per culture (1.66) and the highest shoot length (1.52cm) were observed in Isd 17 and Isd 40 respectively. Similar response was also obtained by [12] for sugarcane variety Isd 18.

Hormonal effect of BA and IAA on shoot initiation

One mg/l of BA with different concentration of IAA (0.25, 0.50 and 0.75mg/l) was used for shoot initiation. Effects of different concentrations of hormones (BA and IAA) on shoot initiation from *in vitro* callus culture of sugarcane are shown in (Table 5).

Table 3: Interaction effect of variety and hormones (cytokinin and auxin) for regeneration of shoot from callus of sugarcane.

Variety and doses of (cytokinin and auxin) hormone (mg/l)	Days to shoot initiation	No. of shoot / culture	Shoot length (cm)
Isd 2-54 × BA1.0+ IBA0.25	9.0	2.60	1.57
× BA1.0+ IBA0.50	12.0	6.13	1.93
× BA1.0+ IBA0.75	15.0	2.46	1.16
LJ-C × BA1.0+ IBA0.25	15.0	2.40	1.45
× BA1.0+ IBA0.50	12.0	5.60	2.32
× BA1.0+ IBA0.75	15.0	1.53	1.06
Isd 17 × BA1.0+ IBA0.25	9.0	2.80	1.12
× BA1.0+ IBA0.50	13.0	4.60	2.53
× BA1.0+ IBA0.75	16.0	2.26	1.03
Isd 37 × BA1.0+ IBA0.25	10.0	2.73	2.13
× BA1.0+ IBA0.50	13.0	4.33	3.71
× BA1.0+ IBA0.75	17.0	2.00	1.91
Isd 40 × BA1.0+ IBA0.25	9.0	2.73	1.52
× BA1.0+ IBA0.50	12.0	6.73	2.03
× BA1.0+ IBA0.75	14.0	2.40	1.00
LSD (5%)	14.28	0.223	0.711

Table 4: Varietal response to application of cytokinin and auxin (BA and IAA) for shoot initiation from in vitro callus culture of sugarcane.

Varieties	Days to shoot initiation	No. of shoots / culture	Shoot length (cm)
Isd 2-54	13.33	2.46	1.81
LJ-C	13.33	2.24	1.77
Isd 17	14.00	1.66	1.84
Isd 37	15.00	1.80	1.83
Isd 40	13.33	2.00	1.52
LSD (5%)	0.745	0.117	0.096

Table 5: Effects of different concentrations of hormones (BA and IAA) for shoot initiation from in vitro callus culture of sugarcane.

Doses of hormone (mg/l)	Days to shoot initiation	No. of shoot / culture	Shoot length (cm)
BA1.0 + IAA 0.25	10.40	1.97	1.52
BA1.0 + IAA 0.50	12.40	2.61	2.37
BA1.0 + IAA 0.75	15.40	1.45	1.37
LSD (5%)	0.638	0.031	0.074

Significant variation among days to shoot initiation for different combination and combination of BA and NAA was observed (Table 5). The minimum days to shoots initiation (10.20) was found in concentration of 1 mg/l of BA and 0.25 mg/l of IAA. The doses of 1mg/l of BA and 0.50mg/l of IAA showed the highest number of shoot per culture (2.61) and the highest shoot length (2.37cm). The concentration of 1 mg/l of BA and 0.75 mg/l of IAA produced the lowest number of shoot per culture (1.45) and shoot length (1.37cm). Our results supported by [12] for sugarcane variety Isd 18 at the combination of 1.0 mg/l BA + 0.5 mg/l IAA.

Interaction effect of varieties and doses of BA and IAA on regeneration of shoot

Days to shoot initiation, number of shoot per culture and shoot length were found to vary due to interaction effect of variety and hormone. One mg/l of BA with different concentrations of IAA (0.25, 0.50 and 0.75) was used for shoot initiation, number of shoot per culture and shoot length. Results of interaction effect of variety and doses of hormones (cytokinin and auxin) on regeneration of shoot from callus of sugarcane are shown in (Table 6).

The doses of 1mg/l of BA and 0.50mg/l of IAA produced the highest number of shoot per culture (23.66) and the highest shoot length (2.81cm). The combination of 1 mg/l of BA and 0.25 mg/l of IAA showed the lowest days to shoot initiation (9.0) and the lowest number of shoot per culture (1.06). The findings of the present study were agreement with the findings of [12] for sugarcane variety Isd 18 at the combination of 1.0 mg/l BA and 0.5 mg/l IAA.

CONCLUSIONS

1mg/l of BA with different concentration (0.25, 0.50 and 0.75 mg/l) of IBA and IAA were used in the experiment clearly revealed that 1.0 mg/l of BA and 0.5mg/l of IBA producing the

Table 6: Interaction effect of variety and hormones (cytokinin and auxin) on regeneration of shoot from callus of sugarcane.

Variety and hormones (cytokinin and auxin)	Days to shoot initiation	No. of shoot /culture	Shoot length (cm)
Isd 2-54 × BA1.0+ IAA0.25	10.00	2.33	1.46
× BA1.0+ IAA0.50	14.00	3.60	2.81
× BA1.0+ IAA0.75	16.00	1.46	1.16
LJ-C × BA1.0+ IAA0.25	9.00	2.00	1.38
× BA1.0+ IAA0.50	14.00	3.22	2.68
× BA1.0+ IAA0.75	17.00	1.20	1.27
Isd 17 × BA1.0+ IAA0.25	10.0	1.73	1.68
× BA1.0+ IAA0.50	15.00	2.00	2.35
× BA1.0+ IAA0.75	17.00	1.26	1.50
Isd 37 × BA1.0+ IAA0.25	12.00	1.73	1.76
× BA1.0+ IAA0.50	15.00	2.06	2.15
× BA1.0+ IAA0.75	18.00	1.60	1.60
Isd 40 × BA1.0+ IAA0.25	10.00	1.06	1.33
× BA1.0+ IAA0.50	14.00	2.20	1.88
× BA1.0+ IAA0.75	16.00	1.33	1.34
LSD (5%)	1.292	0.204	0.166

highest number shoots per culture and the highest shoot length which showed in the variety Isd 40 and Isd 37 respectively. But IAA is not better response in maximum number of shoots per culture and shoot length.

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