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# *In vitro* Clonal Propagation of Sugarcane (*Saccharum officinarum*) Variety Isd 31

M.Z. Karim, R. Alam , R. Baksha, S.K. Paul, M.A. Hossain and A.B.M. Mafizur Rahman
Biotechnology Laboratory, Bangladesh Sugarcane Research Institute, Ishurdi-6620, Pabna, Bangladesh

**Abstract:** Leaf sheath were used as explants to induce callus on modified MS media supplemented with 2, 4-D as growth regulators. Different concentrations of BAP, IBA, NAA and IAA were used to regenerate shoots and a concentration of 1.0 mg/l BAP and 0.5mg/l IBA was found superior in the optimum production of multiple shoots. NAA (5mg/l) showed best performance in the production of roots. The plantlets were successfully transferred to soil with 70 percent survivability.

**Key words:** *In vitro*, clonal propagation, callus, sugarcane leaf sheath

## Introduction

Sugarcane is a member of the Gramineae family and belongs to the genus *Saccharum*. It is an important cash-cum industrial crop of Bangladesh. Sugarcane is emerging as one of the most important crop plants because of its ability to produce cheap food in the form of sugar and gur that lend itself for the production of energy and many by-products, all of which are of great economic value. In Bangladesh sugarcane yield levels found decreasing recently. Using conventional method usually 10-15 years of work is needed to complete a selection cycle and an improved variety can be planted commercially. To meet the future need of our sugar industry it is urgent to increase cane productivity without further area expansion. Production of pathogen free seeds and rapid multiplication of improved varieties assumes considerable importance. During the last two decades the techniques of plant tissue culture have been developed as a new and powerful tool for crop improvement (Carlson, 1975; Razdan and Cocking, 1981). Micro propagation of sugarcane may be used to produce a large number of elite plantlets within a short time period. The present study was undertaken to establish a protocol for large-scale clonal propagation of Isd 31 elite sugarcane variety of Bangladesh through *in vitro* culture.

## Materials and Methods

The experiment was conducted at Biotechnology Laboratory in Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during the period of 2000-2001. Leaf sheath explant of variety Isd-31 was collected from 3-5 months old field grown plants at BSRI field. The explants were treated with savlon for 6 minutes and washed thoroughly with sterile distilled water. For surface sterilization the material was taken under laminar air flow cabinet and disinfestation was done with 0.1% $HgCl_2$ for different duration of time. The explants were then aseptically cultured on modified MS medium (Murashige and Skoog, 1962) as recommended by Heinz and Mee (1969) supplemented with 3.0 mg/l 2,4-D and coconut water 10% for callus induction. For rooting MS (strength both of organic and inorganic salts) medium supplemented with different concentrations of auxin was used. After 3-4 weeks, culture calli were transferred to MS medium supplemented with different combinations and concentrations of cytokinin and auxin for shoot formation. General composition of all culture media were 3% sucrose, 0.6% agar, pH was adjusted to 5.7 before addition of agar and then autoclaved at 1-2 kg/cm$^2$ for 15 minutes at 121°C. All the cultures were incubated at 25± 2°C and kept under 14 hours photoperiod of fluorescent tube light.

## Results and Discussion

Five different concentrations of 2,4-D ( 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) along with coconut milk on MS media were used to initiate callus. In the process of callusing the callus tissue secreted polyphenols into the medium as a result growth of the calli slowed down and calli could not survive if grown on the same medium for more than 6 weeks. By rapid transfers of the callus to the fresh medium at 30 days of interval this problem could be overcome.

Table 1: Effect of different concentrations of 2, 4-D on callus initiation from the leaf sheath explant of sugarcane var. Isd 31.There were 20 explants in each treatment and data were recorded after 3-4 weeks of culture on MS medium+ 10% (v/v) coconut milk.

| Concentrations of 2, 4-D (mg/l) | No. of explants showed callusing | % of explants with callus initiation |
|---|---|---|
| 0.5 | 4 | 20 |
| 1.0 | 8 | 40 |
| 2.0 | 11 | 55 |
| 3.0 | 14 | 70 |
| 4.0 | 7 | 35 |

Poor callusing = 20-50% ; Considerable callusing = 51-85%; Intensive callusing = 86-100%

Table 2: Effect of three different concentrations of BAP along with auxin (0.5mg/l) NAA/IBA/IAA on regeneration & shoot growth. Data were recorded after 4-6 weeks of culture

| Hormonal concentration (mg/l) | No. of shoots/ culture | No of usable shoots/culture | Shoot length (cm) |
|---|---|---|---|
| $BA_{0.5}$+ $IBA_{0.5}$ | 12 | 9 | 3.70 |
| $BA_1$+ $IBA_{0.5}$ | 19 | 13 | 4.95 |
| $BA_2$+ $IBA_{0.5}$ | 11 | 8 | 3.65 |
| $BA_{0.5}$+ $NAA_{0.5}$ | 10 | 4 | 2.50 |
| $BA_1$+ $NAA_{0.5}$ | 12 | 9 | 3.66 |
| $BA_2$+ $NAA_{0.5}$ | 9 | 4 | 3.02 |
| $BA_{0.5}$+ $IAA_{0.5}$ | 7 | 3 | 2.61 |
| $BA_1$+ $IAA_{0.5}$ | 4 | 2 | 2.70 |
| $BA_2$+ $IAA_{0.5}$ | 3 | 1 | 2.05 |

Table 3: Effect of different concentration of auxins for root formation from the *in vitro* plantlet. .Data were recorded after 4-6 weeks of culture.

| Media | Hormonal concentration (mg/l) | | No. of roots /explant | Root length (cm) |
|---|---|---|---|---|
| $MS_1$ | IBA | 1.0 | 4 | 0.65 |
| | | 3.0 | 9 | 0.69 |
| | | 5.0 | 13 | 0.90 |
| | | 7.0 | 12 | 0.85 |
| | NAA | 1.0 | 5 | 0.49 |
| | | 3.0 | 13 | 0.50 |
| | | 5.0 | 17 | 0.95 |
| | | 7.0 | 8 | 0.87 |
| | IAA | 1.0 | 3 | 0.40 |
| | | 3.0 | 4 | 0.43 |
| | | 5.0 | 5 | 0.42 |
| | | 7.0 | 2 | 0.65 |
| $MS_2$ | IBA | 1.0 | 5 | 0.35 |
| | | 3.0 | 11 | 0.42 |
| | | 5.0 | 16 | 0.65 |
| | | 7.0 | 9 | 0.80 |
| | NAA | 1.0 | 8 | 0.45 |
| | | 3.0 | 15 | 0.52 |
| | | 5.0 | 21 | 0.62 |
| | | 7.0 | 12 | 0.67 |
| | IAA | 1.0 | 4 | 0.39 |
| | | 3.0 | 6 | 0.40 |
| | | 5.0 | 7 | 0.31 |
| | | 7.0 | 3 | 0.33 |

$MS_1$= ½ strength of Murashige & Skoog's medium.
$MS_2$= $^1/_2$ strength both of organic and inorganic salts of MS medium.

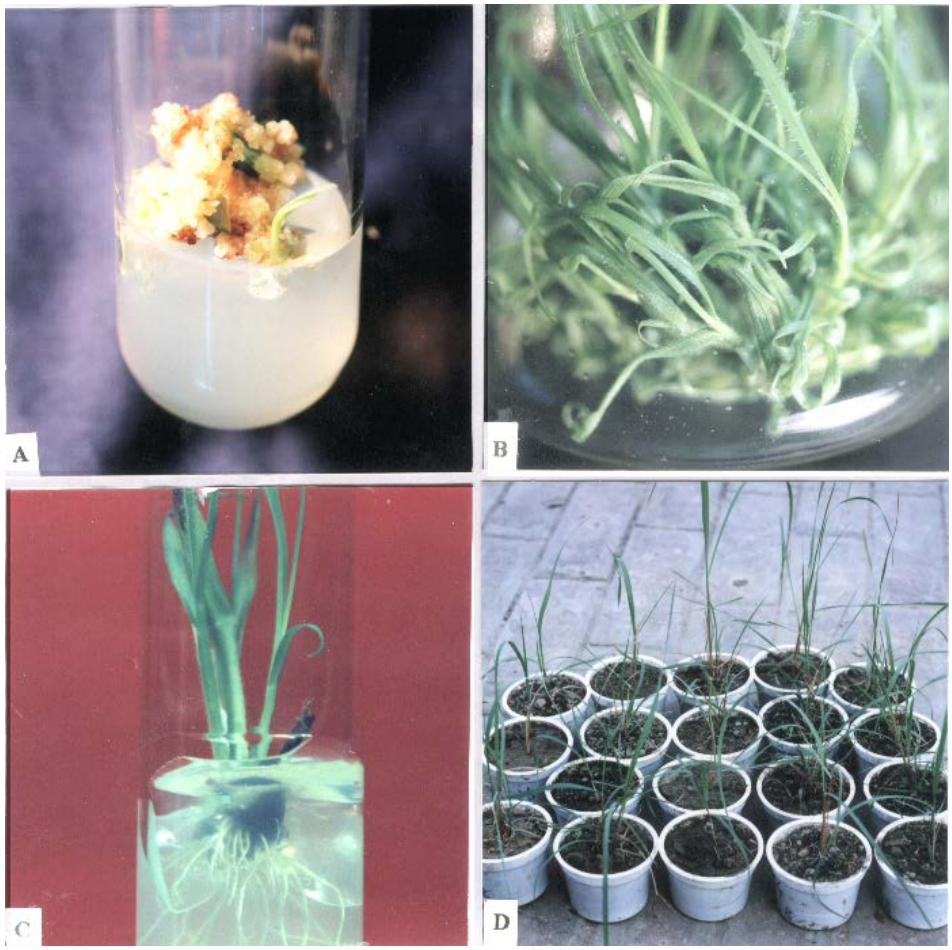


Fig. 1: Plantlet regeneration from callus in sugarcane. A: Callus induction and development from leaf sheath explants on MS medium supplemented with 3 mg/l 2,4-D after 3 weeks of culture. B: Shoot multiplication on MS medium supplemented with 1 mg/l BAP + 0.5 mg/l IBA C: Root formation in regenerated shoot in ½ strength MS+ 5.0mg/l NAA. D: Regenerated plantlets established in the soil.

Among different concentrations of 2,4-D 3.0mg/l produced the maximum amount of regenerative callus from the leaf sheath explant (Table 1). These findings collaborated with Barba *et al.* (1977) reports on callus formation when medium was supplemented with 2,4-D ranging from 0.5-5.0mg/l for sugarcane. Modified MS medium supplemented with 2,4-D for callus induction of sugarcane was also reported by Islam *et al.* (1982) and Hossain *et al.* (1996). Taylor *et al.* (1992) established callus on leaf explant tissue taken from a range of 18 genetically diverse sugarcane cultivars by culture on MS medium containing 13.4M 2,4-D. Begum *et al.* (1995) found that 3-5mg/l 2,4-D produced high percentage of callus in Bangladeshi sugarcane varieties (viz., Nagarbari, L.Jeba, Isd-16, Isd-20 and Clone I.123). The combinations of phytohormones often determine the course of morphogenesis i.e. shoot organogenesis and embryogenesis (Yutaka *et al.*, 1998). For shoot regeneration and multiplication three different concentrations and combinations of BAP along with auxin (0.5mg/l) NAA/IBA/IAA were tested. The best results on shoot formation and multiplication were found with 1.0 mg/l BAP + IBA 0.5 mg/l (Table 2). In this combination, the highest number of shoots per culture was 19. The result of this study is inconsistent with the hypothesis of Skoog and Miller (1957). Larkin (1982) used BAP combined with IAA in modified MS medium for shoot induction in sugarcane. He found that shoot primordia induced, elongated and formation of shoots were profuse in the modified MS medium supplemented with BAP 1.0 mg/l + IAA 0.5 mg/l. High level of cytokinin and a low level of auxin were essential for regeneration of shoots. In the regeneration of shoots BAP+ IBA was more effective than the combinations of BAP+ NAA and BAP+ IAA. Islam *et al.* (1982) also reported the positive effects of BAP and IBA combination on shoot formation in sugarcane. Microshoots of the *in vitro* grown proliferating culture were isolated and individually cultured on MS medium and modified MS medium ($MMS_1$ and $MMS_2$) supplemented with auxin viz., NAA, IBA and IAA for root formation. Among these auxins NAA (5mg/l) showed best performance (21 roots/shoot), IBA showed effective result but IAA was found of low performance (Table 3). Nand and Singh (1994) reported that root can be easily induced on cultured shoots by their transfer to another medium with or without NAA, where optimal growth was observed with a half strength modified MS medium. Healthy and well established rooted plantlets were transferred to soil for acclimatization (Fig. 1A-D). Among the regenerates transplanted 70% of them survived and acclimatized successfully on the soil. Therefore, *in vitro* propagation of sugarcane may be more beneficial for inducing characteristic changes with the view to select superior somaclonal variations.

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